ADRENOCEPTORS WITHIN THE CENTRAL NERVOUS SYSTEM CONCERNED IN THE MODIFICATION OF ARTERIAL BLOOD PRESSURE AND HEART RATE

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SUMMARY.

Initial experiments using hypertensive rats indicated that the antihypertensive effect of α-methyldopa given either systemically or intracerebroventricularly (icv) was mediated by the formation of α-methylnoradrenaline in the central nervous system.

Blood pressure and heart rate responses were recorded after icv infusions of α- and β-adrenoceptor agonists and antagonists in conscious, unrestrained, normotensive cats, rabbits and rats.

In the majority of cats used, icv infusions of α-adrenoceptor agonists (noradrenaline, adrenaline in the presence of propranolol, phenylephrine, α-methylnoradrenaline, methoxamine and clonidine) produced hypotension and bradycardia which were abolished by icv phentolamine. The α-adrenoceptor antagonist itself generally induced hypertension and tachycardia. It was found that the clinically used antihypertensive agents, α-methyldopa and clonidine, produced hypotension and bradycardia by stimulating central α-adrenoceptors.

A comparison was made between the central effects of noradrenaline and α-methylnoradrenaline in the cat with respect to the false transmitter theory. Icv α-methylnoradrenaline induced similar sized responses but of a longer duration than those of icv noradrenaline.

α-Adrenoceptor stimulation by clonidine led to bradycardia resulting from a decreased efferent sympathetic

and increased efferent vagal outflow to the heart.

In 4 cats icv noradrenaline produced hypertension and bradycardia, these effects also being abolished by icv phentolamine. Although both blood pressure responses of noradrenaline were inhibited by icv phentolamine the receptors involved may be slightly different as clonidine always produced hypotension in the cats that responded with hypertension to noradrenaline.

Opposite effects to the α-agonists were generally observed to icv administrations of β-adrenoceptor agonists (isoprenaline, adrenaline given after phentolamine, salbutamol and isoetharine) in conscious cats, rabbits and rats. Tachycardia was always obtained and was usually accompanied by hypertension although hypotension, biphasic responses and an absence of blood pressure effects were occasionally observed. All these responses were inhibited by icv infusions of β-adrenoceptor blocking agents.

Peripheral adrenergic neuron blockade with bethanidine completely blocked the icv effects of β-agonists indicating that a change in efferent vagal tone played no part in the tachycardia and adrenal catecholamine secretion no part in the pressure responses.

Icv dopamine always induced large pressor effects with small tachycardias which were inhibited by central β-adrenoceptor or dopamine receptor blockade. In 4 cats, the initial stimulant effects were followed by hypotension and bradycardia. These latter effects were due to α-adrenoceptor stimulation as they were absent after icv

phentolamine and were probably caused by noradrenaline formation since disulfiram, a dopamine β-hydroxylase inhibitor, prevented the depressant effects of dopamine.

In a large number of conscious cats and rabbits, 7 clinically useful β-blocking agents infused icv induced potent hypotensive effects with bradycardias. Results were obtained which indicated that these depressant effects were produced by central β-adrenoceptor blockade. Thus, it was demonstrated that it was possible for these compounds to exert centrally mediated cardiovascular depressant effects once introduced into the brain and that the central nervous system may provide a site of action, in part at least, for the mediation of their antihypertensive effects.

All the β-blockers with the exception of ICI 66082 produced initial rises in blood pressure and heart rate. These effects were investigated and found to be due to either local anaesthetic or intrinsic β-sympathomimetic activity or both and were blocked by central β-blockade.

The central effects of parasympathetic agonists were investigated. Icv carbachol was the most potent agonist and produced pronounced pressor effects and tachycardias which were inhibited by both nicotinic and muscarinic blocking agents and also β-blocking and adrenergic neuron blocking agents given icv. The possibility of a link between central cholinergic and sympathetic mechanisms involved in cardiovascular control is discussed.

It was concluded that the experimental model used in this project (i.e. the conscious cat) would be an excellent

one in the screening of future antihypertensive agents (e.g. β-blockers), especially those which act centrally. The results of the putative central neurotransmitters given icv to conscious cats are discussed in the light of their possible roles in central cardiovascular control. The mechanisms by which α-methyldopa, clonidine and α-adrenoceptor blockers produced their centrally mediated effects are also discussed.

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HISTORICAL INTRODUCTION

HYPERTENSION: Recognition as a disease.

Research into human hypertension began in the late 1820's and 1830's as a result of Richard Bright's investigation into the association between albuminuria and dropsy (Bright, 1836), which developed the broad concept of systemic vascular disease. In 1872, Gull & Sutton hypothesised that a vascular disease, which they called "arterio-capillary fibrosis", was the "primary and essential condition in the morbid state called chronic Bright's disease with contracted kidneys". Mahomed (1879, 1881) reported the first systematic investigation of blood pressure during life. He observed that a raised blood pressure may either precede or follow renal disease and he proposed the idea that "what is the cause in one case may be the result in another".

Von Basch (1893), Huchard (1893) and Allbutt (1895) independantly reported that hypertension may occur without variable course. Von Basch termed the condition as "latent arteriosclerosis" without renal involvement. Huchard indicated that "presclerosis" preceded arteriosclerosis and Allbutt presented his idea of hypertension as "senile plethora". In 1915, Allbutt used terms such as "hyperpiesia" and "hyperpiesis" to distinguish a hypertensive disease from the hypertension resulting from nephritis. From the publication by Frank (1911) the term "essential hypertension" has evolved.

In 1914, Volhard & Fahr classified essential hypertension into benign and malignant phases. Keith, Wagener

and Kernohan (1928) precisely described the various stages of hypertensive disease based on changes in the optic fundi. In malignant hypertension, one of the major findings was papilloedema and they correlated this form of hypertension with an early death.

Early studies on hypertension were hampered by the fact that only crude blood pressure measurements could be made. In 1856, Faivre was first to measure the arterial blood pressure of man by directly cannulating an artery with a catheter attached to a calibrated mercury manometer. Besides the problems encountered with this technique, it was found unsuitable for studying a large number of individuals. An indirect method of arterial blood pressure measurement was developed by Riva-Rocci in 1896. Development of his device has led to the modern day sphygmomanometer. Korotkov in 1905, discovered a series of sounds from the brachial artery as the raised pressure in an occluding cuff was allowed to slowly fall on deflation. By detecting these Korotkov sounds, quick, reliable and accurate systolic and diastolic pressure readings can be taken. Using this principle, Friedman & Freed (1949) measured the indirect blood pressure of the rat by inflating and deflating a cuff placed around its tail and monitoring the pulse in the caudal artery, distal to the cuff. Developments and modifications of this method allow quick industrial screening of potential antihypertensive compounds.

The initial studies on hypertension misled clinicians because they believed that an elevated pressure forced blood

through thickened arteries and arterioles, thus ensuring adequate perfusion of the tissue. Consequently, they assumed that reduction of the blood pressure could only be detrimental to the hypertensive individual.

At least since Janeways's clinical investigation into hypertension, it has been known that persons with elevated blood pressure tend to die prematurely (Janeway, 1913). The most common cause of death in these persons is heart disease (including mainly coronary artery disease, congestive heart failure and left ventricular failure); the next most common cause is apoplexy, due to cerebral thrombosis and haemorrhage, and uraemia is the least common cause.

Despite the contention of Pickering (1960) that essential hypertension is only a deviation from the norm, there is sufficient evidence to indicate that essential hypertension constitutes a true clinical entity (Platt, 1960). Cases of secondary hypertension (i.e. hypertension of a known origin, see later) account for a very small percentage of the hypertensive population and the disorder designated essential hypertension includes the majority of patients suffering from a disease characterised by specific haemodynamic and pathological features which follow a definite course. Any doubt as to essential hypertension being a specific disease is dispelled by the vast number of experimental reports in which a disorder with the same haemodynamic and pathological findings and the same clinical course can be induced in animals normally free from the disease (Grollman, 1963). Hence, essential or primary

hypertension is accepted as a clinical entity despite the virtual impossibility of establishing a diagnosis of the disease in its earliest stages before morphological alterations and an unequivocal chronic rise in diastolic pressure are established.

The distinction between normotension and hypertension is now known to be quantitative and not qualitative. Arterial pressure is a quantity and the dangerous consequences that are related to its elevation are quantitative; the higher the pressure, the worse the prognosis.

Arterial blood pressure is influenced by anger, fear, pain, temperature and exercise. Richardson, Honour, Fenton Stott & Pickering (1964) observed large fluctuations in blood pressure throughout day and night. Hence, as there is a large variation of blood pressures throughout the population and so many factors cause blood pressure to fluctuate, the problem arises at what point is a person classified as a hypertensive individual and at what point does this subject need antihypertensive therapy.

Robinson & Brucer (1939) suggested that the dividing line between normotension and hypertension was the relatively low level of 120/80 mmHg. Ayman (1934) proposed the level of 140/80mmHg. Various dividing lines have been proposed ranging from the suggestion of Robinson & Brucer (1939) to the relatively higher levels of 180/110mmHg as proposed by Evans (1956).

Pickering (1960,1961) concluded that essential hypertension was merely a deviation of the blood pressure

from its average level and that essential hypertension simply represents the right-hand side of the bell-shaped distribution curve of normal blood pressure values of the population. Pickering has challenged the validity of a dividing line for over 25 years and has described the dividing line as a fallacy (See reviews by Pickering, 1968, 1970). The separation of "normal" and "abnormal", using 140/90 mmHg as the dividing line was purely arbitrary. Blood pressure levels in the population are thought by Pickering to be a continuum and one could equally well use some other arbitrary value.

Although there is still a problem concerning the definition and fixation of a specific dividing line, essential hypertension is generally graded into sub-sections depending upon the severity of the condition (see Freis, 1974). Julius & Schork (1971) defined borderline or labile hypertension as blood pressure readings averaging between 150/90 and 160/110 mmHg with occasional normal readings and no evidence of target organ damage. Freis (1974) defined mild hypertension as diastolic pressure persistently between 90 and 104 mmHg, moderate hypertension as diastolic pressure between 105 and 114 mmHg and moderately severe hypertension as having a diastolic pressure in the range of 115 to 129mmHg. A person with severe hypertension, defined by Freis (1974) as having a persistently raised diastolic blood pressure above 130mmHg, may be close to entering or may already be in the accelerated or malignant phase of hypertension. According to Freis (1974), hypertension is not usually treated until it reaches the moderate stage, although some persons with labile or mild hypertension undergo drug therapy depending upon

risk factors such as age, sex, race, target organ damage, family history, hyperlipoidemia and hyperglycaemia. At the present time, there is no evidence to suggest that antihypertensive drug treatment of labile or mild hypertensives prevents the subsequent development of established essential hypertension. Some borderline hypertensive persons revert back to normotensive states and probably no more than a quarter of patients with borderline hypertension actually develop established essential hypertension.

Aetiology of Essential hypertension.

1. Secondary Hypertension.

Since the classical work of Goldblatt in the 1930's on renovascular hypertension (see review by Goldblatt, 1947), there has been a concentrated effort to determine the aetiological basis of essential hypertension using both animal and human studies. Hypertension, which mimics the major symptoms of human essential hypertension, has been successfully induced by various methods in several animal species (see later). However, even with the development of better blood pressure measurement techniques in man and animals, efficient, accurate biochemical assays and numerous epidemiological and natural history studies, the definite primary factors initiating the development of essential hypertension are still unknown. Work has been successful in uncovering the causes of a few rare types of hypertensive diseases. When the raised arterial pressure is due to known pathological factors then this hypertension is termed -

secondary hypertension.

There are forms of hypertension in which only the systolic pressure is raised. Systolic hypertension is caused firstly by an increased stroke output of the left ventricle found in such conditions as complete heart block, aortic reflex, Paget's disease of bone, fever, thyrotoxicosis and pregnancy, secondly by an increased rigidity of the aorta due to a degenerative disease of the aortic wall and thirdly due to a decreased capacity of the aorta in coarction. In most forms of secondary hypertension both the systolic and diastolic pressures are raised. Kidney and urinary tract diseases (for classification and examples see Pickering, 1972), diabetes, phaeochromocytoma, Cushing's syndrome, primary aldosteronism, coarction of the aorta, pre-eclamptic toxaemia of pregnancy, post-toxaemic hypertension and miscellaneous conditions affecting the nervous system are the main causes of secondary hypertension. Incidents of secondary hypertension account for approximately 10% of the hypertensive population.

2. Neural factors in Essential hypertension.

Prior to 1970, hypertensive patients were classified as either having essential (primary) or secondary hypertension. However, in recent years, persons suffering from essential hypertension have been subdivided into groups with low, high and normal plasma volumes (Tarazi, Dustan, Frohlich, Gifford & Hoffman, 1970) and those with low, high and normal plasma renin levels (Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard & Buhler, 1972). Thus, the

problem of finding initiating factors has been further complicated.

Most of the work implicating sympathetic nerve disfunction in essential hypertension has been performed in animals. Denervation of the carotid sinus and aortic arch leads to an increase of the activity of peripheral sympathetic neurons by removing a major source of inhibition of central vasomotor tone. Thus, a hypertension results which is accompanied by a tachycardia (Heymans & Neil, 1958; Uvnas, 1960 a). Reduction of noradrenaline levels and increased noradrenaline turnover occurs in the heart and adrenal glands of rabbits after deafferentation (De Quattro, Nagatsu, Maronde & Alexander, 1969). Deafferentation in rabbits also causes an increased noradrenaline turnover in the bulbospinal areas of the sympathetic nervous system (Chalmers & Wurtman, 1971).

Neurogenic factors play at least a contributory role in other hypertensive animal models (De Champlain, Krakoff & Axelrod, 1967; Willard & Fuller, 1969). Ayitey-Smith & Varma (1970) effectively prevented the onset of experimental hypertension in rats by immunosypathectomy. Grewal & Kaul (1971) observed in rats with renal artery stenosis hypertension that ablation of sympathetic nerve fibres with 6-hydroxydopamine and extirpation of the adrenal medullae lowered the blood pressure to normal levels. Similar results were obtained in DOCA/saline uninephrectomised hypertensive rats by Finch (1971) and De Champlain & Van Ameringen (1972). In rats made hypertensive with bilateral

lesions of the nucleus tractus solitarius (i.e. hypertension resulting from central deafferentation of baroreceptors by destruction of their primary synapse within the brain), Doba & Reis (1974) found that the hypertension was due to an increased sympathetic discharge inducing a raised peripheral resistance and increased catecholamine release from the adrenals.

The sympathetic nervous system has been suspected of playing a pathogenic role in the genesis of human hypertension for many years but confirmatory evidence has been sparse. There is some direct evidence demonstrating increased sympathetic nerve activity in patients with sustained hypertension (Walsh, Heyman & Moronde, 1969; Smirk, 1971). However, Frohlich, Tarazi, Ulrych & Dustan (1967) found conflicting results in that the sympathetic nerve responsiveness was inversely proportional to the severity and duration of the hypertension.

Several investigators have measured urine and plasma noradrenaline and serum dopamine β-hydroxylase levels in an attempt to demonstrate the degree of sympathetic nerve activity in hypertension (see De Quattro & Miura, 1973). Several workers have observed no abnormalities between the excretion rates of noradrenaline in normotensive and hypertensive individuals (see Brunjes, 1964) and Sundin (1956) and Birke, Duner, Von Euler & Plantin (1957-58) reported decreased catecholamine excretion in hypertensive persons. In contrast, Boake & Rieke (1965) Ikoma (1965), De Quattro & Sjoerdsma (1968) and Nestle & Doyle (1968)

observed that in unmedicated hypertensive patients with normal renal function urinary levels of noradrenaline were elevated. Engelman, Portnoy & Sjoerdsma (1970) and De Quattro & Chan (1972) found blood levels of noradrenaline to be elevated in 75% and 25 - 30% respectively of their essential hypertensive patients examined. Louis, Doyle & Anavekar (1973) described a direct relationship between raised plasma noradrenaline levels and diastolic blood pressure. Louis et al.(1973) also demonstrated a direct correlation between the fall in diastolic pressure and decreased plasma noradrenaline concentration after treatment with ganglion blocking agents in essential hypertensive patients.

Noradrenaline turnover rate in man has served to implicate abnormal catecholamine metabolism as a causative factor in hypertension. Gitlow, Mendlowitz, Wilk, Wilk, Wolf & Naftchi (1964) reported an enhanced plasma clearance of noradrenaline in hypertensive patients.

The development of a wide range of effective antihypertensive drugs which impair peripheral sympathetic nerve function adds much weight to the concept of the neurogenic component of hypertensive diseases being due to an overactive peripheral sympathetic nervous system. The drugs which have been successfully used as antihypertensive agents are discussed later in the Introduction.

McCubbin, Green & Page (1956) first showed that in renal hypertensive dogs there is an upward resetting of the baroreceptor mechanism and that this might account, at least in part, to the neurogenic component of hypertension.

This phenomenon has been confirmed by other workers (McCubbin & Page, 1963; Kezdi & Wennemark, 1964; Aars, 1968). The resetting of the carotid sinus buffer mechanism follows the hypertension and although it helps to maintain a high blood pressure it does not appear to contribute to its onset.

Evidence that the central nervous system is involved in the initiation and maintenance of essential hypertension has grown rapidly in recent years. In an attempt to simulate normal human stresses, Folkow & Rubenstein (1966) produced hypertension by chronic stimulation of the defence alarm areas in the lateral hypothalamus of the rat. Folkow, Hallback, Lundgren, Sivertsson & Weiss (1973a) are now of the opinion that essential hypertension is triggered by intermittent bouts of hypothalamic stimulation which increases cardiac output in genetically predisposed persons. In turn, the increased blood flow is counteracted by an increased peripheral resistance which was seen within days in rats and within an unknown period in man. Folkow stated that "essential hypertension might be the result of a complex interaction between the centrally elicited pressure and output increases and a structural adaptive response of heart and precapillary vessels if the pressor responses were repeated for a sufficient period of time," (see Page, 1974).

Dock (1940) found that pithing of the brain and spinal cord lowers the blood pressure of hypertensive animals to the same low values that are found in normotensive

animals after pithing. Taquini (1963) also observed that destruction of the central nervous system of hypertensive dogs by pithing caused a dramatic fall in blood pressure.

Destruction of central adrenergic neurons by centrally administered 6-hydroxydopamine has been shown to prevent the development of hypertension following buffer nerve section in rabbits (Chalmers & Reid, 1972), renal hypertension in rabbits (Lewis, Reid, Chalmers & Dollery, 1973), renal and spontaneous hypertension in rats (Haeusler, Finch & Thoenen, 1972) and DOCA/saline hypertension in rats (Haeusler et al., 1972; Dargie, Dollery & Lewis, 1975). In established neurogenic and renal hypertension in rabbits, intracisternal 6-hydroxydopamine reduced the blood pressure to control normotensive levels (Chalmers & Reid, 1972; Lewis et al., 1973) suggesting that central adrenergic neurons are important in the initiation and maintenance of hypertension in rabbits. In contrast to this work in rabbits Haeusler et al.(1972) observed that, in three experimental models of hypertension in rats, icv 6-hydroxydopamine produced only small transient falls in pressure when administered during the established phase of hypertension. Hence, as the induction of hypertension in rats could be prevented by 6-hydroxydopamine pretreatment, they suggested that a centrally located neurogenic trigger mechanism is necessary for the initiation of DOCA/saline, renal and spontaneous hypertension in rats.

Yamori, Lovenberg & Sjoerdsma (1970) observed a decreased synthesis and content of noradrenaline in the brain

stem and hypothalamus of spontaneously hypertensive rats. A decreased turnover rate of noradrenaline in the brain stem was also found in DOCA/saline rats (Nakamura, Gerold & Thoenen, 1971). As noradrenaline has been shown to possess a depressor action in the brain stem (see later in the Introduction), Yamori et al.(1970) proposed that an unnaturally low noradrenaline concentration in the brain stem might be one of the factors in the pathogenesis of hypertension.

Further evidence that lends support for the involvement of the central nervous system in the induction and maintenance of hypertension is that hypertension can be induced by prolonged stimulation of the hypothalamus of rats (Folkow & Rubenstein, 1966), and by lesioning the nucleus tractus solitarius (Doba & Reis, 1974) and area postrema (Ylitalo, Karppanen & Paasonen, 1974). In addition, clinically active antihypertensive agents such as ∝-methyldopa, clonidine and propranolol (see later in Introduction) have been shown to exert their blood pressure lowering effects, in part at least, through an action within the central nervous system.

3. Humoral factors

Goldblatt, Lynch, Hanzal & Summerville (1934) demonstrated that persistent hypertension could be induced by renal ischaemia, produced by constriction of the renal arteries. They postulated that essential hypertension was due 'figuratively to an indefinite number of clips' on the renal vessels leading to a release of a humoral pressor

agent from the kidneys. Braun-Menédez, Fasciolo, Le Loir & Munoz (1939) and Page & Helmer (1939) indentified this pressor substance to be angiotensin. Angiotensin is formed from renin; the latter substance being released from the ischaemic kidney (see Page & McCubbin,1968; Davis, 1973).

An enormous literature has now been compiled concerning the central and peripheral cardiovascular actions of angiotensin and its involvement in hypertension. With respect to its role in hypertension,indirect actions of angiotensin involving the sympathetic nervous system and direct effects on vascular smooth muscle have been postulated (see Page & McCubbin, 1968; Owen, 1969; Page, 1974). The peripheral effects of angiotensin will not be discussed.

Bickerton & Buckley (1961), using cross circulation experiments in dogs, observed that angiotensin was able to produce a central nervously mediated pressor response. This finding has been confirmed by numerous workers (Nashold, Mannarino & Wunderlich, 1962; Smookler, Severs, Kinnard & Buckley, 1966; Severs, Daniels, Smookler, Kinnard & Buckley, 1966; Scroop & Lowe 1968). Smookler et al.(1966) suggested that central noradrenergic mechanisms were important in the mediation of the pressor effect of icv administered angiotensin. Yamori confirmed this suggestion after observing a diminution of the pressor response to icv angiotensin in rats depleted of central noradrenaline following icv 6-hydroxydopamine, (see Yamori, Ooshima & Okamoto, 1973). Joy & Lewis (1971) reported that a direct action on the brain stem contributed towards the pressor effect seen after intravenous

angiotensin in the rabbit. They proposed that if the central reponse to angiotensin depends upon central catecholaminergic pathways then a probable humoral link exists between the manipulated kidney and the central nervous system in experimental renal hypertension in rabbits.

Other humoral agents make the understanding of humoral mechanisms in hypertension rather complicated. Elevated angiotensin blood levels promote the stimulation of aldosterone secretion from the adrenal cortex causing the retention of sodium and thus tending further to increase the blood pressure (Genest, Nowaczynski, Koiw, Sandor & Biron, 1960; Laragh, Angers, Kelly & Liberman, 1960; Davis, Ayers & Carpenter 1961). Aldosterone may actively participate in the maintenance of certain hypertensive persons, as Tarazi et al.(1970) found that those persons in whom the hypertension is volume dependant respond best to treatment with the aldosterone antagonist, spironolactone and thiazide diuretics.

In recent years, other humoral agents such as some of the prostaglandin series and bradykinins have been postulated as being involved in the humoral mechanisms of hypertension (see Page, 1974).

4. Genetic factors.

A hereditary mechanism has been established as probably the most important factor in the aetiology of essential hypertension from studies involving families and close relatives of patients suffering from severe hypertension (Weitz, 1923; Thomas, 1952, 1959; Miall & Oldham

1963; Platt, 1963). The hereditary mechanism is not explicable by either multifactorial inheritance or by the action of a single dominant gene but is best explained as a result of a single gene with incomplete dominance and a frequency of 0.24, which in the homozygous form gives rise to severe hypertension and in the heterozygous form produces more moderate elevations in pressure (Platt, 1963).

Pickering (1967) considered that arterial blood pressure is inherited polygenically as a tendency to high and low pressures at all ages. He does not regard the rate of rise of blood pressure with age to be inherited and considers that environmental factors determine arterial pressures in middle age and beyond.

Relatively rare conditions such as phaeochromocytoma might be produced as a result of a simple genetic mechanism. Certain forms of secondary hypertension, such as that accompanying pyelonephritis, have an apparent hereditary pattern which can be explained by the fact that they are mediated through an auto-immune mechanism (White & Grollman, 1964), which also shows a familial tendency.

If most cases of essential hypertension are of genetic origin, then one would expect that the incidence of essential hypertension should vary in different population groups. Epidemiological surveys tend to support this contention (for references see reviews by Grollman, 1969; Henry & Cassel, 1969).

Smirk & Hall (1958) and Okamoto & Aoki (1963) developed colonies of spontaneous hypertensive rats in which their structural characteristics are genetically programmed. These findings greatly enhance the theory that essential hypertension is an inheritable disease. Dahl, Heine & Tassinari (1962) successfully bred rats that were salt-sensitive. They have shown that in such rats the hypertension and early death from vascular disease is dependent upon their genetically induced super-sensitivity to salt. The haemodynamic and pathogenic conditions of spontaneously hypertensive rats resemble those found in human essential hypertension (Okamoto, 1969).

5. Excessive salt intake.

In both experimental animals and humans, reductions in blood pressure may be induced by sodium deficient diets (Murphy, 1950; Corcoran, Taylor & Page, 1951). Grollman, Harrison, Mason, Baxter, Crompton & Reishman (1945) found that hypertension could be induced in animals given a high salt intake. The relative salt intake of different population groups differs greatly. Epidemiological studies have produced divergent results when relating hypertensive blood pressure levels to respective salt intakes. Meneely (1954), Dahl (1961), Isaacson, Moldin & Jackson (1963) and Maddocks (1964) are some of the surveys that have proposed that a habitually high salt intake accounts for the variable incidence of hypertension in different populations.

That there was no evidence of a direct correlation

between the salt intake of subjects and their varying levels of blood pressure was shown by Schnecklock, Corcoran, Stuart & Moore (1962). (see also review by Henry & Cassel, 1969). It is now generally accepted that while a high salt intake probably plays an important role in the deterioration of those suffering from hypertension it would appear not to commonly determine the onset of the condition.

6. Obesity.

Obese individuals in civilised communities are prone to have higher blood pressures than persons of the correct weight (Doyle & Lovell, 1961; Aleksandrow, 1967; see review by Whyte, 1963). Natives living in primitive conditions have usually a very low incidence of essential hypertension and have a relatively low dietary fat intake (Whyte, 1958; De Wolf & Whyte, 1958; Whyte, 1963; Henry & Cassel, 1969). It is generally believed that obesity represents an added detrimental factor in hypertension but probably does not initiate the condition.

A strong correlation has been shown between the presence of elevated plasma lipid levels, of which most attention has been paid to cholesterol, and atherosclerosis. Persons with high plasma cholesterol levels are especially prone to the development of coronary heart disease (Eder, 1970). Reduction of body weight and plasma lipid levels decrease the incidence of cardiac and vascular diseases (Eder, 1970).

7. Haemodynamic picture in essential hypertension.

It is now known that the haemodynamic picture in essential hypertension varies in groups of subjects of different ages, with different degrees of blood pressure elevation and with different degrees of complications (Lund-Johansen, 1973). In early essential hypertension (borderline or mild hypertension), theraised pressure is mainly maintained by an increased cardiac output while the calculated total peripheral resistance remains normal (Varnauskas, 1955; Widimsky, Fejfarova & Fejfar, 1957; Julius & Conway, 1968). The elevated cardiac output was more often observed in young persons than middle-aged or old subjects. Most investigators have reported that the high resting cardiac output is due to an increased heart rate (Eich, Peters, Cuddy, Smulyan & Lyons, 1962; Sannerstedt 1966; Lund-Johansen, 1967).

The mechanisms which produce the raised cardiac output, heart rate and oxygen consumption in subjects with mild or borderline hypertension, are unknown although an increased activity of the sympathetic nervous system has been suggested (Lund-Johansen, 1973). Ulrych, Frohlich, Dustan & Page (1968) indicated that an increased responsiveness of the cardiac β-adrenoceptors may account for the raised parameters since propranolol given to borderline hypertensives lowered heart rate and cardiac output. Julius Pascual & London (1971) concluded that a decreased parasympathetic tone may also exist in subjects with borderline hypertension.

In subjects with well-established essential hypertension, of long duration, the raised pressure is maintained in the presence of a low or normal cardiac output (Freis, 1960; Glezer, 1963; Sannerstedt, 1966; Amery, Julius, Whitlock & Conway, 1967; Lund-Johansen, 1967). As the hypertension becomes more severe, the cardiac output tends to be very low and the peripheral resistance very high (Sannerstedt, 1966; Lund-Johansen, 1967). Since subjects with this haemodynamic pattern represent the majority of those persons who receive antihypertensive drug therapy it appears necessary to use drugs that reduce peripheral resistance without further depressing cardiac output (Gilmore, Weil & Chidsey, 1970; Lund-Johansen, 1970).

It is not known whether all subjects investigated, showing the two different haemodynamic pictures for labile and well established hypertension, represent persons with the same disease but at different stages. However, the observations by Eich, Cuddy, Smulyan & Lyons (1966) and Bello, Sevy, Harakal & Hillyer (1967) tend to support the theory that a haemodynamic change does take place, but more studies are still needed to provide conclusive evidence.

Production of Experimental Hypertension.

Chanutin & Ferris (1932) induced chronic renal hypertension by a method involving partial ablation of the kidneys. However, this method was found to be applicable only to the rat and the relative success rate of producing hypertension was small as a large number of rats died due

to the harsh surgical procedures.

Goldblatt et al.(1934) successfully induced reliable sustained hypertension after permanently constricting both renal arteries. Temporary hypertension, which gradually returns to normal after 4 - 6 weeks, has been produced by constriction of one main renal artery. Removal of the clamp or excision of the kidney with the constricted renal artery causes the blood pressure to return to normal within 24 hours (Goldblatt, 1940). This form of temporary hypertension may be made permament by the subsequent removal of the contralateral kidney (Goldblatt, 1937-1938; Katz, Mendlowitz & Friedman, 1938), by constriction of the renal artery of the contralateral kidney (Goldblatt et al., 1934) or by occlusion of the ureter of the contralateral kidney (Goldblatt, 1947).

Page (1939a) induced hypertension in dogs after wrapping the kidneys in cellophane. This method induces an inflammatory response forming a thick fibrous capsule around the treated kidney. Wrapping the kidneys with silk or collodion also induces perinephritis and hypertension in dogs (Page, 1939, a, b). A simplified technique developed by Grollman (1944) led him to report a higher incidence in the mouse, rat, rabbit and dog. A cotton thread or tape is passed around the pole and body of the kidney in a figure of 8 ligature, and by drawing it taut produces distortion of the manipulated kidney. Removing or ligating the remaining kidney quickens the development of hypertension.

Neurogenic hypertension was produced in dogs by Heymans (1938). He induced persistent hypertension after sectioning all four afferent nerves to the central nervous system from the baroreceptors. This procedure curtails the afferent nervous activity to the medullary cardiovascular regions from the baroreceptors and in turn leads to an increased efferent sympathetic and decreased parasympathetic tone to the periphery. Doba & Reis (1973) observed that in the rat bilateral lesions of the nucleus tractus solitarius, a nucleus lying dorsolaterally in the medulla oblongata, result in the rapid development of hypertension. Confirmation of this finding has been made by de Jong, Nijkamp & Bohus (1975) using normotensive rats. Hypertension occurs as a consequence of central deafferentation of the baroreceptors by destruction of their primary synapse within the brain (see later in Introduction).

That a combination of desoxycorticosterone acetate (DOCA) and high sodium chloride intake produced pronounced nephrosclerosis and hypertension in rats was first described by Selye, Hall & Rowley (1943). They observed that the pathological findings and the malignant hypertension produced by this method closely resembled the hypertensive heart disease of renal origin seen in man.

Hypertension has been reported by Farris, Yeakel & Medoff (1945), Medoff & Bongiovanni (1945) Hudak & Buckley (1961) and Rosecrans, Watzman & Buckley (1966) after subjecting rats to repeated multiple or single stressful situations for long periods. The hypertension induced in

rats by auditory excitation persisted for several months after exposure to the noises had ceased (Smirk, 1949). Marwood & Lockett (1973) produced sustained hypertension, which was relatively quick in onset, after exposing rats to complete silence. They termed this type of hypertension in rats as "sound-withdrawal" hypertension.

The development of genetic spontaneously hypertensive rats (Smirk & Hall, 1958; Okamoto & Aoki, 1963) has produced an experimental model of hypertension which closely resembles essential hypertension in man (Okamoto, 1969). Thus, this hypertensive model is the one most extensively used in the industrial screening of anti-hypertensive compounds.

Folkow and colleagues have shown that spontaneously hypertensive rats exhibit a greater cardiovascular response to stress than normotensive rats. Spontaneous hypertensive rats showed a hyper-reactivity of central autonomic structures mediating emotional behaviour during stress (Folkow, Hallback & Weiss, 1973 b; Hallback & Folkow, 1974). Folkow et al. (1973b) suggested the hyper-reactivity to be a genetically linked factor predisposing to hypertension in these rats, rather than a consequence of the hypertensive state. Yamori, Matsumoto, Yamabe & Okamoto (1969) were able to augment spontaneous hypertension in rats by subjecting them to chronic stress.

The Development of antihypertensive therapy.

The first major contribution to the treatment of

patients suffering from malignant hypertension was made by Page & Heuer (1937 a, b). They observed that surgical sympathectomy lowered the raised blood pressure, reversed symptoms such as retinal exudates, papilloedema and inverted T waves and reduced the size of their enlarged hearts. It was realised that other effective measures must be found as sympathectomy was a harsh irreversible process characterised by severe side effects and occasionally patients were observed to regress back into hypertensive states. However, the years up to 1950 were relatively unfruitful in producing advances in the treatment of hypertension.

That a low sodium diet was relatively beneficial in the treatment of hypertension was first reported by Allen (1920). However, the significance of this observation was not realised until Kempner (1948) introduced the rice-fruit diet and found that the blood pressure was lowered in 500 patients. This therapy was not widely accepted as it was a monotonous and tasteless diet,which could not be endured for long periods.

Megibow, Pollak, Stollerman, Roston & Bookman (1948) and Gubner (1950) respectively demonstrated that mercurial diuretics enhanced the anti-hypertensive effects of low salt diets and potassium thiocyanate but the significance of these results was not realised until the introduction of chlorthiazide in 1957 (see later).

Freis & Wilkins (1947) used large doses of pentaquine, an antimalarial agent, with some success in the treatment

of malignant hypertension. They showed that in doses larger than those needed in the treatment of malaria, pentaquine exhibited sympathetic blocking activity. As pentaquine showed many severe side effects its usefulness was cut short.

In the late 1940's and early 1950's several antihypertensive agents were developed and introduced clinically; these being the veratrum alkaloids, ganglion blocking agents, reserpine and hydralazine. This time represented the start of the present era of effective drug treatment of essential hypertension. In this section, the peripheral mechanisms of these compounds will only be briefly covered and more emphasis will be made to their central actions.

Veratrum Alkaloids.

Using the relatively recently produced pure veratrum alkaloid extracts, Krayer & Acheson (1946) demonstrated that veratrum alkaloids were suitable to undergo clinical trials as effective antihypertensive agents. However, the hypotensive properties of veratrum had been known for several years.

Veratrum alkaloids produce their cardiovascular depressant effects by sensitising or stimulating peripheral receptors which lead to a reflex lowering of blood pressure and heart rate. That the hypotension due to veratrum was reflex in nature was suggested by Von Bezold & Hirt (1867) but was not confirmed until 1939 by Jarisch & Richter.

Intravenously administered veratrum predominantly stimulates endings of afferent vagal fibres in the coronary sinus and left ventricle (see Dawes & Comroe, 1954; Juhász - Nagy & Szentiványi, 1961) producing reflexly elicited hypotension and bradycardia. This response of increasing afferent vagal activity by the veratrum alkaloids is called the Bezold-Jarisch reflex. Additional 'hypotensive' receptors, which are stimulated by veratrum, have been identified in the left ventricle, both auricles, the pulmonary vascular system and the baroreceptors in the aortic arch and carotid sinus (see review by Kupchan & Flacke, 1967).

Atropine or vagotomy abolishes the bradycardia seen after stimulation of the Bezold-Jarisch reflex by veratrum, indicating that it is mainly due to a raised efferent vagal tone from the central nervous system. However, the hypotension is unaffected since it results from a decreased efferent sympathetic tone to the vasculature (see Nickerson 1970b).

It is not known with certainty whether veratrum possesses direct effects within the central nervous system, which could lead to hypotension and bradycardia, in addition to the indirect effects resulting from the change in afferent inflow. Goldring & O'Leary (1954a,b) observed changes in sustained potentials and evoked potentials were obtained after local application of veratrum to central neurons. These effects appear to represent a local direct effect of the alkaloids upon central nervous system structures.

Because the compensatory mechanisms are 'reset' rather than blocked, the hypotension seen after veratrum does not have a major postural component (Freis, Stanton, Culbertson, Little, Halperin, Burnett & Wilkins,1949). The hypotension is usually accompanied by a reduction in peripheral resistance without greatly altering the cardiac output (Brest, 1969).

The main disadvantage encountered with these alkaloids is that they produce severe emetic reactions. They cause nausea and vomiting by stimulating receptors in the region of the nodose ganglia of the vagus (Borison & Fairbanks, 1952). As the doses of veratrum that produce antihypertensive effects and severe vomiting are so similar, the alkaloids were not greatly used clinically.

However, at the present time, with the general trend of antihypertensive therapy being the use of low doses of a combination of drugs, there has been a recent renewed interest in the veratrum alkaloids.

Ganglion Blocking Agents.

The pharmacology of hexamethonium (a bisquaternary ammonium compound) was described by Paton & Zaimis (1948, 1949). They observed that these compounds caused blockade of autonomic ganglia by competitively antagonising the action of acetylcholine on nicotinic receptors within the ganglia.

Arnold & Rosenheim (1949) observed in man that these compounds produced sharp falls in blood pressure and

Smirk (1950) reported that hexamethonium quickly reversed the symptoms of malignant hypertension. In 1956 and 1958 mecamylamine, a secondary amine, and pempidine, a tertiary amine, were respectively introduced as orally active ganglion blocking agents (see Lee, 1967).

By virtue of their pharmacological actions in totally blocking the peripheral autonomic nervous system, patients receiving ganglion blocking therapy experienced severe side effects such as dry mouth, lack of visual accommodation, constipation, impotence and difficulty in micturation due to parasympathetic blockade and weakness and faintness in the erect position due to orthostatic hypotension resulting from sympathetic blockade. Hence, antihypertensive therapy with these compounds was unpleasant and they were superceded by compounds which blocked only the sympathetic nervous system. Trimethaphen, a potent ganglion blocking agent with a very rapid onset and brief duration of action, is still used in severe hypertensive emergencies and to produce controlled hypotension in some surgical procedures.

Reserpine (Rauwolfia)

Sen & Bose (1931), Chopra, Gupta & Mukherjee (1933) and Bhatia (1942) demonstrated hypotension with simultaneous tranquillising activity and sedation with Rauwolfia extracts. Vakil (1949) observed that in man Rauwolfia extracts produced antihypertensive effects. In 1952, reserpine was isolated and found to be the most active alkaloid, possessing both hypotensive and sedative actions (Bein, 1953).

Reserpine induces antihypertensive effects associated with bradycardia. The bradycardia results from a blockade of the sympathetic nervous system (either by a central or peripheral action) which also leads to a predominance of the vagus nerve to the heart. Reserpine produces a depletion of the stores of neurohumoral transmitter substances in peripheral and central adrenergic nerves with a consequent impairment of neuronal function (Carlsson, Rosengren, Bertler & Nillson, 1957; Muscholl & Vogt, 1958). The mechanisms by which reserpine depletes transmitter stores are fully discussed by Brodie & Shore (1957), Stjarne (1964) and Iversen, Glowinski & Axelrod (1965).

It has been suggested that reserpine exerts its antihypertensive action, at least in part, by acting within the central nervous system. Bein (1955) observed in cats that reserpine induced a decrease in the electrical activity of preganglionic sympathetic cardioaccelerator nerves together with a bradycardia. McCubbin & Page (1958) reported that reserpine produced a slow, progressive and marked diminution in efferent splanchnic nerve activity in both cats and dogs. However, Iggo & Vogt (1960) noted no change in the electrical activity of cervical sympathetic nerve fibres nor did they observe bradycardia in response to reserpine. Administration of 1.5 mg of reserpine into the lateral cerebral ventricles or cisterna magnas of cats and dogs produced hypotension, bradycardia and inhibition of the carotid occlusion response (McCubbin, Kaneko & Page, 1960; Kaneko, McCubbin & Page, 1960); van Zwieten, Bernheimer & Hornykiewicz (1966) observed

hypotension after vertebral artery infusions of reserpine into cats. The hypotension occurred in the absence of any reduction in catecholamine content of peripheral tissues. Bein (1955) observed in cats that reserpine reduced the pressor response to electrical stimulation of the medullary vasomotor centres although he reported the influence of reserpine on medullary centres to be far less than on rostrally situated structures. In cross-circulation experiments in dogs, Wang, Kanai, Markee & Wang (1964) failed to note any change in the excitability of the medullary vasopressor area to electrical stimulation after reserpine.

Although the central mechanism of reserpine is not known it is probable that it acts by releasing catecholamines (5-hydroxytryptamine and noradrenaline) from their bound forms in the brain as shown by Brodie, Olin, Kuntzman & Shore (1957). Holzbauer & Vogt (1956) observed that reserpine caused the release of noradrenaline from the hypothalamus of the cat leading to reduced noradrenaline levels. It may be that the endogenous noradrenaline liberated in the central nervous system stimulates central α-adrenoceptors thus reducing peripheral sympathetic outflow and consequently lowering blood pressure and heart rate (see later in Introduction; van Zwieten, 1973).

Carlsson (1966) hypothesised that there are several central sympathetic centres acting selectively on different parts of the peripheral sympathetic nervous system. He proposed that drugs could act selectively on these centres and since orthostatic reactions after reserpine are

relatively rare reserpine could act partially through a central mechanism. However, at the present time the relative importance of the peripheral and central actions of reserpine in mediating its antihypertensive effect is still unknown.

Internationally, reserpine is still extensively used in drug combinations in the treatment of mild to moderately severe hypertension (Simpson, 1971; Gifford, 1974). Side effects such as nasal stuffiness, increased appetite, diarrhoea, drowsiness, lethargy, decreased libido, nightmares and fluid retention occur during reserpine therapy. However, more seriously, reserpine may occasionally lead to severe depression resulting in suicide (Brod, 1972).

Hydralazine.

Freis & Finnerty (1950) first demonstrated that hydralazine was a clinically active antihypertensive agent in man. Early studies with hydralazine attributed its hypotensive effect successively to a specific renal vasodilatation and to an action within the central nervous system (see Moyer, 1953). Freis, Rose, Higgins, Finnerty, Kelly & Partenope (1953) and Åblad (1963) observed that hypotension due to hydralazine was the result of direct vasodilatation leading to a large reduction in peripheral resistance. They observed that the vasodilatation was not uniform; the vascular resistance in the coronary, splanchnic, renal and cerebral circulation being decreased more than in the skin and muscle.

Kirpekar & Lewis (1958) suggested that hydralazine produces direct vasodilatation by inhibiting the Kreb cycle within the vascular smooth muscle. Hydralazine probably chelates with trace elements which form part of the enzymes within the cycle. Kirpekar & Lewis (1959) also observed that hydralazine interfered with the normal oxidative processes in the brain.

Hydralazine inhibited the pressor effects after stimulation of the afferent nerves without interfering to the same extent with the pressor reactions produced by stimulation of an efferent sympathetic nerve. This lead Bein, Gross, Tripod & Meier (1953) to suggest a central mode of action for hydralazine. The increase in cardiac output induced by hydralazine was abolished by hexamethonium (Stein & Hecht, 1955). Tachycardia was induced after intracerebroventricularly administered hydralazine in dogs and was abolished by peripheral ganglionic blockade or β -adrenoceptor blockade (Gupta & Bhargava, 1965).

Craver, Barrett, Cameron & Yonkman (1951), Tangri & Bhargava (1960) and Ingenito, Barrett & Procita (1969) demonstrated centrally mediated hypotensive effects of hydralazine. Baum, Shropshire & Varner (1972) showed that oral administration of low doses of hydralazine to rats caused a reduced efferent sympathetic outflow from the central nervous system. However, Reis & van Zwieten (1967) suggested that the hypotensive effects of hydralazine were mainly due to peripheral mechanisms as the falls in blood pressure observed after vertebral artery and intravenous

infusions of hydralazine were not significantly different.

Initially, hydralazine was given in combination with either ganglion blockers or reserpine as these compounds reduced the reflex tachycardia induced by hydralazine. In recent years, hydralazine has been administered in combination with propranolol and diuretics (see Koch-Weser, 1974). Propranolol prevents the tachycardia and also reduces the increased levels of renin caused by hydralazine (Ueda, Yaki & Kaneko, 1968). Diuretics help to reduce the sodium retention often seen with hydralazine therapy (Moyer, 1953). Propranolol and diuretics also enhance the antihypertensive effects of hydralazine. Relatively few side effects occur with hydralazine and postural hypotension does not occur (Åblad, 1963).

Diuretics

Freis & Wilson (1957) and Hollander & Wilkins (1957) independantly demonstrated that chlorthiazide was anti-hypertensive and also enhanced the effects of ganglion blockers and reserpine.

Today, diuretics are often the first type of compound to be used in the treatment of mild hypertension as most of the diuretics, e.g. mercurials, benzothiadiazides, furosemide and ethacrynic acid, possess antihypertensive properties. (see de Stevens, 1967). However, the mechanisms by which these compounds produce their hypotensive effects or enhance the actions of other antihypertensive agents have not been fully elucidated (Tobian, 1967), although the physiological

sequence of events obtained during diuretic treatment is well documented (see reviews by de Stevens, 1967; Tobian, 1967; Nickerson, 1970b; Dustan, Tarazi & Bravo, 1974). The mechanisms of action of diuretics will not be discussed in this thesis.

The introduction of diuretics marked a great advance in the development of antihypertensive therapy. These compounds themselves possess few side effects and they allow smaller doses of other more potent antihypertensive compounds e.g. guanethidine, α-methyldopa and propranolol to be used, thus reducing the side effects obtained with these latter compounds.

α-Adrenoceptor blocking agents.

With the realisation that the majority of the severe side effects encountered with ganglion blockade were due to parasympathetic blockade, a firm trend developed in the mid-1950's to use anti-adrenergic compounds in the treatment of hypertension. The first compounds tested as anti-hypertensives were reserpine and α-adrenoceptor blocking agents. However, results with these latter compounds were variable and clinically disappointing. (Werner & Barrett, 1967; Nickerson, 1970a).

Phentolamine, a potent α-adrenoceptor blocking agent, has found wide acceptance as a diagnostic agent in the detection of phaeochromocytoma as it has been shown to be an intrinsically safe compound which rarely produces false-positive tests (Helps, Robinson & Ross, 1955; Roth, Flock, Kvale, Waugh & Ogg, 1960).

Adrenergic Neuron Blocking Agents.

Complete blockade of the sympathetic nervous system can be achieved by administering a combination of α-and β-adrenoceptor blocking agents. This combination has generally been found to be unsatisfactory as considerable side effects usually occur (Mull & Maxwell, 1967). However, although Beilin & Juel-Jensen (1972) and Majid, Meeran, Benaim, Sharma & Taylor (1974) respectively observed that phenoxybenzamine and phentolamine were inactive when given alone, they produced significant additive effects when given together with propranolol and oxprenolol. These groups of workers suggested that further investigations should be undertaken to evaluate the clinical potentialities of this combination therapy.

The pharmacology of the first adrenergic neuron blocking agent, bretylium, was described by Boura & Green (1959). They observed that bretylium impaired the function of adrenergic neurons without affecting the parasympathetic and central nervous systems. Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim (1959), Smirk & Hodge (1959) and Dollery, Emslie-Smith & McMichael (1960) demonstrated that bretylium possessed antihypertensive properties. Clinically, bretylium was disappointing as it was short lasting, produced severe exercise and orthostatic hypotension (Boura et al., 1959) along with other side effects due to blockade of the sympathetic nervous system and rapid tolerance developed. (Dollery et al., 1960).

In 1959, Maxwell, Mull & Plummer synthesised guanethidine and as a result of favourable clinical trials by Frohlich & Freis (1959) and Page & Dustan (1959) this adrenergic neuron blocking agent is widely used in the treatment of moderate to severe hypertension. Although guanethidine produces postural hypotension, compared to bretylium it induces fewer side effects, development of tolerance is not a problem and it possesses a long duration of action (see review, Boura & Green, 1965).

The adrenergic neuron blocking agent, bethanidine, was introduced by Boura, Copp, Green, Hodson, Ruffell, Sim, Walton & Grivsky (1961) and its hypotensive effects and pharmacological properties were observed to be between those of bretylium and guanethidine (Boura & Green, 1962).

Determination of the exact mechanism by which these compounds produce their adrenergic neuron blockade has been complicated by the findings that they produce catecholamine release and depletion of varying degrees and are all potent local anaesthetics (Boura & Green, 1965). Intravenously administered bretylium, bethanidine or guanethidine produce pressor effects which are attributed to indirect sympathomimetic actions. The pressor actions are further increased by the findings that bretylium, bethanidine and to a lesser extent guanethidine are reversible monoamine oxidase inhibitors (McCoubrey, 1962; Kuntzman & Jacobson, 1963). Because of their indirect sympathomimetic effects and their ability to potentiate

the effects of exogenous catecholamines during adrenergic neuron blockade, these compounds are contraindicated in persons suffering from phaeochromocytoma (Genest, 1960).

Noradrenaline depletion by guanethidine (guanethidine being a more potent depletor than bretylium or bethanidine) appears not to be the mechanism by which it produces adrenergic blockade as the block may reach its peak before maximum depletion occurs (Burn, 1961; Cass & Spriggs, 1961). The mechanism by which this class of compounds produce their adrenergic neuron blockade is to prevent the release of noradrenaline in response to nerve stimulation. Impairment of sympathetic neurons does not always correlate directly with their systemic local anaesthetic potencies (Rand & Wilson, 1967). However, the possibility exists that their local anaesthetic actions account for part of their blocking effects under certain conditions, e.g. high dose levels (Boura & Green 1965). The mechanisms by which adrenergic neuron blockers produce their impairment are fully discussed in reviews by Boura & Green (1965), Carlsson & Waldeck (1965), Nickerson (1970a) and Laverty (1973).

Bretylium and bethanidine do not readily pass through the blood brain barrier and hence are not accumulated in the brain (Boura, Copp, Duncombe, Green & McCoubrey, 1960; Boura, Duncombe, Robson & McCoubrey, 1962). Also guanethidine is almost totally ionised at physiological pH and hence would not be expected to enter the brain (Boura & Green, 1965). However, variable effects on brain amines in several animal species have been reported. Although

Cass, Kuntzman & Brodie (1960) and Kuntzman, Costa, Gessa & Brodie (1962) observed no change in the brain content of noradrenaline and 5-hydroxytryptamine of rabbits after systemic guanethidine, Sanan & Vogt (1962) reported that guanethidine reduced hypothalamic levels of noradrenaline in some, but not all of the cats and rabbits used in their study. Systemic guanethidine administered daily for 7 days decreased hypothalamic levels of noradrenaline in cats but not in rats (Dagirmanjian, 1963). The adrenergic neuron blocking agents, guanoxan and guanoclor, can cause depletion of noradrenaline from the hypothalamus during chronic administration (Lawrie, Lorimer, McAlpine & Reinert, 1964; Davey & Reinert, 1965).

Guanethidine administered icv or intracisternally to dogs produced hypotension and bradycardia (Kaneko, McCubbin & Page, 1962). They suggested that a central component may contribute to the antihypertensive effect of guanethidine as small amounts may enter the brain after chronic treatment. Jaju, Tangri & Bhargava (1968) reported hypotension after bretylium administered icv to dogs.

α-Methyldopa

Oates, Gillespie, Udenfriend & Sjoerdsma (1960) first demonstrated that α-methyldopa possessed potent antihypertensive effects in man. It lowered blood pressure in both the standing and supine positions and α-methyldopa did not cause exercise hypotension, and postural hypotension was limited to only a relatively small number of subjects

(Dollery & Harington, 1962). Today, ∝-methyldopa is extensively used in the treatment of moderately severe to severe hypertension and as with guanethidine is often given in drug combination therapy (Simpson 1971; Gifford, 1974). ∝-Methyldopa does not exert as many side effects as guanethidine (Oates, Seligmann, Clark, Rousseau & Lea, 1965).

∝-Methyldopa is a potent inhibitor of dopa decarboxylase (Sourkes, 1954) and Oates et al. (1960) observed that dopa decarboxylase inhibition accompanied the hypotensive effects in man. Hence, the fall in blood pressure produced by ∝-methyldopa was attributed to its ability to reduce tissue noradrenaline stores by dopa decarboxylase inhibition. This mechanism has since been rejected as ∝-methyldopa was the only compound with dopa decarboxylase inhibiting properties to actively lower blood pressure (Sourkes & Rodriguez, 1967; Laverty, 1973).

The identification of ∝-methyldopamine and ∝-methylnoradrenaline as metabolic products of ∝-methyldopa in the brains and hearts of mice pretreated with ∝-methyldopa (Carlsson & Lindqvist, 1962) was followed by the report that ∝-methyldopa or its metabolites, ∝-methyldopamine or ∝-methylnoradrenaline, restored responses to sympathetic nerve stimulation and to indirectly acting sympathomimetic amines in reserpine treated animals and isolated tissues (Day & Rand, 1963a; 1964). Day & Rand (1963a) suggested that since they found ∝-methylnoradrenaline to be a less potent pressor amine than the natural transmitter,

noradrenaline, then its substitution for noradrenaline in peripheral sympathetic nerves would lead to a partial sympathetic nerve blockade consequently reducing heart rate and blood pressure. This 'false transmitter hypothesis' received support from the important observation by Muscholl & Maître (1963) that in animals pretreated with α-methyldopa stimulation of the sympathetic nerves to the heart released a mixture of noradrenaline and α-methylnoradrenaline. Evidence conflicting with the hypothesis has mainly centred around the lack of significant impairment of sympathetic function in animals treated with α-methyldopa, the small difference in many tissues between the α-adrenoceptor agonistic potencies of noradrenaline and α-methylnoradrenaline and the relative lack of antihypertensive properties of amino acids which consequently produced weaker 'false transmitters' than α-methylnoradrenaline.

Day & Rand (1964) summarised the weaker potencies of α-methylnoradrenaline compared with those of noradrenaline in several animal species and isolated tissues. Holtz & Palm (1967) indicated that α-methylnoradrenaline possessed weaker intrinsic activity than noradrenaline and Brunner, Hedwall, Maître & Meier (1967) observed that the relative pressor activity of α-methylnoradrenaline in the conscious rat, was less than for noradrenaline. However, several reports have indicated that in terms of their pressor effects, noradrenaline and its α-methylated analogue are equipotent in pithed rats (Maître & Staehelin, 1963), dogs (Conradi, Gaffney, Fink & Vangrow, 1965), and cats, (Haefely, Hurlimann

& Thoenen, 1966; Kadzielawa, 1967). Trinker (1971) observed that α-methylnoradrenaline and noradrenaline were equipotent as pressor amines in dogs and rats but in rabbits α-methylnoradrenaline was slightly weaker than noradrenaline.

On the basis of the 'false transmitter hypothesis' a substance which produces a weaker transmitter than α-methylnoradrenaline in vivo should be a more potent anti-hypertensive agent than α-methyldopa. The amino acid α-methyl-meta-tyrosine is readily converted in the body to α-methyl-meta-tyramine and then to metaraminol. This latter amine can serve as a false neurotransmitter of much lower potency than either noradrenaline or α-methylnoradrenaline (Udenfriend & Zaltzman-Nirenberg, 1963; Stone, Porter & Torchiana, 1965; Torchiana, Wenger, Stavorski, Ludden & Stone, 1966; Brunner et al.,1967). However, α-methyl-meta-tyrosine was found to be only weakly antihypertensive in rats (Stone, Porter, Watson & Ross, 1961) and man (Horwitz & Sjoerdsma, 1964).

The evidence concerning the mechanism by which α-methyldopa lowered blood pressure was further complicated by Farmer (1965) as he observed that α-methyldopamine reduced the response of the nictitating membrane of the cat to post-ganglionic sympathetic nerve stimulation and suggested that this metabolite may be responsible for producing the sympathetic blockade and thus mediates the antihypertensive effects of α-methyldopa.

α-Methyldopa has been shown to reduce plasma renin

activity (Mohammed, Fasola, Privitera, Lipicky, Martz & Gaffney, 1969). Thus, this effect of α-methyldopa may contribute to its blood pressure lowering effect in hypertensive patients with raised plasma renin activity.

A direct depression of arteriolar responsiveness to circulating and endogenously released catecholamines by α-methyldopa was demonstrated in the rat by Altura (1974) and he proposed that this was an important peripheral effect which greatly contributed to its antihypertensive action.

The peripheral mechanisms of α-methyldopa have been extensively reviewed and discussed by Muscholl (1966), Stone & Porter (1966), Kopin (1968) and Henning (1969b).

Evidence has steadily accumulated to indicate that α-methyldopa may have important effects within the central nervous system. Clinically, α-methyldopa often causes sedation and disturbances in sleep patterns, e.g. nightmares. In rats (Day & Rand, 1963b) and mice (Benfey & Varma, 1964), α-methyldopa reversed reserpine-induced sedation. The actions of α-methyldopa in the brain has been reviewed by Sourkes (1965).

Jaju, Tangri & Bhargava (1966) were the first workers to show that α-methldopa given intracerebroventricularly (icv) to dogs caused transient falls in blood pressure. However, it was the work of Henning and colleagues that has provided the most clear-cut evidence in favour of the central nervous system as a primary site for the

blood pressure lowering effects of α-methyldopa (Henning & van Zwieten, 1968; Henning, 1969a; for review see Henning 1969b). Firstly, Henning & van Zwieten, (1968) demonstrated in the cat that vertebral artery infusions of α-methyldopa produced pronounced hypotensive effects Secondly, the antihypertensive effect of α-methyldopa in the rat was not affected by inhibition of dopa decarboxylase in peripheral tissues, whereas it was completely abolished by inhibition of the enzyme in the central nervous system (Henning, 1969a). Since beginning this thesis, Henning & Rubenson (1971) observed that the conversion of α-methyldopamine to α-methylnoradrenaline is necessary centrally for the mediation of the antihypertensive effect of α-methyldopa in rats since inhibition of central dopamine β-hydroxylase also antagonised α-methyldopa. That α-methyldopa produces a centrally mediated anti-hypertensive effect in humans is supported by the findings of Sjoerdsma, Vendsalu & Engelman (1963). They observed that inhibition of peripheral dopa decarboxylase with MK 485 failed to alter the antihypertensive effect of α-methyldopa.

Further recent reports in rats (Day, Roach & Whiting, 1972; Finch & Haeusler, 1973) and cats (Ingenito, Barrett & Procita, 1970; Kale & Satoskar, 1970; Heise & Kroneberg, 1972; 1973; Torchiana, Lotti, Clark & Stone, 1973) convincingly show that α-methyldopa is able to produce a potent centrally mediated hypotensive effect. Heise & Kroneberg (1972, 1973) reported that the hypotension produced by icv α-methyldopa and α-methylnoradrenaline was abolished

by the icv administered α-adrenoceptor antagonists, yohimbine or phentolamine.

The central mechanism by which α-methyldopa lowers blood pressure is discussed in terms of central α-adrenoceptor stimulation and the relative central potencies of noradrenaline and α-methylnoradrenaline in Section 2, Chapters 1 and 2 of the experimental results.

Although it has been shown that α-methyldopa produces pronounced centrally mediated antihypertensive effects, the peripheral depressant actions of α-methyldopa upon sympathetic nerve function, plasma renin activity and arteriolar reactivity cannot be ignored as they probably contribute in part to its antihypertensive effect. The relative importance of the central and peripheral actions of α-methyldopa in man are as yet unknown.

Monoamine oxidase inhibitors.

The hypotensive effects of these compounds were discovered during their use in the treatment of depression Both hydrazine monoamine oxidase inhibitors, including iproniazid, pheniprazine, phenelzine and nialamide and the non-hydrazine inhibitors such as the Harmala alkaloids, tranylcypromine and pargyline have been shown to exert anti-hypertensive actions (see Zbinden, Randall & Moe, 1960; Schoepke & Swett, 1967).

Pargyline was first shown to be a potent antihypertensive agent by Horwitz & Sjoerdsma (1961). The fall in pressure was not seen until several weeks after the start of

pargyline treatment and was observed to continue for several days after discontinuation of treatment. Several mechanisms accounting for the hypotensive actions of the monoamine oxidase inhibitors have been made but none are completely satisfactory.

It seemed paradoxical that a compound capable of inhibiting a degradation pathway of physiological pressor amines should produce hypotension in man. However, monoamine oxidase inhibition appeared to parallel the antihypertensive activity in man and thus a causal relationship between these two effects was postulated for pargyline by Horwitz & Sjoerdsma (1961); the hypotension finally being the result of a selective decreased sympathetic nerve activity. However, Bryant, Schvartz, Torosdag, Fertig, Fletcher, Schwartz & Quan (1963) observed that the demethylated derivative of pargyline, although a potent monoamine oxidase inhibitor, did not lower blood pressure in man.

A ganglion blocking action, an adrenergic blocking action or a bretylium like effect and accumulation of transmitter at the receptor sites leading to a decreased sensitivity to noradrenaline are mechanisms which have been proposed to explain the antihypertensive effects of the monoamine oxidase inhibitors. None of these suggestions are fully satisfactory (see Schoepke & Swett, 1967; Nickerson, 1970b).

Kakimoto & Armstrong (1962) demonstrated that chronic monoamine oxidase inhibition was associated with an

accumulation of octopamine in many tissues of the rabbit. Kopin, Fischer, Musacchio, Horst & Weise (1965) observed that following the administration of ^{3}H-tyramine and ^{3}H-meta-tyramine their respective β-hydroxylated derivatives ^{3}H-octopamine and ^{3}H-meta-octopamine, were rapidly formed in the hearts and salivary glands of rats. The formation of these derivatives was greatly enhanced after monoamine oxidase inhibition. As octopamine has only approximately 1% of the activity of noradrenaline (Lands & Grant, 1952), Kopin et al.(1965) suggested that the antihypertensive effect obtained with chronic treatment with monoamine oxidase inhibitors was due to partial sympathetic blockade produced by the consequent replacement of noradrenaline by octopamine and related compounds, which act as false, inactive neurotransmitters.

It has been known for many years that monoamine oxidase inhibitors are active within the brain and that inhibition of central monoamine oxidase enzymes generally lasts longer than in peripheral organs (Pletscher, 1966) Although the mechanism by which these enzyme inhibitors cause psychomotor stimulation and changes in mood is still not fully understood. Spector, Hirsch & Brodie (1963) showed that central stimulation and behavioural effects observed after pargyline in several animal species were associated with an increased brain noradrenaline content. Since monoamine oxidase is believed to predominantly regulate the metabolism of intraneuronal monoamines, it has been suggested that an increase of intraneuronal amines following

monoamine oxidase inhibition leads to an increase of extra-neuronal amines as a consequence of a 'spillover' of amines onto central receptors from completely filled intraneuronal stores (Costa & Brodie, 1964; Pletscher, 1968). Strada & Sulser (1972) demonstrated that 'spillover' of noradrenaline onto hypothalamic adrenergic receptor sites occurred after systemic administration of monoamine oxidase inhibitors in the rat.

Falls in blood pressure to noradrenaline administered directly into the brains of several animal species have been reported by numerous investigators (see later in Introduction). Hypotension, probably due to indirectly centrally released noradrenaline, was observed by Hoyer & van Zwieten (1972) after intravertebral artery infusions of amphetamine in the cat. Yamori, de Jong, Yambe, Lovenberg & Sjoerdsma (1972) reported that l-dopa administered to spontaneously hypertensive rats in the presence of a monoamine oxidase inhibitor and a peripheral dopa decarboxylase inhibitor produced an increase in the brain stem noradrenaline level and a corresponding fall in blood pressure. This inverse correlation of raised brain stem noradrenaline content and hypotension was highly significant. Hence, from indirect evidence, it seems probable that part of the antihypertensive effect of monoamine oxidase inhibitors may result from an action within the central nervous system. Experiments must now be performed to directly disprove or substantiate this suggestion.

Monoamine oxidase inhibitors have been found to possess several side effects including incompatibilities with a large number of foodstuffs and clinically useful drugs; for details see reviews by Goldberg (1964), Blackwell, Marley, Price & Taylor (1967), Schoepke & Swett (1967), Jarvik (1970) and Nickerson (1970b). Because of side effects and incompatibilities pargyline is rarely used in the treatment of hypertension.

Mebutamate

It was reported by Dunsmore, Dunsmore, Bickford & Goldman (1957) and Boyd, Huppert, Mulinos & Hammer (1959) that the tranquilliser, meprobamate, exerted a mild hypotensive action in some patients suffering from essential hypertension. Ingestion of large amounts of meprobamate with suicidal intent or due to inadvertent overdosage usually produced marked hypotension (Shane & Hirsch, 1956; Charkes, 1958; Aitchison, 1960). Berger, Douglas, Kletzkin, Ludwig & Margolin (1961) developed mebutamate by replacing the propyl group in the meprobamate molecule with a secondary - butyl group. They observed that mebutamate lowered blood pressure in both normotensive and hypertensive animals and hypertensive humans. Pressor effects elicited by electrical stimulation of the medulla, hypothalamus and mesencephalon were reduced by mebutamate. In their cross circulation experiments, Berger et al.(1961) confirmed that the site of action of mebutamate was within the central nervous system and that the hypotension was produced by a reduced peripheral resistance; the cardiac output remained unaltered.

Mebutamate was shown to possess few peripheral effects and Berger et al.(1961) suggested that the antihypertensive action of mebutamate was due to a direct depressant effect on the vasomotor centres in the brain stem. Mulinos, Saltefors Boyd and Cronk (1961) and Corcoran & Loyke (1962) also reported that mebutamate was an effective antihypertensive agent. However, in contrast to these workers Bryant (1962) and Porter, Baird & Griswald (1962) observed little or no antihypertensive activity after mebutamamte. The latter workers demonstrated that there was no significant difference between the effects obtained with mebutamate, meprobamate or a placebo and that mebutamate differed from active compounds such as reserpine in that it did not have an appreciable effect when added to thiazide diuretic therapy. Drowsiness was observed as a side effect in a high percentage of cases in all studies. As mebutamate did not offer advantages over other more potent antihypertensive agents already in use, it was not accepted clinically.

Propranolol and other β-adrenoceptor antagonists.

Since the original observation by Prichard & Gillam (1964) that the β-adrenoceptor blocking agent propranolol effectively lowered elevated arterial pressure, several groups of investigators have confirmed this finding for chronic propranolol treatment. (Prichard & Gillam, 1966; 1969; Frohlich, Tarazi, Dustan & Page, 1968; Zacharias & Cowen 1970; Hansson, Malmcrona, Olander, Rosenhall, Westerlund, Åberg & Hood, 1972; Lydtin, Kusus, Daniel, Schierl, Ackenheil,

Kempter, Lohmöller, Nicklas & Walter, 1972; Zacharias, Cowen, Prestt, Vickers & Wall, 1972). However, conflicting results have been reported concerning the efficacy of propranolol in the treatment of hypertension. In contrast to the observations of Prichard & Gillam (1964, 1966, 1969), that propranolol was as potent as either α-methyldopa or guanethidine in lowering blood pressure Paterson & Dollery (1966), Richards (1966) and Waal (1966) found that propranolol produced only mild antihypertensive effects, no greater than those obtained with thiazide diuretics. Humphries & Devlin (1968) observed that propranolol was completely ineffective in the treatment of hypertensive Jamaicans and similarly, Seedat & Reddy (1971), found propranolol to be relatively ineffective in the treatment of hypertensive South African negroes.

It is generally accepted that acute administration of propranolol to hypertensive individuals results in little or no effect on blood pressure (Hamer & Sowton, 1965; Harrison, Griffin & Fiene, 1965; Ulrych, Frohlich, Dustan & Page, 1968) and that chronic propranolol treatment produces a slow and gradual return of the blood pressure to normal levels; normotensive levels being reached between 2 - 8 weeks from the start of the propranolol treatment (see previous references). However, Hansson, Zweifler, Julius & Ellis (1974) by taking home measurements of blood pressure observed that the peak antihypertensive effects of propranolol occurred after approximately 7 days of treatment.

Although most of the work concerning the anti-hypertensive properties of β-adrenoceptor blocking agents has been done using propranolol, hypotensive actions have been reported for several other β-adrenoceptor blocking agents such as alprenolol (Furberg & Michaelson, 1969; Tibblin & Åblad, 1969; Bengtsson, 1972), INPEA (Gilfrich, Rahn, & Schmahl, 1969) oxprenolol (Dorph & Binder, 1969; Eisalo, Luomanmäki & Heikkilä, 1969; Forrest, 1973; Gysling & Regoli, 1973; Muiesan, Motolese & Colombi, 1973), practolol (Prichard, Boakes & Day, 1971; Frohlich & Bhatia, 1972; Tarazi, Savard, Dustan & Bravo, 1972; Esler, 1974; Sundquist, Anttila & Arstila, 1974), pindolol (Collins & King, 1972; Morgan, Louis, Dawborn & Doyle, 1972; Thorpe, 1972; Persson & Ulrich, 1973), sotalol (Frohlich & Bhatia, 1972; Sundquist et al., 1974), tenormin or ICI 66082 (Amery, Billiet, Joossens, Meekers, Reybrouck & van Mieghem, 1973; Hansson, Åberg, Jameson, Karlberg & Malmcrona 1973; Dollery, Lewis & Myers, 1975) and tolamolol (Nordenfelt, Olsson & Persson, 1974). All β-adrenoceptor blockers have been well tolerated by individuals under treatment and these compounds do not produce orthostatic or exercise hypotension. Asthma and cardiac failure may be induced by β-blockers in patients prone to these conditions. However, the incidence can be kept very low by careful selection of patients. Other side effects mainly consist of psychiatric effects such as dreams, hallucinations, insomnia and depression although they are relatively rare (see review by Simpson, 1974).

Several factors have made the indentification of the

mechanism by which β-adrenoceptor antagonists lower blood pressure very difficult. Within a group of hypertensive individuals the doses of a particular β-blocking agent needed to produce similar antihypertensive effects vary greatly and the time taken to reach the maximum anti-hypertensive effect also varies. The β-adrenoceptor antagonists appear to be more potent in lowering the blood pressure in humans than in animals. Animal studies attempting to elucidate the mechanism of action of β-adrenoceptor blocking agents have been relatively disappointing.

Farmer & Levy (1968) reported that dose levels of propranolol and sotalol causing effective blockade of β-adrenoceptors had no hypotensive effect after acute or chronic administration to conscious hypertensive dogs and rats, although bradycardia did occur. Chronically administered propranolol lacked antihypertensive properties in Grollman hypertensive rats (Menard, Alexandre, Guidicelli, Auzan & Chevillard, 1973). Pressor effects have been observed after acute administrations of large doses of propranolol to normotensive rats (Dasgupta, 1968; Yamamoto & Sekiya, 1969, 1972; Regoli, 1970) and DOCA/saline hypertensive rats (Lydtin & Sommerfeldt, 1972; Dusting & Rand, 1974). The pressor effects to propranolol in rats have been attributed to release of adrenal catecholamines together with blockade of β-adrenoceptors subserving vasodilatation leading to a greater vasoconstrictor effect of the released amines. Dusting & Rand (1974) suggested that the pressor effects seen with propranolol may also be due to noradrenaline released from

adrenergic neurons. However, in spontaneously and pinealectomised hypertensive rats, falls in blood pressure have been reported after large doses of β-adrenoceptor antagonists (Roba, Lambelin & de Schaepdryver, 1972; Varva, Tom & Greselin, 1973; Karppanen, 1974). More recently, Dusting & Rand (1974) observed substantial antihypertensive effects after low doses of propranolol given chronically to DOCA/saline hypertensive rats.

Several mechanisms have been suggested to explain the antihypertensive actions of β-adrenoceptor blockers, but none are wholly satisfactory. Firstly, the reduction in cardiac output does not correlate well with the development of hypotension as the cardiac output is reduced both after acute and chronic administrationsof propranolol, whereas hypotension only occurs after chronic propranolol treatment (Ulrych et al., 1968; Frohlich et al., 1968; Prichard, Shinebourne, Fleming & Hamer, 1970; Lydtin et al., 1972). Tarazi and Dustan (1972) observed that the cardiac output was reduced in all patients given propranolol but antihypertensive effects occurred in only half the cases. Tarazi & Dustan (1972) suggested that in hypertensive patients with an elevated cardiac output the reduction in cardiac output may contribute to the fall in pressure. The peripheral resistance is reflexly increased due to the pronounced fall in cardiac output (Ulrych et al., 1968; Frohlich et al., 1968). However, after chronic propranolol treatment Tarazi & Dustan (1972) observed a relative fall in peripheral resistance and suggested that the fall in pressure after propranolol could be due to haemodynamic

adaptive changes.

Prichard & Gillam (1966, 1969) suggested that the fall in blood pressure observed after propranolol may be due to resetting baroreceptors at a lower level as a consequence of cardiac β-adrenoceptor blockade. They observed that in addition to lowering cardiac output propranolol reduced the reflex cardiac component in response to pressor stimuli. Such a mechanism would also account for the lack of orthostatic and exercise hypotension observed during treatment with β-adrenoceptor antagonists (see previous references).

The report by Waal (1966) casts some doubt as to whether the antihypertensive effect of propranolol was actually due to β-adrenoceptor blockade. Waal (1966) observed that in some patients the antihypertensive effect of propranolol paralleled an antihypertensive effect of quinidine. This observation along with the suggestion that the doses of propranolol used clinically were too high for the hypotension to be just due to β-adrenoceptor blockade led her to propose that the antihypertensive effect of propranolol was due to a non-specific membrane stabilising (local anaesthetic) effect (Waal, 1966). Gilfrich et al. (1969) showed that INPEA in single doses (oral or intravenous) had equal antihypertensive activity in both the d - and l - isomers (only the l - isomer of INPEA possessing β-adrenoceptor blocking properties indicating that some property other than β-adrenoceptor blockade is responsible for producing the antihypertensive effects). However,

Waal-Manning (1970) demonstrated that the l - isomer of propranolol actively lowered blood pressure whilst the d - isomer, which has no β-adrenoceptor blocking activity (Fitzgerald, 1969), was inactive. β-Adrenoceptor blocking agents such as INPEA, practolol and sotalol, which do not possess local anaesthetic actions (Fitzgerald 1969), have been shown to lower blood pressure (see previous references). Thus, the situation is unclear and it may be possible that in certain circumstances some other anti-hypertensive action, which is not mediated by β-adrenoceptor blockade, becomes detectable.

Day, Owen & Warren (1968) demonstrated that propranolol exerted adrenergic neuron blocking actions in isolated tissues. Although they suggested that propranolol may produce its adrenergic neuron blockade by a local anaesthetic action, they proposed that propranolol might be slowly accumulated in peripheral adrenergic neurons, thus explaining the slow development of its antihypertensive effect, and hence cause a reduction in sympathetic vasomotor tone which would tend to reinforce any hypotensive effect produced by the blockade of cardiac β-adrenoceptors. Barrett & Nunn(1970), Ganguly & Bhattacharya (1970) and Mylecharane & Raper (1970, 1973), in vitro, and Eliash & Weinstock (1971), in vivo, have also reported the adrenergic neuron blocking actions of propranolol. Mylecharane & Raper (1973) and Eliash & Weinstock (1971) showed that low concentrations of propranolol had a typical guanethidine-like action, which

suggested that some action other than local anaesthesia was responsible for mediating the blockade. Dusting & Rand (1974) failed to observe any adrenergic neuron action with low doses of propranolol in the pithed rat. The adrenergic neuron blockade produced by high doses of propranolol appeared to be due to its local anaesthetic activity (Barrett & Nunn, 1970; Ganguly & Bhattacharya 1970; Mylecharane & Raper, 1973).

Barrett & Nunn (1970) could not demonstrate adrenergic neuron blockade with practolol. However, Lewis (1974) observed that chronic administration of practolol to the pithed rat reduced the response to electrical stimulation and potentiated the response to intravenously administered noradrenaline. Lewis (1974) suggested that chronic practolol treatment may reduce the release of noradrenaline from the sympathetic nerve ending by an adrenergic neuron blocking action. In the treatment of essential hypertension with practolol, Esler & Nestel (1973) observed a reduction in the responsiveness of the sympathetic nervous system and thus proposed that this effect may be related to the antihypertensive actions of β-adrenoceptor blocking agents.

Adrenergic neuron blockade may contribute to the antihypertensive actions of propranolol and other β-adrenoceptor blockers in experimental animals but would appear to be of little importance in man as these compounds produce no postural or exercise hypotension unlike the classical adrenergic neuron blocking agents (see previous references).

That a reduced renin release produced by β-adrenoceptor blockers contributes to their antihypertensive effects has been intensely investigated in recent years, producing controversial results. The sympathetic nervous system exerts a modulatory influence upon renin release (see review by Davis, 1973). Winer, Chokshi, Yoon & Freedman (1969) indicated that both α- and β- adrenoceptors were involved in the mediation of renin secretion in man. However, the increased renin release produced by stimulation of the medulla oblongata in dogs was unaffected by phenoxybenzamine but was blocked by systemically administered propranolol (Passo, Assaykeen, Goldfien & Ganong, 1971). Collective evidence that strongly indicates that β-adrenoceptors are mainly responsible in the mediation of renin release has been reviewed by Davis (1973).

Bühler, Laragh, Baer, Vaughan & Brunner (1972) observed that propranolol reduced plasma renin activity in man and in addition found that propranolol produced a larger antihypertensive effect in hypertensive patients with a high plasma renin activity than those with normal and low plasma renin activities. Hence, these workers suggested a causal relationship between the reduced renin levels and antihypertensive effects obtained with propranolol in high renin essential hypertension. However, conflicting results have been reported by several investigators monitoring plasma renin activity during chronic propranolol therapy. Michelakis & McAllister (1972)

observed in essential hypertensives that during 6 days of propranolol treatment the plasma renin activity was rapidly decreased whereas the blood pressure remained unaffected. In addition, Tarazi, Dustan, Frohlich & Bravo (1972), Hansson (1973) and Stokes, Weber & Thornell (1974) failed to find a consistent relationship between the anti-hypertensive effects and reduced plasma renin activity values in patients with essential hypertension receiving propranolol.

In addition to propranolol, alprenolol (Castenfors, Johnsson & Orö, 1973), oxprenolol (Salvetti, Arzilli & Baccini, 1973), practolol (Salvetti et al. 1973), and tenormin (Aberg, 1974) have been shown to reduce plasma renin activity in man. Oxprenolol (Weber, Thornell & Stokes, 1974) and tenormin (Johns & Singer, 1974) reduced plasma renin activity in rabbits and cats respectively. However, in contrast, Amery, Billiet & Fagard (1974) and Dollery et al. (1975) did not effectively reduce plasma renin activity in man although antihypertensive effects were recorded and similarly, Esler (1974) found that practolol did not lower plasma renin activity. Pindolol was found to increase plasma renin activity in both humans and rabbits even though pindolol produced equipotent anti-hypertensive effects as propranolol (Stokes et al., 1974; Weber et al., 1974).

These results, besides casting doubt upon the relative importance of inhibiting renin release by β-adrenoceptor antagonists in the mediation of their anti-

hypertensive effects, have also produced a controversy as to whether renin release is mediated via β_1 - or β_2 - adrenoceptors. Further experiments must be undertaken to clarify both points.

Tarazi et al.(1970) subdivided hypertensive patients into groups with high, normal and low plasma volumes. Tarazi, Frohlich & Dustan (1971) and Julius, Pascual, Abbrecht & London (1972) observed that propranolol induced a reduction in the plasma volumes of hypertensive patients. Thus, it is possible that the reduction of plasma volume may partially contribute to the antihypertensive patients with high plasma levels.

The central nervous system provides a possible site for the cardiovascular depressant effects of β-adrenoceptor antagonists. The central cardiovascular effects of these compounds have not received much attention until recent years, although other central actions of β-adrenoceptor blocking agents are well documented both in animals and humans. Propranolol is both depressant and anticonvulsant in mice (Leszkovszky & Tardos, 1965; Murmann, Almirante & Saccani-Guelfi, 1966). Masuoka & Hansson (1967) demonstrated in the rat that systemically administered ^{14}C - labelled propranolol rapidly crossed the blood-brain barrier and reached a concentration in brain tissue fifty times higher than that in the blood. Bainbridge & Greenwood (1971) found propranolol to have significant tranquillising actions in rats. They could not attribute the tranquillising effects of propranolol to central β-adrenoceptor blockade

since the d - isomer of propranolol was as effective as the racemate.

In man propranolol has been used effectively in the treatment of Parkinsonism (Owen & Marsden, 1965) and Abramsky, Carmon & Lavy (1971) showed that patients treated with both propranolol and l - dopa had more relief of tremor than those given l - dopa alone. Weber & Reinmuth (1972) reported propranolol to be of significant benefit in the treatment of migraine and propranolol has also been found to be active in the treatment of anxiety (Granville-Grossman & Turner, 1966; Wheatley, 1969). Wheatley (1969) demonstrated that propranolol was as effective as chlordiazepoxide in the treatment of anxiety states and caused fewer side effects. In man, the anti-anxiety effect of propranolol appears to be due to β-adrenoceptor blockade as d-propranolol was inactive against anxiety (Bonn & Turner, 1971). Oxprenolol has been shown to effectively reduce emotional tachycardia (Imhof, Blatter, Fuccella & Turri, 1969) and propranolol improves the tension and depression shown by abstinent alcoholics (Carlsson & Johansson, 1971). The report that practolol is effective in anxiety states (Bonn, Turner & Hicks, 1972) is the main argument against a central mechanism of action of β-adrenoceptor blockers in anxiety, as only small amounts of the water-soluble blocker enter the central nervous system (Scales & Cosgrave, 1970). However, it is still not known whether the small amount of practolol which enters the brain is sufficient to produce

central β-adrenoceptor blockade.

Centrally mediated side effects, such as vivid dreams, hallucinations, insomnia and depression have been reported during clinical antihypertensive studies with β-adrenoceptor blocking agents. (Prichard & Gillam, 1969; Collins & King, 1972; Hansson et al., 1972; Morgan et al., 1972; Zacharias et al., 1972). These and other central actions of β-adrenoceptor antagonists are described in the review by Greenblatt & Shader (1972).

During their investigation into the central cardiovascular effects of isoprenaline Gagnon & Melville (1967) occasionally observed small blood pressure and heart rate reductions after icv pronethalol in anaesthetised cats. Propranolol administered into the third ventricles of anaesthetised cats treated with gallamine produces marked hypotension and bradycardia (Waite, personal communication). Direct administration of propranolol in the rat brain produced pronounced bradycardia (Lavy & Stern, 1970). Ito & Schanberg (1974) observed that only high doses of propranolol administered via the cisterna magna to anaesthetised rats produced depressor effects; low doses of propranolol by this route induced pressor effects. Hypotension and bradycardia occurs in anaesthetised dogs after central administration of propranolol via the carotid (Stern, Hoffman & Braun, 1971) and vertebral (Stern et al., 1971; Carter, Mitchell & Poyser, 1974) arteries, and the lateral and fourth cerebral ventricles (Srivastava, Kulshretha, Singh & Bhargava, 1973; Carter et al., 1974).

However, in unanaesthetised dogs Conway & Lang (1974) failed to observe any long lasting reductions in blood pressure and heart rate after icv propranolol.

However, in anaesthetised cats there is some doubt as to whether the hypotensive effect of propranolol is due to central β-adrenoceptor blockade as both dl- and d- propranolol administered icv produced falls in blood pressure; whilst only icv dl-propranolol produced a significant bradycardia (Kelliher & Buckley, 1970). They observed that the perfusion of either dl or d-propranolol through the cerebroventricular system generally produced an increase in adrenaline levels along with a relative depletion of noradrenaline in various brain areas. Kelliher & Buckley (1970) suggested that the centrally mediated cardiovascular effects of propranolol were due not to central β-adrenoceptor blockade but to the changes in brain adrenaline and noradrenaline levels. Offerhaus & van Zwieten (1974) observed in anaesthetised cats that the hypotension seen after intravenous and vertebral artery infusions of dl-propranolol were not significantly different thus indicating that propranolol probably lacked marked central hypotensive properties. However, they observed that d- and dl-alprenolol produced significantly greater hypotensive effects when given via the vertebral artery compared to intravenous administration. Hence, they proposed that alprenolol possesses a significant central hypotensive action which is independant of central β-adrenoceptor blockade.

In the anaesthetised cat, Garvey, Ram, Woodhouse & Booker (1972) demonstrated that intracarotid propranolol produced hypotension, bradycardia and reduced spontaneous peripheral sympathetic nerve activity. These workers observed that these actions of propranolol were centrally mediated as the time course of propranolol levels in the hypothalamus and its projection areas correlated closely to the development of the cardiovascular effects. Central administration of propranolol abolished the pressor response, tachycardia and increase in peripheral sympathetic nerve activity induced by hypothalamic stimulation but the cardiovascular effects of intravenous adrenaline and isoprenaline remained unaffected.

Dollery, Lewis, Myers & Reid (1973) and Reid, Lewis, Myers & Dollery (1974) using conscious rabbits found that only dl - and l- propranolol were active after icv injection in abolishing the cardiovascular effects of icv isoprenaline and themselves causing hypotension and bradycardia; the d- isomer of propranolol was inactive on both accounts.

The central mechanism by which propranolol induces hypotension and bradycardia is unclear. Only Dollery et al. (1973), Reid et al.(1974) and Day & Roach (1974) have suggested that propranolol acts via central β-adrenoceptor blockade. It should be noted that their studies were undertaken using conscious animals whereas those workers that have demonstrated a non-specific local anaesthetic action used anaesthetised animals.

Srivastava et al.(1973) observed that propranolol administered onto the floor of the fourth ventricle, besides producing hypotension, reduced the pressor effects resulting from electrical stimulation of the medullary vasomotor loci. They suggested that the hypotension and bradycardia observed in the anaesthetised dog after icv propranolol was due to a central inhibition of sympathetic tone probably resulting from some action other than a central local anaesthetic effect . This is supported by the findings of Garvey et al.(1972) who observed a reduced peripheral sympathetic nervous activity after intracarotid propranolol.

Korczyn & Goldberg (1974) found that intravenous propranolol abolished the pressor effects induced by carotid occlusion, chemoreceptor stimulation with lobeline and sciatic nerve stimulation. They proposed that the depression of these hypertensive reflexes by propranolol are of central origin and may contribute to its anti-hypertensive actions.

Winer (1974) observed that the central administration of propranolol in conscious renal hypertensive dogs produced pronounced antihypertensive effects but the elevated plasma renin activity remained unaltered thus indicating that the mechanism whereby propranolol causes renin suppression is not of central origin and that the centrally mediated antihypertensive action of propranolol is unrelated to its antirenin effect.

Three studies have attempted to correlate central levels of propranolol with its cardiovascular depressant actions. Garvey et al.(1972) observed that in anaesthetised cats an intracarotid injection of propranolol results in hypotension and propranolol being concentrated mainly in the hypothalamus and its projection areas; the lowest concentration of propranolol appeared in the cerebral cortex. Bakke, Dollery, Lewis, Myers & Reid (1974) have shown that propranolol levels in the hypothalamus, midbrain and medulla-pons regions of the rabbit brain after intravenous dosage were similar to the level observed after icv dosage at the time of the maximum blood pressure reduction. Offerhaus & van Zwieten (1974) demonstrated a significant correlation between the concentration of propranolol in both plasma and cerebrospinal fluid and the maximal hypotensive effect observed after vertebral artery infusions of propranolol. No such correlations were demonstrated after intravenous infusions of propranolol. However, the hypotension showed a significant correlation with the alprenolol concentration in the cerebrospinal fluid after intravenously administered alprenolol. After prolonged subcutaneous dosage of propranolol in cats, propranolol was observed not to accumulate in the cerebrospinal fluid (Offerhaus & van Zwieten, 1974).

All the groups of workers that have studied the central cardiovascular effects of propranolol have reported that both the hypotension and bradycardia develop rapidly

after the introduction of the β-adrenoceptor blocker into the brains of various species. However, in clinical studies, although central effects are observed relatively early in the treatment of hypertension with β-adrenoceptor blockers, the development of hypotension is seen much later. These effects are difficult to explain for those β-adrenoceptor blocking agents which are known to enter the brain relatively easily.

The central mechanisms of action of several β-adrenoceptor antagonists and their relative clinical significance are discussed in Chapter 4 of the Experimental Results.

Clonidine.

The numerous studies that have been carried out on this compound, which was originally developed as an α-adrenoceptor stimulant to be used in the local treatment of rhinitis and conjunctivitis, have proved it to be an important advance in the development of new antihypertensive compounds. Research into the mode of action of clonidine has revealed a new and active line in the search for new antihypertensive agents as well as providing useful knowledge about the central control of blood pressure and heart rate.

Clonidine has been shown to be antihypertensive in man (Bock, Heimsoth, Merguet & Schoenermark, 1966; Michel, Zimmerman, Nassehi & Seraphim, 1966; Davidov, Kakaviatos & Finnerty, 1967; Ng, Phelan, McGregor, Laverty,

Taylor & Smirk, 1967) and hypotensive in animals (Hoefke & Kobinger, 1966; Kobinger & Walland, 1967a; Boissier, Guidicelli, Fichelle, Schmitt & Schmitt, 1968; Rand & Wilson, 1968).

Low doses of clonidine administered systemically produce a brief rise in blood pressure followed by a gradual fall accompanied by bradycardia (Hoefke & Kobinger 1966; Kobinger & Walland, 1967a; Boissier et al., 1968; Rand & Wilson, 1968). Boissier et al. (1968) demonstrated that the transient hypertension was present in normal, reserpinised and in adrenalectomised animals and was accompanied by contraction of the cat's nictitating membrane. The above groups of workers demonstrated that these effects were a result of direct peripheral α-adrenoceptor stimulation by clonidine as they were abolished by phentolamine and phenoxybenzamine. The pressor effects are absent in man and animals after oral or slow intravenous infusion of clonidine (Laverty, 1973).

Since the structure of clonidine is similar to that of the vasodilator and α-adrenoceptor blocking agent tolazoline, it is not surprising that these properties have also been demonstrated for clonidine. Shaw, Hunyor & Korner (1971a) observed that clonidine possessed direct vasodilator properties as they still obtained a significant blood pressure reduction with clonidine, due to a decreased peripheral resistance, after complete blockade of the autonomic nervous system with phenoxybenzamine, propranolol and atropine in unanaesthetised rabbits. Peripheral

∝-adrenoceptor blocking effects of clonidine were shown by Ng et al.(1967) and Boissier et al.(1968). Rand & Wilson (1968) showed that clonidine did not possess adrenergic neuron blocking effects as stimulation of the sympathetic nerves to the heart and intestines were unaffected by clonidine. However, clonidine has been found to possess local anaesthetic activity (Laverty, 1973). Naylor, Price, Swann, McInnes, Race & Lowe (1968) have fully described the peripheral actions of clonidine. These peripheral effects do not seem to be sufficiently intense nor do they occur at sufficiently low doses to completely explain the antihypertensive effects of clonidine.

Clonidine, like many other antihypertensive compounds, reduces plasma renin activity in man. It has been suggested that the decrease in plasma renin activity by clonidine may be due to an inhibition of sympathetic nervous system activity (Hökfelt, Hedeland & Dymling, 1970). However, it is not known whether this action of clonidine on plasma renin activity contributes to its antihypertensive action.

During antihypertensive therapy with clonidine in man, the cardiac output is markedly decreased in the acute stage of treatment. The decreased cardiac output is mainly due to a reduced stroke volume (Reubi, Vorburger & Bütikofer, 1970). During chronic treatment with clonidine, little change is observed in the cardiac output and renal haemodynamics and a reduced peripheral resistance largely

accounts for the fall in blood pressure (Reubi et al., 1970). The reduced peripheral resistance is predominant in the upright position (Brest, 1970). Clonidine does not produce postural or exercise hypotension (see previous references; Muir, Burton & Lawrie, 1969) and the reflex mechanisms responsible for the cardiovascular adjustments to the valsalva manoeuvre are not abolished (Kroetz, McRaven, Kioschos & Kirkendall, 1970).

Side effects encountered with clonidine treatment are sedation and depression (see previous references). These effects along with the lack of postural and exercise hypotension and the inability to satisfactorily explain the hypotensive effect of clonidine in terms of a peripheral nervous, or direct, site of action has led to a vast number of reports investigating the central nervous system as a likely site of action. Doses of clonidine, which were too small to have any significant effect on blood pressure and heart rate when given intravenously, caused pronounced hypotension and bradycardia when administered into the cisterna magna (Kobinger & Walland, 1967a,b; Schmitt, Schmitt, Boissier, Guidicelli & Fichelle, 1968; Dollery & Reid, 1973), the lateral ventricle (Sherman, Grega, Woods & Buckley, 1968; Schmitt & Schmitt, 1969), the third ventricle (Schmitt, 1970) the vertebral artery (Sattler & van Zwieten, 1967; Constantine & McShane, 1968; Katic, Lavery & Lowe, 1972; Schmitt, Schmitt & Fénard, 1973b) and the rat hypothalamus (Struyker-Boudier & van Rossum, 1972). In cross-circulation

experiments performed in dogs, low doses of clonidine injected into the cephalic circulation induced a hypotension in the trunk of the recipient dog connected to the head only by the spinal cord (Sherman et al., 1968). The above groups of workers reported that after the administration of clonidine directly into the central nervous system, the initial transient pressor response observed after intravenous clonidine was absent.

Kobinger & Walland (1967 b) observed that intracisternal clonidine in the dog induced a fall in cardiac output along with the hypotension and bradycardia whilst the peripheral resistance and stroke volume remained relatively constant. The hypotension and bradycardia seen after clonidine given either centrally or peripherally is associated with a marked reduction in the efferent nervous discharge rate in the splanchnic and cardiac sympathetic nerves (Kobinger & Walland, 1967b; Hukuhara, Otsuka, Takeda & Sakai, 1968; Schmitt et al., 1968; Sherman et al., 1968; Klupp, Knappen, Otsuka, Streller & Teichmann, 1970; Woodhouse, Ram & Garvey, 1972; Loew & Waite, 1974).

The bradycardia produced by clonidine after central or peripheral administration appears, like the hypotension, to be predominantly of central origin. Naylor Price, Stone & Lowe (1969) demonstrated that clonidine did not induce bradycardia when given to isolated heart muscle or denervated hearts in situ indicating that it has no direct depressant action. However, a direct

depressant effect upon the sino-atrial node has been shown by Scriabane, Stavorski, Wenger, Torchiana & Stone (1970). Although the bradycardia is mainly produced as a result of a reduced sympathetic outflow to the heart from the central nervous system (see previous references) several workers have demonstrated that the bradycardia is also due, in part, to an increased efferent vagal activity to the heart (Boissier et al., 1968; Robson & Kaplan 1969; Robson, Kaplan & Laforce, 1969; Naylor & Stone, 1970; Kobinger & Walland, 1972a,b; Woodhouse et al., 1972). However, Rand & Wilson (1968) were able to completely block the clonidine bradycardia with systemic propranolol pretreatment; propranolol did not affect the the hypotension. Thus they indicated that in their experiments the bradycardia was mediated only through the sympathetic nervous system.

Several reports have been made attempting to localise the cardiovascular depressant effects of clonidine to a particular brain area. The primary site of action of clonidine appears to be within the medulla oblongata by virtue of transection experiments (Hukuhara et al., 1968; Schmitt & Schmitt, 1969) and its potent effects observed after direct administration to this area via the cisterna magna and vertebral artery (see previous references). Bousquet & Guertzenstein (1973) observed pronounced hypotension after specific administration of clonidine onto the ventral surface of the brain stem of cats. Shaw, Hunyor & Korner (1971b) observed both medullary and

diencephalic actions of clonidine in the rabbit. Clonidine induced hypotension and bradycardia after injections into various parts of the rat hypothalamus (Struyker-Boudier & van Rossum ,1972). The largest effects of clonidine were found in the far posterior hypothalamus at the level of fasciculus mamillotegmentalis and the decussatio-supramamillaris. Klevans, Kepner & Kovacs (1973) suggested that clonidine influences resting blood pressure via an action within the forebrain of the cat in addition to its effect at the medullary level.

The mechanism by which clonidine acts within the central nervous system is still relatively unclear. Intact central monoaminergic neurons appear to be necessary for the mediation of the central cardiovascular effects of clonidine (Dollery & Reid, 1973). These workers inhibited the hypotensive effects of clonidine in rabbits by destroying central noradrenergic neurons with 6-hydroxy-dopamine pretreatment. Schmitt, Schmitt & Fénard (1973c) also prevented the central actions of clonidine by lesioning the sympatho-inhibitory area of the medulla.

The first specific central mechanism for clonidine was proposed by Schmitt et al. (1968). They suggested that the hypotension and bradycardia resulted from a central stimulation of α-adrenoceptors by clonidine. However, results involving the effects of clonidine after peripherally and centrally administered α-adrenoceptor blocking agents are inconsistent. Inhibition of the depressant effects of clonidine by intravenous phentolamine

and phencxybenzamine was shown by Hoefke & Kobinger (1966). Kobinger & Walland (1967a, b) and Nayler et al.(1968). In contrast, Schmitt & Schmitt (1970) observed that intravenous phentolamine and tolazoline only weakly antagonised the effects of clonidine whereas phenoxybenzamine was completely ineffective. Schmitt & Schmitt (1970) suggested that the blockade of clonidine with systemically administered α-adrenoceptor blockers, obtained by the above groups of investigators, was due to a masking effect produced by peripheral α-adrenoceptor blockade. However, Bolme & Fuxe (1971) blocked the actions of clonidine with intravenous phenoxybenzamine; the inhibition occurring after the fall in blood pressure due to phenoxybenzamine had fully recovered. Bolme & Fuxe (1971) suggested that the blockade was due to central and not peripheral α-adrenoceptor blockade.

Schmitt & Schmitt (1970) could not antagonise the effects of clonidine in the dog with icv administered phentolamine. However, the α-adrenoceptor antagonists piperoxan and yohimbine administered either systemically or centrally were found to abolish all the central effects of clonidine (Schmitt et al., 1971, 1973b). That central phentolamine lacks activity against clonidine, as reported by Schmitt & Schmitt (1970), has not been confirmed in other studies. Phentolamine injected via the vertebral artery in dogs antagonised the blocking effects of clonidine on the carotid occlusion reflex, whereas intravenous tolazoline and phenoxybenzamine did not(Katic,

et al., 1972). They suggested that very high levels of α-adrenoceptor antagonists must be obtained in the medulla in order to block the effects of clonidine. Bucher, Buckingham, Finch & Moore (1973) observed that icv phentolamine abolished the antihypertensive effects of peripherally administered clonidine in hypertensive rats.

Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson (1970) indicated that clonidine was able to directly stimulate noradrenaline receptor sites in all parts of the rat brain and spinal cord. Bolme & Fuxe (1971) confirmed this finding as they were able to inhibit the hypotension and bradycardia of peripherally administered clonidine with the noradrenaline blocking agents haloperidol and phenoxybenzamine but found that dopamine receptor blocking agents pimozide and spiroperidol were ineffective.

Schmitt et al. (1971, 1973b) proposed that clonidine might produce its cardiovascular depressant effects by stimulating baroreceptor pathways within the central nervous system. The baroreceptor fibres originating in the carotid sinus have their first synapse in the nucleus tractus solitarii (Crill & Reis, 1968; Seller & Illert, 1969) and the nucleus reticularis paramedialis (Miura & Reis, 1969a). Noradrenaline containing neurons have been shown to be present within these areas (Dahlstrom & Fuxe, 1964). Thus, Schmitt et al. (1971, 1973b) suggested that a second intermediary noradrenergic neuron connects the baroreceptor input to the bulbospinal sympathetic neuron

(the third neuron). The α-adrenoceptors could be situated on the membrane of the third neuron and stimulation of the α-adrenoceptors on this neuron could accordingly inhibit or depress sympathetic outflow to the heart and peripheral vasculature.

Recent reports by Haeusler (1973b, 1974b) using anaesthetised cats indicate that clonidine produces its cardiovascular depressant effects by a prolonged central activation of the baroreceptor reflex. Haeusler observed that clonidine and bilateral sinus nerve stimulation produced similar depressant effects on the immediate increase in peripheral sympathetic nerve activity and rise in blood pressure and heart rate to electrical stimulation of the posterior hypothalamus and fastigial nucleus of the cerebellum (Haeusler, 1973b). Low doses of clonidine, which induced little or no depression of sympathetic nerve activity and blood pressure, increased the hypotension observed after bilateral sinus nerve stimulation. Clonidine also potentiated the bradycardia seen after stimulation of the depressor baroreceptor reflex. Hypothalamic stimulation produced a general increase in sympathetic nerve activity. Both clonidine and bilateral sinus nerve stimulation preferentially reduced the increased activity in the adrenergic vasomotor fibres induced by hypothalamic stimulation without affecting the evoked contractions of the nictitating membrane (Haeusler, 1974b).

Haeusler (1973a) found that the depressor effect

of sinus nerve stimulation, like that of clonidine, was abolished after injections of various α-adrenoceptor blocking agents into the lateral ventricles of rats and cats, further supporting the view that clonidine produces its effects via central activation of the baroreceptor reflex and that central α-adrenoceptors are involved in mediating this reflex.

Robson & Kaplan (1969) demonstrated that intravenous clonidine potentiated the vagal reflex bradycardia elicited in dogs by the pressor agents angiotensin and noradrenaline. In dogs, Kobinger & Walland (1972a) could only obtain a facilitation of the vagal reflex bradycardia, induced by noradrenaline or angiotensin, after clonidine administered into the cisterna magna; intravenous clonidine was ineffective. In a later paper, Kobinger & Walland (1972b) observed that the facilitation of the vagal reflex bradycardia by clonidine was inhibited by centrally administered phentolamine. Thus, they concluded that vagal reflex bradycardia is facilitated by an action of clonidine on α-adrenoceptors within the central nervous system. Kobinger & Walland (1973) suggested that the efferent vagal activity to the heart is influenced and regulated by noradrenergic neurons within the central nervous system and that the induced bradycardia partially resulting from an increased efferent vagal activity and the potentiation of reflex bradycardia of pressor agents, observed after intracisternal clonidine, is due to clonidine mimicking the action of the

natural transmitter, noradrenaline, on central α-adrenoceptors. Hence, it may be possible that virtually the same central mechanism is involved in the induction of a reduced efferent sympathetic tone and an increased efferent vagal tone by clonidine.

That clonidine produced cardiac slowing by a direct central activation of the barcreceptor pathway was confirmed by Korner, Oliver, Sleight, Chalmers & Robinson (1974) using the conscious rabbit. They observed that clonidine altered heart rate by directly stimulating the central medullary cardiac motoneurons that receive inputs from the arterial and cardio-pulmonary baroreceptors. Clonidine produced only slight activation of cardiac motoneurons not receiving baroreceptor inputs. Aars (1972) had previously suggested that the bradycardia induced by clonidine was a consequence of peripheral baroreceptor resetting. Thus, clonidine would tend to induce enhanced afferent arterial baroreceptor discharges at a given arterial blood pressure. Aars (1972) demonstrated that the resetting of baroreceptors by clonidine results from dilatation of the aortic wall probably due to an α-adrenolytic effect of clonidine. Korner et al.(1974) observed that only after high doses did clonidine produce peripheral baroreceptor resetting in conscious rabbits. However, at these high doses the peripheral mechanism only reinforced the direct central action of clonidine.

In the cat, dog and rabbit experimental evidence has been described which indicates that the cardiovascular

depressant effects of clonidine are mediated through prolonged activation of central α-adrenoceptors situated on central adrenergic neurons within the baroreceptor reflex. Besides facilitating carotid sinus nerve stimulation to produce an enhanced hypotension and bradycardia (see previous references), clonidine also reduces the blood pressure and heart rate increases due to carotid occlusion (Katic et al., 1972). Both actions of clonidine are prevented by prior central α-adrenoceptor blockade. As clonidine produces a prolonged reduction in the sympathetic tone to the cardiovascular system and an increased efferent vagal tone to the heart from the central nervous system via stimulation of the depressor baroreceptor reflex, one might expect the pressor effects produced by an opposing action (i.e. stimulation of the pressor baroreceptor reflex by carotid occlusion) to be relatively reduced by clonidine. Hence, these experimental results make it very difficult to explain why in man orthostatic and exercise hypotension are absent during antihypertensive therapy with clonidine.

The mechanism involving the stimulation of medullary α-adrenoceptors has now been generally accepted to account for the centrally mediated antihypertensive effects of several other compounds. These compounds include α-methyldopa, l - dopa, monoamine oxidase inhibitors, amphetamine and related anorectic agents, imipramine and reserpine (see van Zwieten, 1973).

In recent years there has been great interest in the presynaptic regulation of noradrenaline release from sympathetic neurons by means of a local feed-back system. It is suggested that neuronal noradrenaline liberated in response to nerve impulses, stimulates α-receptors situated on the presynaptic neuronal membrane and leads to an inhibition of further noradrenaline release in response to continuing nerve impulses (Häggendal, 1970; Farnebo & Hamberger, 1971a; Starke, 1971, 1972; Enero, Langer, Rothlin & Stefano, 1972; McCullock, Rand & Storey, 1972; and see short review by Langer, 1974).

Low concentrations of clonidine have been found to decrease the stimulation evoked release of noradrenaline from peripheral sympathetic neurons (Werner, Starke & Schumann, 1970; Farnebo & Hamberger, 1971a; Armstrong & & Boura, 1973). Farnebo & Hamberger (1971b) suggested that a similar feed-back mechanism operates in central as well as peripheral noradrenergic neurons. They found that the α-adrenoceptor blocking drugs phenoxybenzamine and phentolamine augment, while clonidine, which stimulates α-adrenoceptors, decreases the stimulation induced overflow of tritium from brain slices loaded with ^{3}H-noradrenaline. Starke and Montel (1973) observed that clonidine reduced the amount of ^{3}H-noradrenaline released by electrical stimulation of rat cerebral cortex slices.

On the basis of this hypothesis, that prejunctional α-receptors mediate a negative feed-back system, the cardiovascular depressant effects of clonidine might be due to a decreased liberation of noradrenaline from specific

central adrenergic nerve endings (Stark & Altmann, 1973). A small number of investigations have been made attempting to clarify whether the hypotensive and bradycardic effects of clonidine occur as a result of stimulation of either pre- or post- synaptic ∝-receptors or both. If clonidine was to act by stimulating only post-synaptic ∝-adrenoceptors then its action would be independent of endogenous noradrenaline. Conversely, if complete depletion of endogenous noradrenaline could be achieved then the spontaneous liberation of the transmitter would be eliminated, then if clonidine acts presynaptically to reduce endogenous noradrenaline release its effects should be absent. Dollery & Reid (1973) observed in the rabbit that central catecholamine depletion and destruction of central noradrenergic neurons, produced by intracisternal 6-hydroxydopamine, completely prevented the response to intracisternal clonidine. However, contrasting results were obtained by Haeusler(1974a,c). Catecholamine depletion obtained with reserpine, ∝-methyl-para-tyrosine and 6-hydroxydopamine pretreatment in cats failed to modify the cardiovascular actions of clonidine. Kobinger & Pichler (1974) also indicated that clonidine acts post-synaptically as they observed in decerebrate rats that clonidine still facilitated vagal reflex bradycardia after the granular stores of noradrenaline had been depleted by reserpine and the noradrenaline synthesis inhibitor ∝-methyl-para-tyrosine.

Reservations should be made concerning these

findings as the exact extent to which noradrenaline stores were depleted was not known in each case. Also in the study performed by Dollery & Reid (1973) it is possible that pre- and post synaptic receptors were destroyed by 6-hydroxydopamine. Thus, this problem concerning the relative importance of pre - and post-synaptic α-receptors still remains unsolved at this time.

To date it has been believed that the central actions of clonidine and other α-adrenoceptor stimulants involve central noradrenergic neurons (see short review by van Zwieten, 1973). Hökfelt, Fuxe, Goldstein & Johansson (1973, 1974) have demonstrated that adrenaline containing neurons exist within the rat brain innervating areas known to be involved in vasomotor control such as the nucleus tractus solitarii, the nucleus motorius dorsalis nervi vagi, the sympathetic lateral column, the locus coeruleus and the hypothalamus. Bolme, Corrodi, Fuxe, Hökfelt, Lindbrink & Goldstein (1974) found that low doses of clonidine stimulated the central adrenaline receptors producing hypotension and respiratory actions and that larger doses of clonidine stimulated noradrenergic receptors producing effects on spinal reflexes. Bolme et al. (1974) observed that the hypotension resulting from stimulation of adrenaline neurons by clonidine was abolished by low doses of the α-adrenoceptor antagonists piperoxan and yohimbine. However, they were able to block the clonidine induced bradycardia with yohimbine but not by piperoxan. Thus these workers suggested that in the rat, the heart rate

is controlled by noradrenaline receptors, or at least by different adrenergic mechanisms than the arterial pressure and respiration; the latter two parameters being controlled by adrenaline receptors.

Besides producing potent hypotensive effects with bradycardia mainly by a central mechanism of action several other central actions have been reported for clonidine, such as pronounced sedation in man (see previous references) and experimental animals (Laverty & Taylor, 1969; Delbarre & Schmitt, 1971), inhibition of salivary secretion (Rand, Rush & Wilson, 1969), production of central hyperglycaemia (Bock & van Zwieten, 1971), induction of sleep (Holman, Shillito & Vogt, 1971), inhibition of food and water intake (Le Douarec, Schmitt & Lucet, 1971, 1972), hypothermia (Tsoucaris-Kupfer & Schmitt, 1972) and possession of antinociceptive properties (Schmitt, Le Douarec & Petillot, 1974). Although the exact site and mode of these actions have yet to be determined many have been reported to be inhibited by central α-adrenoceptor blockade.

Analogues of clonidine show similar hypotensive and sedative properties of varying degrees (Laverty, 1969). Two other compounds, which are chemically related to clonidine, Wy-8678 (2,6-dichlorobenzylideneaminoguanidine acetate) and xylazine (BAY 1470, 2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazin) appear to possess similar effects to those of clonidine in reducing sympathetic outflow by stimulation of α-adrenoceptors within the

central nervous system (Baum & Shropshire, 1970; Baum, Shropshire, Rowles, van Pelt, Fernandez, Echfeld & Gluckman 1970; Schmitt, Fournadjiev & Schmitt, 1970). These compounds also exhibit similar side effects to clonidine However, although both Wy-8678 and xylazine exhibit potent centrally mediated cardiovascular depressant effects, the central component does not contribute to their overall hypotensive effect to such a large extent as clonidine, as they have been shown to possess powerful peripheral adrenergic neuron blocking actions (see previous references).

Until the recent development of clonidine and related compounds no directly acting α-sympathomimetic agents able to cross the blood-brain barrier were known. As mentioned earlier, clonidine produces many side effects due to central α-adrenoceptor stimulation. Research at the present time is mainly concerned with a search for similar compounds, able to produce specific centrally mediated antihypertensive effects, which are divorced from sedation and other central effects.

CENTRAL CONTROL OF THE CARDIOVASCULAR SYSTEM.

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS.

Medullary or Bulbar Pathways.

During the past century a determined effort has been made to understand how the brain influences and controls the peripheral cardiovascular system. Relatively crude ablation or transection techniques along with electrical stimulation of various brain areas were the first methods used, as the initial experiments were mainly concerned with identifying the essential brain regions involved in cardiovascular control. In 1908, the Horsley-Clarke stereotaxic instrument was developed (see Ranson, 1934) which allowed responses to be recorded after electrical stimulation of specific brain regions. This advancement together with the recent developments of histochemical fluorometric techniques and microiontophoresis has placed more emphasis upon mapping various pathways and identifying the relevant neurotransmitters.

Dittmar (1870) observed that after separating the cord and medulla from the rest of the brain a reflex pressor effect could still be induced by central stimulation of the sciatic nerve. He concluded that the centre for vasoconstriction was situated in the bulbar region. A year later, Owsjannikow (1871), using rabbits and cats, found that successive caudal transections of the brain stem had no effect on blood pressure until he reached a pontile level just behind the caudal border of the inferior colliculi.

As sections were made more caudally the blood pressure gradually fell until after sectioning at a point 4 - 5 mm above the calamus scriptorius the arterial pressure fell to the low level normally obtained by section of the cervical cord. He also observed that as the transections of the medulla were made caudally so the pressor reflexes were reduced until they were completely absent. Dittmar (1873) combined transection experiments with a microscopical study and concluded that the vasomotor area was bilateral and extended approximately 3 mm above the point of the calamus scriptorius to the fovea superior and included the diffuse part of the superior olive.

Using cats, Ranson & Billingsley (1916) explored the floor of the fourth ventricle with a needle electrode and found two discrete areas; a pressor area situated at the apex of the ala cinerea and a depressor point located in the area postrema just lateral to the obex. Wang & Ranson (1939a) observed cardiovascular responses after electrical stimulation in the depths of the pons and bulbar regions using an accurate stereotaxic instrument. The maps obtained by Monnier (1939), Wang & Ranson (1939a) and Alexander (1946) concerning the distribution of the central pressor points are in general agreement. They were found most often in the anterior end of the medulla in the dorsomedial and ventrolateral quadrants of the reticular formation, shifting slightly in the middle areas of the medulla to a central and dorsomedial concentration and as one approaches the caudal levels of the medulla,

the pressor points are observed to shift almost entirely to the most lateral portions of the dorsal and ventral areas of the bulb. The distribution of the described depressor points appears to be more variable. Alexander (1946) and Amoroso, Bell & Rosenberg (1954) placed the depressor points exclusively in a medial position, while most other workers observed a greater dispersal, with a more central concentration. (See Bard, 1960; Oberholzer, 1960 and Smith, 1965). However, it is generally agreed that the depressor points occupy a more caudal position than do the pressor ones.

It is now known that the brain receives inputs, concerning the state of the peripheral cardiovascular system, from the systemic arterial baroreceptors (arising mainly from the carotid sinus and aortic arch, while minor groups are located in the common carotid and subclavian arteries) pulmonary arterial baroreceptors, cardiac mechanoreceptors, arterial chemoreceptors, lung stretch receptors and somatic, visceral and special sensory inputs (see Heymans & Neil, 1958; Korner, 1971). After entering the medulla the cardiorespiratory afferent fibres in the IX (Glossopharyngeal) and X (Vagus) cranial nerves pass rostrocaudally through the middle and posterior thirds of the tractus solitarius and relay in the tractus and the medial part of the nucleus of the tractus (Bonvallet & Sigg, 1958; Cottle, 1964; Humphrey, 1967; Crill & Reis, 1968; Miura & Reis, 1969a; Seller & Illert, 1969). Electrical stimulation of the carotid sinus and aortic depressor nerves induced maximal unitary activity to be recorded just lateral to

the obex in the region of the area postrema (Humphrey, 1967; Crill & Reis, 1968). All afferent baroreceptor fibres appear to pass through the region of the area postrema since its bilateral destruction completely abolishes reflex responses (Humphrey, 1967; Oberholzer, 1960). Recently, using young normotensive and spontaneously hypertensive rats, Ylitalo, and colleagues, (1974) observed extremely high blood pressure levels after ablation of the area postrema.

Bilateral projections from these initial synapses are sent to the nuclei of the medial reticular formation that lie in the bulbar depressor area (Torvik, 1956; Brodal, 1957; Rossi & Zanchetti, 1957) as well as sending projections to the lemniscal system (Miura & Reis, 1969a). The medial reticular nuclei also receive afferent projections from the cerebellum (Brodal, 1957; Brodal & Gogstad, 1957; Miura & Reis, 1969b), the vestibular nuclei (Brodal & Godstad, 1957), the rostral parts of the bulbar reticular formation (Brodal, 1957) and the somatic afferent pathways in the spinal cord (Valverde, 1961).

The bulbar pressor area in the reticular formation receives long and indirect connections from the primary cardiorespiratory afferents (Humphrey, 1967; Miura & Reis, 1969a). This pressor region also receives collaterals from many long ascending and descending relays passing through the medial reticular formation and from the cerebellum, trigeminal sensory nuclei and diencephalon (Scheibel, Scheibel Mollica & Moruzzi, 1955; Brodal, 1957;

Smith, 1965).

The cardiac vagal effector neurons in the bulb are located in the nucleus ambiguus (Gunn, Sevelius, Puiggari & Myers, 1968; Thomas & Calaresu, 1974). Thomas & Carlaresu, (1974) suggested the nucleus ambiguus is the site of origin of the efferent cardioinhibitory vagal neuron and that reflex excitement of the vagus probably involves an interneuron linking the nucleus ambiguus with the nucleus tractus solitarius; the nucleus in which the efferent baroreceptor and chemoreceptor fibres initially synapse.

With the exception of the sympathetic cholinergic vasodilator neurons to skeletal muscles (Uvnas, 1960b; see later), the bulbospinal efferent neurons can be reflexly stimulated through the main cardiorespiratory inputs. Thus, it is possible in decerebrate animals, through appropriate changes in input from the periphery, to induce reflex vagal and sympathetic cardioinhibitory effects, cardiac sympathetic stimulation, changes in neural constrictor tone in gastrointestinal, cutaneous, muscle and renal vascular beds and increased secretion of adrenal catecholamines (Wang & Ranson, 1939a; Lindgren, Rosen & Uvnas, 1959; Lindgren, 1961; Korner, Uther & White 1969; Uther, Hunyor, Shaw & Korner, 1970). Localised electrical stimulation of the medullary depressor area produces an increased efferent vagal tone and a generalised inhibition of the peripheral sympathetic nervous system, as seen after elevation of the carotid sinus pressure (Wang & Ranson, 1939a; Lindgren & Uvnas, 1952; Folkow,

Lisander, Tuttle & Wang, 1968). In contrast, stimulation of the pressor area induces an increase in the efferent sympathetic tone and the release of adrenal catecholamines; thus mimicking the response seen after reducing the carotid sinus pressure (Chen, Lim, Wang & Yi, 1937; Wang & Ranson, 1939a; Lindgren, Uvnas & Rosen, 1959; Chai & Wang, 1962).

Alexander (1946) demonstrated that a pool of neuronal elements in the pressor area of the medulla contribute to the excitatory state and tonic activity of the sympathetic nervous outflow to the heart and blood vessels and that these same neurons are essential for pressor reflexes. It is now evident that the bulbar depressor area is 'something' different from the old concept of a medullary 'vasodilator' centre through which vasodilator nerves are reflexly stimulated. Evidence clearly indicates that reflex vasodilatation, however evoked, is accomplished by a central inhibition of the prevailing tonic efferent discharge of the sympathetic vasoconstrictor nerves and that the vasodilator nerves are not involved in such a reflex (Alexander, 1946; Frumin, Ngai & Wang, 1953; Lindgren & Uvnas, 1954; Uvnas, 1954; Folkow, 1955; Bard, 1960). Lindgren & Uvnas (1954) found that destruction of the bulbar 'vasodilator' area abolished depressor reactions to buffer nerve stimulation but not those to stimulation of afferent spinal nerves, suggesting that this region consists merely of fibres which carry inhibitory impulses of baroreceptor origin

to vasoconstrictor nerves.

The medullary or bulbar reticular formation contains the most essential central apparatus for the control of the cardiovascular system. In the intact animal, normotensive blood pressure levels depend upon the activity of the bulbar pressor area and as there is yet no reason to suppose that this activity is dependant on afferent inputs, it would appear to be maintained by an intrinsic activity. The nature of the depressor area, however, remains relatively obscure and its activity is probably dependant upon afferent inputs (See Bard, 1960).

Supramedullary Pathways.

The medullary cardiovascular centres can be influenced by higher brain areas allowing further control of the cardiovascular system, thus enabling the animal or individual to cope with various environmental stresses. Cardiovascular 'centres' above the medulla can be regarded as groups of interneurons between the afferent and efferent limbs of the bulbar autonomic mechanisms. Projections of the IX and X cranial nerves travel to higher brain levels with the main ascending pathways from the medial bulbar reticular formation. (Dell, 1952; Brodal, 1957). The ascending pathways pass through the mesencephalon (mid-brain) and the medial forebrain bundle to cross the hypothalamus and send fibres to the septum, amygdala and basal ganglia. Fibres from the mesencephalon also pass through the midline group of thalamic nuclei, projecting

on one hand to the fronto-orbital cortex via the dorsomedial thalamic nuclei and on the other hand to the cingulate gyrus and limbic structures via the anterior thalamic nuclei. (Rose & Woolsey, 1948; Brodal, 1957; Doty, 1967).

Projections from the IX and X afferent cranial nerves terminate in suprabulbar regions from which autonomic effects can be obtained by electrical stimulation. Depressor effects have been produced after electrical stimulation of the fronto-orbital cortex (Delgado & Livingston, 1948; Wall & Davis, 1951), the temporal cortex, piriform lobe and parts of the amygdala (Kaada, Pribram & Epstein, 1949; Wall & Davis, 1951; Wall, Glees & Fulton, 1951; Reis & Oliphant, 1964), the septal region (Covian, Antunes-Rodrigues & O'Flaherty, 1964) and the cingulate gyrus (Kaada et al., 1949; Löfving, 1961). However, with localised stimulation of other regions of the amygdala and septum pressor effects, tachycardia and increased release of adrenal catecholamines have been reported (Wall & Davis, 1951; Hilton & Zbrozyna, 1963; Covian et al., 1964; Reis & Oliphant, 1964; Bromley & Holdstock, 1969).

The descending pathways from the fronto-orbital cortex all traverse through the hypothalamus, while the pathways from the temporal lobe descend partly through the hypothalamus and partly through a more direct temporal-tegmental bulbar tract (Wall & Davis, 1951; Hirata, 1965). There is a convergence onto individual hypothalamic units from cells originating in the septum, amygdala, hippocampus

and midbrain tegmentum (Daly & Scott, 1964).

Early investigations into the exact location of the descending efferent pathways from the hypothalamus and diencephalic regions were in conflict. Beattie, Brow & Long (1930) followed descending degenerated pathways after lesioning the posterior hypothalamus and the rostral end of the midbrain. They concluded that fibres descending around the margin of the periventricular grey matter and in the medial longitudinal fasciculus might constitute the efferent pathway from the hypothalamus. At lower levels, these fibres became more medially placed and were followed directly to the thoracic spinal cord. Beattie et al. (1930) also observed fibres situated more laterally which became lost in the rostral portion of mesencephalic tegmentum. Magoun, Ranson & Hetherington (1938), Wang & Ranson (1939b) and Magoun (1940) confirmed the descending pathways reported by Beattie et al. (1930) but suggested that a more important efferent pathway runs from the lateral hypothalamus through the tegmentum of the midbrain and pons to traverse the lateral part of the reticular formation of the medulla and descend in the ventrolateral column of the spinal cord.

The role of the hypothalamus in cardiovascular control has been intensely investigated. Houssay & Molinelli (1925) first demonstrated that electrical stimulation of the hypothalamus produced an increased release of catecholamines from the adrenal gland. Pressor effects and general sympathetic excitement have been obtained after

hypothalamic stimulation (Karplus & Kreidl, 1927; Kabat, Magoun & Ranson, 1935; Ranson & Magoun, 1939; Bronk, Pitts & Larrabee, 1940). Depressor effects have been reported after stimulation of the anterior hypothalamus (Ranson & Magoun, 1939; Wang & Ranson, 1941; Löfving, 1961; Folkow, Langston, Öberg & Prerovsky, 1964; Gellhorn, 1964).

Activation of the depressor region, which lies ventral to the anterior commissure and extends caudally through the dorsal hypothalamus (Ranson & Magoun, 1939; Löfving, 1961) causes profound inhibition of the sympathetic outflow to the heart and vasculature and it may be an important pathway for inhibitory influences from the fronto-orbital cortex (Folkow, Johansson & Öberg, 1959; Folkow et al., 1964).

Posterior hypothalamic stimulation in rats induced biphasic pressor responses (Eferakeya & Bunag, 1974). They observed that the primary stage was due to an increased sympathetic activity and the second phase due to stimulation of catecholamine release from the adrenal medulla. They also reported that the renin/ angiotensin system was not involved in the mediation of the acute pressor effects seen after short term hypothalamic stimulation. Haas, Goldblatt, Rowland & Vrtunski (1974) observed that acute mesencephalic stimulation in dogs induced large pressor responses. The renal artery blood flow was reduced by over 50% and the renin content in renal venous blood was increased fourfold. However,

they concluded that the increase in renin release played an insignificant part in the pressor effect induced by acute mesencephalic stimulation.

There appears to be some lack of agreement as to the exact hypothalamic areas which yield the various changes in arterial blood pressure. Pitts, Larrabee & Bronk (1941) observed that stimulation of a particular hypothalamic point may lead to a depressor or pressor effect depending upon the stimulation frequency. This is probably due to threshold differences of neurons belonging to different functional groups but intermingled in the region of the electrode tip. However, highly specific responses have been obtained after electrical stimulation of the hypothalamus. Beattie et al. (1930) were able to induce cardiac arrhythmias. Folkow & von Euler (1954) observed that the composition of adrenal secreted catecholamines, i.e. the relative amounts of adrenaline and noradrenaline present, could be varied by altering the exact site of hypothalamic stimulation. In unanaesthetised dogs Rushmer & Smith (1959) and Rushmer, Smith & Franklin (1959) obtained changes in left ventricular function, that closely resembled those exhibited by the same animal during muscular exercise, after electrically stimulating highly localised diencephalic areas (especially in the subthalamus, fields of Forel or zona incerta).

The ventral division of the diencephalon is involved in such diverse neural activities as the expression of emotion, body temperature regulation, water

balance, food intake and the control of secretion of various trophic hormones from the par distalis of the pituitary gland. Several of these activities under hypothalamic control, especially emotional behaviour and temperature, involve changes in the circulatory system.

Ranson (1940) demonstrated that after placing lesions in the rostral hypothalamus and pre-optic area the animal is no longer able to protect itself from overheating but shows little or no impairment of regulation against environmental cold. Injuries limited to the caudal portion of the hypothalamus produce a permanent poikilothermic state. Evidence supporting an anterior location for the heat loss mechanism was produced by Magoun, Harrison, Brobeck & Ranson (1938). They showed that when the anterior hypothalamus was locally warmed panting and sweating occurred. They observed that cutaneous vasodilation was accompanied by vasoconstriction in visceral areas, especially in the kidneys. The circulatory adjustments are integrated with specific somatic activity (e.g. panting) to bring about the appropriate heat loss. The more caudally located mechanism in the hypothalamus concerned with the production and conservation of heat, activated by exposure to cold, induces a different integrated response. Shivering, tensing of the muscles and postural adjustments are combined with sympathetically induced changes such as vasoconstriction, piloerection and secretion of catecholamines from the adrenal medulla.

When activated, the hypothalamus can lead to an integration of cardiovascular and other visceral changes with a specific pattern of overt behaviour that appears to be essential for a full display of anger or rage. Bard (1928) found that the integrity of the caudal hypothalamus was necessary to express anger in decorticate cats. Stimulation of the hypothalamus, especially in its lateral portions, but not elsewhere in the rostral brain stem produced a response similar to sham rage in the decorticate cat (Ranson, 1937). Notable pressor effects and tachycardias occur during sham rage in the decorticate cat due to a generalised increase of efferent sympathetic discharge. Hess & Brugger (1943) obtained defence reactions in conscious cats after hypothalamic stimulation.

Eliasson, Folkow, Lindgren & Uvnas (1951) showed that sympathetic cholinergic vasodilator fibres to the vessels in skeletal muscle, fibres which do not participate in the baroreceptor reflexes (Uvnas, 1954), could be readily activated by hypothalamic stimulation. They noted concomitant vasoconstriction in the skin and intestine and other signs of an increased sympathetic activity characteristic of fear and rage reactions. It is now known that the sympathetic cholinergic vasodilator pathway originates in the anterior sigmoid gyrus of the motor cortex and passes to the spinal cord via the hypothalamus, midbrain tegmentum and medulla oblongata (Lindgren, Rosen, Strandberg & Uvnas, 1956; Uvnas, 1960b.)

Abrahams, Hilton & Zbrozyna (1960) concluded from the results obtained in cats after electrical stimulation of the brain stem that a localised region of the hypothalamus acts as an integrative centre for the defence reaction. Abrahams, Hilton & Malcolm (1962) observed that evoked potentials were obtained in all parts of the integrative centre for the defence reaction in the hypothalamus and midbrain, in response to cutaneous, auditory and visual stimuli. Abrahams et al. (1960, 1964), using acutely decerebrate and conscious cats, proposed that the muscle vasodilatation occurs at just a stage of a whole complex reaction for it to fulfil the role of a preparatory response, in preparation for the muscular exertion needed in fight or flight.

There appears to be some evidence that suggests that the vasodilator pathway can be activated independantly of the defence reaction. Uvnas (1960b)stated that he had not observed rage or fright responses on stimulation of any other part of the pathway other than the hypothalamus, although the cardiovascular responses were always of the same type.

Rushmer et al. (1959) although having obtained cardiac responses in conscious dogs similar to those seen during exercise, failed to notice any external signs of distress after electrically stimulating an area dorsal to the peduncle at the level of the hypothalamic mammillary bodies.

In his review, Hilton (1966) diagrammatically represented the brain stem centre integrating the defence reaction. Impulses ascend via the lemniscal pathway and enter the defence centre by the extra-lemniscal sensory pathway. The lemniscal pathway also sends afferents to the defence centre via the amygdala. He suggested the existence of several efferents each responsible for a particular component of the whole response, thus allowing the centre to exert its integrative function by activating one efferent pathway after another, in a given sequence, as the level of excitement within the centre is increased.

Electrical stimulation of the amygdala had previously been shown to elicit defence reactions in cats (Magnus & Lammers, 1956; Hilton & Zbrozyna, 1963). Hilton & Zbrozyna (1963) observed defence reactions in response to electrical stimulation of the afferent pathway to the amygdala from the hypothalamus, in the stria terminalis, and the efferent pathway from the amygdala to the hypothalamus, which constitutes a narrow ventral band of fibres probably corresponding to the ventral amygdalofugal pathway described by Nauta (1961).

Anxiety in human subjects produces cardiovascular effects similar to those observed for the defence reaction in the cat. Heart rate, cardiac output and blood pressure rise while the total peripheral resistance is often reduced due to active vasodilation in skeletal muscle (Blair, Glover, Greenfield & Roddie, 1959). Blair et al.

(1959) exposed volunteers to a frightening situation and found that the muscle vasodilatation was mediated by the atropine sensitive vasodilator nerve fibres of the sympathetic outflow. Mild electrical stimulation of the medial hypothalamus in man has been reported to induce feelings of restlessness, anxiety, depression, fright and horror, while stronger stimulation of the posterior hypothalamus produced severe rage reactions (Heath & Mickle, 1960; Sem-Jacobsen & Torkildsen, 1960).

Bard (1960) stated that suprabulbar influences must inhibit baroreceptor function in order to maintain an elevated heart rate in combination with a raised blood pressure, as happens during periods of stress and exercise. However, experimental evidence is not in total agreement concerning the extent to which suprabulbar structures influence baroreceptor function. Wilson, Clarke, Smith & Rushmer (1961), despite the text figure which showed an obvious reduction of the cardiac slowing component of the baroreceptor reflex during hypothalamic stimulation, concluded that the reflex effects were independant and simply additive. Reis & Cuénod (1962, 1965) using decerebrations, decerebellations, brain stem transections and electrical stimulation provided evidence that baroreceptor responsiveness was under the tonic and phasic control of supramedullary structures. Hilton (1963) observed that by stimulating the hypothalamic defence area depressor effects and bradycardia could not be obtained by baroreceptor stimulation.

Gebber & Snyder (1970) attempted to assess whether the suppression of baroreceptor activity by the hypothalamus was due to the increased sympathetic activity produced by hypothalamic stimulation overriding the maximal cardiac inhibitory effects of the baroreceptor reflex or whether supramedullary neural pathways are activated which functionally inhibit the compensatory baroreceptor reflex. They found that every location in the hypothalamus that induced a pressor response and tachycardia to electrical stimulation also inhibited the baroreceptor induced bradycardia, thus contradicting the belief that the inhibitory influence is produced only by stimulation of the hypothalamic defence area. Gebber & Snyder (1970) observed that hypothalamic stimulation blocked the bradycardia but had no effect on the depressor component suggesting the presence of a suprabulbar system which functions to inhibit only the vagal bradycardia induced by baroreceptor activation. This is in contrast to the results of Hilton (1963). In addition, Gebber & Snyder (1970) were able to facilitate the hypothalamically produced pressor effect by carotid occlusion and vice versa, thus demonstrating suprabulbar modulation of the baroreceptor response to either increased or decreased systemic pressure. Klevens & Gebber (1970) noted that stimulation of the septal, preoptic or anterior hypothalamic areas, which produced little or no change in blood pressure and heart rate, markedly facilitated the reflex bradycardia evoked by noradrenaline injection, sinus stretch and aortic nerve stimulation.

Hilton & Spyer (1969,1971) were able to produce an identical reponse to the depressor baroreceptor reflex (i.e. vagal activation and sympathetic inhibition) when stimulating a depressor area within the anterior hypothalamus. Bilateral lesions destroying the anterior hypothalamic depressor area reduced the response to baroreceptor afferent stimulation and conversely, lesions of the medullary depressor area, which spared a large part of the nucleus tractus solitarius, also reduced but did not abolish the baroreceptor reflex. By lesioning both areas the depressor reflex could be totally abolished. Hilton & Spyer (1969, 1971) concluded that the whole brain stem extending from the hypothalamus through the midbrain to the medulla constitutes a functional unit which integrates the response to baroreceptor afferent stimulation. Hilton & Spyer (1969) and Spyer (1972) recorded electrical activity of several single hypothalamic neurons in response to increase of carotid sinus pressure. Spyer (1972) found 21 neurons that responded, with either activation or inhibition, to high levels of pressure and concluded that the anterior hypothalamus plays an important role in the integration of the baroreceptor reflex.

Medullary regions which produce cardiovascular effects are also influenced by the cerebellum. The paramedian reticular nucleus which receives inputs from the carotid sinus nerve (Miura & Reis, 1968, 1969a) and probably from the aortic depressor nerve (Crill & Reis, 1968), is innervated by and projects to the cerebellum

(Brodal & Torvik, 1954; Brodal & Gogstad, 1957). Electrical stimulation of the fastigial nucleus in the cerebellum produces a rapid rise in blood pressure and heart rate (Zanchetti & Zoccolini, 1954; Muira & Reis, 1969b; Achari & Downman, 1970). In the experiments of Zanchetti & Zoccolini, (1954) the pressor effects were always part of the patterned autonomic and somatic outburst of sham rage and were shown to be dependant upon the integrity of the hypothalamus. In contrast, Miura & Reis (1969b) and Achari & Downman(1970) failed to observe rage reactions after stimulation of the fastigial nucleus and the pressor responses were produced independantly of the hypothalamus. The powerful pressor effects appeared to be mediated via direct fastigio-bulbar tracts, which enter the brain stem in the restiform body and pass through most portions of the vestibular nuclei, as bilateral lesions of this pathway invariably abolished the response to fastigial nucleus stimulation (Miura & Reis, 1969b). Miura & Reis (1969b) suggested that as the paramedian reticular nucleus was the only nucleus essential for the mediation of the fastigial nucleus reponse, this nucleus may be the principal site of tonic and phasic interactions of the cerebellum with the carotid sinus baroreceptor vasomotor reflexes, previously shown by Moruzzi (1940) and Reis & Cuénod (1965) and more recently by Achari & Downman (1970) and Achari, Al-Ubaidy & Downman (1973).

Doba & Reis (1972) attempted to clarify the functional significance of the fastigial pressor response

and found that the cardiovascular and haemodynamic responses of fastigial stimulation closely resembled the compensatory reflex response in assuming an upright posture. This finding supports the already well known involvement of the cerebellum in posture and motor control and thus some integration of thecardiovascular system by the cerebellum would appear to be essential.

CENTRAL NEUROTRANSMITTERS

Catecholamines

Von Euler (1946) and Holtz (1950) first detected noradrenaline and adrenaline in the mammalian brain. These substances were supposed by these workers to occur in the cerebral vasomotor nerves. Using chromatographic techniques Vogt (1954) determined the relative concentrations of noradrenaline and adrenaline (sympathin) within specific areas of the dog brain and found them to be unevenly distributed. The highest concentrations of the catecholamines were found in diencephalic, mesencephalic and medullary regions and also in the area postrema. All cortical areas, including those which project to the hypothalamus, contained very little noradrenaline.

That dopamine was present in the brain was suggested by Montagu (1957) and subsequently proved indendantly by von Euler (1958) and Carlsson, Lindqvist, Magnusson & Waldeck (1958). Bertler & Rosengren (1959) demonstrated that the central distribution was uneven.

The presence of catecholamines in the central nervous system has been comprehensively reviewed (Bertler & Rosengren, 1959; Carlsson, 1959; Vogt, 1959) and the possible role of these compounds as central neurotransmitters has also received considerable discussion (Bradley 1968; Crossland, 1968; Hebb, 1970; Vogt, 1973).

Accurate mapping of central catecholamine containing neurons was made possible by the development of histochemical fluorescence techniques by Falk, Hillarp, Thieme & Torp (1962). With this method it has been possible to directly visualise the distribution of noradrenergic, dopaminergic and 5-hydroxytryptaminergic cell bodies and nerve terminals in the brain and spinal cord (see Dahlstrom & Fuxe, 1964, 1965; Fuxe, 1965). Recent developments in the field of histochemical investigations has lead to the introduction of immunofluorescence techniques which allow direct visualisation of cellular dopamine β-hydroxylase and other enzymes in the brain (Hartman & Udenfriend, 1972). Immunofluorescent localisation of dopamine β-hydroxylase has proved to be more sensitive than the formaldehyde fluorescence technique for tracing fine preterminal noradrenergic axons, especially in the forebrain (Hartman, Zide & Udenfriend, 1972). Catecholaminergic pathways (noradrenaline, adrenaline, dopamine) and 5-hydroxytryptamine in the brain have been described in detail by Dahlstrom & Fuxe (1964, 1965), Fuxe, (1965), Ungerstedt (1971a,b) Bolme, Fuxe & Lindbrink (1972) and Livett (1973).

(a) Noradrenaline

Noradrenergic pathways have been mapped by the techniques described above after placement of selective stereotaxic lesions in the brain (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971a). 6-Hydroxydopamine has proved useful in mapping pathways as it produces selective degeneration of dopamine and noradrenaline pathways without damage to other neuronal systems (Utretsky & Iversen 1969). After stereotaxic lesioning with 6-hydroxydopamine, the axon on the cell-body side of the lesion will show enhanced fluorescence, while those on the terminal side will have little if any fluorescence. Thus, the origin and terminal distribution of noradrenaline fibre tracts can be determined after placement of lesions at different levels in the brain.

The identification of noradrenaline containing cell bodies has lead to them being designated in the form A1 to A7. A relatively small number of noradrenergic cell bodies in the pons and medulla give rise to large ascending and descending tracts. There are two systems of descending pathways arising from the most caudal group of cell bodies (A1) situated in the ventromedial part of the reticular formation in the caudal medulla; one system terminates in the ventral horn and the other in the sympathetic lateral column and dorsal horn of the spinal cord. A further descending pathway originates from the more rostral locus coeruleus (A6) and innervates the lower brain stem nuclei.

Two ascending pathways are known to exist; one dorsal noradrenergic pathway and one ventral pathway which are clearly separated in the reticular formation in the cranial midbrain (Fuxe, Hökfelt & Ungerstedt, 1970). The vast majority of the ascending axons in the dorsal pathway originate from the locus coeruleus (A6) which is entirely built up of noradrenergic cell bodies. The dorsal pathway runs within the dorsal medial part of the reticular formation in the mesencephalon and after passing through the septal area innervates the cortical areas (neocortex and hippocampus). The dorsal bundle from A6 also innervates areas of the amygdala and anterior hypothalamus as it ascends to the cortex (see Bolme et al., 1972; Livett, 1973). A pathway from A6 also innervates the cerebellum. The dorsal system would appear to be of great importance for the regulation of the responsiveness of cortical areas to various incoming stimuli and for the attention mechanisms in the cortex.

The main ventral pathway originates from the A1 group which then follows the course of the medial forebrain bundle all the way to the olfactory bulb. A ventral pathway also arises from the cell groups A5 and A7. The ventral bundle, which comprises of ascending fibres from the cellgroups A 5 and A7 in the pons as well as from the cell groups A1 and A2 (the latter group in the rat corresponding to the solitary-vagal complex in the cat), gives off branches to the lateral mammillary nuclei, the lateral and ventral hypothalamus and to large parts of the limbic forebrain (including the anterior medial amygdaloid

complex, the ventral medial septum and cingulum). Before reaching the medial forebrain bundle the ventral pathway passes through the area ventralis tegmenti where the fibres join the great number of nigrostriatal dopaminergic neurons.

(b) Dopamine

With the development of specific antibodies to dopa decarboxylase immunofluorescent techniques have been used to localise dopaminergic cell bodies (Goldstein, Fuxe & Hökfelt, 1972). These cell bodies do not fluoresce with dopamine β-hydroxylase antisera and thus these two antisera are used to differentiate noradrenergic and dopaminergic neurons. The largest dopaminergic pathway in the brain is the nigrostriatal pathway, which originates in the substantia nigra (cell groups A8 and A9) and innervates the caudate putamen (Anden, Carlsson, Dahlstrom, Fuxe, Hillarp & Larsson, 1964b). The fibre tract ascends through the lateral and mid-hypothalamus and spreads out forming a very dense mesh work of very fine dopaminergic terminals to innervate the neostriatum.

The second pathway originates from dopaminergic cell bodies surrounding the nucleus interpeduncularis (A10) and ascends via a medial route, passing directly through the central amygdaloid nucleus to innervate part of the limbic forebrain, particularly the nucleus accumbens and olfactory tubercle.

There also exists a short dopaminergic intra-hypothalamic system which has its cell bodies in the

arcuate nucleus in the hypothalamus. These axons extend into the external layer of the media eminence (see Ungerstedt, 1971; Bolme et al., 1972; Livett, 1973).

(c) 5-Hydroxytryptamine.

Amin, Crawford & Gaddum (1953, 1954) demonstrated the presence of 5-hydroxytryptamine in the brain and that its distribution closely resembled that of sympathin (noradrenaline and adrenaline mixture). High concentrations of 5-hydroxytryptamine occur only in the suprachiasmatic nucleus (Fuxe, 1965). 5-Hydroxytryptamine containing terminals have been found in the spinal cord (Fuxe, 1965) and traced to cell groups arising from the caudal nucleus of the raphe system in the medulla oblongata (Dahlstrom & Fuxe, 1965).

The 5-hydroxytryptamine terminals, which surround the presynaptic sympathetic cells in the lateral horn of the spinal cord, have been proposed to have an inhibitory function (Andén, Carlsson & Hillarp, 1964a). However, De Groat & Ryall (1967) showed that noradrenaline applied iontophoretically inhibited the activity of sympathetic neurons in the lateral horn, whereas 5-hydroxytryptamine similarly applied excited these neurons. Thus, Ryall (1967) proposed that the descending noradrenergic bulbospinal pathway was sympatho-inhibitory and the 5-hydroxytryptamine pathway sympatho-excitatory.

Hare, Neumayer & Franz (1962) and Neumayer, Hare & Franz (1974) suggested that the monoaminergic bulbospinal pathways consisted of a noradrenergic excitatory and an

opposing 5-hydroxytryptamine inhibitary pathway. In line with these suggestions, Ito & Schanberg (1972) observed the development of hypertension after depletion of central 5-hydroxytryptamine with p-chlorophenylalanine. Coote & Macleod (1974) obtained evidence that indicated the presence of both noradrenergic and 5-hydroxytryptaminergic pathways descending from the medulla to the spinal cord, both of which were inhibitory to sympathetic outflow.

The different suggestions concerning the functions of the descending noradrenergic and 5-hydroxytryptaminergic pathways are not surprising as microiontophoretic application of noradrenaline onto cells of the brainstem caused excitation or inhibition or firing, or had no effect at all (Bradley & Wolstencroft, 1962).

(d) Adrenaline

Adrenaline was shown to be present in the brain, along with noradrenaline, by von Euler (1946), Holtz (1950) and Vogt (1954). Vogt (1954) observed that adrenaline accounted for 13.7 and 6.5% of the total concentration of hypothalamic sympathin in the dog and cat respectively.

In recent years, using immunohistochemical techniques, Hökfelt et al.(1973, 1974) have demonstrated the existence of adrenaline containing neurons within the rat brain innervating such structures as the hypothalamus, locus coeruleus, nucleus tractus solitarius, nucleus motorius dorsalis nervi vagi and the sympathetic lateral column. Although the significance of these adrenaline containing neurons is not known Bolme et al. (1974)

indicated that they may be involved in vasomotor and respiratory control.

Acetylcholine

As for the catecholamines, evidence supporting a central transmitter role of acetylcholine is mainly indirect and is based upon the presence and distribution of acetylcholine, choline acetylase and choline esterase (Curtis, Tyall & Watkins, 1965). Feldberg & Vogt (1948) investigated the functional role of the choline acetylase enzyme system and found that the concentration varied greatly within different brain regions suggesting that only certain neurons made use of acetylcholine as their transmitter substance.

The distribution and functional role of acetylcholine as a central neurotransmitter has been extensively reviewed (see Gaddum, 1962; Koelle, 1963; Salmoiraghi, Costa & Bloom, 1965; Shute & Lewis, 1966; Votava, 1967; Bradley, 1968).

Two main pathways containing choline esterase have been found in the rat forebrain (see Shute & Lewis, 1966). The dorsal tegmental pathway mainly arises from the nucleus cuneiformis, situated in the dorsolateral part of the mesencephalic reticular formation, and its fibres are distributed to the tectum, pretectal area, geniculate bodies and to the non-specific and specific nuclei of the thalamus. The cells of the anterior thalamic nuclei also contain choline esterase. They can be regarded as forming part of a cholinergic limbic system which includes other

choline esterase containing neurons, such as those of the mid-brain tegmental nuclei receiving impulses from the hippocampus.

The fibres of the ventral tegmental pathway originate mainly from the substantia nigra and ventral tegmental area of the midbrain and traverse the hypothalamus and subthalamus to reach the basal forebrain areas, from where more fibres spread and project to all areas of the cortex and olfactory bulb. Shute & Lewis (1963) suggested that this ascending cholinergic reticular system probably forms the basis of electrocortical arousal, and so is identical with the 'ascending reticular activating system' designated by neurophysiologists. This hypothesis is supported by the finding of Kanai & Szerb (1965), in which they obtained an increased release of acetylcholine from the cerebral cortex during arousal.

The hypothalamic cells with a very high choline esterase content probably represent relays on the ventral tegmental pathway of the ascending cholinergic reticular system. The relative concentrations of true choline esterase within finite nuclei and areas of the hypothalamus was determined by Fuxe (1965).

Acetylcholine was shown to have facilitatory or depressant effects when applied to neurons in the brain stem (Bradley & Wolstencroft 1962) and many other brain regions (see Salmoiraghi et al., 1965). Both nicotinic and muscarinic actions of acetylcholine have been reported (Andersen & Curtis, 1964; Curtis & Ryall, 1964).

There is some reason to suppose that cholinergic and monoaminergic neurons may produce contrary effects by influencing synaptic transmission in specific pathways as acetylcholine and monoamines have respectively produced facilitatory and inhibitory effects at certain synapses (Marrazzi, 1953; Marrazzi & Hart, 1955; Comis & Whitfield, 1966). In unanaesthetised animals, Bloom, Costa & Salmoiraghi (1965) observed that spontaneous activity of caudate cells was in most instances enhanced by acetylcholine and depressed by noradrenaline and dopamine.

PHARMACOLOGICAL CONSIDERATIONS.

Effects of central administration of probable central neurotransmitters on blood pressure and heart rate and their modes of action.

The initial pharmacological studies were undertaken to investigate the properties of the proposed neurotransmitters mainly in behavioural changes and temperature control (see Rothballer, 1959; Feldberg & Fleishhauer, 1965). However, since 1960 the central effects of these substances on blood pressure and heart rate and their modes of action have been extensively investigated.

Noradrenaline.

Bradycardia and hypotension were reported after noradrenaline administration into the lateral cerebral ventricles (icv) of dogs (Kaneko et al., 1960; McCubbin et al., 1960; Bhargava, Mishra & Tangri, 1972), cats (Nashold, Mannarino & Wunderlich, 1962; Share & Melville, 1963;

Gagnon & Melville, 1966; 1968; Smookler, Severs, Kinnard & Buckley, 1966; Schmitt & Fénard 1971), rabbits (Toda, Matsuda & Shimamoto, 1969) and rats (Baum & Shropshire, 1973). Cardiovascular depressant effects to noradrenaline have been reported after administration into the cisterna magnas of cats (Sinha & Schmitt, 1974) and dogs (Kaneko et al., 1960; McCubbin et al., 1960), the third ventricles of cats and dogs (Schmitt, 1970) and the anterior hypothalamus of baboons (Toivola & Gale, 1970) and rats (Struyker Boudier, Smeets, Brouwer & van Rossum, 1975) and after perfusion of the third and fourth ventricles of cats (Heise & Kroneberg, 1973).

The cardiovascular depressant effects of centrally administered noradrenaline were abolished by central pretreatment with α-adrenoceptor blocking agents (Bhargava et al., 1972; Heise & Kroneberg, 1973) but remained unaffected by β-adrenoceptor blockers given centrally (Bhargava et al., 1972).

Share & Melville (1963) observed that the bradycardia due to icv noradrenaline in cats was due to an increased efferent vagal tone to the heart as well as a decreased sympathetic outflow. However, Bhargava et al. (1972) found that in vagotomised dogs the bradycardia was unaffected suggesting that it was mediated completely by a reduced efferent sympathetic tone.

The depressor effects and bradycardia seen after icv noradrenaline in cats were reversed to hypertension and tachycardia after pretreatment with reserpine (Share &

& Melville, 1963; Smookler et al., 1966; Gagnon & Melville,1968) and icv imipramine and chlorpromazine (Gagnon & Melville, 1968). The latter group of workers concluded that the reversal was not entirely due to brain noradrenaline depletion but more probably due to the blockade of neuronal uptake of noradrenaline, thereby preventing the usual central cardiovascular inhibitory effects of the amine.

Reports have been made that noradrenaline administered alone centrally induced cardiovascular stimulant effects. Tachi (1962), using dog cross-circulation experiments, observed pressor effect in recipient dogs to noradrenaline administered to donor dogs and concluded that the pressor effect of noradrenaline was due to a central action which was nervously mediated to the periphery.

Gagnon & Melville (1966) reported that very small doses of noradrenaline (0.01 and 0.1 µg) injected icv produced centrally mediated pressor responses and tachycardia. They observed that central reserpine reduced these vasomotor excitatory effects and that central β-adrenoceptor blockade, induced by icv pronethalol, completely abolished the increases in blood pressure and heart rate due to icv noradrenaline.

Tachi (1962) and Gagnon & Melville (1966) did not suggest a site of action for the central pressor response to noradrenaline. However, since 1970, Philippu and Przuntek and colleagues have investigated the pressor region of the

posterior hypothalamus. Philippu, Heyd & Burger (1970) observed in anaesthetised cats that electrical stimulation of the nuclei posterior, ventromedialis and anterior medialis caused an enhanced release of noradrenaline from the hypothalamus. Acetylcholine also enhanced the release of hypothalamic noradrenaline. Philippu, Przuntek, Heyd & Burger (1971) labelled the hypothalamus with ^{14}C-noradrenaline. Superfusion of the hypothalamus with noradrenaline enhanced the release of radioactive amines from the hypothalamus and also caused dose dependant pressor effects. Phentolamine did not influence the noradrenaline released from the hypothalamus or the rise in blood pressure, whereas imipramine potentiated the pressor response and abolished the release of ^{14}C-noradrenaline from the posterior hypothalamus by noradrenaline.

In a further paper, Philippu and Przuntek observed that the pressor effects obtained with posterior hypothalamic stimulation were inhibited by superfusion of bretylium and amethocaine and were also abolished after central pretreatment of 6-hydroxydopamine (Przuntek, Guimaraes & Philippu, 1971). Thus, they suggested that the pressor effect to electrical stimulation of the posterior hypothalamus was due to release of endogenous noradrenaline and mediated via central noradrenergic neurons.

In contrast to their findings with phentolamine (Philippu et al., 1971), superfusion of the posterior hypothalamus with the α-adrenoceptor blockers tolazoline and piperoxan abolished the pressor effects of posterior

hypothalamic stimulation (Phillipu, Roensberg & Przuntek, 1973). Thus, the pressor responses due to noradrenaline in the posterior hypothalamus appear to be mediated by α-adrenoceptors in this area. This finding is in contrast to the observation of Gagnon & Melville (1966) who suggested a β-adrenoceptor mediated pressor response for noradrenaline. Although the latter workers did not suggest a site of action for noradrenaline in their experiments, it seems probable that the response obtained due to such a low dose of noradrenaline administered into the lateral ventricles was due to an action on the hypothalamus as this is the first major structure noradrenaline would contact as it passed through the cerebroventricular system.

However, in a recent communication Philippu (1975) reported that superfusion of the posterior hypothalamus of cats with l-propranolol or sotalol diminished the pressor responses to hypothalamic stimulation. D-propranolol was ineffective. Thus, he concluded that both α- and β-adrenoceptors are present in the cat hypothalamus and are involved in the mediation of hypothalamic pressor reponses.

Garvey et al. (1972) demonstrated that pressor effects and tachycardias, in response to hypothalamic stimulation, could be produced by central β-adrenoceptor stimulation as these effects were abolished after central propranolol pretreatment. Thus, evidence has been produced that indicates the involvement of both α- and β-adrenoceptors, as defined for the peripheral autonomic

nervous system, in mediating hypothalamic pressor effects whilst α-adrenoceptors appear to be involved in the production of hypothalamic and medullary depressor responses.

However, experiments in rats using centrally administered noradrenaline have produced conflicting results. In contrast to the hypotension, bradycardia and reduced sympathetic outflow observed by Baum & Shropshire (1973) after noradrenaline administered into the right lateral ventricles of rats anaesthetised by pentobarbital, Kleinrok, Jagiello-Wójitowicz & Zebrowska-Lupina (1972) obtained pressor responses to noradrenaline given into the right lateral ventricle in pentobarbital, chloralose or urethane anaesthetised rats. Ito & Schanberg (1974) observed transient pressor effects after noradrenaline administered into the cisterna magna and directly onto the surface of the brain stem of chloralose/urethane anaesthetised rats. Data from sequential transections of the brain stem and from direct application of noradrenaline and phenylephrine on the brain stem surface after transections led Ito & Schanberg (1974) to suggest that the pressor effect elicited by the α-adrenoceptor agonists originates in a region of the medulla localised between the inferior cerebellar peducle and the caudal end of the obex. Hence, it may appear in rats that α-adrenoceptors in the medullary region mediate pressor responses. In addition, Ito & Schanberg (1974) have proposed that medullary β-adrenoceptors are responsible for depressor effects in the rat.

de Jong, Nijkamp & Bohus (1975) and Struyker

Boudier, et al. (1975), have recently observed potent falls in blood pressure and heart rate after the discrete application of noradrenaline onto the nucleus tractus solitarius in the medulla oblongata of anaesthetised rats. Phentolamine applied at this same site prevented the inhibitory action of noradrenaline (de Jong et al., 1975). These workers observed that the most effective site within the nucleus tractus solitarius in producing hypotension to either electrical stimulation or noradrenaline administration comprised of the middle-caudal part of the nucleus at the obex level. Thus, in contrast to Ito & Schanberg (1974), de Jong et al. (1975) and Struyker Boudier et al. (1975) have reported that depressor effects can be produced from a discrete area within the medulla oblongata of the rat by stimulation of α-adrenoceptors by noradrenaline.

Dopamine

McCubbin et al. (1960) failed to produce cardiovascular effects in dogs after icv administered dopamine, except after inhibition of monoamine oxidase when icv dopamine produced hypotension and bradycardia. Using rats, Baum & Shropshire (1973) reported that icv dopamine induced hypotension, bradycardia and a reduced efferent sympathetic outflow. However, as these effects of icv dopamine were observed in the absence of a dopamine β-hyroxylase inhibitor it was probable that they were due to the formation of noradrenaline.

A large increase of central neuronal dopamine

content, achieved after administration of the dopamine β-hydroxylase inhibitor FLA63, failed to influence the blood pressure of conscious rats (Henning & Rubenson, 1970) indicating that in cardiovascular control dopamine mainly acts as a precursor for noradrenaline.

However, Bolme & Fuxe (1971) obtained indirect evidence that central dopaminergic neurons were involved in the control of the cardiovascular system since the dopamine receptor blocking agents, pimozide and spiroperidol produced hypotension which was potentiated by clonidine. Thus, they suggested that there is an interaction between central dopamine and noradrenaline neurons in the control of the cardiovascular system. That central dopamine containing neurons influence blood pressure is also indicated by the observation of Barbeau, Gillo-Joffroy, Boucher, Nowaczynski & Genest (1969) that in certain forms of Parkinsonism, a condition whereby the nigro-striatal pathway is depleted of dopamine, the blood pressure is often low. Hence, it would appear that dopamine neurons may exert a pressor or vasoconstrictor tone to the periphery.

Adrenaline

Nashold et al. (1962) observed in anaesthetised cats that icv adrenaline produced hypotension, which was potentiated when given in combination with a monoamine oxidase inhibitor. Adrenaline administered icv to anaesthetised rabbits induced hypotension and bradycardia (Toda et al., 1969). Pretreatment of rabbits with reserpine reversed the adrenaline-induced hypotension to a hypertension

and abolished the bradycardia. In conscious rabbits, icv adrenaline produced pressor effects in combination with tachycardia followed by hypotension and bradycardia.

In dog cross-circulation experiments and superfusion of the cat posterior hypothalamus, adrenaline produced hypertension and tachycardia, as did noradrenaline (Tachi, 1962; Philippu et al., 1971).

5-Hydroxytryptamine

The central effects of 5-hydroxytryptamine on the cardiovascular system were not investigated in this project. However, 5-hydroxytryptamine administered icv or into the cisterna magna of anaesthetised or conscious dogs produced hypotension and bradycardia (Kaneko et al., 1960; McCubbin et al., 1960). Depletion of central 5-hydroxytryptamine stores with p-chlorophenylalanine induced hypertension in rats (Ito & Schanberg, 1972).

Recently, de Jong et al. (1975) have also suggested that 5-hydroxytryptamine may play an inhibitory role in the central control of blood pressure in the rat. They observed an association between a significant rise in blood pressure and brain stem depletion of 5-hydroxytryptamine after treatment of p-chlorophenylalanine to normotensive and spontaneously hypertensive rats.

Acetylcholine

Various cardiovascular reponses have been obtained after icv acetylcholine in several animal species. Pressor effects were observed by Suh, Wang & Lim (1935) after

administration of acetylcholine into the cisterna magna and onto the floor of the fourth ventricles of anaesthetised cats and dogs. Bhawe (1958) observed both pressor and depressor responses after large doses of acetylcholine injected icv into cats and dogs. Pressor effects to icv acetylcholine were also reported by Dhawan, Gupta, Dixit & Chandra (1965) and Sinha, Dhawan, Chandra & Gupta (1967) in anaesthetised dogs.

Lang and Rush (1973) using conscious dogs, produced pressor effects and tachycardias after icv acetylcholine. The cardiovascular effects of icv acetylcholine were abolished by icv atropine thus indicating that they were as a result of stimulation of central muscarinic receptors.

Experiments involving the central administration of nicotine and other parasympathomimetic agents have provided evidence indicating the presence of central nicotinic receptors capable of mediating cardiovascular effects when stimulated (see Armitage & Hall, 1967a,b; Brezenoff & Jenden, 1970; Lang & Rush, 1973). As this represents a relatively small section of the project the effects of stimulating central nicotinic and muscarinic receptors are discussed more fully in Section 5, Chapter 1 of the Experimental Results.

In the peripheral sympathetic nervous system, Burn, & Rand, (1959,1965)hypothesised that acetylcholine was released initially from the adrenergic nerve endings and subsequently mediated the release of noradrenaline. From

studies using the electron microscope, acetylcholine appears to be associated with large, translucent vesicles within synaptic terminals of the central nervous system, whilst smaller, more dense vesicles seem to contain catecholamines (see Salmoiraghi et al., 1965). However, synaptic terminals of central junctions have often been found to contain more than one type of vesicle indicating that more than one transmitter may be stored in a central nerve ending.

A possible link may exist between the central cardiovascular effects seen with cholinomimetic and sympathomimetic compounds. Dhawan et al. (1965) proposed that the central response to icv tyramine in anaesthetised dogs was due to the release of acetylcholine as well as of noradrenaline. They observed that icv carbachol and tyramine produced similar pressor effects and the response to tyramine was reversed to a depressor effect after icv atropine. Thus, Dhawan et al. (1965) proposed that the pressor effect of icv tyramine was due to endogenously released acetylcholine and in the presence of atropine the hypotension was due to the noradrenaline released.

Scrimal, Jaju, Sinha, Dixit & Bhargava (1969) observed a biphasic pressor/depressor response after icv choline. The initial hypertension was abolished by icv atropine and appeared to be mainly due to central muscarinic stimulation leading to an increased outflow of catecholamines from the adrenal medulla. Adrenalectomy greatly reduced the initial pressor effect of icv choline. The depressor effect of choline resembled

the hypotension observed with icv isoprenaline and was likewise absent after pretreatment with icv β-adrenoceptor blocking agents. Icv reserpine and i.v. tetrabenazine also inhibited the hypotension of icv choline. Scrimal et al. (1969) thus suggested that the presence of catecholamines appear necessary for the mediation of the hypotensive effect of icv choline and when released activate central β-adrenoceptors.

As stated earlier, Philippu et al. (1970) observed that acetylcholine enhanced the release of noradrenaline from the hypothalamus. Philippu, Demmeler & Roensberg (1974) observed that carbachol enhanced the pressor effect of posterior hypothalamic stimulation without influencing the resting arterial pressure. The potentiation was abolished by central hexamethonium pretreatment suggesting the involvement of nicotinic receptors. They concluded that more than one transmitter was involved in the hypothalamic regulation of arterial blood pressure.

AIM OF PROJECT

With the use of conscious normotensive cats, rats and rabbits and conscious hypertensive rats, previously cannulated for drug administration into the cerebroventricular system, experiments were performed to investigate the centrally-mediated blood pressure lowering effect of α-methyldopa, clonidine, propranolol and several other β-adrenoceptor blocking agents. Cardiovascular responses were also recorded in conscious animals after central administration

of peripherally classified α- and β-adrenoceptor agonists and antagonists. It was hoped to further elucidate mechanisms by which the clinically active antihypertensive agents lower blood pressure through actions within the brain in terms of proposed central α- and β-adrenoceptors. The central cardiovascular effects of the proposed neurotransmitters, noradrenaline, dopamine and adrenaline, are described and their possible role in central cardiovascular control will be discussed.

The possibility of a link between central cholinergic and adrenergic nervous mechanisms affecting the peripheral cardiovascular system will be examined.

EXPERIMENTAL METHODS

1. Production of hypertension in rats

Male Wistar rats (Fison's strain) whose body weight at the operation was within the range of 50-60g, were made hypertensive by a modification of the method of Selye et al. (1943). Anaesthesia was induced using a 2:1 mixture of nitrous oxide: oxygen, and 3.5% halothane, the latter being reduced to 1.5% to maintain anaesthesia. The right kidney was removed and a pellet containing 25 mg desoxycorticosterone acetate (DOCA) implanted beneath the skin at the back of the neck in an aseptic operation. For 14 days following the operation drinking water was replaced by 1% sodium chloride in distilled water. The rats were considered hypertensive when their systolic pressures were a minimum of 170 mmHg (1mmHg = 133 Pa) on each of 3 separate recording sessions. All the rats used became hypertensive by these criteria within the period of 14 - 21 days following the operation.

2. Measurement of blood pressure in conscious rats.

(a) Indirect method in hypertensive restrained rats.

The method used was a modification of that described by Friedman & Freed (1949). Rats were initially placed in restraining cages, which were produced in 4 sizes (University of Reading Instruments Department) and capable of holding rats weighing between 100 - 450 g; rats weighting less than 100 g were held in glass restraining cages (Physics Department, University of Aston in Birmingham) The restrained rats were then placed in a specially designed wooden cabinet of dimensions 88 x 62 x 37 cms

which was fitted with ventilation holes and perspex doors. A thermomstat (Associated Electrical Industries Ltd., adjustable bimetal thermostat type TS3) situated centrally on one side of the cabinet controlled three 100 watt electric light bulbs positioned inside the cabinet near the base, to provide a constant temperature of 33.5°C ± 0.5°C in all parts of the cabinet. Three expanded metal shelves were positioned across the full width of the cabinet about 20 cm apart and an electric fan, which rested on the top shelf, circulated the air. Three restrained rats were placed on each of the two lower shelves. To prevent urine and faeces falling onto the lights and also to restrict the degree of disturbance caused to the rats by the continual on-off switching of the lights a metal screen was positioned over the whole of the heat source.

An occluding cuff (Scientific & Reseach Instruments Ltd.),consisting of a perspex cylinder and end clamps holding a latex rubber tube in such a manner that the amount of cuff in contact with the rat's tail was 18mm in length,was placed at the base of the rat's tail. This length enabled the most accurate systolic blood pressure readings to be taken (Maistrello & Matscher, 1969). A silicon semi-conductor strain gauge (Ether type 3A - 1A - 350P) mounted in a perspex clip (Scientific Research Instruments Ltd.) was positioned distally to the occluding cuff; this is demonstrated in Fig. 1.

The occluding cuffs were previously checked to establish that a pressure of not more than 5 mmHg completely occluded the lumen of the cuff otherwise a falsely high

systolic pressure reading would be obtained (R. Whiting, personal communication). The cuffs were connected by polyvinylchloride tubing to a simple luer fitting which enabled any one of six cuffs to be connected to an air line system. A cylinder of compressed air was used as the pressure source to inflate the occluding cuff; the cylinder tap being used to regulate the rate of inflation. The rate of deflation was adjusted by means of a screw clip compressing a rubber tube which formed a side arm of the air line system. The pressure within the system was measured using a blood pressure transducer (Devices/C.E.C. type 4-327-L221) which was connected to a D.C.2 preamplifier within a Devices M.2 electronic recorder. The voltage output, which was proportional to the pressure of the system, was monitored either on a 2 channel oscilloscope (Cossor Instruments Ltd., Oscillograph Model 1049 MkIIIA) or a permanent record obtained on heat sensitive paper.

The pulse in the caudal artery was detected by a strain gauge which formed one arm of a Wheatstone bridge circuit. A 2 pole 6 position switch enabled any of the 6 strain gauges to be selected (see Fig. 2) . The bridge voltage supply was derived from a 4.5 volt battery. The output from the bridge was fed to a Devices A.C.1 preamplifier within the M.2 recorder and as with the pressure the output was monitored on the oscilloscope or recorded on heat sensitive paper. A block diagram representing the complete system is shown in Fig. 3.

The restrained rats were left in the preheated cabinet for 15 - 20 minutes before blood pressure measurements

were taken. When a suitable pulse was obtained the occluding cuff was slowly inflated and the point at which the pulse disappeared noted. The air supply was turned off and the pressure slowly fell due to the slow leak and the point of reappearance of the pulse was also noted. This procedure was repeated twice and the average of these 6 readings was taken as the systolic blood pressure of the rat. No readings were taken when the rat's tail was moving. An example of the tracing demonstrating pressure and pulse changes during a typical reading is illustrated in Fig. 4.

During the development of DOCA/saline hypertension, the systolic blood pressure of each rat was measured at least once every 2 days, thus conditioning the rats to the restraining cages and experimental conditions and so preventing undue stress and false pressure readings during actual experiments.

(b) Direct method in normotensive unrestrained rats.

Rats weighing 175-225 g, which were implanted with icv cannulae one week earlier, were prepared for blood pressure and heart rate recording by the method described by Popovic & Popovic (1960).

Anaesthesia was induced with the halothane 3.5% in a 2:1 mixture of nitrous oxide and oxygen and maintained by 1.0-1.5% halothane in the same oxygen and nitrous oxide mixture. Both the back and front of the neck were shaved and swabbed with 0.5% chlorhexidine gluconate solution (Hibitane, ICI). A small incision approximately 7.5 mm in

length was made at the back of the neck and a larger incision (2 cm in length) made at the front. Two lengths of polyethylene tubing filled with heparinised saline (25 units / ml), one of PP 30 (Portex Plastics) which was attached to a blood pressure transducer and the other of PP 25 (Portex Plastics) which was attached to a syringe containing heparinised saline, was passed under the skin on the right side from the back of the neck through to the front.

The left common carotid artery was located and cannulated with the PP 30 tubing. The cannula was secured in place with 3 cotton ties. The blood pressure and heart rate were monitored during the cannulation procedures on a M.2 Devices electronic recorder. The heart rate was computed by a Neilson instantaneous ratemeter 2751 (Devices Instruments Ltd.) which was triggered by the blood pressure pulse. The left jugular vein was cannulated with the PP 25 tubing and was securely tied in position using cotton thread.

A little penicillin and sulphathiazole powder was sprinkled onto the open wounds at the front and back of the neck. The two incisions were then closed with thread stitches and the closed wounds dusted again with penicillin and sulphathiazole powder.

1.0 ml of heparinised saline was slowly injected into both the venous and arterial cannulae. The venous cannula was cut leaving approximately 3.0 cm protruding from the neck and sealed with a pin. A similar length of the arterial cannula was left protruding from the neck but was heat sealed.

The first experiment, in which blood pressure and heart rate responses were recorded to icv drug administrations, was performed 24 hours after the arterial and venous cannulations. The arterial cannula was clamped and cut just behind the heat seal. Polyethylene tubing of size PP 60 (Portex Plastics) filled with heparinised saline and attached to a blood pressure transducer, which was itself connected to a M.2 Devices electronic recorder, was pushed over the arterial cannula and the clamp removed. The blood pressure and heart rate were then recorded. Remote intravenous administrations were made after joining the venous cannula to a hypodermic syringe attached to PP 25 tubing filled with heparinised saline by replacing the pin with a broken off hypodermic needle.

By keeping the rats well heparinised, blood pressure and heart rate recordings could be made for 7 to 14 days after the cannulations.

3. Measurement of blood pressure in conscious normotensive unrestrained cats.

Cats of either sex and weighing 2 - 5 kg were prepared for blood pressure and heart rate recordings by a similar method as described by Day & Owen (1970). For at least 10 days prior to the operation, the cats were subjected to considerable individual attention and familiarised with the room and cages which were to be used for experiments.

Anaesthesia was induced using a 4:1 mixture of

nitrous oxide: oxygen, and 3.5% halothane, the latter being reduced to 1.5% to maintain adequate anaesthesia.

The cat was shaved along the back of the neck and from the base of the mouth to the top of the chest; the shaved areas were then swabbed with 0.5% hibitane solution. A transverse incision, approximately 3 cm in length was made at the base of the back of the neck and a longitudinal incision of approximately 4 cm on the right side of the trachea at the front.

The arterial cannula consisting of a long length of polyvinylchloride tubing, size PP 90 (Portex Plastics) was connected to a blood pressure transducer and this in turn to a Devices M.2 electronic recorder. The arterial cannula was filled with heparinised saline (50 units/ml). The venous cannula, which comprised of a long length of PP 30 filled with heparinised saline, and the arterial cannula were passed beneath the skin from the incision at the back of the neck to the front on the left side of the neck.

The venous tubing had previously been looped around a thin glass rod 3 times and the loops made permanent by immersing in very hot water. The loops were tightly tied together with several cotton ties. A hypodermic needle was passed through the skin approximately 1.5 cm. behind the transverse incision at the back of the neck and attached to the PP 30 venous cannula. The PP 30 was carefully pulled back through the skin until the loops rested just beneath the skin; the loops were then secured to the muscle at the back of the neck with

a cotton tie. This procedure ensured that the venous cannula was not easily pulled out by the cat after the cannulation.

The right carotid artery was located and carefully dissected free of surrounding tissue. The arterial cannula was measured to the required length by placing the end of the tubing at the base of the sternum and after following the approximate course of the cannula a cotton thread was tied around the tubing at the point of cannulation of the artery. The artery was tied off craniad and the cannula inserted and loosely tied into the artery. The bull-dog clip was removed as the cannula was slowly fed into the artery until the thread on the tubing reached the cannulating point; the blood pressure and heart rate were continuously monitored. When the cannula reached the end of the inominate artery it either passed into the required position in the aorta or into the heart. On rare occasions it was evident that the cannula had entered the left ventricle as the pulse pressure became very wide (the diastolic pressure falling to 0 mmHg) as the recorder monitored the pressure within the left ventricle. If this occurred, the tubing was withdrawn and fed back into the artery until it entered the aorta. In occasional operations the cannula became kinked and this was detected either by the pulse recording being lost or reduced. Also in these cases, the latter part of the cannulation usually became very difficult. The cause of kinking of the cannula was not determined but was probably due to the cannula being pushed

against the wall of the aorta and being forced back upon itself. In these instances, the cannula was completely withdrawn from the carotid artery and the artery was re-cannulated with another length of tubing. This procedure was carried out until it was thought that the cannula was lying in the correct position in the aorta. The cannula was then tied onto the artery with 4 cotton ties.

The transverse (facial) vein was located and carefully dissected free of surrounding tissue. From the point of cannulation, the venous tubing was measured and cut so as to allow approximately 6 - 7 cms of tubing to lie within the right jugular vein. The transverse vein was tied off and the venous cannula slowly fed into the transverse vein and pushed down into the jugular vein. The tubing was securely tied into the transverse vein with 3 or 4 ties. By cannulating this vein, there was minimal interference with the normal blood flow through the right jugular vein. 2.5 mls of heparinised saline (50 units/ml) was flushed into the venous cannula.

The front incision was then stitched together with several cotton ties, which were removed after healing of the wound (usually 10 - 14 days). The venous cannula at the back of the neck was then cut so that approximately 7 - 8 cms was permitted to hang freely and the cannula was sealed with a pin.

The arterial cannula was then closed by means of rubber-tipped artery clamps and disconnected from the transducer. The cannula was cut off close to the back of the

neck and attached to a valve of the type described by Day & Whiting (1972). The valve was placed under the skin at the back of the neck and pushed back through a small incision made approximately 2.5 cm above the transverse incision allowing the valve to protrude through the skin remotely from the original incision and leaving the base of the valve beneath the skin. 4 ties originally tied to the base were sutured through the skin and tied in pairs, thus anchoring the valve and preventing rotation. The original transverse incision was stitched together with cotton ties, which were also removed 10 - 14 days after the operation. The valve was reconnected with the transducer and the arterial cannula flushed with 2.5 mls of heparinised saline. The valve was then disconnected from the transducer and covered with a dust cap. At this time the intracerebroventricular cannula was implanted into the left lateral ventricle (see later for method). The whole operative procedure lasted 90 minutes. Just prior to the end of anaesthesia, the cat was injected intramuscularly with benethamine penicillin G (237.5 mg), procaine penicillin (125 mg) and sodium penicillin G (150mg), which was half the contents of a single dose vial of Triplopen (Glaxo Labs Ltd, Greenford) and provided immediate and long-term antibiotic protection (i.e. for at least 3 days).

The cats were watched during the recovery from anaesthesia and were prevented from scratching at their wounds. They were then transferred to a warm room. During the first 7 post-operative days both the arterial and venous

cannulae were flushed in daily with 2 - 3 mls of heparinised saline (50 units/ml). Subsequently the cannulae were flushed with heparinised saline on alternative days and before starting each experiment. Figs. 5a and 8d illustrate the position of the arterial and venous cannulae (and also the icv cannula) in a cat fully recovered from the operation.

The cats were allowed 3 - 4 days for recovery from the operation, after which the process of training them to sit or lie quietly during experiments and to accept icv infusions and i.v. injections of solutions at room temperatures without alarm, began. In most cases, experiments were started approximately 7 days after post-operative training. The cats were successfully trained to sit or lie quietly for periods of at least 6 hours. Fig. 5b shows a cat lying quietly in its cage during an experiment.

The valve dust-cap was removed for experiments and replaced by the valve top (connector) which opened it and which was attached to a blood pressure transducer (Devices/C.E.C, type 4-327-L221), which itself was connected to an M.2 Devices electronic recorder. The heart rate was recorded by means of a Neilson tachygraph unit (Devices Instruments Ltd., type 2751) connected to the recorder and triggered from the blood pressure pulse.

As soon as the cat was put into its experimental cage and connected to the blood pressure transducer both the arterial and venous cannulae were immediately flushed with

sterile 0.9% $^{w}/_{v}$ saline solution (see later). The cat was then left in its cage for 1.5 - 2.0 hours, after which time the blood pressure and heart rate were observed to be at control resting levels. At this time the experiment was started and drugs were administered either centrally or systemically.

4. Measurement of blood pressure in anaesthetised cats.

The 6 cats used for these experiments had previously been cannulated so allowing blood pressure and heart rate recordings and i.v. and icv drug administrations under conscious conditions.

In 3 cats responses to icv drug infusions were observed under halothane anaesthesia. Anaesthesia was induced with halothane(3.5%) in a 4:1 mixture of nitrous oxide and oxygen and maintained by reducing the halothane to 1.5% in the same gaseous mixture. The arterial valve was attached to a blood pressure transducer and the blood pressure and heart rate recorded by the same method as described for the conscious cats. The arterial and venous catheters were flushed with heparinised saline and the icv. cannula with normal saline at least 60 minutes before induction of anaesthesia. Compounds were infused icv after the blood pressure and heart rate had been at a constant level for at least 10 minutes. After these experiments, the animals were allowed to recover.

Experiments were performed in the other 3 cats under chloralose anaesthesia. Anaesthesia was induced and maintained by halothane in the same concentrations as

described above. The arterial valve was immediately connected to a transducer and the blood pressure and heart rate monitored. Chloralose, 80mg/kg, (as a 1.0% solution in saline) was slowly injected into the venous cannula. During the chloralose injection, the halothane content of the gaseous anaesthetic was gradually reduced and when about half the chloralose had been given the gaseous anaesthetic was completely withdrawn.

The icv cannula was then flushed with normal saline and the experiment using icv drug infusions started between 1.0 - 1.5 hours after the chloralose administration. The cats were not allowed to recover from these experiments.

5. Measurement of blood pressure in conscious normotensive unrestrained rabbits.

Unlike the cats, New Zealand white rabbits, weighing 2 - 4 kg, were not trained to experimental conditions before the operations. Initially, rabbits underwent one operation in which i.v., i.a. and icv cannulae were implanted. However, it was found that these rabbits rarely survived for more than 12 - 24 hours after the operation.

Therefore, it was decided to implant the icv cannula in a small operation (see later in the methods) at least 5 days before cannulation of the carotid artery and jugular vein. Rabbits were anaesthetised by halothane 4.0% in a 4:1, nitrous oxide: oxygen mixture and anaesthesia was maintained with halothane 2.5% in the same gaseous mixture. The carotid artery and jugular vein were cannulated by exactly the same method used for the cats, in

an operation which usually lasted about 45 minutes. Again, it was observed that the rabbits did not seem to stand up to this shortened operation very well and had usually died within 2 - 4 days after the operation. Hence, it was usual that only one experiment was performed in each rabbit and this was carried out 24 hours after the second operation.

6. Administration of drugs into the brain.

(a) Icv injections in rats.

Hayden, Johnson & Maickel (1966) described an indwelling cannula guide which was simple to construct and implant. Sparkes and Spencer (1971) made a further simplification of this method and the cannula guides used in this study were similar to those described by these authors.

The cannula guides were made from sheet perspex 6.35 mm (0.25 inch) thick; the final dimensions of each block being 6 x 7 x 6.35 mm. Each block was drilled through the centre to accept a 27 gauge needle, and one end of this hole was enlarged to a depth of 2.0 mm to accept a 6.0 mm length of 20 gauge stainless steel tubing, leaving 4.0 mm protruding (Fig. 6) . This was secured in place with epoxy cement ("Araldite", Ciba Ltd, Duxford, Cambridge), the curing of which was aided by heating to 50°C; this ensured a patent air-tight joint. The other end of the block was drilled out to a depth of 3.0 mm and the resulting cavity was filled with a cold-setting silicone rubber solution (Silescol S.R. 300, Esco Rubber Ltd, London).

This was allowed to cure for at least 7 days, after which a stilette, constructed of 20 gauge stainless steel wire, was passed down the centre of the cannula guide. Prior to implantation in the rat, the stilette was withdrawn and replaced several times in order to remove any swarf in the lumen of the guide. After implantation it remained in position except when injections were being made via the cannula guide and was replaced after the experiment had been performed.

Male normotensive and hypertensive (see part 1 of methods) Wistar rats weighing 175 - 225 g were used. It has been found that the skull does not grow sufficiently after this stage to dislodge the cannula guide or significantly alter the position of various brain structures with respect to the topographical features of the skull, which were used to implant the guide in the correct location.

The rats were anaesthetised with the usual halothane, nitrous oxide and oxygen mixture previously described. The animal was then secured by means of ear bars similar to those used in stereotaxic instruments. The fur was shaved from the top of the head and the area swabbed lightly with a 1.0% solution of chlorhexidine gluconate in 70% industrial methylated spirits.

A mid-sagittal incision was then made in the scalp and in the underlying connective tissue from the eyes to the ears (about 2.0 - 2.5 cm long). The skin was retracted with small weighted hooks and the underlying tissue cleared from the skull to each side. The surface

of the skull was thoroughly dried with cotton wool. Using a No. 2 round dental burr (in a dental drill) a hole was drilled in the skull according to the co-ordinates used by Calcutt (1972). The injection site was 2.0mm lateral to the bregma, which is the point of intersection of the coronal and sagittal sutures, on the coronal suture. 3 holes were then drilled around this, one in the frontal bone on the ipsilateral side to the injection site, one in the parietal bone on the ipsilateral side and one in the contralateral parietal bone. Small stainless steel screws (No. 2113, Wiseman & Co. Ltd., London), were screwed into these 3 holes such that they were firmly embedded in the skull but not protruding sufficiently within the cranium to compress the dura.

The cannula guide was then perpendicularly lowered into position between the 3 screws, such that the guide needle entered the hole prepared for it in the skull; the block rested firmly on the skull. Dental acrylic cement ("Sevriton No. 3", Amalgamated Dental Ltd., London) was then built up around the base of the perspex block and the 3 anchoring screws (Figure 6). After the cement had hardened (2 - 3 minutes), the skin was sutured around the block and a prophylactic injection of sodium penicillin G (48 mg) and streptomycin (40 mg) (Crystamycin Forte, Glaxo) given i.p. and the animal allowed to recover from the anaesthetic. The rats were used experimentally not sooner than 5 days after the implantation.

In hypertensive rats which had their blood pressures

measured by the indirect method, icv injections were made by holding the rat steady with one hand and removing the stilette from the cannula guide with a pair of forceps. The injection cannula was constructed from 27 gauge needle, 12.0 mm from the tip to the shoulder of the boss, with an acute bevel on the point. This was attached to a 50 µl Hamilton microsyringe and was inserted down the lumen of the cannula guide until the boss rested on the top surface of the rubber plug. An injection volume of 10.0 µl was then slowly injected over a period of 10.0 seconds, the needle withdrawn and the stilette replaced. 20 µl was injected icv to 6 rats on one single occasion (see Section 1, Chapter 2 of Experimental Results).

Remote icv injections were made in normotensive rats in which their blood pressures and heart rates were measured directly. The stilette was removed from the guide and replaced with the injection cannula. The latter consisted of a 16.0 mm length of 27 gauge needle tubing. 12.0 mm from the bevelled tip a piece of dried epoxy resin prevented the needle from entering the cannula guide beyond this point; the bead of resin rested on the top surface of the rubber plug. The needle was previously attached to a 50 µl Hamilton microsyringe by a length of PP 30 tubing. The syringe, PP 30 tubing and injection cannula were filled with the drug solution before being connected to the cannula guide. An injection volume of 10.0 µl was then remotely administered over a period of 10.0 seconds, when the conscious unrestrained rat had

recovered from being disturbed by connection of the injection cannula into the guide.

The top edge of the lateral ventricle lies some 5 - 6 mm from the top surface of the skull at the injection point; since the tubing on the bottom of the guide projects 4 mm below the block, there remains 1 - 2 mm of tissue between the tip of the guide and the ventricle. The 12.0 mm injection cannula projects below the tip of the cannula guide for a distance of 1 - 2 mm, which is sufficient to pass through the remaining brain tissue into the ventricular space.

(b) Icv infusions in cats

The most widely used cannula for injecting drugs into the lateral ventricles of cats is the 'Collison' cannula (C.F. Palmer Ltd, London) described by Feldberg & Sherwood (1953). In initial cat experiments, the Collison cannula was found to suffer from several minor defects. Hence, this lead to the development of a modified cannula, which was used successfully throughout the project. Fig. 7a shows a comparison of the 'Collison' cannula with the modified cannula and Fig. 7b fully illustrates the dimensions of the modified cannula.

In most mature cats, the body of the commercial Collison cannula was too short so that it was often covered by skin up to the level of the screw threads for the cap. This made it difficult to hold the cannula body firmly whilst removing the stilette and sometimes lead to inadvertent loosening of the cannula from the skull. In addition,

complete tightening of the screw-cap damaged the skin surrounding the cannula, hindered healing and caused the cat discomfort. The internal fluid volume of the Collison cannula, which is approximately 40 µl, is unnecessarily large and means that a considerable proportion of any drug dose may remain in the cannula after injection.

As can be seen from Fig. 7a the length of the cannula body has been increased so that the screw threads for the cap are well clear of the skin even in the post-operative period when the skin is somewhat swollen. The direction of the screw-threads on the cannula base for attachment to the skull and on the cannula top for attaching the cap have been reversed with respect to each other (as clearly demonstrated in Fig. 7b). Any lateral pressure on the cannula body when removing the cap therefore tends to tighten the cannula on the skull. In order to provide firmer anchorage of the cannula to the skull a flange has been added to the base which can be embedded in dental acrylic during fixing to the skull. The rubber diaphragm for drug injection of the Collison cannula, as seen in C_1 in Fig. 7a, has been omitted and drugs are introduced through the special connector cap (C_2 in Fig. 7a) which is constructed from a No. 16 hypodermic needle passed through and soldered to a screw-cap.

In comparison to the large dead - space in the Collison cannula, the internal fluid volume of the modified cannula is only 15 µl and the actual dead - space when the

injection connector or stilette is in place is a good deal less.

The modified cannula, which like the original, is made from stainless steel , is approximately twice as heavy (5.8 g compared with 2.8 g) as the Collison cannula. This was not found tc be a problem in practice and once implanted the cats showed very little interest in it.

The modified cannula was implanted by basically using the method previously described for the rat and in cats by Feldberg & Sherwood (1953). The cannulating procedure is clearly demonstrated in Figs. 8a to 8g. Fig. 8a shows a right lateral sagittal incision exposing the bregma. The co-ordinates were measured with dividers and the site of cannulation marked. The co-ordinates were varied with respect to the size of the cat being cannulated. The first co-ordinate was marked between 4 - 6 mm caudal to the bregma on the sagittal suture; the second co-ordinate, giving the site of cannulation, was measured laterally on the left side of the sagittal suture from the first co-ordinate. The co-ordinates used in the cat demonstrated in Figs. 8a and 8b were 6 mm caudal to the bregma and 4 mm lateral to this point on the sagittal suture.

A hole was then drilled in the skull and is shown in Fig. 8b. The dura was punctured with a sharp cataract knife and the hole was tapped to provide the thread in the skull which fits the lower thread of the cannula (Fig. 8c) A hypodermic needle (No. 23g) was inserted

into the central hole in the tapping block (Fig. 8c) and then carefully pushed into the brain tissue to an approximate depth of 6 mm, thus providing a track for the cannula to follow when inserted into the hole.

Figs. 8d and 8e illustrate the cannula tightly screwed into the skull. It was screwed into the skull and stopped at the point when cerebrospinal fluid welled up the cannula with respiration and pulse movements. The cannula was positioned so that the opening in the shaft of the cannula pointed towards the foramen of Monro.

Dental acrylic cement was carefully placed around the cannula flange, thus further ensuring a steady fixation of the cannula onto the skull (Fig. 8f) . After complete hardening of the cement, the skin was pulled over the cannula and the cannula was pushed through a small incision; hence the skin directly around the cannula did not need to be stitched. The remote lateral sagittal incision was stitched up with cotton thread. The stitches were removed after about 10 days after complete healing of the wound. The implanted cannula is shown in Fig. 8g.

A hypodermic needle (No. 25g), the diameter of which was much smaller than that of the cannula track, was inserted into the cannula and 0.2 - 0.3 mls of sterile normal saline solution were flushed into the cannula. Because of the loose fit of the needle in the cannula most of the saline was observed to reflux back up the cannula. The cap with the stilette was replaced and the cat allowed to recover from the anaesthetic.

This flushing procedure was carried out every day for the first 5 post-operative days and subsequently every alternate day. The cannula was flushed out in this way at the beginning of an experiment. Icv drug administrations were not started for at least 2 hours after flushing out the cannula.

All icv drug infusions were made remotely. The connector was attached to the syringe which itself was fixed onto a constant infusion pump, via polyethylene tubing. Drugs were infused in a volume of 100 µl at a rate of 20 - 25 µl/minute. This rate of infusion is approximately the same as the rate of production of cerebrospinal fluid.

(c) Icv infusions in rabbits

The icv cannula used in rabbits was made from a No. 19G hypodermic needle. The top of the needle was filed down so that the height of the cannula resting upon the skull measured 10 mm. The needle shaft was cut leaving a length of 9.0 mm from the boss and was then filed down to a length of 7.5 mm. The cavity within the cannula was filled with cold rubber solution (Silescol S.R. 300) and allowed to cure for 7 days. Stilettes and injection needles were made from hypodermic needles (No.23g) and were similar to those described in the section concerning the rat icv cannula guides. The length of the stilette was 17.5 mm and the holes in the needles were filled with araldite. The injection needle measured 18.0 mm from the araldite bead to the bevelled tip.

The cannula was implanted into the left lateral cerebral ventricle by the same method used for the rats. The co-ordinates used for the cannulation site were 4 mm caudal to the bregma (and coronal suture) and 2 mm laterally to the left of the sagittal suture. Dental acrylic cement was placed around the cannula and over 3 anchoring screws.

Icv drug administration in rabbits was performed in exactly the same method described previously for cats. However, because of the nature of the type of icv cannula used in the rabbit, it could not be flushed out with sterile normal saline like the cannula used in the cat. Hence, 100 µl of sterile normal saline was infused 2 - 3 hours before the start of the experiment using icv drug infusions.

(d) Intracisternal (icm) infusions in cats.

A cisternal cannula guide was made from a No. 0 hypodermic needle based upon the one described by Feldberg, Myers & Veale (1970). The needle shaft was cut and filed to measure 18 mm from the boss and the length of the top of the needle was 12 mm. The needle of a No. 1 hypodermic needle was cut from the boss and the top was filed clean and the point of the needle bevelled. The cannula guide was cemented to the skull (i.e. the interparietal and supra-occipital bones) by the method described by Feldberg et al. (1970). The guide was so positioned that it rested just above the atlanto-occipital membrane. At this point, the injection needle (gauge No. 1) was inserted into the guide

until it punctured the membrane; cerebrospinal fluid then rose up the needle. In this position, the injection needle was permanently fixed into the guide with a quick setting cement ("Araldite Rapid", Ciba Ltd., Duxford, Cambridge). The needle extending into the cisterna magna was capped with a sealed piece of polyethylene tubing.

Experiments involving icm drug infusions were not performed for at least 5 days after the operation. Remote icm infusions were made by the same method as used for the icv infusions in cats with the exception that icm infusion volumes were reduced to 50 µl compared to 100 µl of the icv infusions. 50 µl sterile normal saline was infused icm at least 2 hours before the start of experiments involving icm drug infusions.

7. Verification of icv administrations

Correct positioning of the icv cannulae was checked in all experimental animals after experiments had been completed. Pontamine sky blue (5 mg/ml in sterile normal saline), a dye which is bound to protein, was administered icv in volumes of 10 µl in rats and 100 µl in cats and rabbits. 15 minutes after dye administrations the animals were killed by overdosage with pentobarbitone sodium (Nembutal), decapitated and the brains dissected out. The brains were then kept in formalin solution until coronal sections were made.

In all rats cannulated using the co-ordinates of Calcutt (1972), dye was detected in both lateral ventricles the third ventricle, the aqueduct and the fourth ventricle.

In almost all cases some staining occurred over the posterior aspect of the cerebellum and the dorsal surface of the medulla indicating that the dye had passed through the ventricular system and entered the cisterna magna via the foramena of Luschka and Magendie. In some cases the dye could be seen staining blood vessels on the ventral surface of the brain.

Dye was also detected throughout the cerebroventricular system in all rabbits used for icv administrations. As with the rats, dye was observed at the base of the cerebellum indicating passage into the cisterna magna.

In these dye studies, it was found that in all cats cannulated icv with the modified 'Collison' cannula administrations into the ventricular system were successful in every case. The typical distribution of pontamine sky blue within the cat brain, 15 minutes after icv infusion of the dye, is demonstrated in Fig. 9a, b and c. Fig. 9a shows the exact position of each coronal section (A to F) as seen in Figs. 9 b and c, with respect to the total length of the whole brain (5.5 cm). Fig. 9b illustrates the faces of the 6 coronal sections, as viewed from the front to the back of the brain. Section B demonstrates the tract of the cannula and the dye can be seen in the left lateral ventricle, the third ventricle, the aqueduct and the fourth ventricle. Fig. 9c shows the staining of the reverse sides of the coronal section, when looking from the back to the front of the brain.

DRUGS USED

Acetylcholine bromide (B.D.H.)

Adrenaline bitartrate (B.D.H.)

α-Methyldopa (Aldomet; Merck, Sharp & Dohme)

α-Methyldopamine (Merck, Sharp & Dohme)

α-Methylnoradrenaline (Corbasil; Hoechst)

dl-Alprenolol (Aptin; Astra)

d-Alprenolol (a gift from Dr. R. Poyser, Beecham Research Labs. to Dr. M.D. Day).

Atropine methylnitrate (B.D.H.)

Bethanidine sulphate (Burrough's Wellcome)

Carbachol chloride (B.D.H.)

Chloralose (B.D.H.)

Clonidine (Catapres; C.H. Boehringer Sohn)

"Crystamycin Forte" (Glaxo)

Desmethylimipramine hydrochloride (Pertofran; Geigy).

Desoxycorticosterone acetate (Organon Labs).

Dexamphetamine sulphate (Sigma)

Dopamine hydrochloride (Sigma)

Guanethidine monosulphate (Ismelin; Ciba)

Haloperidol (Janssen)

Halothane (Fluothane; I.C.I.)

Heparin (Evans Medical)

Hexamethonium bromide (Kock-Light Labs.)

Isoetharine (a gift from Dr. R. Poyser, Beecham Research Labs. to Dr. M.D. Day)

Isoprenaline sulphate (B.D.H.)

Lignocaine hydrochloride (B.D.H.).

McNeil A-343 (McNeil)

Metaraminol bitartrate (Aramine; Wyeth)

Methoxamine hydrochloride (Vasoxyl; Burrough's Wellcome)

Nicotine hydrogen tartrate (B.D.H.)

Noradrenaline hydrochloride (Sigma)

Oxprenolol (Trasicor; Ciba-Geigy)

Pempidine tartrate (May & Baker)

Phenylephrine hydrochloride (Boots Pure Drug Co.)

Phentolamine methane sulphonate (Rogitine; Ciba)

Pimozide (Janssen)

Pindolol (LB-46; Visken; a gift from Dr. R. Poyser, Beecham Research Labs to Dr. M.D. Day)

Pontamine Sky blue (B.D.H.)

Practolol (Eraldin; I.C.I.)

Procaine hydrochloride (B.D.H.)

dl-Propranolol hydrochloride (Inderal, I.C.I.)

d-Propranolol (I.C.I. 47,319; I.C.I.)

l-Propranolol (I.C.I. 47,320; I.C.I.)

Ro4-4602 (seryl-2,3,4-trihydroxybenzyl-hydrazine; Hoffman-La Roche & Co. Ltd).

Salbutamol (Allen & Hanburys)

Sodium diethyldithiocarbamate (B.D.H.)

Sodium pentobarbitone (Nembutal; Abbott Labs).

Sotalol (MJ 1999; Mead Johnson)

Tenormin (I.C.I. 66082; I.C.I.)

Tetraethylthiuram disulphide (Disulfiram; B.D.H.)

Tetramethylammonium chloride (B.D.H.)

"Triplopen" (Glaxo)

U-14,624 (1-phenyl-3-(2-thiazolyl)-2-thiourea; Upjohn & Co.)

All doses quoted in the text are expressed in terms of the salts except those of salbutamol, haloperidol, pimozide, d- and l-propranolol, d-alprenolol and practolol. All compounds were dissolved in sterile 0.9% W/v sodium chloride solution ("Steriflex", Allen & Hanburys), unless otherwise stated in the text and sterile water for injections was used. In the cases of α-methyldopa and methoxamine, icv administrations were made using the respective commerical injection forms, 'Aldomet' and 'Vasoxyl'. Tetraethylthiuram disulphide (disulfiram) was suspended in sterile normal saline solution with compound powder of tragacanth. Disulfiram was always administered i.p.

The 'Aldomet' commercial injection vehicle was prepared according to the formula stated by Merck, Sharpe & Dohme Ltd. Each 5ml of vehicle contained citric acid anhydrous (25.0mg), sodium bisulphite (16.0mg), disodium edetate (2.5mg) and monothioglycerol (10.0 mg) Sodium hydroxide was added to adjust the pH to 4.2 and the volume made up to 5 mls with water for injections. Methylparaben, 7.5 mg (0.15%) and propylparaben, 1.0 mg (0.02%) were added as preservatives.

Means and standard errors of means were calculated for each group of results and analysed by the Students' t - test. In all cases, $p \leqslant 0.05$ was considered significant.

FIG. 1.

Photograph demonstrating positions of strain gauge and cuff on tail of a restrained rat housed in heated cabinet.

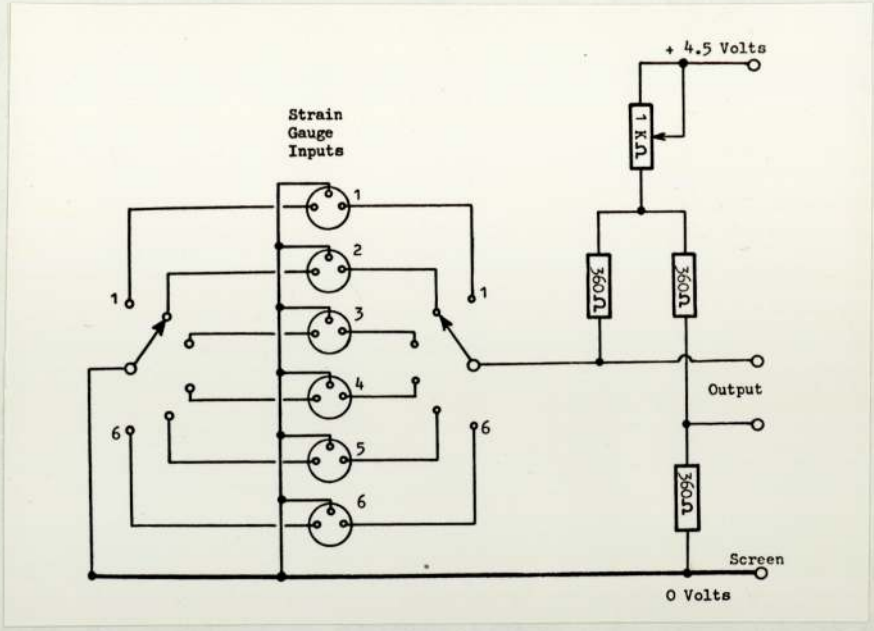


FIG. 2.

Wheatstone bridge circuit as used with pulse detectors. A switch enables one of 6 strain gauges to be connected into the bridge circuit. The output of the bridge is taken to the input of a medium gain A.C. amplifier, e.g. E.C.G.

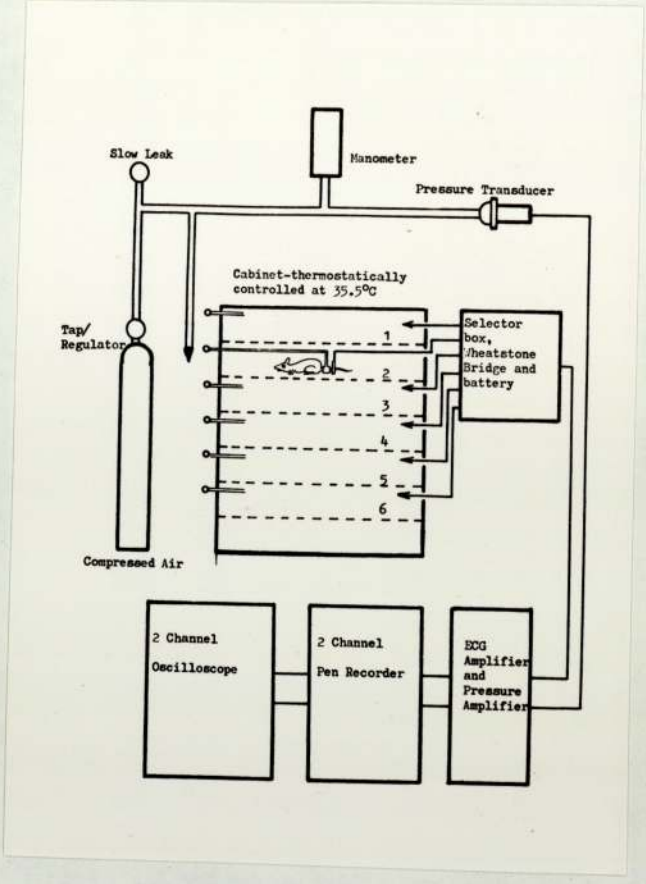


FIG. 3.

Block diagram of the apparatus used for measuring the systolic blood pressure of a rat by the tail cuff method.

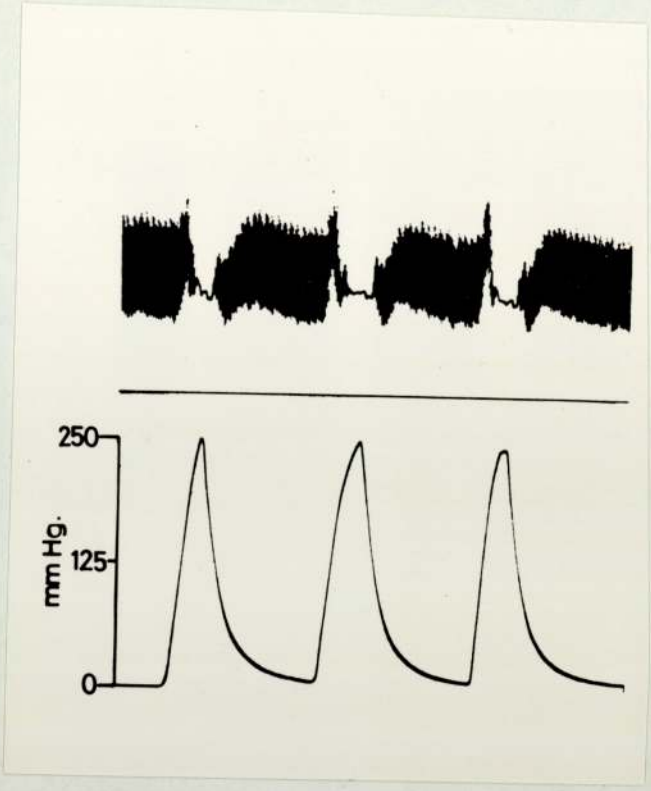


FIG. 4.

A course of 3 sets of systolic blood pressure readings obtained on a Devices Electronic Recorder. The upper trace represents the pulse of the caudal artery of a restrained, conscious rat and the lower trace represents the pressure in the occluding cuff (0 - 250 mmHg).

FIG. 5a.

Photograph of conscious cat in recording cage illustrating the positions of the arterial, venous and intracerebroventricular (icv) cannulae. In this photograph the dust cap has been removed from the blood pressure valve and replaced with the valve connector which connected the valve to the blood pressure transducer and electronic recorder by means of the soft polyethylene tubing shown. Also, it can be seen that the stilette of the icv cannula has been replaced with a connector cap. The cap was attached to a syringe, which was fitted to an infusion pump, via polyethylene tubing.

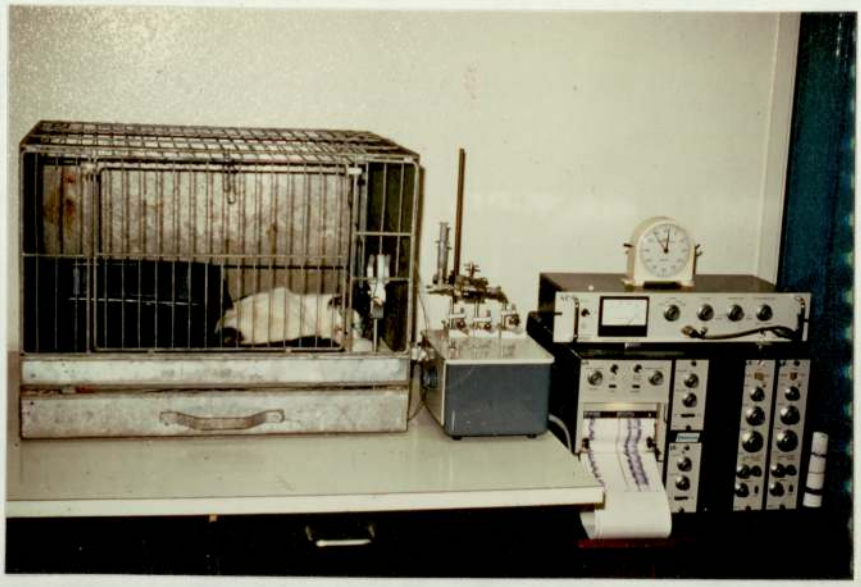


FIG. 5b.

Photograph taken during a typical experiment in which the blood pressure and heart rate of a conscious, normotensive cat was continuously monitored. The photograph was taken during an icv infusion.

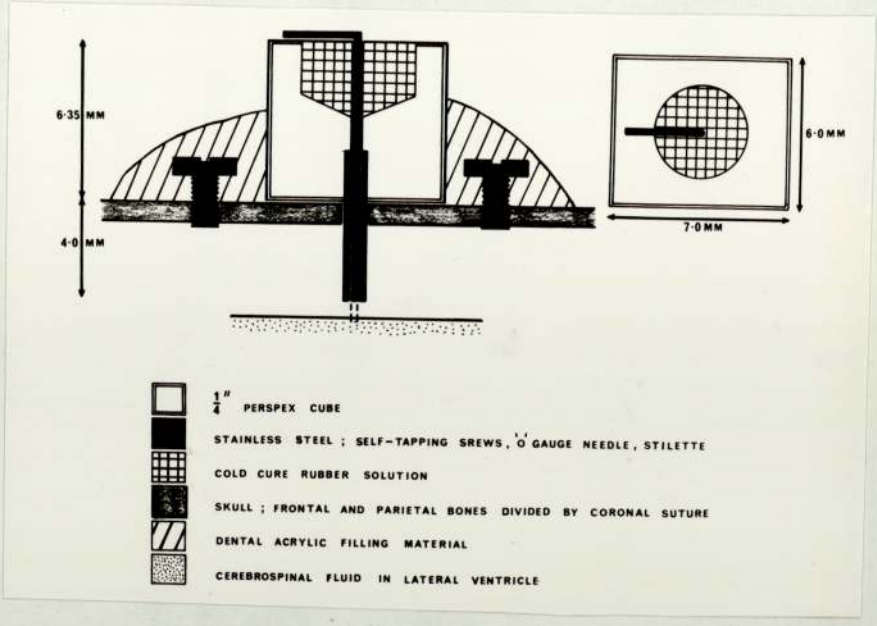


FIG. 6.

Diagram of icv cannula guide used in rats showing vertical section and plan view.

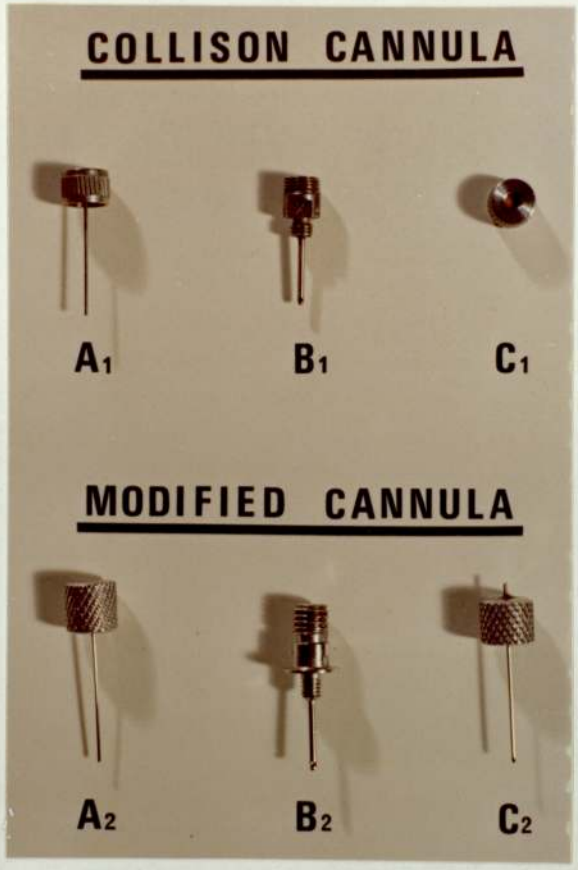


FIG. 7a.

This photograph shows the differences between the commercially available Collison cannula and the modified icv cannula used in the cat experiments of this thesis. A_1 and A_2 illustrate the stilette caps of the Collison and modified cannulae respectively, B_1 and B_2 the cannula bodies and lastly the injection cap C_1 of the Collison cannula has been replaced with a connector cap C_2.

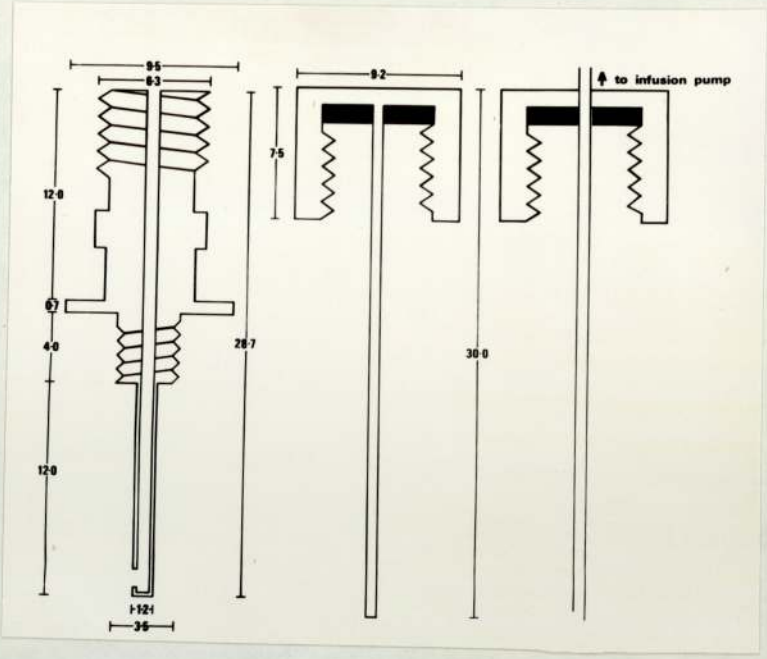
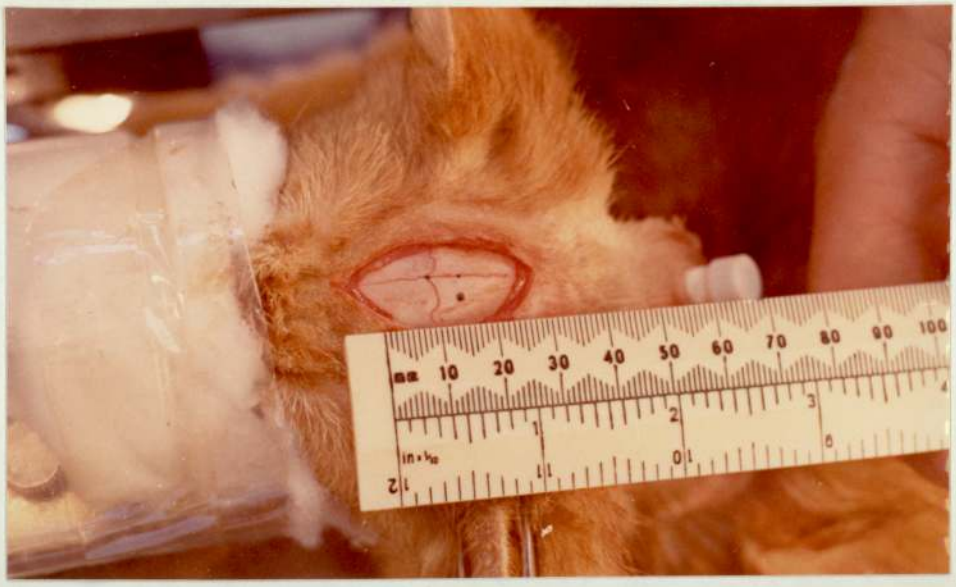


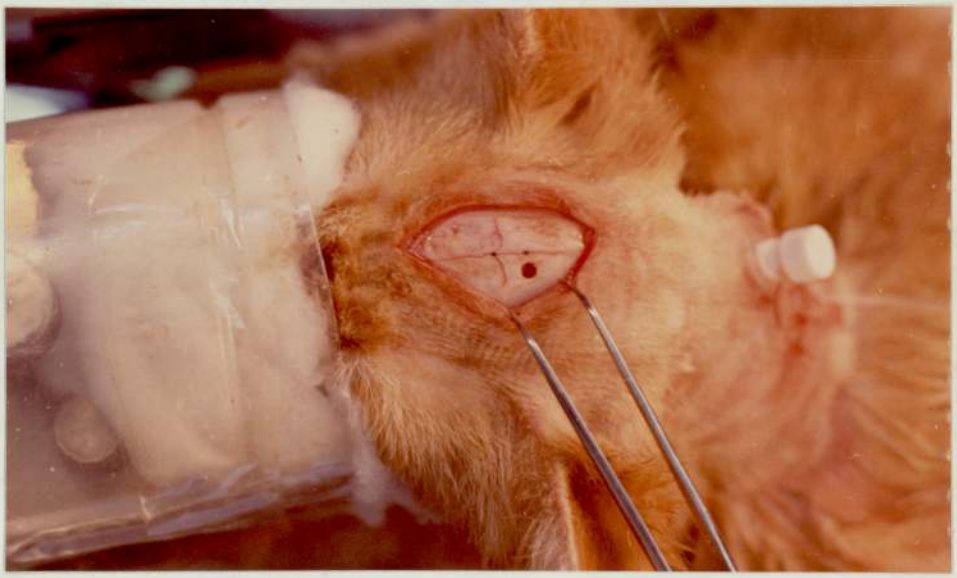
FIG. 7b.

Cross-sectional and dimensional diagram of the modified cannula with stilette and connector cap for icv infusions in cats. All measurements in mm.

a



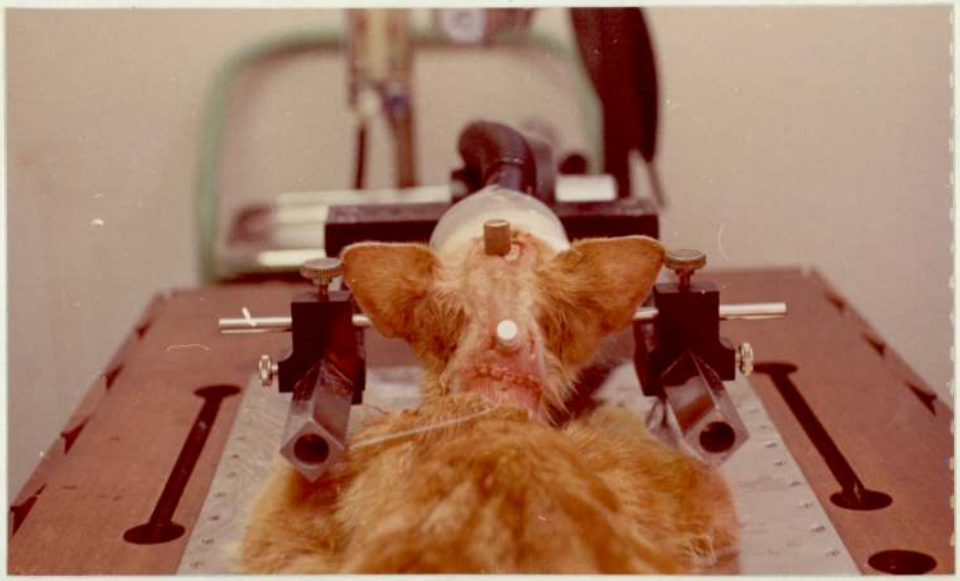
b



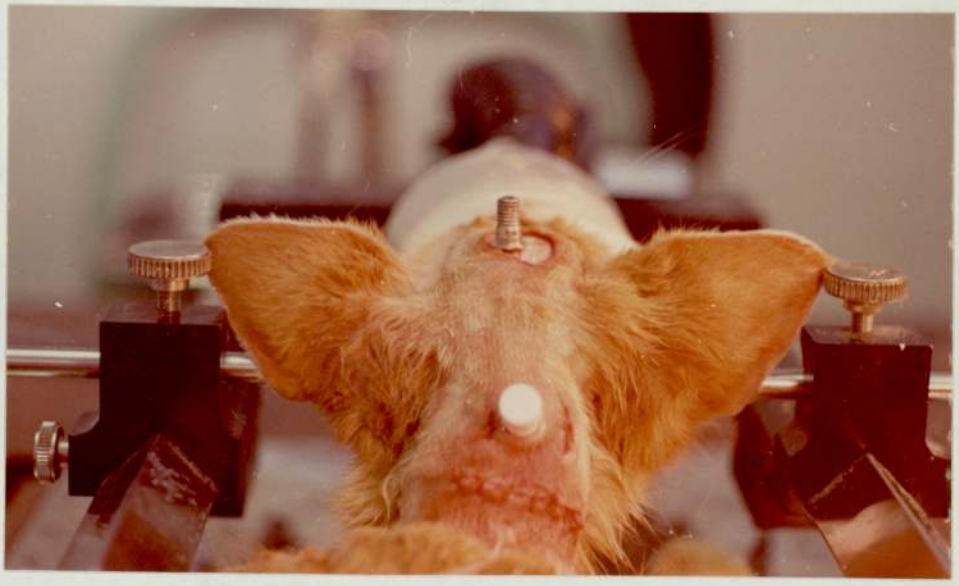
FIGS. 8a - g.

A series of photographs illustrating the icv cannulation procedure of a halothane anaesthetised cat.

c

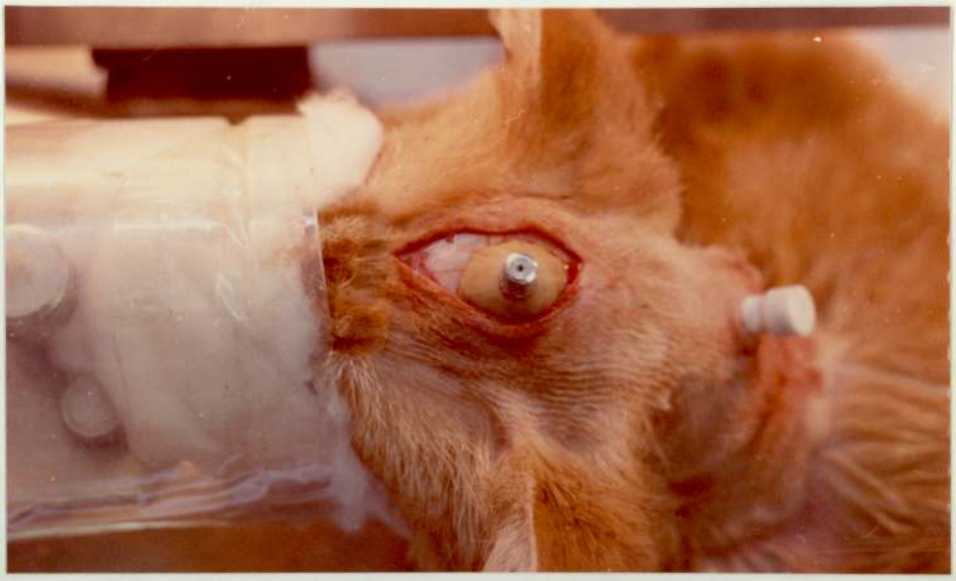


d

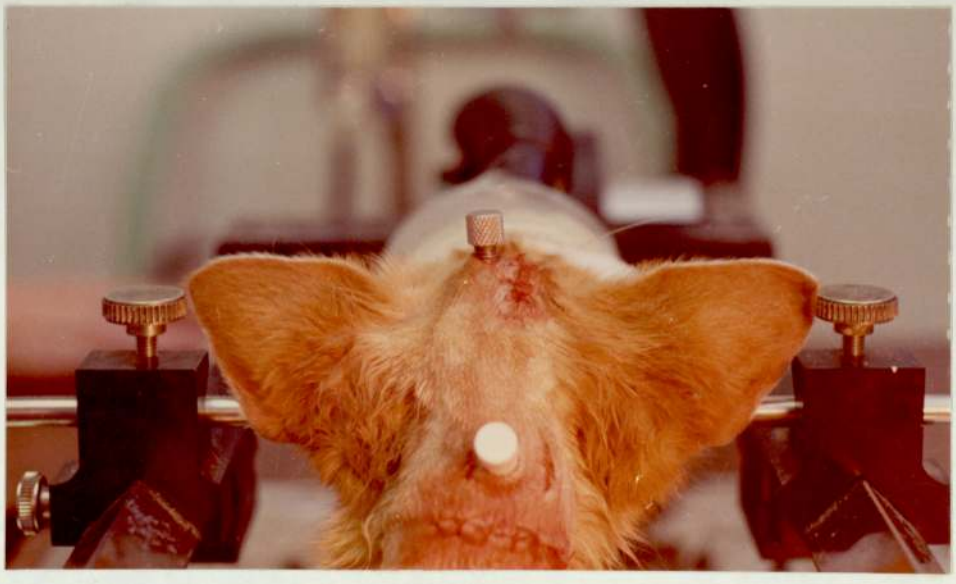


e

f



g



a

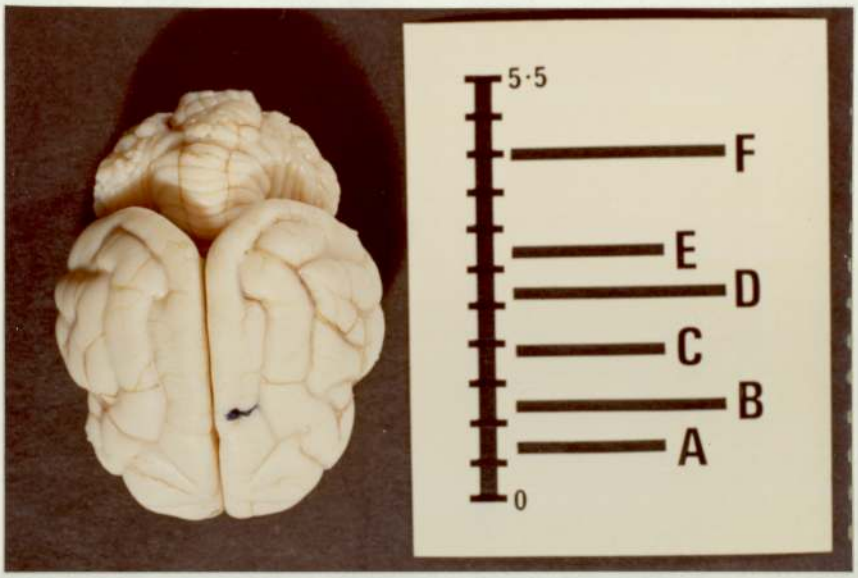
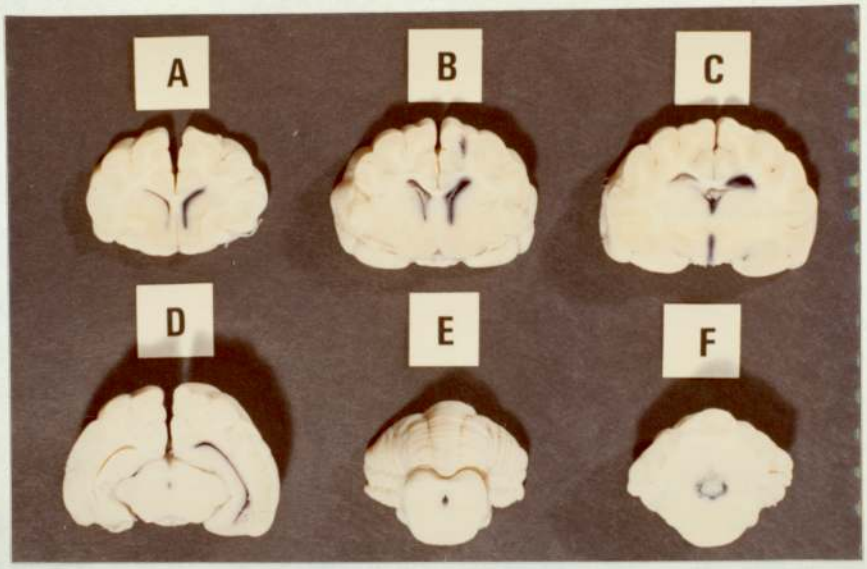


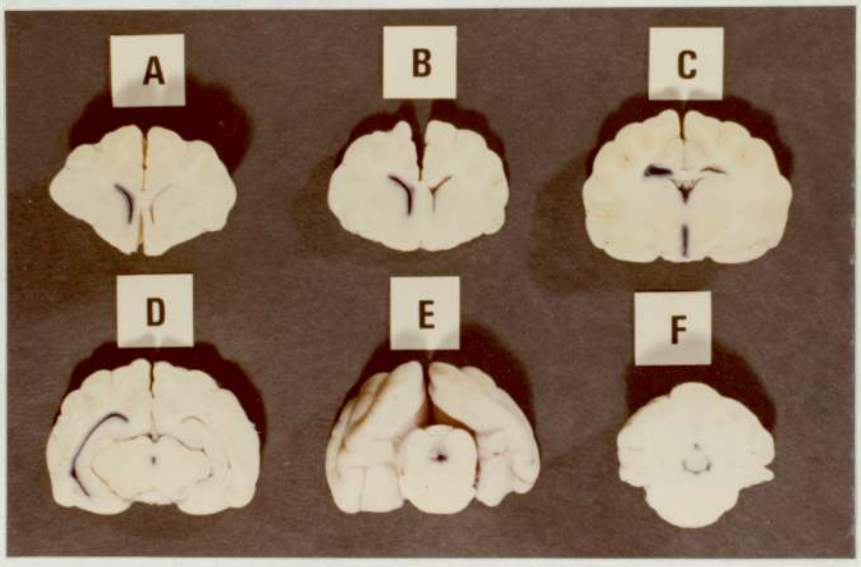
FIG. 9a

Photograph taken vertically above a cat brain, previously infused with 100 µl pontamine sky blue (5mg/ ml) and kept in formalin solution for 7 days, illustrating the positions of 6 coronal sections (A - F) taken of this brain in relation to the total length of the brain, 5.5 cms. Section B was taken through the cannulation site. The cat was killed 15 minutes after the dye infusion and the brain dissected from the skull immediately.

b



c



FIGS. 9b and c. These photographs illustrate the faces of each coronal section. Fig. 9b shows the faces of the 6 sections as viewed from the front to the back of the brain, i.e. from A to F. Fig. 9c shows the staining on the reverse sides of each section, when looking from the back to the front of the brain, i.e. from F to A.

EXPERIMENTAL RESULTS

SECTION 1. The effects of enzyme inhibitors of catecholamine synthesis on the antihypertensive activity of α-methyldopa.

CHAPTER 1. A study using peripherally administered α-methyldopa.

Carlsson & Lindqvist (1962) detected α-methyldopamine and α-methylnoradrenaline in the brains and hearts of mice pretreated with α-methyldopa demonstrating that α-methyldopa was metabolised, in vivo, in a similar manner to the natural precursor dopa, in the noradrenergic pathway (Fig. 10). After pretreatment with α-methyldopa, stimulation of adrenergic neurons resulted in a release of α-methylnoradrenaline (Muscholl & Maitre, 1963).

α-Methyldopa or its metabolites, α-methyldopamine and α-methylnoradrenaline, restored responses to sympathetic nerve stimulation and to indirectly-acting sympathomimetic amines in reserpine treated animals and isolated tissues (Day & Rand, 1963a, 1964). In the experiments of Day & Rand (1963a, 1964), α-methylnoradrenaline was observed to be a less potent pressor amine than noradrenaline in several animal species. These workers suggested that substitution of the α-methylated metabolite of noradrenaline for the natural transmitter in peripheral sympathetic nerves would lead to a partial sympathetic nerve impairment producing lowering of the blood pressure and heart rate.

Farmer (1965) reduced the response of the nictitating membrane to post-ganglionic sympathetic nerve

stimulation in cats with either α-methyldopa or α-methyldopamine suggesting that the antihypertensive effect observed after α-methyldopa could be due to an anti-sympathetic action of α-methyldopamine.

Evidence that the metabolism of α-methyldopa within the human central nervous system is important in the mediation of its antihypertensive effect was presented by Sjoerdsma et al. (1963). These workers observed that pretreatment, in hypertensive patients, with α-methyldopa hydrazine (MK485), a dopa decarboxylase inhibitor which is unable to enter the brain (Porter, Watson, Titus, Totaro & Byer, 1962), failed to affect the hypotensive activity of α-methyldopa. However, Davis, Drain, Horlington, Lazare & Urbanska (1963) found divergent results in the rat. They noticed that the dopa decarboxylase inhibitor N-2-hydroxybenzyl-N-methylhydrazine (NSD1039) antagonised the antihypertensive action of α-methyldopa.

Henning (1969a) further investigated the effects of dopa decarboxylase inhibitors on the hypotensive action of α-methyldopa in hypertensive rats. He found that the central decarboxylation of α-methyldopa was essential in order to produce its antihypertensive effect. He observed that MK485 (an inhibitor of peripheral dopa decarboxylase enzymes only) did not affect the fall in blood pressure due to α-methyldopa administered systemically whilst pretreatment with seryl-2,3,4-trihydroxybenzyl-hydrazine (Ro4-4062), administered in doses sufficient to inhibit both peripheral and central dopa decarboxylase (Pletscher & Gey, 1963) prevented the antihypertensive action of α-methyldopa.

A series of experiments was performed using inhibitors of both dopa decarboxylase and dopamine β-hydroxylase enzymes in an attempt to find the necessary metabolite needed to mediate the antihypertensive action of α-methyldopa. Since this study was undertaken, Henning & Rubenson (1971) found that central inhibition of dopamine β-hydroxylase with bis(4-methyl-1-homopiperazinyl-thiocarbonyl)disulphide (FLA-63) abolished the fall in blood pressure due to α-methyldopa, thus demonstrating that the central formation of α-methylnoradrenaline is necessary for the antihypertensive effect of α-methyldopa in the rat.

Results

1. Effect of dopa decarboxylase inhibition with Ro4-4602 on the antihypertensive activity of α-methyldopa administered systemically to hypertensive rats.

In all experiments of this study, the effects of α-methyldopa alone or in combination with enzyme inhibitors were observed in groups of 6 DOCA/saline hypertensive rats.

The mean systolic blood pressure of a group of hypertensive rats was markedly reduced from 187 ± 3 to 129 ± 2 mmHg by a single intraperitoneal dose of α-methyldopa (200 mg/kg). The maximum fall in blood pressure occurred 4.5 hours after the α-methyldopa administration and had returned to pretreatment levels within 24 hours (Fig. 11). α-Methyldopa (200 mg/kg, i.p.) produced sedation of the rats at this dose level.

In a second group of hypertensive rats, the mean blood pressure was unaffected by 3 i.p. doses of Ro4-4602 (50 mg/kg). This dose regimen of Ro4-4602 (total dose 150 mg/kg given over a period of 4 hours) was shown by Kuruma, Bartholini, Tissot and Pletscher (1972) to inhibit peripheral but not central dopa decarboxylase.

It can be seen from Fig. 11 that in combination with α-methyldopa (200 mg/kg, i.p. administered 30 minutes after the first dose of Ro4-4602, 50 mg/kg, i.p.) this low dose regimen of Ro4-4602 did not significantly alter the antihypertensive action of α-methyldopa . The fall in mean systolic blood pressure after α-methyldopa in this group at 4.5 hours was not significantly different ($p > 0.05$) from the blood pressure course observed with α-methyldopa given alone.

The vehicle in which the commercial 'Aldomet' injection of α-methyldopa is formulated contains a number of chemical constituents (see methods) and was therefore tested for possible effects on blood pressure. When injected i.p. to 6 hypertensive rats in equivalent volumes to those used for α-methyldopa, the vehicle produced no effect on blood pressure.

Fig. 12 illustrates a similar series of experiments in which Ro4-4602 was administered, in the same pattern as described above, at an increased dose of 200 mg/kg per injection. This dose regimen was used by Henning (1969a). This treatment of Ro4-4602 (total dose of 600 mg/kg administered over a period of 4 hours) has been

shown by Kuruma et al. (1972) to be sufficient to inhibit both central and peripheral dopa decarboxylase enzymes.

In the groups of hypertensive rats that received Ro4-4602 (3 x 200 mg/kg, i.p.) in combination with the 'Aldomet' injection vehicle, administered 30 minutes after the first Ro4-4602 dose, the mean blood pressure was observed to fall from 186 $\pm$ 2 to 150 $\pm$ 1 mmHg after 6.75 hours. The reduced mean systolic blood pressure, due to Ro4-4602 (3 x 200 mg/kg, i.p.), had returned to pretreatment levels within 24 hours.

Ro4-4602 (3 x 200 mg/kg, i.p.) markedly reduced the antihypertensive effect of α-methyldopa (200 mg/kg, i.p.). The mean systolic blood pressure of this group remained at a higher level than that observed with Ro4-4602 administered in combination with the 'Aldomet' injection vehicle (Fig. 12). At 6.75 hours the blood pressures of these two groups were significantly different ($p < 0.0005$) Hence, there would appear to be a mutual antagonism between the antihypertensive effects of α-methyldopa and Ro4-4602.

The blood pressure course of the group of rats that received the 'Aldomet' vehicle alone paralleled the blood pressure time course of the group of rats that received the Ro4-4602 and α-methyldopa combination, for the first 4 hours. At this time the systolic blood pressure was observed to fall in the group of rats that received the enzyme inhibitor and α-methyldopa combination. After approximately 7 hours, the difference in the blood

pressures of the two groups was highly significant ($p < 0.0005$).

2. Effect of dopamine β-hydroxylase inhibition on the antihypertensive activity of α-methyldopa.

Three dopamine β-hydroxylase inhibitors were used in this study. These were disulfiram, sodium diethyldithiocarbamate and U-14,624. Disulfiram and its reduction product in vivo, diethyldithiocarbamate (DDC), were shown to be effective inhibitors of central and peripheral dopamine β-hydroxylase by Mussachio, Goldstein, Anagnoste, Poch & Kopin (1966) whilst Johnson, Boukma & Kim (1970) reported that U-14,624 (1-phenyl-3-(2-thiazolyl)-2-thiourea) was a more specific inhibitor of central than peripheral dopamine β-hydroxylase.

The effect of disulfiram on the antihypertensive effect of α-methyldopa (200mg/kg, i.p.) was observed after three different dose levels of disulfiram (25,50 and 100 mg/kg).

Disulfiram systemically administered 2 hours before α-methyldopa produced a dose dependant inhibition of the antihypertensive effect of α-methyldopa. Disulfiram (25 and 50 mg/kg, i.p.) respectively produced a 43 and 69% inhibition of the blood pressure depressant response of α-methyldopa (200 mg/kg, i.p.). When administered 2 hours before the 'Aldomet' vehicle the 25 mg/kg dose of disulfiram produced no effect on the mean systolic blood pressure, whilst 5 hours after the 50 mg/kg dose of disulfiram, the mean systolic blood pressure had fallen from 189 ± 2 to 160 ± 3 mmHg.

Disulfiram (100 mg/kg, i.p.) completely inhibited the antihypertensive effect of α-methyldopa (200 mg/kg, i.p.) This is illustrated in Fig. 13. This dose of disulfiram administered 2 hours before the 'Aldomet' vehicle, produced a moderate fall in the mean systolic blood pressure of this group. As with disulfiram (50 mg/kg, i.p.), the peak effect occurred 5 hours after administration and was of a similar magnitude; disulfiram (100 mg/kg, i.p.) induced the blood pressure to fall from 191 $\pm$ 2 to 159 $\pm$ 2 mmHg. Thus, the extent of the antihypertensive effect and its time course were almost identical for the 50 and 100 mg /kg doses of disulfiram.

Fig. 13 illustrates that at the time of the greatest inhibitory effect of disulfiram on the antihypertensive response of α-methyldopa (i.e, 6.5 hours after the disulfiram administration) the blood pressure of the group of rats that received disulfiram in combination with the 'Aldomet' vehicle was already reverting back to pretreatment levels; the disulfiram had previously induced its own antihypertensive effect. Hence, it may be that the inhibition of α-methyldopa and the fall in systolic pressure induced by disulfiram are produced by different actions of disulfiram.

Replacement of disulfiram by DDC (100 mg/kg, i.p.) in another series of experiments also produced a complete abolition of the antihypertensive response to α-methyldopa (Fig. 14). DDC (100 mg/kg, i.p.) injected 2.25 hours before the 'Aldomet' vehicle produced a fall in blood pressure

of similar size to that observed with disulfiram (100 mg /kg). DDC (100 mg/kg) caused the mean blood pressure to fall from 183 ± 2 to 151 ± 3 mmHg. The onset of the antihypertensive effect due to DDC was much quicker than for disulfiram. The maximum fall in blood pressure after DDC occurred 2 hours after administration of the enzyme inhibitor (compare Figs. 13 and 14).

The third dopamine β-hydroxylase inhibitor used was U-14,624. U-14,624 (200 mg/kg, i.p.) itself produced a fall in mean systolic blood pressure. The peak effect was observed 6 hours after administration of the U-14,624 and was a mean fall of 23 ± 5 mmHg. The blood pressure returned to hypertensive pretreatment levels after 10 hours. U-14,624 (200 mg/kg, i.p.) was found to abolish the antihypertensive action of α-methyldopa when given 2 hours before α-methyldopa. However, as the dopamine β-hydroxylase inhibitory effects of U-14,624 were long lasting (Johnson et al., 1970) it was decided to administer α-methyldopa to U-14,624 pretreated rats, at a time when the blood pressure effects of U-14,624 had ended. The antihypertensive effect of α-methyldopa was still completely absent when administered 12 hours after the dopamine β-hydroxylase inhibitor (Fig. 15).

Discussion

The results described in this Chapter strongly support the work of Henning (1969a) and Henning & Rubenson (1971). α-Methyldopa possess a central antihypertensive action in the rat and in order for this effect to be observed

α-methylnoradrenaline must be formed within the central nervous system.

However, using systemically administered enzyme inhibitors, in doses that are not totally specific to either peripheral or central enzymes, it is impossible to conclude, from these experiments, that the antihypertensive effect observed after peripherally administered α-methyldopa consists totally of a central action. For example, in the experiments using Ro4-4602 to evaluate the action of α-methyldopa two dose levels were used. It is not possible to assume that total inhibition of peripheral dopa decarboxylase enzymes occurred in the hypertensive rats used in these experiments after the low dose regimen of Ro4-4602 as no biochemical analysis was performed. Ro4-4602 (3 x 50 mg/kg) did produce a slight but statistically insignificant reduction in the antihypertensive response to α-methyldopa. The larger dose regimen of Ro4-4602 abolished the antihypertensive effect of α-methyldopa but total inhibition of both peripheral and central enzymes probably occurred.

Disulfiram and DDC inhibit both peripheral and central dopamine β-hydroxylase (Mussachio et al., 1966) and again one cannot separate a peripheral component of α-methyldopa, if present, from the central one. However, U-14,624 was reported by Johnson et al. (1970) to be a more specific inhibitor of central enzymes but still peripheral dopamine β-hydroxylase inhibition occurs.

Using similar dose levels of α-methyldopa, Ro4-4602

and disulfiram in Goldblatt hypertensive rats, Cohen, Wepierre, Jacquot & Rapin (1974) obtained similar results.

In addition, Nijkamp, Ezer & de Jong (1975) have recently confirmed that the antihypertensive effects and reductions in heart rate observed after systemic α-methyldopa administration to renal hypertensive rats were of central origin and due to the formation of the metabolites of α-methyldopa. Peripheral dopa decarboxylase inhibition with Ro4-4602 failed to alter the cardiovascular depressant effects of α-methyldopa whilst central dopa decarboxylase inhibition prevented the falls in pressure and heart rate after systemic α-methyldopa.

Ro4-4602 produced a significant antihypertensive effect only when given in the high dose regimen; the low dose regimen of Ro4-4602 was inactive (compare Figs. 11 and 12). Since the high doses of Ro4-4602 are reported to inhibit both peripheral and central dopa decarboxylase enzymes and the low doses only peripheral enzymes (see Henning, 1969a; Kuruma et al., 1972) and also as the antihypertensive effects of α-methyldopa and Ro4-4602 when given in combination, appeared to be mutually antagonistic (see Fig. 12), the antihypertensive effect of Ro4-4602 was probably caused by a central action of Ro4-4602. This is discussed in more detail in the discussion of the next Chapter.

Evidence supporting a peripheral mode of action for α-methyldopa is briefly discussed in the Historical Introduction.

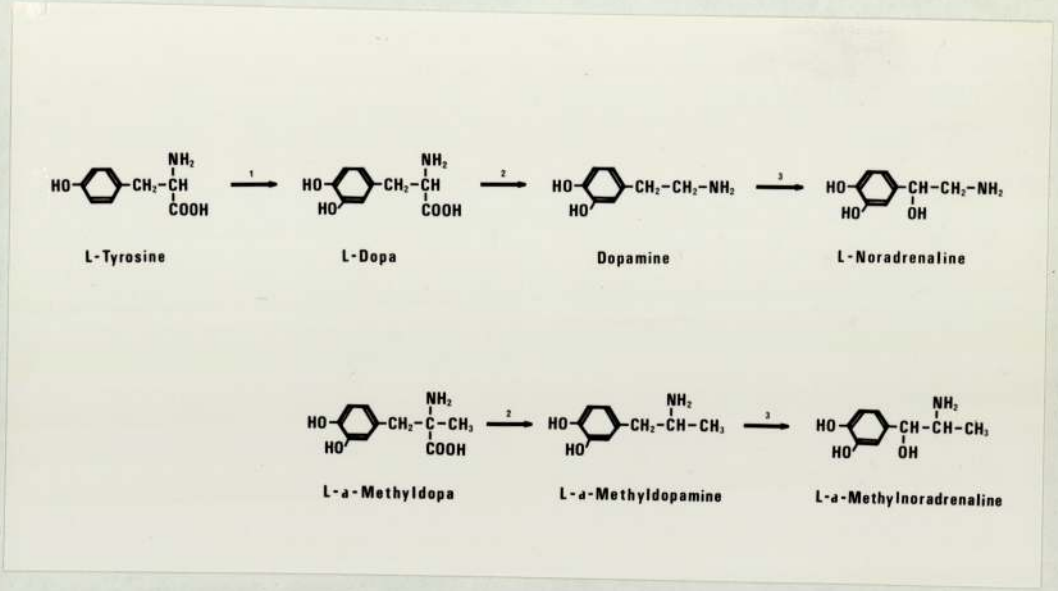


FIG. 10.

Diagram illustrating the natural synthetic pathway of l-noradrenaline produced from l-tyrosine. The lower pathway shows the synthesis of l-α-methylnoradrenaline after substitution of l-α-methyldopa for l-dopa in the natural pathway. Enzymes 1,2 and 3 are tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase respectively.

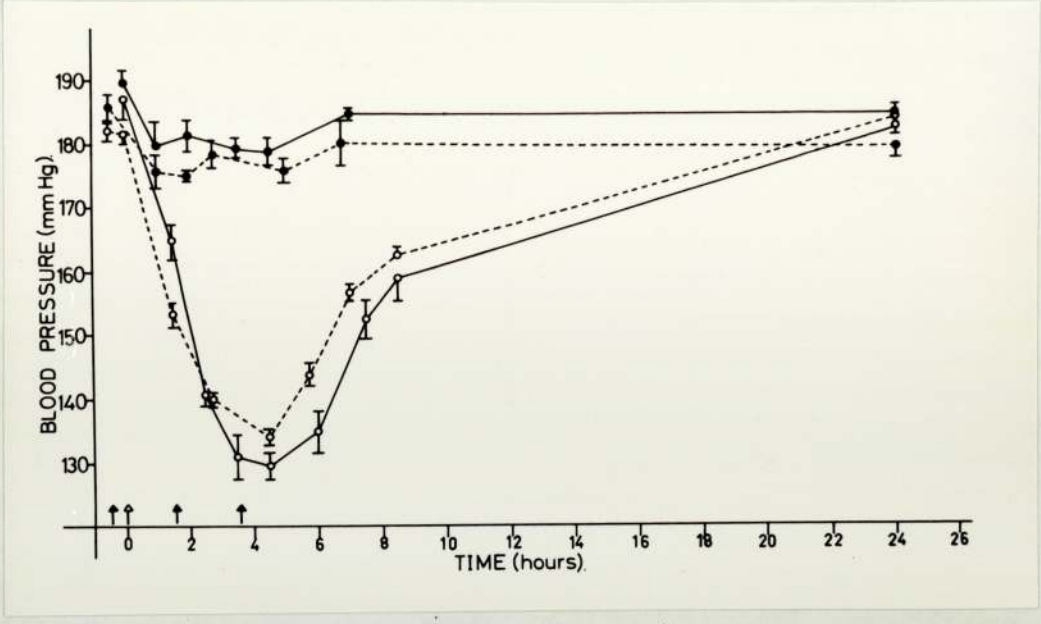


Fig. 11. Effect of peripheral dopa decarboxylase inhibition with Ro4-4602 (3 x 50 mg/kg, i.p.) on the antihypertensive action of ∝-methyldopa (200 mg/kg, i.p.). ∝-Methyldopa or equivalent volumes of the 'Aldomet' vehicle, which consisted of the commercial formulation in which ∝-methyldopa is dissolved, were injected at 0 hours (i.e. at point of open arrow). Ro4-4602 (50 mg/kg, i.p.) was injected on 3 occasions at 2 hourly intervals (i.e. at the points of the closed arrows). Each point represents the mean systolic blood pressure with the standard error of the mean of a group of 6 hypertensive rats. Key; O——O represents group that received ∝-methyldopa alone; ●——● group with 'Aldomet' vehicle alone; ●- -● group with Ro4-4602 and 'Aldomet' vehicle; O- -O group with Ro4-4602 and ∝-methyldopa.

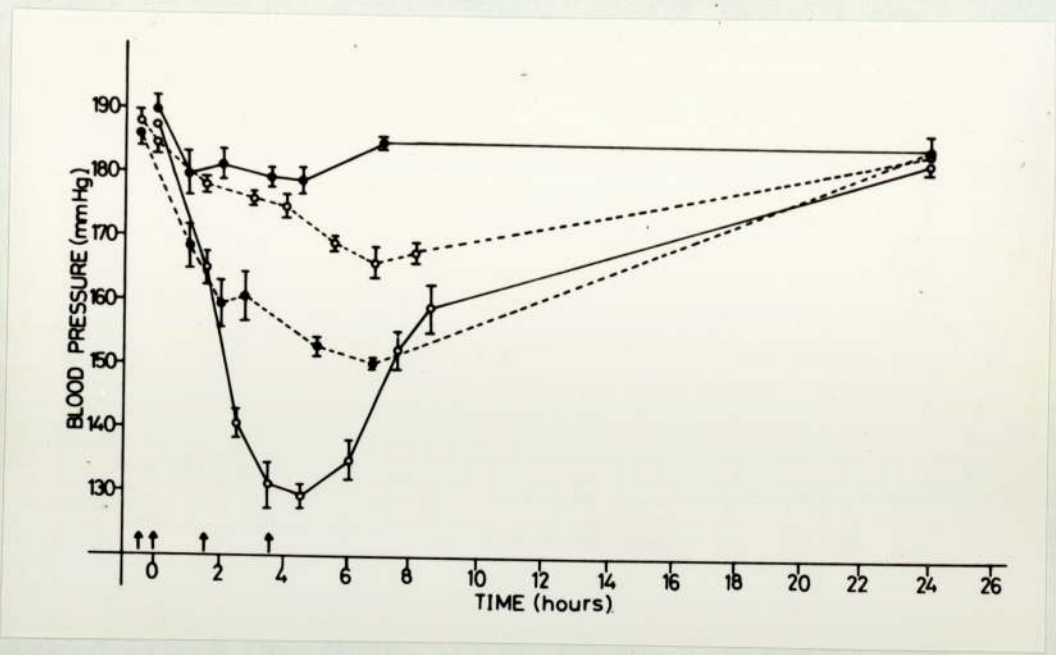


Fig. 12. Effect of simulataneous peripheral and central dopa decarboxylase inhibition with Ro4-4602 (3 x 200 mg /kg, i.p.) on the antihypertensive action of α-methyldopa (200 mg/kg, i.p.). The times of dose administrations and group numbers are as described in Fig. 11. Key; O——O represents group that received α-methyldopa alone; ●——● group with 'Aldomet' vehicle alone; ●- -● group with Ro4-4602 and 'Aldomet vehicle; O- -O group with Ro4-4602 and α-methyldopa.

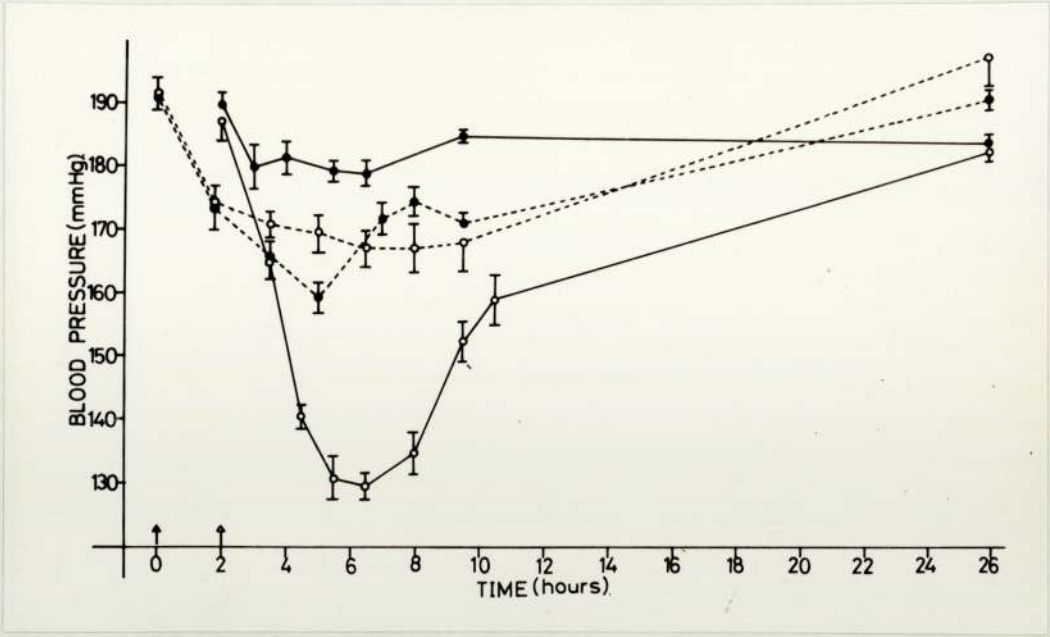


Fig. 13. Effect of simultaneous peripheral and central dopamine β-hydroxylase inhibition with disulfiram (100 mg /kg, i.p.) on the antihypertensive action of α-methyldopa (200 mg/kg, i.p.). Disulfiram was injected at 0 hour (at the point of closed arrow), 2 hours prior to the α-methyldopa or 'Aldomet' vehicle administrations (at the point of open arrow). Each point represents the mean systolic blood pressure with the standard error of the mean of a group of 6 hypertensive rats. Key; O——O represents group that received α-methyldopa alone; ●——● group with 'Aldomet' vehicle alone; ●--● group with disulfiram and 'Aldomet' vehicle; O--O group with disulfiram and α-methyldopa.

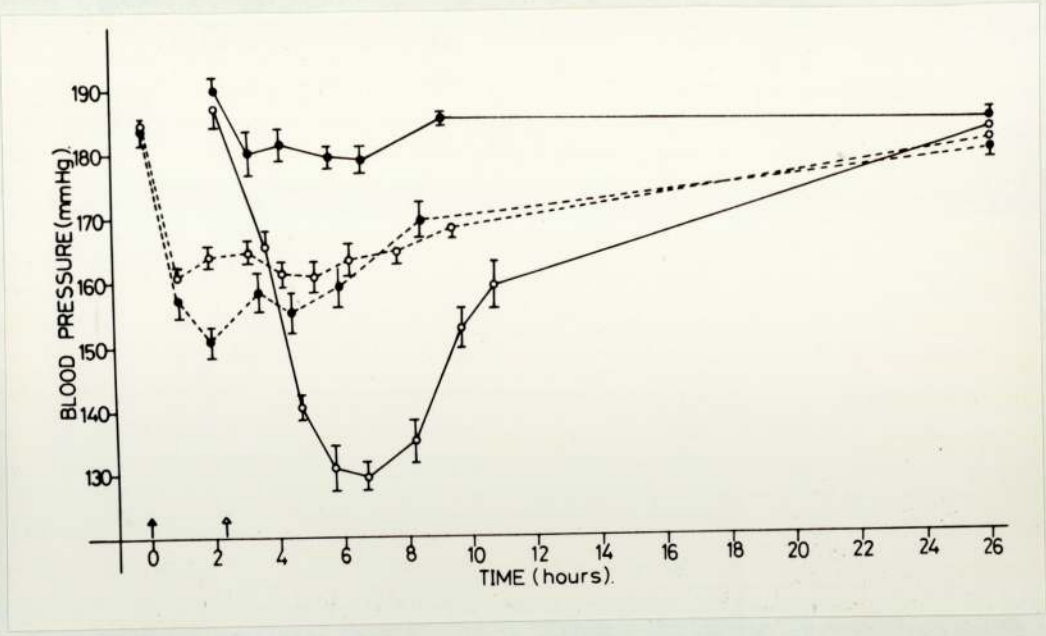


Fig. 14. Effect of simultaneous peripheral and central dopamine β-hydroxylase inhibition with sodium diethyldithiocarbamate (DDC, 100 mg/kg, i.p.) on the antihypertensive action of α-methyldopa (200 mg/kg, i.p.). DDC was injected at 0 hour (at the point of closed arrow), 2.25 hours prior to the α-methyldopa or 'Aldomet' vehicle administrations (at the point of open arrow). Each point represents the mean systolic blood pressure with the standard error of the mean of a group of 6 hypertensive rats. Key; O——O represents the group that received α-methyldopa alone; ●——● group with 'Aldomet' vehicle alone; ●- -● group with DDC and 'Aldomet' vehicle; O- -O group with DDC and α-methyldopa.

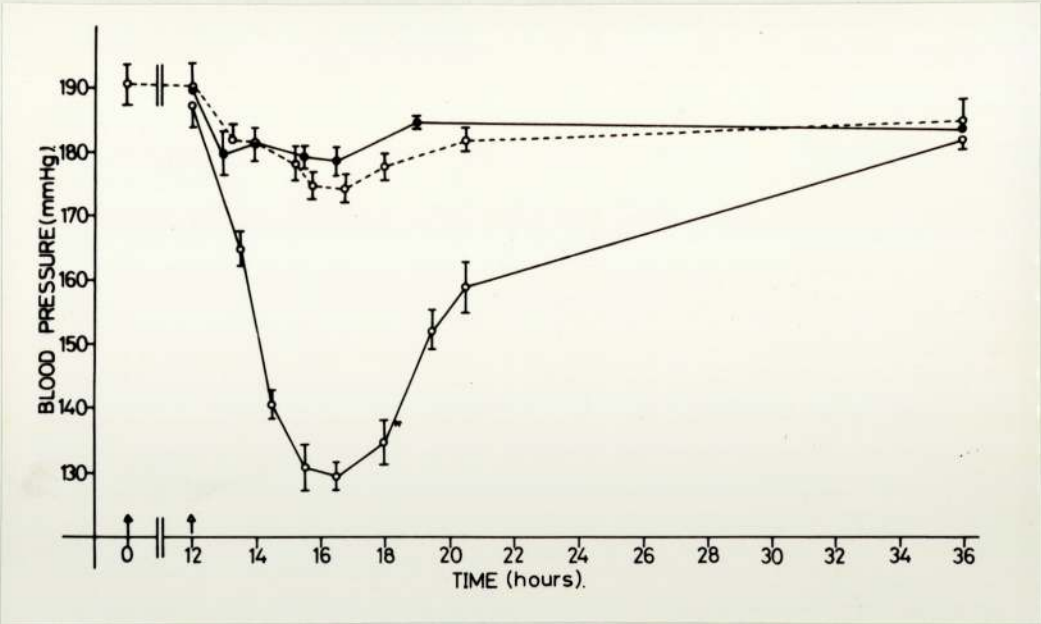


Fig. 15. Effect of preferential central dopamine β-hydroxylase inhibition with U-14,624 (200 mg/kg, i.p.) on the antihypertensive action of α-methyldopa (200 mg/kg, i.p.) U-14,624 was injected at 0 hour (at the point of closed arrow), 12 hours prior to the α-methyldopa or 'Aldomet' vehicle administrations (at the point of open arrow). Each point represents the mean systolic blood pressure with the standard error of the mean of a group of 6 hypertensive rats. Key; O——O represents the group that received α-methyldopa alone; ●—● group with 'Aldomet' vehicle alone; O--O group with U-14,624 and α-methyldopa.

CHAPTER 2. A study of the central action of α-methyldopa in hypertensive rats.

Before this study was started only 4 reports were found in the literature describing effects on blood pressure and heart rate obtained after administration of α-methyldopa into the central nervous system. Jaju et al.(1966) administered α-methyldopa (20 mg/kg) into the cerebral ventricles (icv) of dogs and observed a fall in blood pressure. They also reported that this dose of α-methyldopa was inactive when administered systemically.

Henning & van Zwieten (1968) and Kale & Satoskar (1970) infused α-methyldopa (20 mg/kg) into the vertebral arteries of anaesthetised cats. In both cases, they observed hypotensive effects after α-methyldopa. Henning & van Zwieten (1968) also observed that this dose of α-methyldopa administered intravenously had no effect on blood pressure. Ingenito et al. (1970) perfused α-methyldopa through the isolated cat brain which had reflex and tonic sympathetic control over the circulations of the body and an isolated perfused hind limb of the same cat. They observed a centrally mediated hypotension, bradycardia and decreased hind limb vascular resistance after brain perfusions of α-methyldopa in concentrations of 25 - 200 µg / ml of perfusing blood.

The results described in Chapter 1 indicate that α-methyldopa is able to mediate an antihypertensive action via a central mechanism. All the previous observations using

centrally administered α-methyldopa were obtained using anaesthetised normotensive animals. It was decided therefore to investigate the effects of centrally administered α-methyldopa in conscious hypertensive rats and test the effectiveness of the previously used enzyme inhibitors administered systemically.

Results.

1. Effect of systemically administered Ro4-4602 on the antihypertensive response to centrally administered α-methyldopa in hypertensive rats.

24 hypertensive rats were prepared for drug administrations into the left lateral cerebral ventricles as described in the methods. α-Methyldopa (0.5 mg, total icv dose) produced a fall in blood pressure of rapid onset. The α-methyldopa was administered in a volume of 10 µl slowly injected over a period of 10 seconds. The maximum antihypertensive effect was observed 1.5 hours after the icv injection. The mean systolic blood pressure of a group of 6 rats fell from 190 ± 3 to 148 ± 2 mm Hg (see Fig. 16).

A larger fall in blood pressure occurred after the icv dose of α-methyldopa was increased to 1.0 mg; this increase was obtained by doubling the injection volume to 20 µl. The peak effect occurred at 1.5 hours after the icv injection and the group mean systolic pressure fell from 191 ± 2 to 138 ± 3 mmHg (mean responses from 3 hypertensive rats).

The low dose level of α-methyldopa was used for this study as the large dose (1.0 mg) of the α-methyldopa appeared to be a toxic one. Half of the 6 hypertensive rats that received α-methyldopa (1.0 mg, icv) became super-sensitive to external stimuli and died. The 3 rats were subjected to a loud noise between 3 - 5 minutes after receiving the icv α-methyldopa. The rats immediately started to convulse and within 60 seconds the 3 rats had died in a position termed opisthotonus (Sayers, personal communication). In this state, the head was bent backwards, the spine was arched and the tail continued the line of the body in a circular manner. Under the same conditions a 20 µl icv injection of the 'Aldomet' vehicle failed to produce any toxic side effects. Thus, it was concluded that the convulsions were due to an excessive dose of α-methyldopa and not to the pressure changes produced within the brain from the 20 µl icv volume or to the relatively low pH of the 'Aldomet' solution (pH 4.2).

The blood pressure lowering effect observed after icv α-methyldopa (0.5 mg) was relatively transient compared to the prolonged antihypertensive response seen after 200 mg/kg, i.p., α-methyldopa (Fig. 16). The mean blood pressure of the group of rats treated with icv α-methyldopa had returned to pretreatment levels within 6 hours, which corresponded to the approximate time of the maximum hypotensive effect observed after α-methyldopa (200 mg/kg, i.p.). The 'Aldomet' vehicle produced no effects on blood pressure when injected icv in volumes of 10 µl and 20 µl (i.e.

equivalent volumes used for the 0.5 mg and 1.0 mg icv doses of α-methyldopa, respectively).

Fig. 16 demonstrates that treatment of hypertensive rats with the low dose regimen of Ro4-4602 (3 x 50 mg /kg, i.p.) reported to inhibit only peripheral dopa decarboxylase enzymes, was completely ineffective in altering the hypotension observed after α-methyldopa (0.5 mg) administered centrally. No significant difference ($p > 0.05$) was obtained between the hypotension observed at 1.5 hours produced by α-methyldopa 0.5 mg icv administered alone and α-methyldopa 0.5 mg icv administered in the presence of Ro4-4602 (3 x 50 mg/kg, i.p.)

In a parallel series of experiments, in which central and peripheral dopa decarboxylase enzymes were inhibited by the high dose regimen of Ro4-4602 (3 x 200 mg/ kg, i.p.), Ro4-4602 was observed to totally abolish the centrally mediated antihypertensive effect of α-methyldopa (Fig. 17). Results shown in Fig. 12 demonstrate a mutual antagonism between the antihypertensive effects of α-methyldopa and Ro4-4602. It can be seen from Fig. 17 that α-methyldopa administered centrally antagonised the blood pressure lowering effects normally observed after systemically administered Ro4-4602 (3 x 200 mg/kg, i.p.).

2. Effect of centrally administered Ro4-4602 on the antihypertensive response to systemically administered α-methyldopa in hypertensive rats.

3 doses of Ro4-4602 each of 0.05 mg were administered icv, in 10 µl volumes, at 2 hourly intervals

to a group of 6 hypertensive rats (i.e. the same pattern as used peripherally). The weights of the 6 rats ranged between 190 - 205 g; thus the central dose of Ro4-4602 was approximately 0.25 mg/kg.

Ro4-4602 administered icv in combination with 'Aldomet' vehicle given peripherally did not produce any significant effect in blood pressure. After substitution of the vehicle with α-methyldopa (200 mg/kg, i.p.), administered 30 minutes after the first icv injection of Ro4-4602, the antihypertensive effect of α-methyldopa was largely inhibited. Fig. 18 shows that α-methyldopa (200 mg/kg, i.p.) produced a small fall in blood pressure, in the group of rats that also received Ro4-4602 (3 x 0.05 mg, icv), at the time which corresponded exactly with the maximum fall observed after α-methyldopa given alone. At this time (4.5 hours), the blood pressure of the group that received icv Ro4-4602 in combination with α-methyldopa was significantly different ($p < 0.025$) from the mean pressure of the group that received icv Ro4-4602 and the 'Aldomet' vehicle given peripherally (Fig. 18).

DISCUSSION

On the introduction of α-methyldopa into the brain it produced a pronounced fall in blood pressure which was rapid in onset. The response observed after a single icv injection of α-methyldopa was transient. The onset and duration of the response to icv α-methyldopa were both much shorter than the responses observed after systemic administration. The differences in the responses is probably due to the different availabilities of α-methyldopa

after icv and i.p. administrations at centrally active sites. For a central mode of action α-methyldopa administered intraperitoneally must be absorbed into the blood and then cross the 'blood-brain' barrier to its sites of metabolism within the brain, thus increasing the time of the onset of its action. A large systemic dose of α-methyldopa would possibly act as a depot for the slow absorption of the substance into the brain accounting for a long duration of action.

If all of the hypotensive action of peripherally administered α-methyldopa was due to a central action, then the same degree of hypotension should be obtained with central α-methyldopa administration. The falls in systolic pressure seen after icv administrations of α-methyldopa (0.5 and 1.0 mg) were not as large as the falls in blood pressure observed after systemic dosage of α-methyldopa. Even at the 1.0 mg dose level of α-methyldopa given icv, which was observed to be an unsuitably high dose, the blood pressure was only reduced to approximately 140 mmHg compared to 130 mmHg after systemic administration.

The toxic effects observed after 1.0 mg α-methyldopa icv were almost certainly not due to an excessive amount of the metabolic products of α-methyldopa, namely α-methyldopamine and α-methylnoradrenaline, as the convulsions occurred within 5 minutes of the icv injections of α-methyldopa. As the equivalent icv volume of the 'Aldomet' vehicle (20 µl) did not produce any side effects, factors such as the volume of 20 µl itself, other constituents

of the 'Aldomet' solution and the pH of the 'Aldomet' solution (4.2) can be excluded from causing the super-sensitivity to external stimuli. Thus, it would seem that an excessively high concentration of α-methyldopa within certain brain areas probably caused the toxic effects.

A single icv injection may not be the most efficient route by which the greatest central effect on blood pressure is produced. The toxic effects were most likely produced by a large concentration of α-methyldopa occurring initially within certain brain regions that are relatively difficult to reach after systemic administration of α-methyldopa. Larger antihypertensive effects could possibly have been obtained after repeated smaller doses of α-methyldopa given centrally to mimic the prolonged depot effect of systemic administration; thus allowing sufficient α-methylnoradrenaline to be formed within the central adrenergic neurons at the active sites.

The low dose regimen of Ro4-4602 was ineffective in antagonising the centrally mediated antihypertensive effect of icv α-methyldopa suggesting that the 3 x 50 mg /kg doses of Ro4-4602 were not large enough to affect central dopa decarboxylase enzymes. However, from these experiments the degree of peripheral dopa decarboxylase inhibition achieved with this low dose regimen of Ro4-4602 is still not known.

The large dose regimen of Ro4-4602 (3 x 200 mg /kg, i.p.) completely abolished the central effects of α-methyldopa thus indicating that this dose regimen was

sufficient to fully inhibit central dopa decarboxylase enzymes.

The central administration of Ro4-4602 (3 x 0.05 mg, icv) appeared not to completely inhibit the fall in blood pressure due to α-methyldopa (200 mg/kg, i.p.). Since no biochemical studies were performed it is impossible to say whether this dose regimen of Ro4-4602 totally inhibited the central decarboxylase enzymes and the fall in pressure resulted from a peripheral action of α-methyldopa or that total inhibition of central enzymes was not fully obtained, thus allowing a small resultant central effect of α-methyldopa. Nijkamp et al. (1975) reported that the falls in blood pressure and heart rate normally seen after systemic α-methyldopa (400 mg/kg) were completely abolished by 3 doses of Ro4-4602 (0.15 mg/kg) administered icv at 2 hourly intervals to renal hypertensive rats (i.e. the same pattern of dosage as used in these experiments using DOCA/saline hypertensive rats).

In the previous chapter, it was observed that the large doses of Ro4-4602 (3 x 200 mg/kg, i.p.) produced a fall in pressure that was antagonised by α-methyldopa (Fig. 12). From Fig. 17, it can be seen that the anti-hypertensive effects normally observed after Ro4-4602 (3 x 200 mg/kg, i.p.) and α-methyldopa (0.5 mg, icv) administered alone, were both absent when given in combination. Thus, the fall in blood pressure observed after the large dose regimen of Ro4-4602 administered peripherally appears to be of central origin.

Since the low dose regimens of Ro4-4602 administered peripherally and centrally (see Figs. 11,16 and 18) did not produce antihypertensive effects when given in combination with the 'Aldomet' vehicle, the antihypertensive response seen with Ro4-4602 (3 x 200 mg/kg, i.p.) was probably due to an action within the central nervous system other than central dopa decarboxylase inhibition. Nijkamp et al. (1975) also reported that their dose regimens of Ro4-4602 used to block peripheral (4 x 50 mg/kg, i.p.) and central (3 x 0.15 mg/kg, icv) dopa decarboxylase did not themselves produce any significant effect on blood pressure and heart rate. It could be possible that with this high dose regimen of Ro4-4602 far more Ro4-4602 entered the brain than was needed for inhibition of central dopa decarboxylase enzymes and that this excessive amount of the inhibitor present in the brain was responsible for the antihypertensive effect.

Henning (1969a) observed that Ro4-4602 (3 x 200 mg/kg, i.p.) produced a significant antihypertensive effect when given alone to renal hypertensive rats. However, the peak effect was not observed until 12 hours after the first Ro4-4602 dose. The blood pressure was still lowered after 24 hours, and had returned to control levels by 48 hours. In the experiments of Henning (1969a), the average blood pressure of the group of rats that received Ro4-4602 and α-methyldopa in combination was slightly higher than the group that received Ro4-4602 alone, although the difference was not significant. The time course of the blood pressures of these groups was very similar. Hence, Henning (1969a) did

not observe a mutual antagonism between the antihypertensive effects of α-methyldopa and Ro4-4602.

It may be that for DOCA/saline uninephrectomised hypertensive rats, the build up of α-methyldopa within the central nervous system, as a result of central dopa decarboxylase inhibition, was able to antagonise the antihypertensive effect of Ro4-4602, which in turn was possibly due to an excessive amount of Ro4-4602 in the brains of these rats.

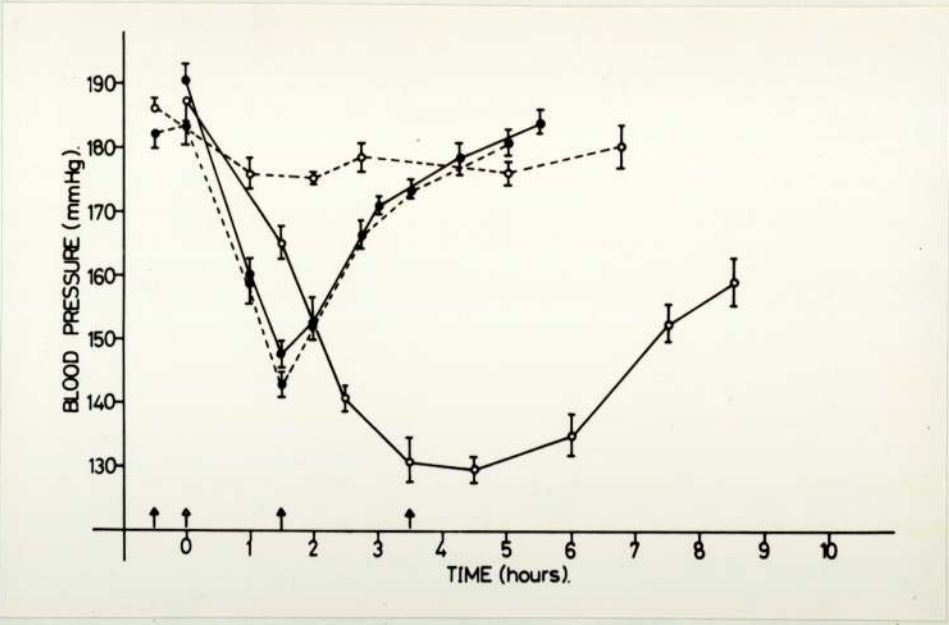


Fig. 16. Effect of peripheral dopa decarboxylase inhibition with Ro4-4602 (3 x 50 mg/kg, i.p.) on the antihypertensive action of centrally administered ∝-methyldopa (0.5 mg, total icv dose). ∝-Methyldopa or equivalent volumes of the 'Aldomet' vehicle were injected either peripherally or centrally at 0 hours (i.e. at point of open arrow). Ro4-4602 (50 mg/kg, i.p.) was injected on 3 occasions at 2 hourly intervals (i.e. at the points of closed arrows). Each point represents the mean systolic blood pressure with the standard error of the mean of a group of 6 hypertensive rats. Key; O——O represents group that received ∝-methyldopa (200 mg/kg, i.p.) alone; ●——● group with ∝-methyldopa (0.5 mg, icv) alone; ●--● group with Ro4-4602 and icv ∝-methyldopa; O--O group with Ro4-4602 and 'Aldomet' vehicle both given peripherally.

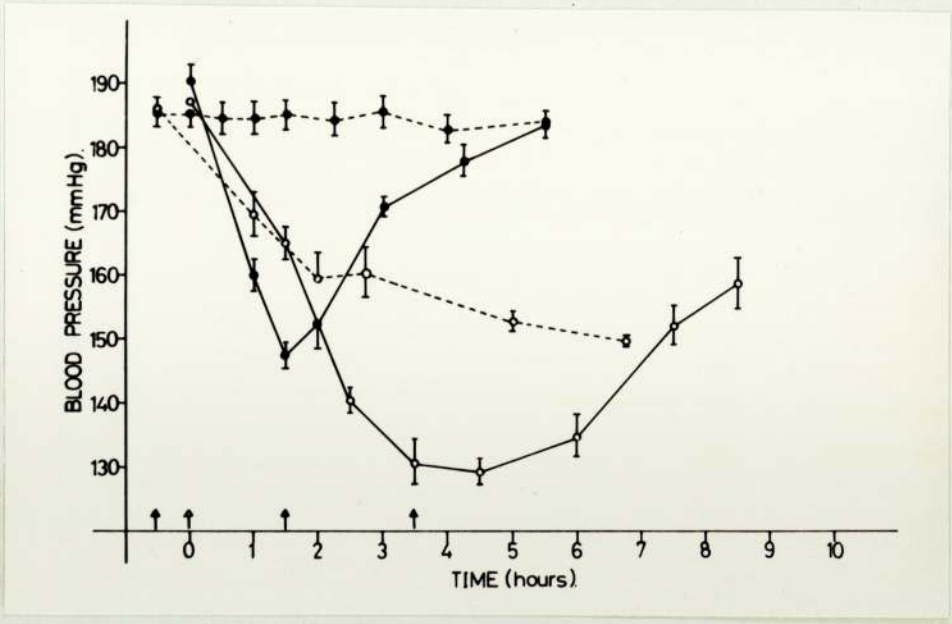


Fig. 17. Effect of simultaneous peripheral and central dopa decarboxylase inhibition with Ro4-4602 (3 x 200 mg /kg, i.p.) on the antihypertensive action of centrally administered α-methyldopa (0.5 mg, icv). The times of dose administrations and group numbers are as described in Fig. 16. Key; O——O represents group that received α-methyldopa (200 mg/kg, i.p.) alone; ●——● group with icv α-methyldopa alone; ●--● group with Ro4-4602 and icv α-methyldopa; O--O group with Ro4-4602 and 'Aldomet' vehicle both given peripherally.

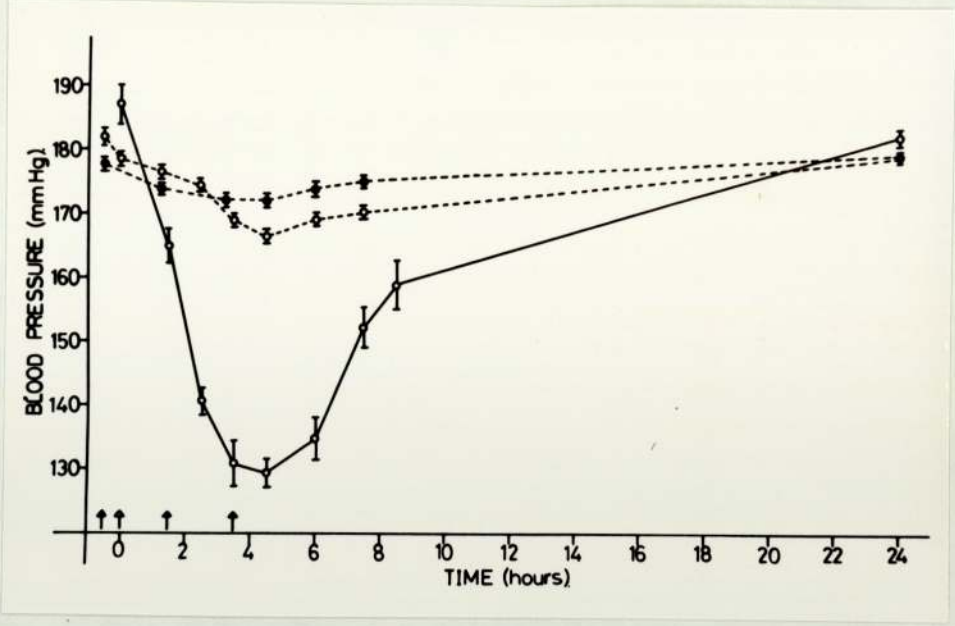


Fig. 18. Effect of central dopa decarboxylase inhibition with Ro4-4602 (3 x 0.05 mg total dose icv) on the antihypertensive action of peripherally administered ∝-methyldopa (200 mg/kg, i.p.). The times of dose administrations and group number are as described in Fig. 16. Key; O——O represents group that received ∝-methyldopa alone; O- -O group with icv Ro4-4602 and ∝-methyldopa; ●- -● group with icv Ro4-4602 and 'Aldomet' vehicle.

SECTION 2.

A study of responses following central administration of α-adrenoceptor agonists in the consious normotensive cat.

CHAPTER 1. The mechanism of action of α-methyldopa administered centrally in the cat.

Following the initial work involving the central administration of α-methyldopa in experimental animals (see Section 1, Chapter 2) two further groups of workers have investigated the central mode of action of α-methyldopa in cats.

Torchiana, and colleagues, (1973) inhibited the hypotension produced by icv α-methyldopa (60 mg total dose) by blockade of central dopa decarboxylase enzymes with Ro4-4602 and central dopamine β-hydroxylase with FLA-63 confirming that in cats, like rats, α-methyldopa must be metabolised to its β-hydroxylated amine, α-methylnoradrenaline to be active centrally. They also found that dopaminergic receptor blockade with spiroperidol did not modify the hypotension due to icv α-methyldopa thus confirming that the fall in pressure in the cat, like the rat, was not due to α-methyldopamine.

Heise & Kroneberg (1972, 1973) perfused α-methyldopa, α-methyldopamine and α-methylnoradrenaline (30 μg/min for 10 minutes for all compounds) into the fourth and parts of the third ventricles of anaesthetised cats. α-Methylnoradrenaline was found to have a more potent

depressor action than α-methyldopa and α-methyldopamine. The hypotensive response of α-methyldopamine and α-methylnoradrenaline were both significantly inhibited by pretreatment with central infusions of the α-adrenoceptor antagonists yohimbine and phentolamine.

Icv pretreatment with phentolamine inhibited the hypotensive effect of systemically administered α-methyldopa in conscious genetic hypertensive rats (Finch & Haeusler, 1973).

Results described in Section 1 and by Finch & Haeusler (1973) are the only cases in which α-methyldopa has been administered centrally in conscious hypertensive animals. All other results using central α-methyldopa, either in the dog or cat, have been obtained using normotensive anaesthetised animals. Experiments are described in this chapter demonstrating the effects of α-methyldopa in the conscious cat, thus eliminating the factor of anaesthesia.

Results

1. Effect of peripherally and centrally administered α-methyldopa on blood pressure and heart rate of conscious cats.

Experiments were performed using 4 normotensive conscious unrestrained cats (weight 2 - 3 kg). The normal resting blood pressure and heart rate of the 4 cats ranged from $^{135}/_{95}$ to $^{119}/_{83}$ mmHg and 155 to 130 beats/min. Each dose level of α-methyldopa (see Table 1) was administered to each cat on two occasions. The mean control responses

are shown in Table 1. After each experiment involving either peripheral or central α-methyldopa administration the cat was not used again for at least three days.

α-Methyldopa (200mg/kg) was administered orally to 4 normotensive cats. However, falls in blood pressure and heart rate were observed in only 3 of these cats. The mean depressor effect and bradycardia were calculated from the results obtained in the 3 cats. Orally administered α-methyldopa (200 mg/kg) produced a mean reduction of the systolic and diastolic pressures of 15.4 $\pm$ 3.4 and 14.8 $\pm$ 3.2 mmHg respectively. The mean bradycardia produced was 19.5 $\pm$ 3.5 beats/minute. The maximum hypotensive effect, observed after orally administered α-methyldopa occurred between 4 and 6 hours after the systemic administration. The bradycardia always paralleled the time course of the hypotension seen after α-methyldopa. In each case the blood pressure and heart rate had returned to pretreatment levels within 24 hours.

Hypotension and bradycardias were observed in all 4 cats after icv administration of α-methyldopa (0.5 - 1.25 mg, total icv dose). The time of the maximum cardiovascular depressant activity after icv α-methyldopa was observed between 45 minutes and 2 hours after the icv infusion. Pretreatment resting levels of blood pressure and heart rate were invariably reached by 7 to 8 hours after the icv dosage of α-methyldopa. The maximum effects seen after 1.25 mg α-methyldopa icv appeared slightly quicker and usually persisted for a longer time than the effects observed after the 0.5 mg icv dose. For the mean

reductions in blood pressure and heart rate seen after icv α-methyldopa see Table 1.

All icv infusions of α-methyldopa were made in 100 µl volumes. The correct α-methyldopa concentration in the infusion solution was obtained by dilution of the original 'Aldomet' solution (containing 50 mg/ ml of α-methyldopa) with the sterile 'Aldomet' injection vehicle.

The blood pressure and heart rate were unaffected in each of the 4 cats, by an icv infusion of 100 µl of 'Aldomet' vehicle solution.

2. Effect of icv phentolamine pretreatment on the cardiovascular responses of either peripherally or centrally administered α-methyldopa.

Icv phentolamine 0.5 mg and 0.25 mg respectively administered 30 minutes before and 1.5 hours after α-methyldopa (200 mg/kg, orally) completely inhibited the hypotension and bradycardia observed in the 3 cats after orally administered α-methyldopa. In 2 cats icv infusions of phentolamine produced slight increases in the resting blood pressure and heart rate. In the remaining cat, icv phentolamine produced no effect on blood pressure and heart rate.

Phentolamine (0.6 mg, icv) administered 30 minutes before α-methyldopa (0.5 mg, icv) completely abolished the blood pressure and heart rate lowering effects of α-methyldopa in 4 cats. The centrally mediated cardiovascular depressant effects observed after 0.75, 1.0

and 1.25 mg α-methyldopa icv were fully inhibited by icv phentolamine (0.75 mg, icv) given 30 minutes before the α-methyldopa.

3. Effect of intravenously administered phentolamine on the cardiovascular responses to either peripherally or centrally administered α-methyldopa.

I.v. phentolamine 2.0 mg/kg and 1.0 mg/kg respectively administered 30 minutes before and 1.5 hours after α-methyldopa (200 mg/kg, orally) inhibited the hypotension and bradycardia due to oral α-methyldopa in 3 cats. Iv. phentolamine produced an initial tachycardia of approximately 25 beats/minute, which was of short duration in all 3 cats and was associated with a fall in mean blood pressure of approximately 10 mmHg. The fall in blood pressure was prolonged and was still observed after the heart rate had returned to normal levels.

Phentolamine (2.0 mg/kg, iv.) administered 30 minutes before α-methyldopa (1.25 mg, icv) abolished the hypotension and bradycardia due to the central administration of α-methyldopa. (For this series of experiments only the large icv dose of α-methyldopa was used).

DISCUSSION

Finch & Haeusler (1973) abolished the anti-hypertensive effect produced after systemic administration of α-methyldopa with icv pretreatment with phentolamine in the conscious hypertensive rat. Similar results have been obtained using the conscious cat. α-Methyldopa

administered orally produced hypotension and bradycardia which were inhibited by either peripheral or central pretreatment with the α-adrenoceptor antagonist phentolamine. The systemic dose of α-methyldopa used in a 2.5 kg cat was one thousand times the dose needed centrally to produce similar depressant effects on blood pressure and heart rate. This provides a strong indication that α-methyldopa produces much of its hypotensive effect via a central mechanism in the cat.

Heise & Kroneberg (1972,1973) inhibited the depressant effects of icv α-methyldopamine and α-methylnoradrenaline with central phentolamine pretreatment. These workers did not carry out phentolamine pretreatment experiments on the effects of α-methyldopa. They found that α-methyldopa produced large variations in its depressant effects. This is in contrast to the responses described in this Chapter. α-Methyldopa icv produced reliable dose-related falls in blood pressure and heart rate within the 4 cats tested. Heise & Kroneberg used chloralose anaesthetised cats pretreated with atropine. Hence the use of chloralose anaesthesia may account for the variability of results obtained after icv α-methyldopa.

The falls in blood pressure, although somewhat variable, obtained by Heise & Kroneberg were larger than the responses obtained in the conscious cat. They perfused a total dose of 0.3 mg of α-methyldopa directly to the area of the hind brain via the third and fourth ventricles. By using dye studies (see methods) it was shown that the

100 µl volume infused into the lateral ventricle used in the experiments of this thesis, was sufficient to reach the fourth ventricle of the cat brain. If the site of action of α-methyldopa surrounds the third and/or fourth ventricle , in order to produce similar effects, larger doses of α-methyldopa would be needed with administrations into the lateral ventricles.

Phentolamine administered intravenously produced a short-lived tachycardia with a more prolonged hypotension. The fall in blood pressure produced by a direct action of phentolamine causing peripheral vasodilatation could have masked the centrally mediated hypotension of α-methyldopa. However, as the heart rate was not lowered by i.v. phentolamine the bradycardia should still have been present unless sufficient phentolamine was able to penetrate into the brain and abolish the effects of α-methyldopa within the central nervous system. (See also the effects of i.v. phentolamine on the central effects of clonidine, Section 2, Chapter 4).

Torchiana et al. (1973) by using enzyme inhibitors, demonstrated that α-methylnoradrenaline must be formed from α-methyldopa in the cat in order to manifest a centrally mediated hypotension. Thus, in both the rat and cat α-methyldopa is able to mediate a fall in blood pressure and heart rate via the central formation of α-methylnoradrenaline which in turn stimulates central receptors. These receptors are inhibited by compounds known to inhibit peripheral α-adrenoceptors, e.g. phentolamine and yohimbine.

Kale & Satoskar (1970) prevented the onset of the hypotension, observed after vertebral artery infusion of α-methyldopa, by pretreatment with either imipramine or reserpine. They suggested that reserpine blocked the hypotensive effect of α-methyldopa by preventing the conversion of α-methyldopamine to α-methylnoradrenaline within the granules of central adrenergic neurons or by inhibiting the granular uptake of these substances. Imipramine probably inhibited the hypotensive action of α-methyldopa by either preventing the uptake of α-methyldopa into central neurons or by an α-adrenoceptor blocking action or by a combination of these effects.

DOSE OF α-methyldopa	Route of administration	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s.e.m.		DECREASE IN HEART RATE beats/min ± s.e.m.	No. of cats used.	TOTAL No. of observations.
		SYSTOLIC	DIASTOLIC			
200 mg/kg	oral	15.4 ± 3.4	14.8 ± 3.2	19.5 ± 3.5	3	6
0.5 mg	icv	12.7 ± 3.6	12.5 ± 3.7	17.5 ± 3.1	4	8
0.75 mg	icv	14.3 ± 2.9	15.4 ± 3.0	23.2 ± 4.6	4	8
1.0 mg	icv	20.6 ± 4.1	20.1 ± 3.8	25.6 ± 4.3	4	8
1.25 mg	icv	22.2 ± 3.0	21.9 ± 3.1	31.8 ± 3.5	4	8

TABLE 1. Maximal arterial blood pressure and heart rate reductions (mean ± standard error of mean) by α-methyldopa administered peripherally and centrally into normotensive conscious unrestrained cats.

CHAPTER 2. Comparison of the centrally mediated cardiovascular effects of icv administered noradrenaline and α-methylnoradrenaline in the cat.

The major criticism of the 'false transmitter' hypothesis suggested by Day & Rand (1963a), to explain the antihypertensive action of α-methyldopa, has centred around the relatively small differences between the α-adrenoceptor stimulant potency of noradrenaline and α-methylnoradrenaline in many animals and isolated tissues (see Historical Introduction for references). However, recent work has now pointed to a central mode of action of α-methyldopa; its antihypertensive effect being mediated by the central production of α-methylnoradrenaline.

Hypotension commonly occurs as a side effect in the treatment of Parkinsonism with l-dopa (Calne, Stern, Laurance, Sharkey & Armitage, 1969; Cotzia, Papavasiliou & Gellene, 1969; Yahr, Duvoisin, Schear, Barrett & Hoen, 1969). McCubbin, Kaneko & Page (1960) first demonstrated that l-dopa was able to produce a centrally mediated hypotension after icv administration into dogs. Several investigators have demonstrated that the hypotension due to l-dopa is centrally mediated in rats (Henning & Rubenson, 1970; Yamori, De Jong, Yamabe, Lovenberg & Sjoerdsma, 1972; Baum & Shropshire, 1973), dogs (Robson, 1971; Kaplan, Barker & La Sala, 1972; Schmitt, Schmitt & Fénard, 1972, 1973a) and cats (Schmitt et al.1972,1973a; Watanabe, Judy & Cardon, 1974).

Henning & Rubenson (1970) found that the hypotension due to l-dopa in the rat, like that of α-methyldopa, was abolished by prior central dopa decarboxylase and dopamine β-hydroxylase inhibition. They found that the hypotension due to l-dopa was unaffected by the pretreatment with the dopamine receptor blocking agent spiroperidol, thus indicating that noradrenaline and not dopamine is the important central mediator of the response to l-dopa.

Peripheral administration of l-dopa in the presence of a peripheral dopa decarboxylase inhibitor (MK485) and a monoamine oxidase inhibitor produced a highly significant inverse correlation between the increase brain stem noradrenaline content and the resulting reduction in blood pressure (Yamori et al., 1972).

Henning, Rubenson & Trolin (1972) localised the action of l-dopa within the rat brain to the lower brain stem. Schmitt, Schmitt & Fénard (1973a) suggested that the hypotension produced by l-dopa in the cat and dog was due to an action within the medulla oblongata and/or the spinal cord.

There are a large number of reports in the literature describing the centrally mediated cardiovascular effects after central administrations of noradrenaline in various animals. The results are variable producing either pressor or depressor effects. The variation in the responses to centrally administered noradrenaline can be accounted for by the responses produced being the resultant effects of

stimulation of different brain areas within different animal species (see Historical Introduction for references).

Heise & Kroneberg (1973) found that α-methylnoradrenaline produced slightly larger falls in blood pressure than those observed with noradrenaline after perfusion of both compounds through the third and fourth ventricles of anaesthetised cats although the difference was not significant.

Results are described in this chapter comparing the size and duration of responses obtained after icv noradrenaline and α-methylnoradrenaline in conscious normotensive cats.

Results.

1. Blood pressure and heart rate responses to icv noradrenaline and α-methylnoradrenaline.

Noradrenaline (15,20 and 30 µg) and α-methylnoradrenaline (15,30 and 40 µg) infused into the lateral ventricles of conscious cats produced dose-related falls in heart rate and systolic and diastolic blood pressures. The mean reductions in blood pressures and heart rate due to these two compounds given icv are presented in Table 2. The hypotensions and bradycardias produced by noradrenaline and its α-methylated analogue can be compared at the 15 and 30 µg dose levels.

Noradrenaline 15 µg icv produced a larger hypotension than did the same dose of α-methylnoradrenaline icv. The reductions in the systolic blood pressure after 30 µg icv

noradrenaline and α-methylnoradrenaline were almost identical, as were the bradycardias. However, α-methylnoradrenaline was observed to lower the diastolic pressure to a greater extent than noradrenaline. The mean decrease in the diastolic pressure after icv noradrenaline 30 µg was 19.1 ± 2.0 mmHg compared to 25.6 ± 2.8 mmHg observed after icv α-methylnoradrenaline 30 µg icv (see Table 2).

Fig. 19 demonstrates an experiment whereby the blood pressure and heart rate effects of icv noradrenaline and α-methylnoradrenaline (30 µg) are compared. Record A shows that noradrenaline (30 µg, icv) produced reductions in blood pressure and heart rate of $^{20}/_{25}$ mmHg and 50 beats/minute. α-Methylnoradrenaline (30µg, icv), in the same cat, produced a hypotension of $^{27}/_{32}$ mmHg and a bradycardia of 50 beats/minute (Record B). Thus, in this cat, icv α-methylnoradrenaline produced a larger hypotension than icv noradrenaline but both compounds produced the same degree of bradycardia. The blood pressure changes produced by the 30 µg icv doses of noradrenaline and α-methylnoradrenaline were not unduly different from the mean values presented in Table 2. However, both compounds, in this cat, produced very large bradycardias. This was probably due to the fact that the resting heart rate of this particular cat was relatively high (approximately 175 beats/minute.)

It can be seen from Fig. 19 that the cardiovascular effects produced by icv α-methylnoradrenaline were much more prolonged than those observed for icv noradrenaline.

Blood pressure and heart rate effects were usually completed within 30 minutes of the icv infusion of noradrenaline and 45 to 75 minutes after icv α-methylnoradrenaline. If one measured the total degree of hypotension, i.e. the area above the response, then α-methylnoradrenaline appears more potent dose for dose than noradrenaline.

Metaraminol (30 µg) was administered centrally to one cat that responded to icv noradrenaline and α-methylnoradrenaline by producing hypotension and bradycardia. Fig. 19, record 3, shows that icv metaraminol did not alter the heart rate and produced a very small rise in blood pressure.

Gagnon & Melville (1966) reported that low doses of icv noradrenaline (0.01 and 0.1 µg) produced hypertension and tachycardia, whilst larger doses of icv noradrenaline (50 µg) induced hypotension and bradycardia in anaesthetised cats. Noradrenaline and α-methylnoradrenaline infused icv in doses of 0.01 and 0.1 µg did not produce any effect on blood pressure and heart rate in each of 5 conscious normotensive cats that normally responded to 30 µg of these compounds with hypotension and bradycardia.

In all cats used in these experiments and those described in further sections, icv infusions of 100 µl of sterile sodium chloride solution (0.9% $^{w}/_{v}$) and sterile water for injections failed to produce any blood pressure or heart rate effects.

2. Effect of icv phentolamine on the cardiovascular effects of icv noradrenaline and α-methylnoradrenaline.

The hypotension and bradycardia produced by icv noradrenaline and α-methylnoradrenaline (15 and 30 μg) were completely abolished by icv phentolamine (0.5 - 0.75 mg) administered 30 to 60 minutes before the agonists. Fig. 20 shows a typical experiment in which the hypotension and bradycardia induced by icv noradrenaline (15 μg) were inhibited by pretreatment with icv phentolamine (0.6 mg). In all but 2 of the cats used, icv phentolamine caused rises in blood pressure and heart rate (as seen in Fig. 20).

In 2 cats, the blood pressure and heart rate fell after icv phentolamine (0.5 - 0.75 mg). In each cat the diastolic blood pressure and heart rate did not fall by more than 15 mmHg and 20 beats/minute respectively. The control hypotensive and bradycardic effects due to icv noradrenaline and α-methylnoradrenaline in these 2 cats were absent after icv phentolamine pretreatment. It is unlikely that the depressant effects of icv phentolamine masked the falls due to the agonists as the hypotension and bradycardias due to the agonists were larger than those observed for phentolamine and at the time of the peak effect of the phentolamine (after approximately 45 minutes) the pressure and heart rate could still have been lowered further.

Phentolamine, after icv administration, was confined to the central nervous system. Responses to intravenous noradrenaline (200 - 400 ng/kg) remained unaltered when given at the time of the maximum inhibition of the icv responses to the α-adrenoceptor agonists. This is demonstrated in Fig. 21 in which the responses to noradrenaline 200 ng/kg, iv) were unaffected by phentolamine (0.6mg, icv).

3. Effect of central β-adrenoceptor blockade on the cardiovascular effects of icv noradrenaline and α-methylnoradrenaline.

Responses to noradrenaline and α-methylnoradrenaline (30 µg, icv) were unaffected by prior icv infusions of the β-adrenoceptor blocking drugs dl-propranolol and dl-alprenolol (0.5 - 1.0 mg). These experiments were performed in 2 cats that responded consistently with depressor effects to icv noradrenaline and α-methylnoradrenaline.

The effect of icv dl-propranolol on the central responses to noradrenaline and α-methylnoradrenaline was tested in one cat. In the other the effects of icv dl-alprenolol were tested. Doses of dl-propranolol and dl-alprenolol were used that were previously found to fully inhibit the blood pressure and heart rate effects of icv isoprenaline (30 µg) in each particular cat.

A control response to noradrenaline (30 µg, icv) produced falls in the diastolic blood pressure and heart rate of 21 mmHg and 25 beats/minute respectively. 45 minutes after dl-propranolol (0.6 mg, icv), the diastolic pressure and heart rate had fallen from 84 mmHg and 155 beats/minute to 66 mmHg and 130 beats/minute. At this time, icv administration of noradrenaline (30 µg) further reduced the diastolic pressure and heart rate to 52 mmHg and 105 beats/minute. 2 days later, a similar pattern of responses was obtained with α-methylnoradrenaline infused centrally after icv pretreatment with 0.6 mg dl-propranolol.

Similarly, α-methylnoradrenaline (30 µg, icv) further reduced the blood pressure and heart rate when given 45 minutes after icv dl-propranolol.

In the second normotensive cat, both icv noradrenaline and α-methylnoradrenaline (30 µg) further reduced the blood pressure and heart rate when infused 45 - 60 minutes after icv dl-alprenolol (0.75 mg). Similar traces were obtained in these experiments to those shown in Fig. 55 which demonstrates the central action of clonidine in the presence of icv dl-propranolol. The significance of these results is discussed in Section 4 of the Results.

4. Pressor effects obtained after icv noradrenaline in conscious cats.

Noradrenaline (15 - 30 µg) administered centrally into 12 cats produced hypotension and bradycardia. However, in a further 3 cats newly cannulated for icv drug administrations, noradrenaline in the same icv dosage produced a rise in blood pressure and bradycardia. A typical response is shown in Fig. 22. The pressor response and bradycardia observed after icv noradrenaline (30 µg) were totally abolished by phentolamine pretreatment (0.6 mg, icv). The abolition of the responses to icv noradrenaline by icv phentolamine is shown in Fig. 22. Although the centrally administered α-adrenoceptor blocking agent abolished the pressor effects of icv noradrenaline, phentolamine itself (0.6 mg, icv) produced hypertension with a tachycardia. In these 3 cats, icv phentolamine produced

pressor effects and tachycardias.

The inhibition of the pressor effects of icv noradrenaline in these cats by icv phentolamine indicates that the response to icv noradrenaline is centrally mediated. These results are in contrast to those described in Section 6 of the results which demonstrate that in some cats the pressor effects of icv noradrenaline are due to the leakage of the agonist from the brain to the periphery.

Pretreatment with dl-propranolol (0.75 mg, icv) in each cat, did not affect the hypertension and bradycardia observed after icv noradrenaline (30 µg) when administered 60 minutes after the dl-propranolol. At the time of the icv noradrenaline administration it was observed that the icv propranolol had reduced the blood pressure and heart rate below normal resting levels.

The pressor effect to icv noradrenaline was examined in the presence of peripheral ganglion blockade produced by pempidine (7.5 mg/kg, i.v.). Noradrenaline was infused icv in each of the 3 cats approximately 30 - 60 minutes after the intravenous injection of pempidine. The degree of ganglionic blockade was determined by assessing the reduction in the pressor response to tetramethylammonium (25 - 100 µg / kg , i.v.).

The pressor effect and bradycardia normally observed after icv noradrenaline was inhibited by pretreatment with i.v. pempidine. Hence, it appears that the pressor response observed after icv noradrenaline in these

3 cats was not due to leakage of the amine into the periphery.

It should also be noted that icv α-methylnoradrenaline produced similar types of responses as did noradrenaline in these 3 cats but, as in the case of the depressor effects, α-methylnoradrenaline produced longer lasting pressor effects than noradrenaline.

DISCUSSION

Heise & Kroneberg (1973) reported that equal doses of noradrenaline and α-methylnoradrenaline administered centrally to anaesthetised cats produced similar blood pressure lowering effects. The results presented in this chapter, from experiments involving conscious normotensive cats, also demonstrate that icv administrations of noradrenaline and α-methylnoradrenaline produced similar falls in blood pressure and heart rate. Thus, it would seem that the centrally mediated cardiovascular depressant effects of α-methyldopa are not brought about by the centrally formed α-methylnoradrenaline acting as a weaker 'false transmitter' but as an equipotent or slightly more potent transmitter than noradrenaline. In the conscious cat, it was observed that icv α-methylnoradrenaline possessed a longer duration of action than noradrenaline. Very recent reports by de Jong et al. (1975) and Struyker Boudier et al. (1975) using anaesthetised rats have confirmed these results in conscious cats. de Jong et al. (1975) observed that α-methylnoradrenaline applied directly onto the nucleus tractus solitarius in the

medulla oblongata of anaesthetised rats produced more potent falls in blood pressure and heart rate than noradrenaline. Struyker Boudier et al. (1975) observed that α-methylnoradrenaline applied to either the nucleus tractus solitarius or the anterior hypothalamus/preoptic area in anaesthetised rats produced much longer lasting falls in blood pressure and heart rate than noradrenaline. α-Methylnoradrenaline is not metabolised by monoamine oxidase enzymes (Blaschko, Richter & Schlossmann, 1937) and this property probably accounts for the prolonged central action of both α-methylnoradrenaline and α-methyldopa.

If this is so then the hypotensive effects produced after treatment with monoamine oxidase inhibitors may be due to a prolonged action of the partly protected noradrenaline within the brain. However, the central mode of action of monoamine oxidase inhibitors was not investigated in the conscious cat because most inhibitors combine irreversibly with monoamine oxidase enzymes. For example, after pargyline full regeneration of monoamine oxidase enzymes occurs after approximately 3 weeks (S. Baker, personal communication).

The depressor effects of icv noradrenaline and α-methylnoradrenaline (30 μg) obtained in these experiments using conscious cats, are of a similar magnitude to the responses observed by Heise & Kroneberg (1973) who perfused a total dose of 300 μg of each of the amines through the third and fourth ventricles of chloralose anaesthetised cats. Thus, it appears that chloralose anaesthesia may cause some degree of insensitivity to the central administration of the two catecholamines.

The hypotension and bradycardia produced by icv noradrenaline and α-methylnoradrenaline were abolished after icv pretreatment with an α-adrenoceptor antagonist. In the conscious cat complete inhibition was obtained with icv phentolamine (0.5 - 0.75 mg). Heise & Kroneberg (1973) reported a reduction in the responses to noradrenaline and α-methylnoradrenaline (300 µg) after central phentolamine (0.9 mg) or almost complete inhibition after central yohimbine (1.14 mg) pretreatment. They found that these doses of phentolamine and yohimbine given centrally did not themselves produce any effects on blood pressure.

Phentolamine administered centrally has produced variable effects on blood pressure and heart rate which appear to be dependant upon the central route of administration and animal species used. Phentolamine administered into the right lateral ventricle (Kleinrok et al.,1972) and cisterna magna (Ito & Schanberg, 1974) of anaesthetised rats and in cross-perfused isolated head experiments in dogs (Hilliard, Bagwell & Daniell, 1972) produced hypotension and bradycardia. Finch (1974) reported that phentolamine (100-200 µg) administered into the lateral ventricles of conscious renal hypertensive cats did not produce any significant changes in the resting blood pressure or heart rate. However, Finch has occasionally observed pressor effects and tachycardias after icv phentolamine in conscious cats (Finch, personal communication). In the conscious normotensive cat, phentolamine administered into the left lateral ventricle produced pressor effects and tachycardias in the majority of occasions. Only 2 cats

responded to icv phentolamine with cardiovascular depressant effects.

Both rises and falls in blood pressure have been recorded after centrally administered noradrenaline (see Historical Introduction for references) and thus variable cardiovascular effects to centrally administered phentolamine and other α-adrenoceptor antagonists might be expected. It should be noted that those workers who obtained depressant effects to central phentolamine in anaesthetised rats also reported pressor effects after central noradrenaline. In anaesthetised dogs most workers have observed hypotension and bradycardia after centrally administered noradrenaline (Kaneko et al., 1960; McCubbin et al., 1960; Schmitt & Fénard, 1971; Bhargava et al., 1972). However, using cross-circulation experiments in dogs, Tachi (1962) produced centrally mediated pressor effects to noradrenaline which may explain why Hilliard et al. (1972) observed hypotension after phentolamine in their cross-perfused isolated dog head experiments.

Baum & Shropshire (1973) demonstrated that the hypotension and bradycardia observed after icv noradrenaline in the rat was associated with a reduced sympathetic outflow from the brain. This has also been demonstrated for the α-adrenoceptor agonist clonidine (see Historical Introduction for references). Thus, it seems likely that the hypertension and tachycardia observed after icv phentolamine in the majority of conscious normotensive cats are due to a reduction of central α-adrenoceptor activity resulting in

an increased sympathetic outflow from the brain.

The cardiovascular depressant effects observed after phentolamine given icv to 2 cats may have been due to a predominant inhibition of central pressor pathways (although it should be remembered that in these 2 cats the hypotensive effect and bradycardia due to icv noradrenaline were fully inhibited by icv phentolamine). A slight agonist action or a direct action of phentolamine on depressant pathways may have also contributed to the falls in blood pressure and heart rate seen with icv phentolamine in these cats.

In early experiments in this series, 2 cats died after receiving icv phentolamine (1.0 mg). This dose of icv phentolamine induced rises in blood pressure and heart rate in both cats and after 60 minutes the depressant responses to icv noradrenaline were inhibited. Approximately 3 hours after the end of the experiments both cats appeared to be very sedated. 12 hours after completion of the experiments both cats were found dead. The cats were relatively newly cannulated and were both healthy at the time of the experiments. On examination of the dead cats, no pathological abnormalities were found. In these laboratories, Cooling has observed similar occurrences in conscious cats receiving icv phentolamine in doses of and greater than 1.0 mg. After these observations, the maximum dose of phentolamine administered during an experiment was 0.75 mg; this icv dosage appeared to have no ill-effects and was sufficient to inhibit the central effects of α

-adrenoceptor agonists.

Gagnon & Melville (1966) reported that very small doses of noradrenaline (0.01 and 0.1 μg, icv) induced pressor effects with associated tachycardias, whilst larger doses (50 μg) produced cardiovascular depression in the same anaesthetised cats. These observations involving the small doses could not be repeated in the present experiments using conscious cats. These low doses of noradrenaline icv failed to produce any change in blood pressure and heart rate.

However, in 3 newly cannulated cats noradrenaline (15 - 30 μg, icv) produced a hypertension associated with a bradycardia. These responses were apparently centrally mediated as they were blocked by a low dose of phentolamine (0.6 mg) given centrally and also by peripheral ganglion blockade using pempidine (7.5 mg/kg, i.v.). Gagnon & Melville (1966) abolished the pressor effect and tachycardia observed after icv administrations of the low doses of noradrenaline by central pretreatment with a β-adrenoceptor blocking agent. Unlike these results, the pressor effect obtained in the conscious cat after icv noradrenaline (15 - 30 μg) was unaffected by prior central β-adrenoceptor blockade (produced by dl-propranolol, 0.75 mg, icv).

These three cats were cannulated by exactly the same procedure as the other 12 cats used in this series of experiments, which responded to icv noradrenaline with hypotension and bradycardia. Hence, it would appear that noradrenaline causes preferential stimulation of

α-adrenoceptors within certain brain areas responsible for mediating pressor effects. The preferential stimulation of pressor pathways may result from a variation in the diffusion of noradrenaline from the cereboventricular spaces in these 3 cats, from the usual spread and diffusion of the amine produced in the majority of cats.

It is possible that the noradrenaline induced stimulation of certain pressor pathways within the brain results in an increased sympathetic outflow to the vasculature, thus producing vasoconstriction and hypertension. In these experiments, at no time did the icv administration of noradrenaline and other α-adrenoceptor agonists (see later) produce any effect on heart rate other than bradycardia. Thus, the bradycardia may result from the actual stimulation of the central pathway of the baroreceptor reflex by noradrenaline (as suggested for clonidine by Haeusler, 1973b, 1974b) and from the normal increase of the afferent part of the baroreceptor arc as occurs in response to a hypertensive effect. The hypertension observed after icv noradrenaline was not due to leakage from the brain.

α-Methylmetatyrosine has been shown to be an active antihypertensive agent in man (Horwitz & Sjoerdsma, 1964; Holtmeier, Kein-Wisenberg & Marongiu, 1966). When α-methylmetatyrosine is metabolised in vivo by dopa

decarboxylase and dopamine β-hydroxylase enzymes the corresponding 'false transmitter' formed is metaraminol. Metaraminol, although normally regarded as a weak pressor substance compared to noradrenaline (Innes & Nickerson, 1970), also possesses antihypertensive properties in man after prolonged oral administration (Crout, Johnston, Webb & Shore, 1965). A comparison was made between the central actions of equal doses of metaraminol, α-methylnoradrenaline and noradrenaline in one cat (Fig. 19). It can be seen that metaraminol possessed no effect on blood pressure and heart rate after icv administration in the same doses as noradrenaline and α-methylnoradrenaline. This further substantiates the suggestion that the centrally mediated reductions in blood pressure and heart rate produced after α-methyldopa are not a result of production of a weaker false transmitter but more likely due to production of an equipotent or more potent false transmitter.

Metaraminol is one of the most effective compounds known to displace and replace noradrenaline from the stores in adrenergic neurons (Iversen, 1967). Thus, if metaraminol releases noradrenaline from central noradrenergic neurons then cardiovascular responses would be expected. However, since no effects were observed, it would appear that the icv dose used (30 μg) was insufficient to release significant quantities of endogenous noradrenaline.

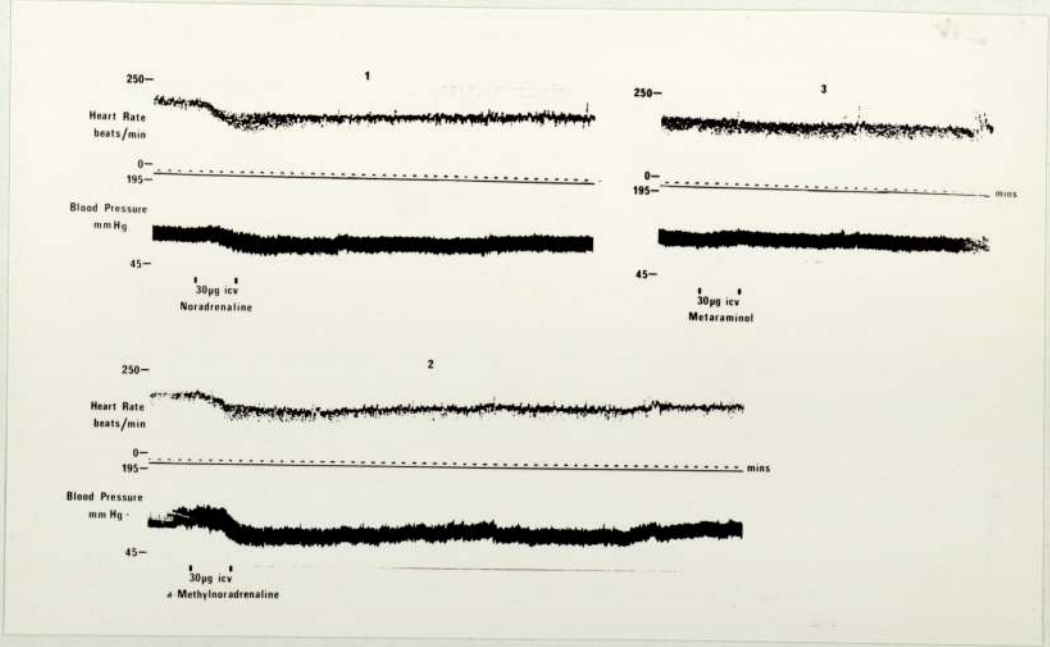


FIG. 19.

Conscious, normotensive, unrestrained cat. Traces 1,2 and 3 respectively illustrate the blood pressure and heart rate responses to noradrenaline, α-methylnoradrenaline and metaraminol in the same cat. Each compound was infused icv in a dose of 30 µg.

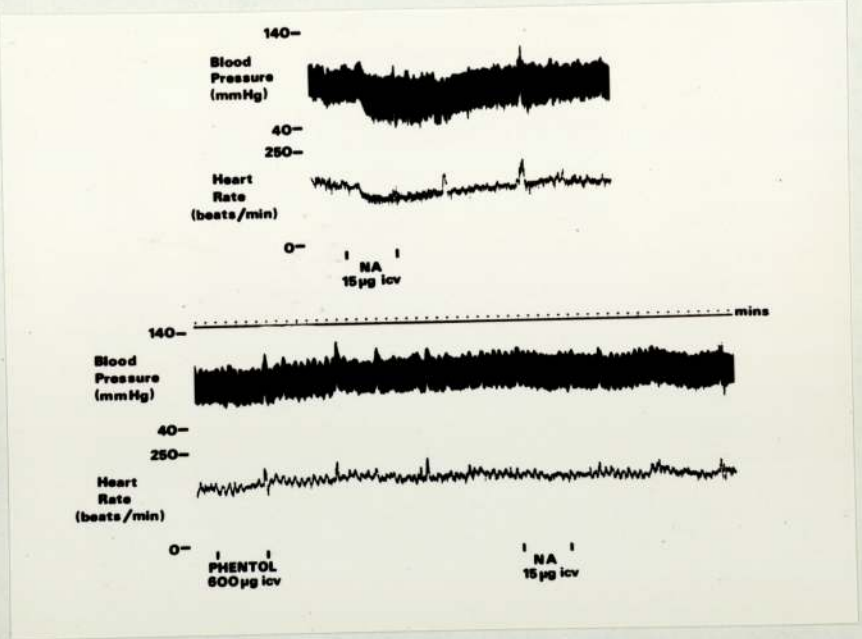


FIG. 20.

Conscious, normotensive, unrestrained cat. Upper trace illustrates lowering of arterial blood pressure and heart rate in response to noradrenaline (15 µg, icv). Lower trace shows abolition of response to icv noradrenaline (15 µg) after phentolamine (600 µg, icv).

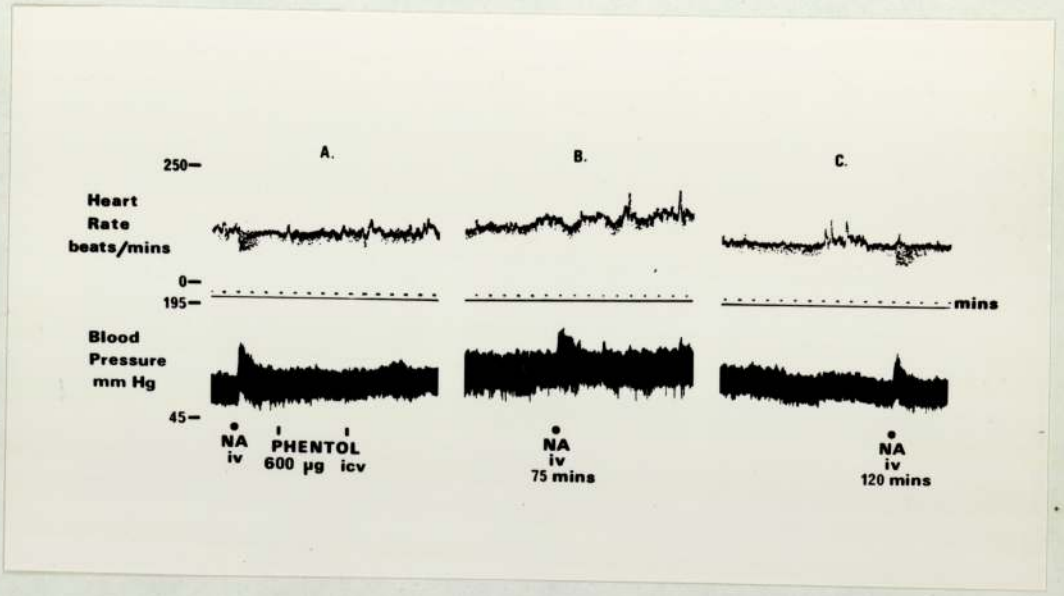


FIG. 21.

Conscious, normotensive, unrestrained cat. Trace A shows pressor response and bradycardia to noradrenaline (200 ng/kg, i.v.) given 3 minutes before phentolamine (600 µg, icv). Traces B and C respectively illustrate responses to noradrenaline (200 ng/kg, i.v.) 75 and 120 minutes after phentolamine icv.

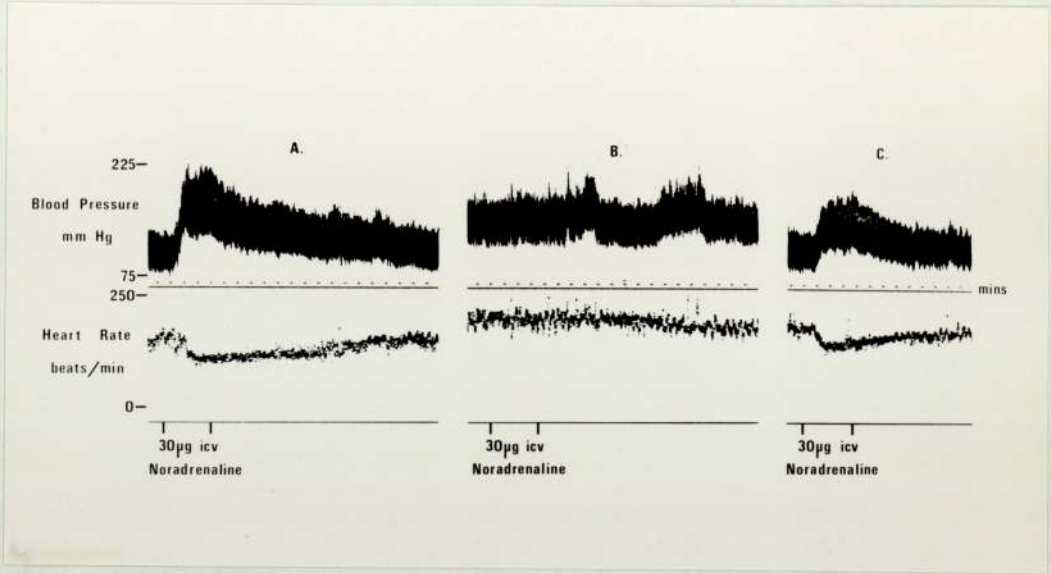


FIG. 22.

Conscious, normotensive, unrestrained cat. Trace A shows a hypertensive effect with bradycardia after noradrenaline (30 µg, icv). The hypertensive response with bradycardia due to icv noradrenaline (30 µg) was absent when given 60 minutes after icv phentolamine (0.6 mg) (Trace B). Trace C illustrates the gradual return of the icv noradrenaline response. Noradrenaline (30 µg, icv) was given 3 hours after icv phentolamine.

AGONIST	TOTAL ICV DOSE µg	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s.e.m.		DECREASE IN HEART RATE beats/min ± s.e.m.	No. of cats used.	No. of responses obtained in each cat.
		SYSTOLIC	DIASTOLIC			
Noradrenaline	15	12.3 ± 3.6	16.2 ± 3.9	20.3 ± 3.1	12	4
	20	19.4 ± 2.9	16.7 ± 2.3	25.7 ± 2.7	12	4
	30	23.6 ± 2.5	19.1 ± 2.0	29.4 ± 2.5	12	5
α-Methylnoradrenaline	15	10.7 ± 2.1	12.1 ± 3.2	15.1 ± 3.6	7	4
	30	24.1 ± 1.8	25.6 ± 2.8	28.6 ± 2.3	7	5
	40	27.4 ± 3.9	30.0 ± 4.3	35.5 ± 5.1	7	3

TABLE 2. Maximal blood pressure and heart rate decreases (mean ± s.e.m.) produced by noradrenaline and α-methylnoradrenaline administered into the lateral cerebral ventricles of normotensive conscious unrestrained cats.

CHAPTER 3.

The effect of other α-adrenoceptor stimulants administered centrally in the conscious cat.

Nashold et al. (1962) observed that icv adrenaline, in anaesthetised cats, produced a depressor effect which could be increased when given centrally with a monoamine oxidase inhibitor. Toda et al. (1969) produced falls in blood pressure and heart rate after icv adrenaline (10 - 200 μg) in anaesthetised rabbits. Systemic treatment with reserpine reversed the adrenaline icv depressor response to a pressor response. They found in the unanaesthetised rabbit icv adrenaline produced hypertension and tachycardia followed by hypotension and bradycardia.

Adrenaline is known to be a potent stimulant of both peripheral α- and β-adrenoceptors. (Innes & Nickerson, 1970). The two groups of workers, mentioned above, did not attempt to investigate whether the responses obtained after icv adrenaline were due to either central α- or β-adrenoceptor stimulation or to a combination of both.

Phenylephrine administered centrally in anaesthetised cats produced pressor effects after low doses but depressor responses and bradycardia after larger doses (Schmitt & Fénard, 1971). Hypertension associated with bradycardia occurred after icv phenylephrine in anaesthetised rabbits (Toda et al., 1969). Bhargava et al. (1972) observed that icv phenylephrine in anaesthetised dogs produced falls in blood pressure and heart rate.

No reports of central administration of methoxamine, a peripheral ∝-adrenoceptor stimulant (Hjort, Randall & De Beer, 1948), were found in the literature.

Experiments were performed to establish the blood pressure and heart rate effects produced after central administration of adrenaline, phenylephrine and methoxamine. An attempt was also made to record the central cardiovascular effects produced by icv amphetamine. The results of centrally administered dopamine, although normally regarded as an ∝-adrenoceptor stimulant (Innes & Nickerson, 1970), are described in Section 3 of the results.

Results

1. Effect on blood pressure and heart rate observed after icv infusions of adrenaline, phenylephrine and methoxamine in cats.

Adrenaline

Adrenaline (60 & 120 µg) was infused icv in 14 cats. When given alone, it produced complex blood pressure responses consisting of both pressor and depressor components and similarly, variable rises and falls in heart rate were observed. In 3 cats, the depressor effect and bradycardia preceded the hypertension and tachycardia whilst in 9 cats the hypertension and tachycardia preceded the hypotension and bradycardia. A biphasic response of the latter type due to icv adrenaline is demonstrated in Fig. 31. Icv adrenaline in the remaining 2 cats produced no effect on blood pressure or heart rate.

8 cats, from this group of 14, were pretreated with icv dl-propranolol (0.5 - 1.0 mg) 60 minutes before icv adrenaline administration. A dose of dl-propranolol was used for each particular cat that had been shown previously to completely inhibit the centrally mediated effects of isoprenaline (30 µg, icv). Icv adrenaline (60 & 120 µg) produced hypotension and bradycardia when administered after icv dl-propranolol. The mean depressant effects on blood pressure and heart rate are summarised in Table 3. As in the previous experiments in which α-methylnoradrenaline or noradrenaline were combined with α-adrenoceptor blocking agents centrally, icv adrenaline further reduced the blood pressure and heart rate to very low levels when given 60 minutes after icv dl-propranolol.

The blood pressure and heart rate lowering effects of icv adrenaline (in the presence of dl-propranolol) were immediate in onset and the responses were observed to be completed usually 45 to 75 minutes after the icv infusion. The 60 µg dose of adrenaline lowered the mean diastolic pressure by a greater extent than the mean systolic pressure. The reductions in diastolic and systolic pressures after the 120 µg dose of adrenaline (icv) were very similar.

The β-adrenoceptor stimulant effects of adrenaline infused centrally are discussed in Section 3.

Phenylephrine

Phenylephrine (75 & 150 µg, icv) produced dose-dependant falls in blood pressure and heart rate when given to 2 cats. Each dose of phenylephrine was infused icv

to both cats on 2 occasions. Thus, the mean reductions in blood pressure and heart rate of icv phenylephrine were calculated from 4 observations, (see Table 3). Icv phenylephrine reduced both the systolic and diastolic blood pressure by similar amounts. The reductions in blood pressure and heart rate after central phenylephrine were quick in onset. The response to icv phenylephrine began at the end of the icv infusion. The maximum reductions occurred between 15 - 25 minutes after the start of the infusion and the blood pressure and heart rate had returned to pretreatment levels within 75 - 90 minutes from the start of the infusion.

Methoxamine.

Dose-dependant falls in blood pressure and heart rate were produced after icv administration of methoxamine (100, 250 µg), in 3 cats. The maximum hypotensions and bradycardias were observed between 15- 30 minutes after the start of the icv infusion. The hypotensions and bradycardias observed for methoxamine were of longer duration than for adrenaline and phenylephrine. Resting pretreatment levels of blood pressure and heart rate were reached between 2 - 3 hours after the icv methoxamine administration.

Dose-response relationships of icv adrenaline, phenylephrine and methoxamine, from the data described in Table 3, are plotted in Fig.23. The blood pressure response curves for phenylephrine and methoxamine overlap and continue in a straight line indicating that icv phenylephrine

and methoxamine produce equipotent effects on blood pressure. The blood pressure curves for adrenaline in the presence of dl-propranolol are approximately parallel with those of methoxamine and phenylephrine, indicating that all 3 compounds may reduce blood pressure by a similar mechanism. Adrenaline was more potent than either phenylephrine or methoxamine in reducing blood pressure and heart rate.

The order of potency in producing bradycardias was adrenaline > phenylephrine > methoxamine. Although phenylephrine and methoxamine produced equipotent effects on blood pressure phenylephrine was more potent than methoxamine in producing centrally mediated bradycardias.

Hoyer & van Zwieten (1972) produced hypotension and bradycardia after vertebral artery infusions of amphetamine in anaesthetised cats. Amphetamine was administered in a dose of 150 µg into the lateral ventricle of one cat. It was found impossible to measure the true blood pressure and heart rate effects as the cat became aroused and restless after the icv infusion of amphetamine. It began to cry and whine and moved about the cage. Its pupils were dilated and the respiration appeared to be increased. These behavioural effects were unexpected as Gaddum & Vogt (1956) had previously reported that amphetamine (500 µg, icv) produced a lethargic state in cats. However, they did observe pupil dilatation and tachypnoea.

2. Effect of icv phentolamine on the centrally mediated cardiovascular effects of adrenaline, phenylephrine and methoxamine in cats.

The hypotension and bradycardia normally observed after icv adrenaline, administered in the presence of icv dl-propranolol, were completely absent after phentolamine (0.6mg, icv). Phentolamine was administered centrally 60 minutes before the icv adrenaline, i.e. phentolamine and dl-propranolol were infused icv at the same time. This combination of α- and β- adrenoceptor antagonists infused centrally together produced an initial hypertension and tachycardia which lasted for approximately 15 minutes and then reverted back to normal pretreatment levels.

The hypotension and bradycardias after icv phenylephrine (150 µg) and methoxamine (100 µg) were also abolished by icv phentolamine (0.6 mg) pretreatment, given 60 minutes before the α-adrenoceptor agonists. The falls in pressure and heart rate observed after methoxamine (250 µg, icv) were greatly reduced after 0.6 mg phentolamine icv and abolished after 0.75 mg.

3. Pressor effects after icv adrenaline.

As stated in part 4, chapter 2 of this section, noradrenaline (15 - 30 µg, icv) produced a centrally mediated hypertensive response accompanied by bradycardia in 3 cats. Adrenaline (60 µg, icv) was administered to 2 of these cats. Administered alone, adrenaline produced very little effect on the blood pressure and heart rate of either cat. Adrenaline (60 µg) was infused icv in each cat 60 minutes after dl-propranolol (0.6 mg, icv). In each cat, icv adrenaline produced hypertension with associated bradycardia. The cardiovascular responses to icv

adrenaline after dl-propranolol pretreatment was inhibited by icv phentolamine (0.6 mg). A hypertensive response with bradycardia due to icv adrenaline given 60 minutes after dl-propranolol is shown in Fig. 24.

DISCUSSION

Adrenaline, phenylephrine and methoxamine produced dose-dependant falls in both blood pressure and heart rate after icv administration in conscious cats. Adrenaline was found to be more potent than either phenylephrine or methoxamine in producing both hypotension and bradycardia. Phenylephrine and methoxamine were equipotent in producing hypotension but at equal doses phenylephrine induced a larger bradycardia than methoxamine (Fig. 23.). These effects were abolished by icv phentolamine.

Biphasic responses in blood pressure and heart rate were observed, in the majority of cats tested, after adrenaline infused icv alone. These resemble the responses to icv adrenaline reported in the conscious rabbit by Toda et al. (1969). However, these workers did not investigate the central mechanism of action of adrenaline in the rabbit. The results obtained from these cats indicate that the depressor component and bradycardia were due to central α-adrenoceptor stimulation by adrenaline as the regular control hypotension and bradycardia responses seen after adrenaline infused icv in the presence of central β-adrenoceptor blockade (induced by dl-propranolol) was inhibited by icv phentolamine, a blocker of peripheral α-adrenoceptors.

However, in 2 cats icv adrenaline, like noradrenaline, induced centrally mediated pressor effects associated with bradycardia. The pressor response was apparently centrally mediated since it was abolished by pretreatment with a small icv dose of phentolamine (0.6 mg). This icv dose of phentolamine has previously been shown not to affect the responses to i.v. noradrenaline, 200 ng /kg (see Fig. 21).

The peripheral mechanism by which the pressor effect was mediated was not investigated. As with noradrenaline, icv adrenaline may have caused vasoconstriction by direct neuronal influence from the central nervous system to the vasculature. Although, it has been shown that electrical stimulation of the hypothalamus can lead to the release of catecholamines from the adrenal medulla (see Historical Introduction for references), experiments were not performed to indicate whether this mechanism contributes to the production of the pressor effects observed after icv adrenaline or noradrenaline.

In contrast to the results described in Section 3 of the results, in which pressor responses were produced due to central β-adrenoceptor stimulation, the hypertension produced in these 3 cats by icv adrenaline and noradrenaline was blocked by icv phentolamine, an α-adrenoceptor blocker. Thus, the results obtained with icv noradrenaline and adrenaline show that these compounds are able to produce two types of centrally mediated blood pressure response,

both of which are inhibited by phentolamine. Bradycardia was always associated with the pressor or depressor response and also appeared to be due to α-adrencceptor stimulation.

In the anaesthetised cat, Schmitt & Fénard (1971) produced hypotension and bradycardia after icv phenylephrine (1 - 2 mg) whilst lower doses of phenylephrine (100 - 300 µg, icv) produced pressor effects. However, results presented in this chapter show that phenylephrine (75 & 150 µg, icv) caused depressor effects with associated bradycardia in the conscious cat. In Chapter 2 of this section, it was shown that similar sized responses to icv noradrenaline and α-methylnoradrenaline in the conscious cat, were obtained using one tenth of the doses used by Heise & Kroneberg (1973) in the anaesthetised cat. Thus, since anaesthesia may appear to reduce the sensitivity of animals to icv drug administration, cardiovascular responses may have been expected in the conscious cat by administering lower doses of phenylephrine centrally (e.g. 10 - 30 µg being one tenth of the icv doses of phenylephrine used by Schmitt & Fénard in the anaesthetised cat). However, in this series of experiments, blood pressure and heart rate effects were not observed when phenylephrine was administered in doses up to 75 µg.

Gagnon & Melville(1966) produced blood pressure stimulation after small icv doses of noradrenaline and depressor effects with larger doses. Schmitt & Fénard (1971) similarly produced opposite blood pressure effects

with 2 dose levels of phenylephrine. After administration into the lateral ventricles, low doses of the amines would be expected to stimulate areas near the site of administration while larger doses would stimulate areas further from the site of administration. The hypothalamus forms the lateral walls and floor of the third ventricle and is easily and quickly reached from the lateral ventricle; the drug solution just passes through the Foramen of Monro from the lateral into the third ventricle. Drugs must pass through the third ventricle and the aqueduct to enter the fourth ventricle and act on pathways within the medulla oblongata. Thus, it may be that the pressor effects due to low doses of amines administered into the lateral ventricles result from hypothalamic stimulation whilst depressor effects occur after stimulation of areas within the medulla.

In normotensive conscious cats, low icv doses of noradrenaline and phenylephrine did not produce any noticeable effects on blood pressure or heart rate. It may be possible that anaesthesia may reduce the activity of the metabolic enzymes, monoamine oxidase and catecholamine-o-methyl transferase or uptake processes, allowing low doses of the amines to be sufficiently protected and active within hypothalamic areas. Pressor effects due to α-adrenoceptor stimulation were observed in 3 cats after administration of normal dose levels of noradrenaline and adrenaline into the lateral ventricles. These effects may have been produced as a result of a reduced passage of the drugs to the hind brain areas. A reduced flow of the infused drug

in the cerebrospinal fluid may have been due to a physical blockage within the ventricular system; thus allowing a greater stimulation of forebrain or hypothalamic areas. However, it should be remembered that in these 3 cats, the cardiovascular effects observed after icv phentolamine (i.e. a hypertension and tachycardia) were identical to those seen in the majority of cats producing cardiovascular depressant effects to α-adrenoceptor agonists. Hence, the pressor effects may be a result of preferential stimulation of medullary pressor pathways by the agonists in these particular cats.

Methoxamine administered into the brains of three conscious cats produced significant falls in blood pressure and heart rate. Although, less potent than adrenaline and phenylephrine in reducing heart rate methoxamine was as potent as phenylephrine in producing hypotension. The responses to icv methoxamine were more prolonged than those observed for icv adrenaline and phenylephrine. The structure of methoxamine is shown in Fig. 25 and it can be seen that methoxamine contains a methyl group attached to the α-carbon atom of the side chain, thus conferring immunity of this compound from monoamine oxidase enzymes. Methoxamine is not metabolised by catecholamine-o-methyl transferase as there are two methoxy groups already attached to the phenyl ring. Thus, these two factors concerning the structure of the methoxamine molecule probably account for the long duration of action seen after icv administration.

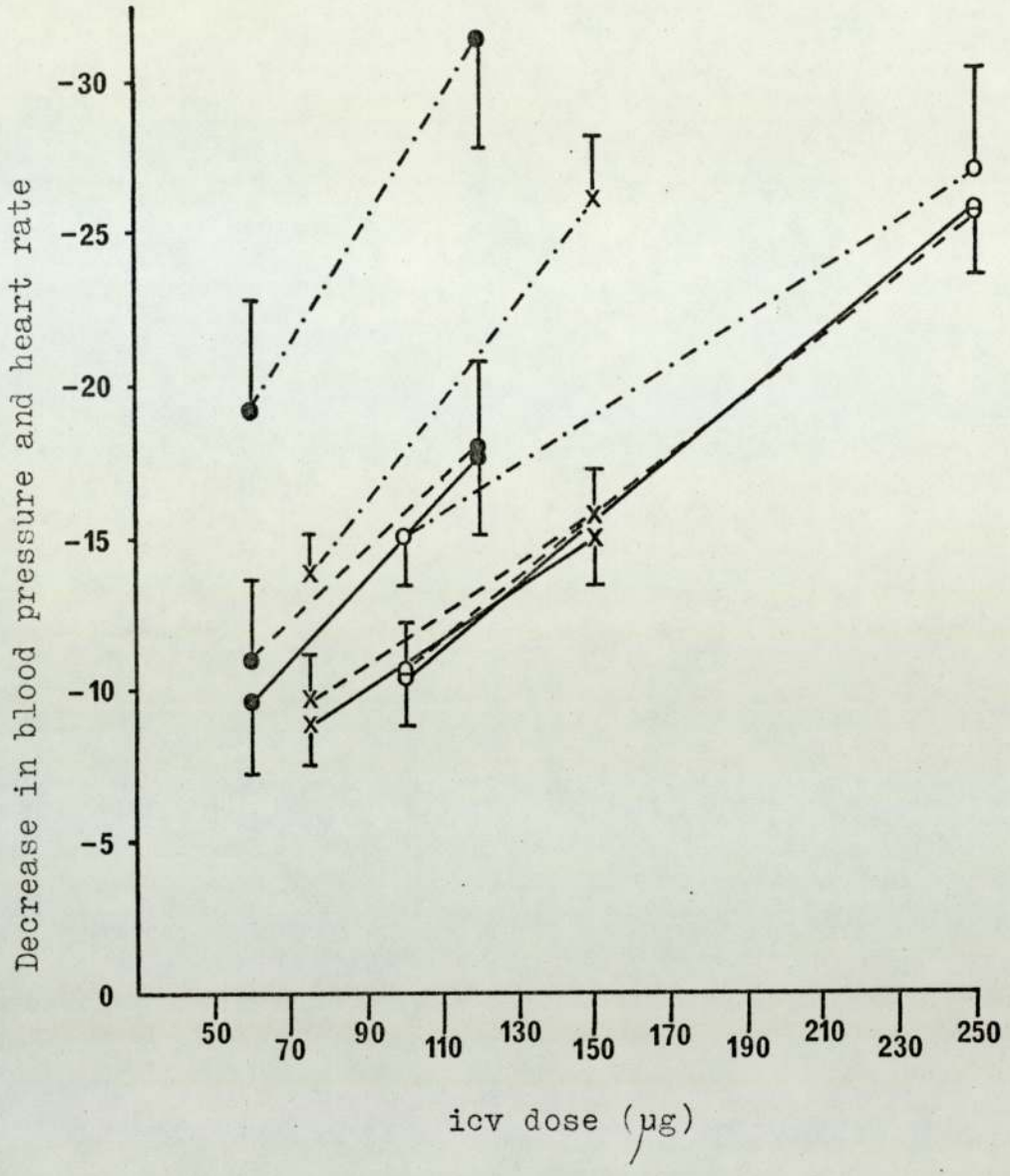


FIG. 23. Dose-response relationships of results obtained with icv adrenaline (60 & 120 µg), phenylephrine (75 & 150 µg) and methoxamine (100 & 250 µg) in conscious cats. Results of icv adrenaline, phenylephrine and methoxamine are shown by ●, X and O respectively. Reductions in systolic blood pressure (mmHg) are represented by (———) diastolic blood pressure by (------) and heart rate (beats/minute) by (-·-·-·). Data is taken from Table 3.

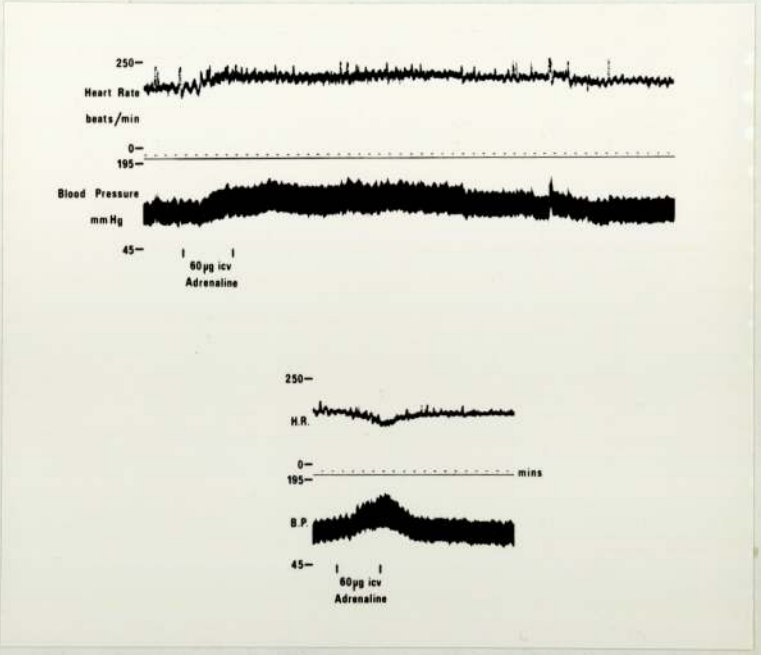


FIG. 24.

Conscious, normotensive, unrestrained cat. Upper trace shows blood pressure and heart rate increases after adrenaline (60 µg, icv) given alone. Lower trace illustrates a transient pressor response with bradycardia after icv adrenaline given 60 minutes after dl-propranolol (0.75 mg, icv).

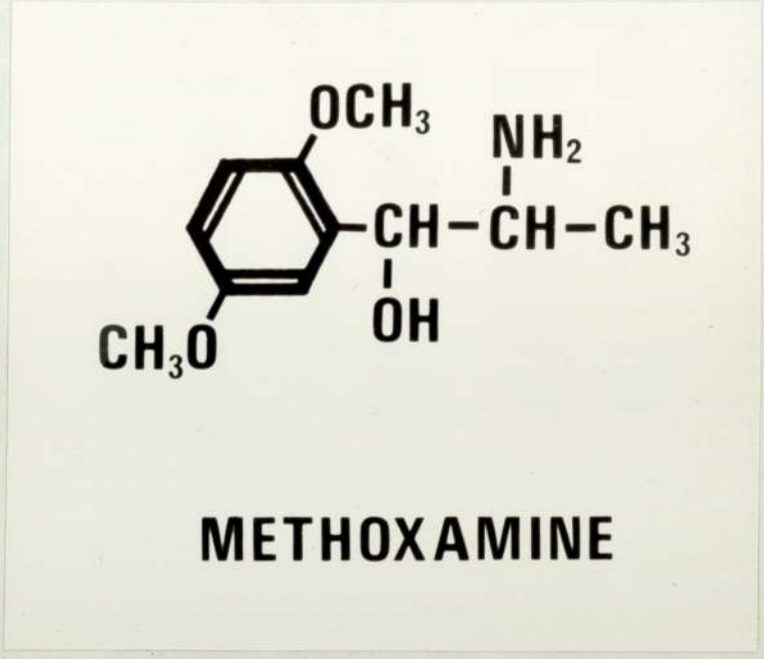


FIG. 25.

Diagram of structure of Methoxamine.

α-adrenoceptor agonist	Total icv Dose µg.	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s.e.m.		DECREASE IN HEART RATE beats/min ± s.e.m.	No. of cats used	TOTAL No. of observations
		SYSTOLIC	DIASTOLIC			
ADRENALINE (1 hour after icv dl-propranolol, 0.5 - 1.0 mg).	60	9.6 ± 2.4	11.0 ± 2.7	19.2 ± 3.5	8	16
	120	17.8 ± 2.5	18.0 ± 2.8	31.4 ± 3.6	7	12
PHENYLEPHRINE	75	8.9 ± 1.3	9.7 ± 1.4	13.8 ± 1.3	2	4
	150	15.1 ± 1.5	15.8 ± 1.5	25.9 ± 2.1	2	4
METHOXAMINE	100	10.4 ± 1.6	10.6 ± 1.6	15.1 ± 1.6	3	6
	250	25.8 ± 2.0	25.7 ± 2.1	27.1 ± 3.3	3	6

TABLE 3. Maximal decreases in blood pressure and heart rate (mean ± s.e.m.) induced by icv adrenaline (after icv dl-propranolol, 0.5 - 1.0 mg), phenylephrine and methoxamine in conscious normotensive unrestrained cats.

CHAPTER 4.

An investigation into the central effects of clonidine on blood pressure and heart rate in conscious cats.

A large number of investigations have been undertaken to reveal the mode of action of the antihypertensive action of clonidine. As a result of this work much knowledge has been provided explaining the central control of blood pressure and heart rate.

Clonidine administered systemically has been shown to produce biphasic changes in blood pressure. A brief hypertension due to stimulation of peripheral α-adrenoceptors (Hoefke & Kobinger, 1966; Kobinger & Walland 1967a,b; Boissier et al., 1968; Rand & Wilson, 1968) followed by a long lasting reduction in blood pressure and heart rate is obtained. The hypotension and bradycardia appear to be produced as a result of reduced central sympathetic outflow to the heart and resistance vessels (Kobinger & Walland, 1967a, b; Hukuhara et al., 1968; Schmitt et al., 1968; Sherman et al., 1968; Klupp et al., 1970; Woodhouse et al., 1972; Loew & Waite, 1974).

Several workers have demonstrated that the bradycardia observed is also due in part, to an increase in efferent vagal discharge (Boissier et al., 1968; Robson & Kaplan, 1969; Robson et al., 1969; Nayler & Stone, 1970; Kobinger & Walland, 1972 a, b; Woodhouse et al., 1972).

The pressor effect normally seen after systemic clonidine is absent after clonidine administered into the brain; only the hypotension and bradycardia persist after

administration of clonidine into the cisterna magna or lateral cerebral ventricles (Kobinger & Walland, 1967b; Schmitt et al., 1968; Sherman et al., 1968; Dollery & Reid, 1973), third cerebral ventricle (Schmitt, 1970), rat hypothalamus (Struyker Boudier & van Rossum, 1972), vertebral artery infusions (Sattler & van Zwieten, 1967; Constantine & McShane, 1968; Katic et al., 1972; Schmitt et al., 1973b) and in cross circulation experiments (Sherman et al., 1968).

The centrally mediated cardiovascular depressant effects were abolished by systemic or intracisternal pre-treatment with the α-adrenoceptor antoagonists yohimbine and piperoxan (Schmitt et al., 1971, 1973b) but not by systemic or icv phentolamine (Schmitt & Schmitt, 1970). Several workers have inhibited the central effects of clonidine with central pretreatmentof α-adrenoceptor antagonists (see van Zwieten, 1973 and Historical Introduction for references). Bolme & Fuxe (1971) demonstrated that the central actions of clonidine were inhibited by the systemic administration of the noradrenaline receptor blocking agents, phenoxybenzamine and haloperidol but unaffected by the dopamine receptor blocking agents pimozide and spiroperidol.

Intact central monoaminergic neurons have been shown by Dollery & Reid (1973) to be essential for the mediation of the central actions of clonidine. These workers inhibited the centrally mediated hypotension and bradycardia of clonidine after destruction of central neurons with 6-hydroxydopamine pretreatment. Schmitt et al. (1973c)

also abolished the central action of clonidine by lesioning the sympatho-inhibitory area of the medulla.

Attempts have been made to localise the central action of clonidine to particular brain areas. The main site of action is thought to be upon medullary sympathetic structures (Sattler & van Zwieten, 1967; Constantine & McShane, 1968; Schmitt & Schmitt, 1969). Bousquet & Guertzenstein (1973) suggested that clonidine produces its cardiovascular depressant effects by an action on 'chemosensitive zones' on the ventral surface of the brain stem. Recent experimentation has revealed that clonidine may also act by an action in the forebrain, e.g. the hypothalamus (Shaw et al., 1971b; Struyker Boudier & van Rossum, 1972; Woodhouse et al., 1972; Klevens et al., 1973).

Robson & Kaplan (1969) demonstrated that an intravenous injection of clonidine increased vagal reflex bradycardia elicited in dogs by the pressor substances noradrenaline and angiotensin. More recently, Kobinger & Walland (1972a) found that clonidine facilitated a vagal cardioinhibitory reflex by an action within the brain. This central effect of clonidine, like the centrally mediated hypotension and bradycardia, was antagonised by central α-adrenoceptor blockade. Thus, noradrenergic receptors are implicated in the reflex activities of the vagus system within the central nervous system of dogs (Kobinger & Walland, 1972b, 1973). Haeusler (1973b, 1974b) has recently suggested that the hypotension and bradycardia

of clonidine is due to a long lasting activation of the central part of the baroreceptor reflex by clonidine. Korner et al. (1974) also found that clonidine alters heart rate mainly through a direct central action on baroreceptor pathways.

In this series of experiments, it was hoped to further substantiate the proposed mechanisms of action of centrally and peripherally administered clonidine. It was also hoped to establish whether or not the clonidine bradycardia is centrally mediated in cats by an increased efferent vagal tone, a decrease in efferent sympathetic tone or a combination of both effects.

Results

1. Cardiovascular effects produced by peripherally and centrally administered clonidine.

Clonidine (5-15μg/kg, i.v.) was administered to 3 cats; it produced, in all cases, an initial rise in blood pressure with an associated reflex fall in heart rate followed by prolonged hypotension and bradycardia. A typical biphasic blood pressure response with associated bradycardia observed after clonidine (10 μg/kg, i.v.) is shown in Fig. 26. The pressor effect and reflex bradycardia were observed immediately after the intravenous injection of clonidine whilst the maximum hypotension and bradycardia were observed approximately 30 - 45 minutes later. The peak falls in blood pressure and heart rate observed in each of the 3 cats after i.v. clonidine (10 μg/kg) were 28/26, 30/32 and 25/24 mmHg and 50, 48 and 45

beats/minute, respectively. Resting normotensive levels of blood pressure and heart rate were noted 2 - 3 hours after the systemic dosage of clonidine.

Clonidine (2.5 - 10 µg) administered into the lateral ventricles of conscious cats produced dose dependant falls in blood pressure and heart rate. The initial pressor effect , normally observed after systemic administration, was completely absent. The absence of the pressor effect after icv clonidine (10 µg) is demonstrated in Fig. 27. The onset of the hypotension and bradycardia was rapid; often starting during the icv infusion. Maximal blood pressure and heart rate reductions usually occurred 15-30 minutes from the completion of the icv infusion. Resting normotensive levels were observed 1.5 - 2.5 hours after the icv administration of clonidine. Effects on blood pressure and heart rate were obtained for 3 doses of icv clonidine (2.5, 5.0 and 10.0µg) from administrations into 5 cats (i.e. one response of each dose in 5 cats) and are summarised in Table 4. The 5 cats used were newly cannulated and in these animals it was observed that responses to particular doses of icv clonidine were very consistent. However, during the investigations reported in this chapter clonidine has been administered to a total of 10 conscious cats. In all cats, icv clonidine produced hypotension and bradycardia.

Comparing the depressant effects of icv clonidine with those observed with icv noradrenaline and α-methyl-noradrenaline it appears that clonidine is approximately

4 times more potent than icv noradrenaline and α-methyl-noradrenaline in producing hypotension and bradycardia. Responses obtained after 20 µg icv noradrenaline and 40 µg α-methylnoradrenaline closely corresponded to the respective depressant effects obtained after 5 and 10 µg icv clonidine.

Clonidine (2.5 - 10.0 µg) was administered centrally to 2 of the 3 cats that responded with hypertension and bradycardia after icv noradrenaline and adrenaline. Clonidine icv produced hypotension associated with bradycardia in both cats. Clonidine failed to produce pressor effects when administered centrally to all cats used in these investigations.

If the doses of clonidine were raised above 15 µg/kg i.v. or 10 ug icv severe retching and vomiting occurred in the conscious cat. Licking and swallowing movements were always observed immediately before vomiting. Occasionally, licking and swallowing occurred with the doses of clonidine used in the cardiovascular studies but vomiting was rarely seen. Retching and vomiting usually occurred within 2 - 5 minutes of intravenous administration and usually within 3 minutes from the end of the icv clonidine infusion.

Clonidine administered either i.v. or icv produced sedation in the conscious cat. The sedation was prolonged, often lasting for 2 to 3 hours after the clonidine administration.

2. Effect of peripheral and central administration of phentolamine on the cardiovascular responses to clonidine.

Intravenous pretreatment with phentolamine (3mg/kg), in 3 cats, abolished the biphasic blood pressure effects and bradycardia observed after clonidine (10 µg/kg, i.v). Clonidine was given intravenously at the time when complete inhibition of the peripheral cardiovascular effects of noradrenaline (200 ng/kg) had been obtained.

Phentolamine (3 mg/kg, i.v.) caused a small hypotension with little or no effect on heart rate in each of the 3 cats used. The diastolic blood pressures of the 3 cats were observed to fall by 15, 12 and 18 mmHg respectively. The peak falls in pressure due to i.v. phentolamine had been reached before the clonidine administration. Although the blood pressure was at a lower resting level after i.v. phentolamine it was higher than the maximum control responses to clonidine and thus if clonidine had produced a response after phentolamine it would have not been obscured. However, as the blood pressures were not further reduced by i.v. clonidine after phentolamine and the bradycardias were also absent, it was assumed that phentolamine had fully inhibited the effects of i.v. clonidine.

The effect of centrally administered phentolamine (0.6 mg, icv) on the cardiovascular effects of clonidine (10 µg/kg, iv) were investigated in 3 cats. Icv phentolamine prevented the onset of the prolonged hypotension and bradycardia observed after i.v. clonidine. However, in all

3 cats, the pressor effect and reflex bradycardia due to i.v. clonidine were still present. Fig. 26 illustrates such an experiment. The upper trace depicts the control response to clonidine (10 µg/kg, iv.). 60 seconds after the clonidine administration, the blood pressure had risen to $^{160}/_{108}$ from an initial level of $^{121}/_{79}$ mmHg. The second part of the pressor effect was due to a slight movement of the cat as it appeared to be making licking and swallowing motions. The cat did not vomit. The heart rate reflexly fell from 190 to 130 beats/minute; the resting heart rate of this cat was relatively high. After 45 minutes the blood pressure and heart rate had fallen to $^{93}/_{63}$ mmHg and 140 beats/minute respectively.

The lower trace shows the second clonidine administration this time repeated 30 minutes after phentolamine (0.6 mg, icv). This dose had previously been shown to adequately inhibit the central cardiovascular effects of icv noradrenaline in this cat. The initial bradycardia and pressor response were still present after the clonidine administration. The pressor effect was almost identical to the control response; the blood pressure rose from $^{120}/_{78}$ to $^{159}/_{100}$ mmHg and the heart rate fell from 185 to 130 beats/minutes. After 45 minutes the hypotension due to iv clonidine was absent and the heart rate had returned to 180 beats/minute.

Clonidine administered centrally produced only prolonged hypotension and bradycardia. The hypotension and bradycardia produced by icv administration of clonidine 10 µg, in 3 cats, were completely absent when icv clonidine

was infused after complete peripheral α-adrenoceptor blockade had been achieved by phentolamine (3mg/kg, iv.). α-Adrenoceptor blockade was obtained when the cardiovascular effects of noradrenaline (200-400 ng/kg, iv.) had been totally abolished; clonidine was infused icv 30 - 60 minutes after the phentolamine.

The cardiovascular depressant effects observed after clonidine (10 µg, icv) were also inhibited by icv phentolamine (0.5 - 0.75 mg) pretreatment in 5 cats. Fig. 27 is a record of one such experiment. The control response to clonidine (10 µg, icv) was a very pronounced hypotension and bradycardia; the maximum effects being 24 mmHg and 32 beats/minute respectively. The depressant effects were observed to start during the icv clonidine infusion and the maximum effects observed approximately 15 - 20 minutes after completion of the icv infusion. When administered 60 minutes after phentolamine (0.6 mg, icv) the hypotension and bradycardia normally seen after icv clonidine were absent. During the abolition of the cardiovascular effects of icv clonidine (seen on the lower trace) cardiovascular changes were observed due to movement of the cat.

It was observed that central pretreatment with phentolamine prevented the emetic reaction of icv clonidine when it was occasionally observed in control responses, due to the use of a slightly large dose of clonidine.

3. Effect of peripheral and central β-adrenoceptor blockade by dl-propranolol on the cardiovascular responses to centrally administered clonidine.

Hypotension and bradycardia were still observed after icv clonidine (10 µg) given during peripheral β-adrenoceptor blockade with dl-propranolol (1.5 mg/kg, i.v.), in 3 cats. The experiments described in parts 3,4,5 and 6 of this chapter were performed in the same 3 cats. Trace A of Fig. 28 demonstrates a control response to clonidine (10 µg, icv). Clonidine produced maximum falls in blood pressure and heart rate of $^{24}/_{24}$ mmHg and 30 beats/minute. The slight increase in blood pressure and heart rate observed soon after the end of the icv infusion was due to excessive licking and swallowing movements associated with a general restlessness of the cat. However, the cat did not vomit.

Trace B (Fig. 28) shows the icv administration of clonidine in the same cat after abolition of the systemic effects of 250 ng/kg iv. isoprenaline given 30 minutes after dl-proproanolol (1.5 mg/kg, i.v.). In the presence of dl-propranolol, clonidine produced a violent retching and vomiting response observed 2 minutes after the end of the icv infusion of clonidine. However, the heart rate still fell below normal levels producing a 25 beat/minute bradycardia. Also icv clonidine produced a $^{12}/_{15}$ mmHg hypotension.

Central administration of dl-propranolol (0.5 - 1.0 mg) produced prolonged bradycardia and hypotension (see Section 4). Clonidine 10 µg was administered icv, to 3 cats, 60 minutes after dl-propranolol (0.75 mg, icv). The pattern of response was as for noradrenaline and α-methylnoradrenaline as described in Section 2, Chapter 2,

part 3. Clonidine icv further reduced the blood pressure and heart rate after icv dl-propranolol to very low levels. Thus, it is likely that clonidine and dl-propranolol produce their depressant effects by different mechanisms of action and the combined effects being additive. The effects of smaller doses of clonidine and dl-propranolol given centrally in combination are discussed in Section 4 Chapter 1, part 2 of the results.

4. Effect of peripheral adrenergic neuron blockade by bethanidine on the cardiovascular responses to centrally administered clonidine.

Bethanidine (5.0 mg/kg, iv.) was administered 3 hours before clonidine (10 µg, icv) in 3 cats. Clonidine was infused centrally when the greatest reduction of the systemic effects of McNeil A-343 (25 µg/kg, i.v.) had been obtained. It was found that the maximum reduction of the pressor effect and reflex bradycardias of McNeil A343 by bethanidine i.v. was between 80 - 85% of control responses (see Fig. 28); the maximum reduction occurring between 3 - 6 hours after the bethanidine administration. A marked bradycardia occurred in each of these cats after icv clonidine, but the hypotensions were not so pronounced. However, in each cat, the blood pressure was slightly further reduced after icv clonidine. Vomiting regularly occurred after icv clonidine in these experiments.

Trace A (Fig. 28) shows the control response to clonidine (10 µg, icv). Record C shows the effect of clonidine after i.v. bethanidine in the same cat. Clonidine (10 µg,

icv) infused 3 hours after bethanidine (5.0 mg/kg, iv) produced a 30 beat/minute bradycardia. At the time of the maximum bradycardia, the blood pressure had fallen by $^{5}/_{8}$ mmHg (c.f. $^{24}/_{24}$ mmHg hypotension observed with the control clonidine icv infusion). However, bethanidine i.v. caused the resting presure to fall from $^{127}/_{82}$ to $^{106}/_{66}$ mmHg at the time of the clonidine icv infusion. Systemic bethanidine did not cause any significant changes in the resting heart rate. As in the experiments using dl-propranolol (i.v) severe vomiting occurred with central clonidine after peripheral adrenergic neuron blockade with bethanidine. Record C (Fig. 28) shows that the cat vomited twice after icv clonidine.

5. Effect of peripherally administered atropine methylnitrate on the cardiovascular responses to centrally administered clonidine.

The effects of atropine methylnitrate (0.5 mg/kg, i.v.) on the central effects of clonidine (10µg, icv) were observed in 3 cats. Clonidine was administered icv after inhibition of the cardiovascular effects of McNeil A-343 (25 µg/kg, i.v.), a potent muscarinic receptor stimulant, had been obtained with atropine methylnitrate (0.5 mg/kg, i.v.). Full blockade of McNeil A-343 usually occurred between 15 - 45 minutes after the atropine administration. In each of the 3 cats used, a large hypotension and bradycardia resulted after icv clonidine, administered during peripheral atropinisation.

Fig. 28 (Trace D) shows pronounced hypotension and bradycardia after icv clonidine in the presence of

peripherally administered atropine. Clonidine (10 µg, icv) reduced the heart rate from 225 to 150 beats/minute A maximum fall in blood pressure of 18/15 mmHg was associated with the bradycardia. The large bradycardia observed after icv clonidine was probably due to atropine methylnitrate inducing very large increases in the resting heart rate.

During the icv infusion of clonidine the cat became restless and it made licking and swallowing motions 2 minutes after the completion of the icv infusion the first pressor effect, observed in Trace D of Fig. 28, was due to the cat retching; 3 minutes later the cat vomited. After these disturbances in the blood pressure and heart rate records, the hypotension and bradycardia can be seen to develop.

6. Effect of peripheral ganglionic blockade by pempidine on the cardiovascular effects of centrally administered clonidine.

The hypotension and bradycardia normally observed after clonidine (10 µg, icv) were absent when clonidine was given 60 minutes after pempidine (7.5 mg/kg i.v.) in 3 cats. At this time, the cardiovascular effects of tetramethylammonium (25 - 100 µg/kg, i.v.) were blocked by the pempidine administration. In the presence of peripheral pempidine, icv clonidine (10 µg) failed to induce vomiting in all 3 cats tested.

DISCUSSION

It was confirmed that in the conscious cat the regular pressor effects observed after intravenous clonidine were due to peripheral α-adrenoceptor stimulation as they were absent after systemic phentolamine pretreatment.

The hypotension and bradycardia observed after i.v. or icv clonidine appeared to be mediated from the central nervous system as these effects were completely inhibited by central pretreatment with small doses of phentolamine. Dollery & Reid (1973) and Schmitt et al. (1973c) respectively showed in the rabbit and cat that the hypotension and bradycardia seen after clonidine are dependant upon the integrity of the central nervous system. However, the exact mechanism by which clonidine produces its centrally mediated cardiovascular depressant effects is still unclear.

Schmitt et al. (1968) suggested that the centrally mediated effects of clonidine were due to an α-sympathomimetic action within the brain. However, Schmitt & Schmitt (1970) found that systemically administered phentolamine and tolazoline were only weak antagonists of the effects of clonidine and phenoxybenzamine was ineffective. They also reported that phentolamine injected into the lateral ventricles of dogs failed to antagonise the central actions of clonidine. Additionally, Schmitt et al. (1973b) found it was difficult to demonstrate an antagonism against clonidine with many other α-adrenoceptor antagonists. However, they did show that piperoxan

and yohimbine, administered either peripherally or centrally, inhibited the depressant effects of clonidine. Schmitt & Schmitt (1970) have suggested that the central α-adrenoceptors involved in the mediation of the hypotension and bradycardia of clonidine may not be identical to the peripherally classified α-adrenoceptors.

Schmitt and co-workers have mainly attempted to inhibit the central actions of clonidine with peripherally administered α-adrenoceptor antagonists. They found it difficult to observe a blockade of clonidine as a large number of the α-adrenoceptor blockers reduced blood pressure and splanchnic discharges themselves after peripheral administration.

The reduction or inhibition of the hypotensive effect of clonidine by peripherally administered phentolamine or phenoxybenzamine (Hoefke & Kobinger, 1966; Kobinger & Walland, 1967a; Nayler et al., 1968) was explained by Schmitt and colleagues as being due to a masking effect produced by a peripheral but not central blockade of α-adrenoceptors. However , Bolme & Fuxe (1971) observed that the blockade of clonidine with peripherally administered phenoxybenzamine occurred after the fall in blood pressure due to phenoxybenzamine had fully recovered to pretreatment levels. They suggested that the blockade of clonidine was due to central and not peripheral inhibition of noradrenaline or α-adrenoceptor sites by phenoxybenzamine.

In the conscious normotensive cat phentolamine (3 mg/kg, i.v.) completely abolished the hypotension and

bradycardia to i.v. clonidine. Phentolamine administered peripherally produced a hypotensive response due to vasodilatation resulting from a direct action on vascular smooth muscle and α-adrenoceptor blockade (Nickerson, 1970a) As it has been reported that the centrally mediated hypotension of clonidine is produced by a reduced sympathetic outflow from the brain to the vasculature (see previous references), then after a full blockade of peripheral α-adrenoceptors the hypotension due to clonidine might be expected to be masked. However, the bradycardia induced by clonidine results both from an increased efferent vagal tone and a decreased efferent sympathetic tone to the heart (see previous references). Hence, if only peripheral α-adrenoceptors were blocked after systemically administered phentolamine then some degree of bradycardia would have been expected after clonidine. In the conscious cat, i.v. phentolamine inhibited both the hypotension and bradycardia of clonidine; i.v. phentolamine also inhibited the cardiovascular depressant effects of α-methyldopa (see Section 2, Chapter 1.) Thus, it would appear that sufficient phentolamine may enter the central nervous system from the periphery to inhibit the central effects of clonidine and α-methyldopa.

The results described in this chapter involving the blockade of the cardiovascular depressant effects of i.v. or icv clonidine by icv phentolamine are in contrast to the findings of Schmitt & Schmitt (1970) using anaesthetised dogs. In their experiments, icv phentolamine

failed to antagonise the central actions of clonidine. However, Kobinger & Walland (1972b, 1973) and Walland, Kobinger & Csongrady (1974) using dogs and Bucher, Buckingham, Finch & Moore (1973) using rats, reported that icv phentolamine prevented all the central actions of clonidine. Schmitt & Schmitt (1970) suggested that the ineffectiveness of icv phentolamine on the clonidine response in dogs was due to the failure of phentolamine to reach the central site of action of clonidine.

Clonidine administered into the lateral ventricles of conscious normotensive cats always produced hypotension and bradycardia. When administered icv to cats in which a centrally mediated hypertension had previously been recorded with icv noradrenaline and adrenaline (see Section 2, Chapter 2, part 4 and Chapter 3, part 3), clonidine still produced hypotension and bradycardia. The pressor effects due to icv noradrenaline and adrenaline, and the hypotension due to icv clonidine, along with the bradycardias observed in the same cats, were inhibited by icv pretreatment with phentolamine.

The central effects of clonidine appear to be relatively non-specific in as much as that vomiting and sedation regularly occurred in the cat. Several other central effects of clonidine, due to its α-adrenoceptor stimulant properties, have been reported (for references see Historical Introduction). However clonidine seems to be able to specifically stimulate brain 'areas' or pathways responsible for the production of hypotension or bradycardia, unlike noradrenaline and adrenaline which are

able to also stimulate pressor 'areas' or pathways.

Since noradrenaline and adrenaline occur naturally within the brain and are probable neurotransmitters it is not surprising that these compounds administered exogenously into the central nervous system are able to produce pressor and depressor effects. However, as clonidine is only able to induce central depressor effects which are blocked by icv phentolamine, as are the depressor and pressor effects of icv noradrenaline and adrenaline, it may be that the central α-adrenoceptors responsible for pressor effects are slightly different from those which produce depressor effects when stimulated.

In cats and dogs, clonidine increases the vagal activity to the heart and also facilitates the vagal reflex bradycardia observed after pressor compounds; these effects are abolished by icv pretreatment with an α-adrenoceptor blocking agent (see previous references). In the conscious cat experiments, pronounced bradycardia due to icv clonidine was still observed after peripheral adrenergic neuron blockade with bethanidine and peripheral β-adrenoceptor blockade with dl-propranolol confirming that an increased efferent vagal activity is an important component in the mediation of the clonidine induced bradycardia.

Share & Melville (1963) reduced the bradycardia due to icv noradrenaline in the cat by vagotomy indicating that other agonists beside clonidine are able to increase the vagal activity from within the brain. In the anaesthetised dog, Bhargava et al. (1972) reported

contrasting results. They completely abolished the bradycardia normally observed after icv noradrenaline, by surgically removing the stellate ganglia; the ganglia contains the sympathetic outflow to the heart. Thus, they suggested that in dogs the bradycardia of icv noradrenaline was totally mediated by a reduced efferent sympathetic outflow to the heart. The mechanism by which noradrenaline and other α-adrenoceptor agonists produce bradycardia was not examined in these experiments using conscious cats.

It is now generally accepted that clonidine produces its centrally mediated hypotension and bradycardia by stimulation of central α-adrenoceptors located in the anterior hypothalamus and probably more importantly in the medulla oblongata. However, the precise central mechanism of action appears to be complicated. Experimental evidence has been presented in recent years implicating an indirect sympathomimetic action for clonidine and also that clonidine may stimulate central presynaptic and postsynaptic α-adrenoceptors.

Intracisternal injections of 6-hydroxydopamine given with the intention of destroying central catecholaminergic neurons abolished the hypotensive effect of clonidine in rabbits (Dollery & Reid, 1973). Additionally the hypotensive action of clonidine has been observed to be antagonised by tricyclic antidepressants in man (Briant, Reid & Dollery, 1973), rabbits (Reid, Briant & Dollery, 1973) and cats (van Spanning & van Zwieten, 1973). These reports indicate that destruction of central adrenergic neurons or inhibition of neuronal uptake prevent the effect

of clonidine and give rise to the possibility of an indirect sympathomimetic action for clonidine. In contrast, Scholtysik & Salzmann (1973) using DOCA/saline hypertensive rats, Hoefke & Warnke-Sachs (1974) using rabbits and Finch (1974) using renal hypertensive cats could not demonstrate an antagonism of the clonidine induced hypotension by desmethylimipramine. In cats severely depleted of noradrenaline by reserpine and α-methyl-p-tyrosine pretreatment Haeusler (1974a,c) observed that clonidine still reduced spontaneous sympathetic nerve activity and also reduced the rise in sympathetic nerve activity elicited after electrical stimulation of the posterior hypothalamus. In these cats, clonidine also induced contraction of the nictitating membranes. Hypotension and bradycardia could not be observed in these cats after clonidine administration as the basal levels were extremely low due to noradrenaline depletion. Kobinger & Pichler (1974) observed that clonidine still induced facilitation of the vagally mediated cardiodepressor reflex to pressor agents in rats pretreated with reserpine and α-methyl-p-tyrosine. Thus, it would appear that clonidine does not act indirectly and would seem highly probable that the interaction between the tricyclic antidepressants and clonidine was mainly due to blockade of central α-adrenoceptors by the former compounds.

A large amount of research has been undertaken to investigate the local feed-back control of stimulation evoked release of noradrenaline from sympathetic nerve fibres. It is suggested that liberated neuronal noradrenaline

acts upon presynaptic α-adrenoceptors thus inhibiting the secretory response to nerve implulses (Häggendal, 1970; Farnebo & Hamberger, 1971a; Starke, 1971, 1972; Enero, Langer, Rothlin & Stefano, 1972).

Low concentrations of clonidine have been found to decrease the stimulation evoked release of noradrenaline from peripheral sympathetic neurons (Werner, Starke & Schümann, 1970; Farnebo & Hamberger, 1971a). Farnebo & Hamberger (1971b) suggested that a similar feed-back mechanism operates in central as well as peripheral noradrenergic neurons. They found that the α-adrenoceptor blocking drugs phenoxybenzamine and phentolamine augment, while clonidine, which stimulates α-adrenoceptors, decreases the stimulation induced overflow of tritium from brain slices loaded with ^{3}H-noradrenaline. Starke & Montel (1973) also found that clonidine reduced the amount of ^{3}H-noradrenaline released by electrical stimulation of rat cerebral cortex slices. Philippu et al. (1974) reported that superfusion of low doses of clonidine enhanced the pressor effect caused by stimulation of the hypothalamus, whilst high concentrations of clonidine tended to reduce the pressor responses to hypothalamic stimulation. Hence they suggested that low doses of clonidine produce enhancement by activating α-adrenoceptors of the hypothalamic area and high doses inhibit the pressor responses by reducing the noradrenaline release via a feed-back mechanism and/or by inhibiting the release of acetylcholine from hypothalamic cholinergic nerve endings (see Section 5, Chapter 2).

From this suggestion of a central negative feed-back system, the hypotension and bradycardia observed after clonidine and other α-adrenoceptor stimulants might be due to reduced liberation of noradrenaline from central noradrenergic nerve endings resulting from presynaptic α-adrenoceptor stimulation. Thus, if clonidine prevented noradrenaline release by presynaptic α-adrenoceptor stimulation then a reduced adrenergic influence within that particular pathway should result. Haeusler (1974c) reasoned that due to noradrenaline depletion with reserpine and α-methyl-p-tyrosine any adrenergic activity approached zero and, thus, could not be further inhibited. Hence, as clonidine was still able to reduce spontaneous sympathetic nerve activity in the splanchnic and renal nerves and also reduced the increased nerve activity produced by hypothalamic stimulation, it was concluded by Haeusler (1974c) that clonidine produces bradycardia and hypotension in vivo by stimulating postsynaptic α-adrenoceptors. Kobinger & Pichler (1974) also concluded that the central cardiovascular effects of clonidine are indepenent of noradrenaline release from central noradrenergic nerve endings.

Haeusler (1973b, 1974b) has recently proposed that the hypotension and bradycardia of clonidine are produced by clonidine activating the central pathway of the baroreceptor reflex. Depending upon the degree of reduced efferent outflow by stimulation of this reflex by clonidine to induce its hypotension and bradycardia, then if it was severe one might expect some degree of orthostatic hypotension to occur. However, clonidine

produces very little, if any, postural hypotension when used clinically (see previous references). Armstrong & Boura (1973) observed that clonidine, in low intravenous doses, inhibited the tachycardia produced by peripheral sympathetic nerve stimulation in the pithed rat; the greatest reduction occurred at low frequencies of nerve stimulation. However, guanethidine inhibited cardiac responses to both low and high rates of stimulation. Armstrong & Boura (1973) suggested that blockade of only the lower rates of adrenergic nerve transmission, both centrally and peripherally, may explain the lack of postural and exertional hypotension observed in clonidine therapy and also why they are commonly observed with guanethidine treatment.

With the recent suggestion of Bolme et al. (1974) that clonidine may produce its cardiovascular depressant effects in the rat by stimulating central adrenaline neurons, previously described by Hökfelt et al. (1973, 1974) as being present in the important regions known to be involved in vasomotor control, the exact mode and site of action of clonidine and other α-adrenoceptor agonists are still in doubt.

Wang & Borison (1950) have shown in the decerebrate cat that there is a bilateral sensory chemoreceptor trigger zone in the reticular formation of the medulla near the fasciculus solitarius lying just below the vagal triangle near the inferior angle of the fourth ventricle Electrical stimulation of this area produced vomiting and

ablation abolished the emetic response to apomorphine and raises the threshold to a number of drugs and metabolic emetic agents to which it is sensitive. It is very probable that clonidine stimulates this area directly to induce its emetic responses. Clonidine may produce emesis due to stimulation of α-adrenoceptors in this area as clonidine induced emesis was never observed after icv phentolamine pretreatment. This finding is in contrast to the report of Finch (1974) who observed clonidine induced vomiting in the conscious renal hypertensive cat after central pretreatment of phentolamine. Finch (1974) also observed that vomiting occurred after pretreatment with haloperidol which is known to antagonise apomorphine induced vomiting (Janssen, Niemegeers & Schellekens, 1965).

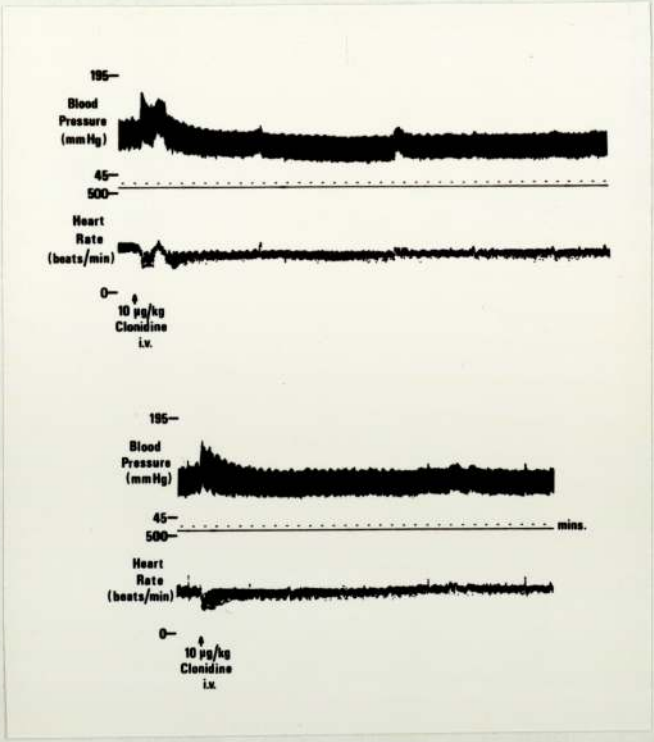


FIG. 26.

Conscious, normotensive, unrestrained cat. Upper trace shows initial transient pressor response with bradycardia followed by prolonged hypotension and bradycardia after clonidine (10 µg/kg, i.v.). Lower trace demonstrates the blockade of the prolonged cardiovascular depressant effect of i.v. clonidine with the initial pressor effect and bradycardia remaining unaffected 30 minutes after icv phentolamine (0.6 mg, icv).

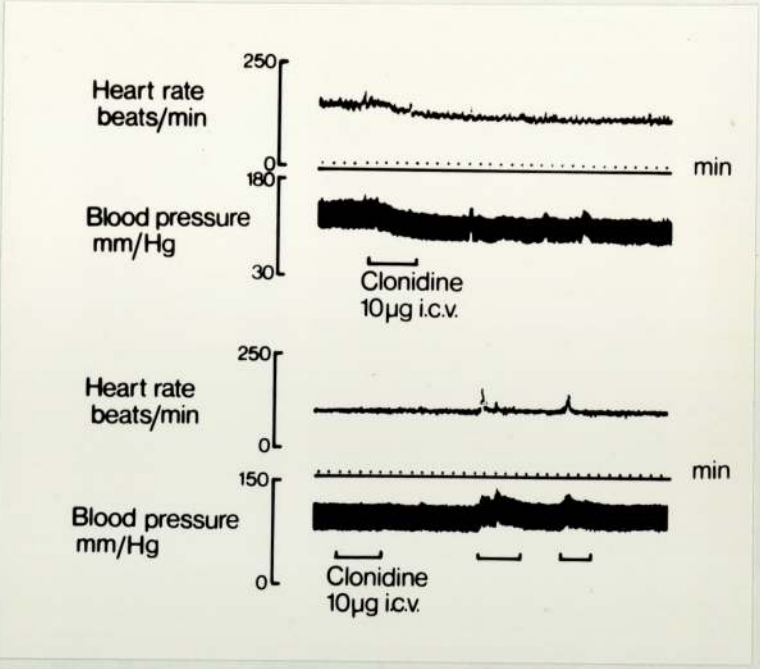


FIG. 27.

Conscious, normotensive, unrestrained cat. Upper trace demonstrates a typical hypotensive response with bradycardia after a 4 minute infusion of clonidine (10 µg, icv). Lower trace shows that both responses to clonidine icv were abolished 60 minutes after phentolamine (0.6 mg, icv). The horizontal bars on the lower trace indicate cardiovascular changes caused by the cat moving.

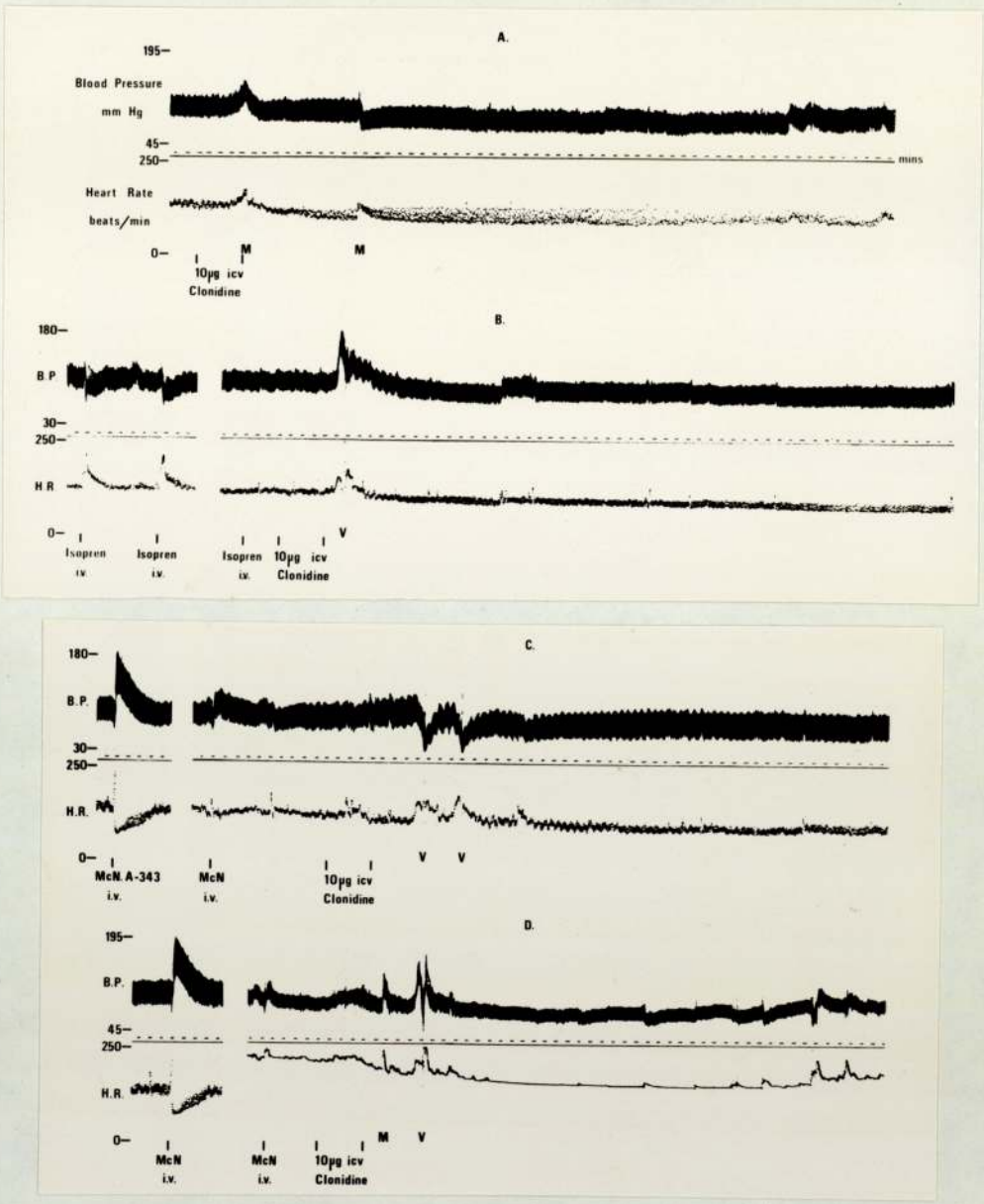


FIG. 28. Conscious, normotensive, unrestrained cat. Trace A. illustrates the control hypotension and bradycardia observed after clonidine (10 µg, icv). Trace B illustrates the response to icv clonidine (10 µg) given 30 minutes after dl-propranolol (1.5 mg/kg, i.v.), a time at which the effects of isoprenaline (250 ng/kg, i.v.) were inhibited. Trace C shows the effect of clonidine (10 µg, icv) given 3 hours after bethanidine (5 mg/kg, i.v.), a time at which the effects of McNeil -A343 (25 µg/kg, i.v.) were greatly inhibited. Trace D shows the effect of clonidine (10 µg, icv) given 30 minutes after atropine methylnitrate (0.5 mg/kg, i.v.), a time at which the effects of McNeil - A343 (25 µg/kg, iv) were almost abolished. Traces B and C demonstrate the vagal component and Trace D the sympathetic component of the clonidine bradycardia. All traces were obtained in the same cat. Cardiovascular effects induced either by the cat vomiting or moving are shown on the Traces by V or M respectively.

TOTAL icv DOSE OF CLONIDINE µg	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s. e. m.		DECREASE IN HEART RATE beats/min ± s.e.m.	TOTAL No. of Observations	No. of cats used.
	SYSTOLIC	DIASTOLIC			
2.5	18.8 ± 2.1	16.0 ± 2.0	15.6 ± 4.3	5	
5.0	21.5 ± 2.6	18.3 ± 2.3	27.4 ± 3.2	5	5
10.0	28.3 ± 1.9	23.7 ± 2.1	35.8 ± 4.7	5	

TABLE 4. Maximal blood pressure and heart rate reductions (mean ± s.e.m.) observed after 3 doses of clonidine administered into the lateral cerebral ventricles of 5 conscious normotensive unrestrained cats.

SECTION 3. A study of responses following central administrations of β-adrenoceptor agonists.

CHAPTER 1.

The cardiovascular effects of icv administrations of β-adrenoceptor agonists and of dopamine in the conscious cat.

Recent evidence favouring the existence of central β-adrenoceptors capable of influencing the peripheral cardiovacular system is fragmentary and somewhat contradictory. Share (1966, 1973) reported that electrical stimulation of the 'vasomotor centre' in anaesthetised cats produced a rise in blood pressure with an associated tachycardia. The pressor response was abolished by icv pretreatment with an α-adrenoceptor antagonist whilst the tachycardia was inhibited by prior icv administration of a β-adrenoceptor antagonist. Garvey, Ram, Woodhouse & Booker (1972), also using anaesthetised cats, found that electrical stimulation of the hypothalamus elicited pressor effects with associated tachycardia and an increase in peripheral sympathetic nerve activity. All of these effects were abolished by central pretreatment with propranolol.

Gagnon & Melville (1966) observed pressor responses after icv injections of low doses of noradrenaline in the anaesthetised cat. Large doses of noradrenaline (icv) produced cardiovascular depressant effects. They suggested that small doses of noradrenaline may have produced the pressor effects by stimulation of central β-adrenoceptors since the responses were abolished by icv pronethalol.

The use of isoprenaline to stimulate

central β-adrenoceptors in dogs, cats and rabbits has led to variable results. The most usual response to icv isoprenaline is tachycardia associated with a fall in blood pressure. These effects have been reported in anaesthetised cats (Gagnon & Melville, 1967), rabbits (Toda et al., 1967) and dogs (Bhargava et al., 1972). Tachycardia in combination with hypotension has been reported after isoprenaline icv to conscious rabbits (Dollery et al., 1973) and in conscious dogs (Conway & Lang, 1974).

Bhargava et al. (1972) concluded that central α-adrenoceptors are concerned with mediating bradycardia and β-adrenoceptors with tachycardia and that both receptors are involved with the production of hypotension.

Schmitt (1970) and Schmitt & Fénard (1971) reported that icv isoprenaline caused mainly reductions in blood pressure, heart rate and splanchnic discharges in anaesthetised cats and dogs. However, hypotension, tachycardia and increased sympathetic nervous activity were occasionally observed after icv isoprenaline but these workers considered this combination of effects to be due to diffusion of isoprenaline to the periphery from the central nervous system.

Day & Roach (1972) reported that pressor responses were often observed in combination with tachycardia after icv isoprenaline in conscious cats. Poyser (personal communication) has noticed both pressor and depressor effects after icv isoprenaline in anaesthetised dogs.

In the present study it was hoped to investigate

more fully the central actions of various β-adrenoceptor agonists in the conscious cat in an attempt to elucidate the role of central β-adrenoceptor influence in cardiovascular control and also to explain why variable results have previously been obtained with icv isoprenaline.

Isoprenaline, the most potent stimulant of peripheral β-adrenoceptors, was mainly used in this study. The effects of adrenaline administered after icv phentolamine were also observed. These 2 β-adrenoceptor agonists are potent stimulators of cardiac, vascular and bronchial β-adrenoceptors. The central actions of salbutamol and isoetharine have also been investigated. These latter compounds are reported to be more active in stimulating β-adrenoceptors in bronchial smooth muscle (β_2-adrenoceptors) than those in cardiac muscle (β_1-adrenoceptors), (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Brittain, Farmer Jack, Martin & Simpson, 1968).

The peripheral cardiovascular actions of dopamine are complicated because the amine has been found to stimulate 3 different receptors; β-adrenoceptors stimulating the heart (McDonald & Goldberg, 1963), α-adrenoceptors causing vasoconstriction (McDonald & Goldberg, 1963; McNay, McDonald & Goldberg, 1966) and dopaminergic receptors causing renal (McDonald, Goldberg & McNay, & Tuttle, 1964) and mesenteric, (Elbe, 1964) vasodilation.

Dopamine is known to be present in the brain and is believed to possess a neurotransmitter function in addition to being a precurser for noradrenaline formation (see short

review by Sourkes, 1971). Because of results obtained after icv administration of dopamine in conscious cats, it has been included in this section and a comparison made between the effects of dopamine and the 4 β-adrenoceptor agonists used.

RESULTS

1. Blood pressure and heart rate effects observed after icv isoprenaline, adrenaline, salbutamol and isoetharine in the conscious cat.

The dose levels used centrally of the agonists were isoprenaline (5 - 60 µg), salbutamol (45,60 µg), isoetharine (75,150 µg) and adrenaline (60,120µg) administered 60 minutes after icv phentolamine.

Isoprenaline was administered icv in 25 cats. In 14 of these cats icv isoprenaline regularly induced dose-related increases in blood pressure whilst in 5 other cats it produced dose-related decreases in blood pressure. 4 cats responded to icv isoprenaline by producing biphasic changes in blood pressure. In 2 cats, icv isoprenaline failed to affect the blood pressure. In all 25 cats, icv isoprenaline produced consistent, dose-related tachycardias. Fig. 29 demonstrates the various types of responses seen after icv isoprenaline.

The single blood pressure changes (either hypotension or hypertension) and the tachycardia after icv isoprenaline had usually reached a peak within 10 minutes from the end of the icv infusion. The blood pressure and heart rate had returned to control levels within 30 - 60 minutes of the

icv administration. The central responses to icv adrenaline were also quick in onset and had subsided within 60 minutes. A typical pressor response due to icv adrenaline (60 µg) administered 60 minutes after icv phentolamine (0.6 mg) is shown in Fig. 30.

In cats that produced pressor effects with icv isoprenaline, rises in blood pressure were also observed after icv salbutamol, isoetharine and adrenaline (see Table 5.)

The responses due to icv salbutamol took approximately 10 - 20 minutes to reach a maximum and control resting levels of blood pressure and heart rate were not attained until 2 - 3 hours after icv administration. The time course of the icv isoetharine response was similar to that observed for salbutamol.

The systolic blood pressure was increased to a greater extent than the diastolic blood pressure by all 4 β-adrenoceptor agonists administered icv. (see table 5) The differences in the effects upon systolic and diastolic pressure can be seen with the larger icv doses of the agonists.

From Table 5, similar blood pressure increases were obtained after icv isoprenaline 15 µg, salbutamol 45 µg and isoetharine 75 µg (i.e. a dose ratio of 1:3:5). Adrenaline was seen to be equipotent with salbutamol with respect to the blood pressure responses obtained at the 60 µg dose level. The order of potency with respect to the tachycardia produced after icv administration was

isoprenaline > salbutamol > adrenaline > isoetharine.

Table 6 summarises the blood pressure decreases and tachycardias obtained with icv isoprenaline in 5 conscious cats. The time course of the blood pressure depressant effects was similar to that described for the pressor responses of icv isoprenaline. Salbutamol was administered icv to 3 cats in which consistent depressor responses had been observed with icv isoprenaline. Salbutamol produced depressor effects in 2 of the 3 cats. The depressant effects are summarised in Table 6. In the third cat, however, salbutamol, unlike isoprenaline, produced a pressor response in combination with tachycardia.

Except for the average blood pressure decreases seen after 15 µg of icv isoprenaline, the diastolic pressure was reduced by a larger degree than the systolic pressure with isoprenaline (30, 60 µg) and with salbutamol (45, 60µg).

By comparing Table 5 with Table 6 it can be seen that the magnitude of the tachycardias which accompanied either hypertension or hypotension, after icv isoprenaline and salbutamol, was similar.

Salbutamol (60 µg, icv) was administered to one cat which was previously shown to be unresponsive to isoprenaline (30 µg, icv) with regard to blood pressure changes. In this cat, salbutamol was also virtually without effect on the blood pressure, but like isoprenaline caused a marked tachycardia (see Fig. 31).

2. Cardiovascular effects of icv dopamine in the conscious cat.

Dopamine (30,45 µg) was administered icv to 10 cats. In 6 cats dopamine (30,45 µg, icv) produced increases in blood pressure and heart rate. The mean results obtained in these cats are summarised in Table 7. The time course of the dopamine responses was similar to that of icv isoprenaline. The maximum effect was observed within 10 minutes and had terminated approximately 30 - 45 minutes after the end of the icv infusion.

Comparing the central effects of dopamine with those of the other β-adrenoceptor agonists (see Tables 5 and 7), it can be seen that dopamine was more potent than the β-agonists in raising the blood pressure, (especially the diastolic pressure) but it was much less effective in causing tachycardia. Figs. 33 and 34 demonstrate typical responses to icv dopamine.

Of the 6 cats that responded to icv dopamine with pressor effects, 2 cats were from the group of 5 cats that produced depressor effects after icv isoprenaline.

In the remaining 4 cats, of the group of 10 in this series, icv dopamine (30, 45 µg) produced an initial rise in blood pressure and heart rate followed by hypotension and bradycardia. The pressor effect and tachycardia were usually observed to have subsided after approximately 30 minutes, after which the blood pressure and heart rate progressively decreased below pre-dose control levels to produce marked hypotension and bradycardia.

The peak falls occurred between 45 - 60 minutes after the icv infusion and the pre-dose resting levels of blood pressure and heart rate were obtained within 1.5 hours of the dopamine administration. Table 8 summarises the initial maximal hypertensive and tachycardic effects followed by the hypotension and bradycardia observed after icv dopamine. Fig. 37 demonstrates a biphasic cardiovascular response seen after icv dopamine (45 µg).

L-α-methyldopamine (30, 45 µg) was administered centrally to 3 cats; 2 of which produced biphasic responses and the third which produced pressor effects and tachycardia after icv dopamine. Icv administration of l-α-methyldopamine induced very similar cardiovascular effects, in all 3 cats as normally observed for dopamine. The duration of the l-α-methyldopamine responses was slightly longer than those with icv dopamine. The duration of the responses with l-α-methyldopamine was extended by approximately 15-20 minutes as compared with the dopamine responses.

3. Effect of central and peripheral β-adrenoceptor blockade on the centrally mediated cardiovascular responses of β-adrenoceptor stimulants and dopamine.

The effect of 7 β-adrenoceptor antagonists administered icv were tested on the icv responses of the β-adrenoceptor agonists and dopamine. The β-adrenoceptor antagonists used icv were dl-propranolol, dl-alprenolol, oxprenolol, pindolol, practolol, sotalol and tenormin (I.C.I. 66082). All the β-adrenoceptor blocking agents used icv completely abolished or reduced the centrally

mediated effects of icv isoprenaline, salbutamol, adrenaline and isoetharine. Table 10b (Section 4) summarises the effectiveness of the 7 blocking agents administered centrally on the tachycardia response produced by icv isoprenaline.

It was found that dl-propranolol and dl-alprenolol were the most effective β-adrenoceptor blocking agents and in the majority of experiments either dl-propranolol or dl-alprenolol have been used. In each of the 25 cats treated with icv isoprenaline, the effect of either dl-propranolol or dl-alprenolol (0.5 - 1.0 mg) was investigated. The effects of central β-adrenoceptor blockade produced by dl- propranolol or dl-alprenolol was investigated in every cat tested with icv salbutamol (5) isoetharine (4) adrenaline (8) and dopamine (10).

All blood pressure effects, including pressor, depressor and biphasic responses, and the tachycardias observed after isoprenaline and salbutamol were reduced or abolished by icv dl-propranolol or dl-alprenolol (0.5 - 1.0 mg) administered 30-60 minutes before the agonist. Complete inhibition of the cardiovascular effects produced by the 5 agonists administered icv could be achieved depending upon the central dosage used of icv dl-propranolol and dl-alprenolol. Within a particular cat, if 0.5 mg dose of dl-propranolol was found not to completely antagonise the effect of icv isoprenaline then total inhibition of isoprenaline was obtained by increasing the dose to 1.0 mg. Since total abolition of the blood pressure and heart rate effects of the agonists given icv were always obtained with

doses of dl-propranolol or dl-alprenolol up to 1.0 mg, the icv doses of the β-adrenoceptor blockers were never increased above 1.0 mg in any cat.

Fig. 32 illustrates a typical experiment in which the pressor response and tachycardia due to isoprenaline (30 μg, icv) was totally inhibited by pretreatment with dl-propranolol administered 30 minutes previously.

The pressor effects and tachycardia observed after icv dopamine were also inhibited by icv dl-propranolol An experiment in which the hypertension and tachycardia to icv dopamine (30 μg)was inhibited by icv dl-propranolol (0.75mg) is demonstrated in Fig. 33. Fig. 34 also illustrates the abolition of the central effects of dopamine by icv dl-alprenolol (0.75 mg).

In the group of 4 cats, that produced biphasic cardiovascular effects to icv dopamine, central β-adrenoceptor blockade abolished the initial stimulant effects but did not affect the secondary hypotension and bradycardia.

The hypertension and tachycardia observed after icv salbutamol and isoetharine were slightly more difficult to completely inhibit possibly due to their persistent effects. However, it was found that icv dl-propranolol or dl-alprenolol (1.0mg) administered 30 minutes before the β-adrenoceptor agonists usually totally abolished the icv effects of these agonists. Icv pretreatment with the $\beta_1$adrenoceptor blocking agents practolol (4.0mg) and ICI 66082 (2.5 mg) given 45 - 60 minutes before either icv salbutamol (45 μg) or isoetharine (75 μg) reduced the centrally

mediated pressor effects and tachycardias due to the peripherally classified β_2-adrenoceptor stimulants in each of 2 conscious normotensive cats.

The active β-adrenoceptor blocking form of propranolol and alprenolol is their l-isomers; the d-isomeric forms of each antagonist possesses little β-adrenoceptor blocking activity but are of similar local anaesthetic potency to the l-isomers (Fitzgerald, 1969). Icv l-propranolol (0.25 - 0.5 mg) was found to produce a similar inhibitory effect on responses to icv β-adrenoceptor agonists as twice the dose of the racemates. However, d-propranolol (0.25 - 0.5 mg, icv) was completely ineffective. D-alprenolol (0.25 - 0.75 mg) did not affect the responses to icv isoprenaline or dopamine. Fig. 34 demonstrates the lack of blocking activity of d-alprenolol (0.75 mg) on the central effects of icv dopamine (30 µg) compared to the total inhibition of dopamine achieved by the same icv dose of dl-alprenolol.

Sotalol (2.0mg), practolol (2.0-4.0 mg) and tenormin (1.0 - 2.5 mg), β-adrenoceptor blocking drugs with little or no local anaesthetic activity, markedly reduced the blood pressure and heart rate increases induced by isoprenaline (30µg, icv) whilst the local anaesthetics procaine and lignocaine given icv were ineffective.

In all conscious cats used in these experiments, isoprenaline and dopamine were infused icv 60 minutes before and after icv administration of 100 µl of sterile 0.9% $^{w}/_{v}$ sodium chloride solution. It was found that the 2 centrally mediated responses of the agonists were almost

identical, thus demonstrating that the icv effects of these compounds were readily repeatable during the course of the experiments. In cats that responded biphasically to icv dopamine, the sodium chloride solution (100 µl) was infused icv after the hypotension and bradycardia had returned to control normotensive levels. Dopamine was repeated centrally 60 minutes after the saline. The biphasic effects of dopamine in these cats were found to be readily repeatable.

Hypotension and tachycardia observed after isoprenaline (200 - 400 ng/kg, i.v.) were unaltered by the icv administration of β-adrenoceptor antagonists indicating that there was little or no leakage of the blockers from the brain and the blocking action of these compounds on the agonists occurred within the central nervous system.

Fig. 33 shows that the peripheral effects of dopamine (30 µg/kg, i.v.) were unaffected by icv dl-propranolol (0.75 mg) at the time of the maximum inhibition of icv dopamine (30 µg). In fact, the reflex bradycardia to dopamine (30 µg/kg, i.v.) was more pronounced when administered after icv dl-propranolol.

Dl-propranolol (1.0 - 1.5 mg/kg, i.v.) administered 30 minutes before isoprenaline (30 µg, icv), a time when abolition of isoprenaline (200 - 400 ng/kg, i.v.) occurred, completely inhibited the centrally mediated blood pressure and heart rate effects of isoprenaline. I.v. dl-propranolol inhibited both the pressor and depressor responses seen after icv isoprenaline. Peripherally administered dl-propranolol caused a usual fall of about 15-20 beats/minute

in the resting heart rate but was invariably without effect on the resting blood pressure.

Practolol, a β-adrenoceptor antagonist with a specificity for cardiac β_1-adrenoceptors, was only tested in 3 cats that produced pressor effects after icv isoprenaline Practolol (5.0 - 7.5 mg/kg, i.v.) produced, like dl-propranolol a marked bradycardia that was initially associated with a small hypertension. The blood pressure quickly returned to normotensive levels and occasionally it was observed to fall slightly below resting levels; the maximum fall in mean blood pressure was 9 mmHg. The pressor effects and tachycardias observed after icv isoprenaline were inhibited by i.v. practolol.

4. Effect of central α-adrenoceptor blockade on the centrally mediated cardiovascular responses to β-adrenoceptor stimulants and dopamine.

Phentolamine (0.5 - 0.75 mg, icv) did not alter the blood pressure and heart rate changes induced by icv isoprenaline, isoetharine and salbutamol. Phentolamine icv did not alter the pressor or depressor effects obtained in the relevant cats with icv isoprenaline (30 μg).

Fig. 35 demonstrates that the hypotension produced after icv isoprenaline was induced by a different mechanism than those obtained with the α-adrenoceptor agonists, as it was unaffected by icv phentolamine pretreatment. Fig. 35 shows 2 responses to icv isoprenaline; the first was the control response to icv isoprenaline and the second response was obtained 60 minutes after icv phentolamine (0.75mg).

The β-adrenoceptor stimulant effects of adrenaline were observed after icv phentolamine pretreatment. As described earlier, icv phentolamine given in excessive doses appeared to produce toxic side effects. Therefore, it was decided not to investigate the effects of adrenaline after a second icv dose of phentolamine.

The pressor effects and tachycardias observed after icv dopamine (30,45 µg) were not reduced by icv phentolamine. However, in the 4 cats that responded biphasically to icv dopamine, phentolamine (0.5 - 0.75 mg, icv) prevented the onset of the hypotension and bradycardia normally seen after icv dopamine but did not affect the initial cardiovascular stimulant effects.

5. Effect of central dopamine receptor blockade on the centrally mediated cardiovascular responses of isoprenaline and dopamine.

Haloperidol and pimozide were used to inhibit central dopamine receptors. Haloperidol is less specific than pimozide and is known to block both noradrenergic and dopaminergic receptors in the central nervous systems of rats (Carlsson & Lindqvist, 1963), cats and dogs (Janssen, 1967). However, pimozide has been shown to block only central dopamine receptors in cats and dogs (Janssen, 1967).

The effects of these blockers (given either i.v. or icv) were investigated in 2 cats which responded with pressor effects and tachycardias to icv dopamine and in 2 cats that responded biphasically to icv dopamine.

Haloperidol (400 µg, icv) completely abolished

all the centrally mediated effects of icv dopamine in all 4 cats tested; i.e. both the initial stimulant and secondary depressant effects were inhibited by haloperidol. Fig. 36 illustrates an experiment in which the pressor effect and tachycardia due to icv dopamine (45 μg) were inhibited by haloperidol (400 μg, icv). The upper record (trace A) illustrates the control response to icv dopamine (45 μg). Icv haloperidol (400 μg) produced a large hypertension and tachycardia which were more prolonged than the responses to icv dopamine. The blood pressure had returned to control level within 60 minutes of the icv haloperidol infusion. Trace C shows the almost total abolition of the cardiovascular effects of icv dopamine infused 60 minutes after the haloperidol.

Haloperidol was dissolved in lactic acid and the volume made up with sterile water for injections to provide the correct concentration. The pH of the solution was adjusted to pH 4.8 with 0.1 N sodium hydroxide solution (added before making up to volume with sterile water). 100 μl of the injection vehicle alone produced a small pressor effect and tachycardia (see trace D, Fig. 36). Thus, the pressor response seen after icv haloperidol was mainly due to the antagonist and not to the vehicle. The haloperidol vehicle solution did not alter the response to icv dopamine.

Initially, a 0.5 mg dose of haloperidol was administered centrally to the first cat used in this series of experiments and convulsions resulted. Consequently the icv dosage of haloperidol was never increased above 400 μg.

Systemically administered haloperidol (1.0mg/kg, i.v.) produced identical effects to those seen after icv administration. A small hypertension and tachycardia were observed immediately after the i.v. injection. These effects were of a short duration. The blood pressure and heart rate had returned to normal resting levels after 60 minutes. At this time, both phases of the blood pressure and heart rate responses to dopamine were abolished.

Since i.v. haloperidol pretreatment was as effective in blocking the icv responses of dopamine as central administration, only the effects of i.v. haloperidol were tested on the responses to icv isoprenaline. In 3 cats that produced pressor effects and in 2 cats that produced depressor effects after icv isoprenaline, haloperidol (1.0mg/kg, i.v.) did not alter the responses to icv isoprenaline, in any of the 5 cats, when infused 60 minutes after the haloperidol.

Pimozide (100 - 200 µg, icv) completely abolished the increases in blood pressure and heart rate in 2 cats when given 60 minutes before icv dopamine (30, 45 µg). In 2 other cats, pimozide icv inhibited the production of the initial hypertension and tachycardias but failed to affect the secondary hypotension and bradycardias normally observed after icv dopamine in these cats. Pimozide was more effective than haloperidol in inhibiting the cardiovascular stimulant effects of icv dopamine.

Icv pimozide induced small initial rises in blood pressure and heart rate but these were of much shorter duration than those for haloperidol. Normal resting

levels were returned within 10 minutes of the central pimozide administration. Pimozide was dissolved in 0.1% w/v tartaric acid and was made to the correct volume with sterile water for injection. As with haloperidol, the pH of the solution was adjusted with 0.1N sodium hydroxide solution. The final pH of the pimozide solution was pH 5·5. 100 µl of the vehicle solution was infused icv and produced no effect on the blood pressure and heart rate or on the responses to icv dopamine.

Pimozide administered peripherally (1.0mg/kg, i.v.) was as effective in blocking the stimulant effects of icv dopamine as it was when administered icv. Fig. 37 demonstrates the abolition of the pressor response and tachycardia after icv dopamine (45 µg) and also the lack of effect on the latter hypotension and bradycardia.

As with haloperidol, pimozide (1.0mg/kg, i.v.) did not diminish the responses to icv isoprenaline (30 µg) in the same 5 cats used for the haloperidol experiments. In a sixth cat in which isoprenaline (30 µg, icv) produced a tachycardia with little effect on blood pressure, isoprenaline infused centrally 45 minutes after pimozide (1.0mg/kg, i.v.) produced a tachycardia associated with an increased systolic pressure and a decreased diastolic pressure. This experiment is illustrated in Fig. 38.

6. Effect of dopamine β-hydroxylase inhibition on the centrally mediated cardiovascular effects of dopamine.

The group of 4 cats that responded to icv dopamine with biphasic cardiovascular effects were pretreated

with disulfiram for 3 days. On the first day of treatment, the cats received 2 x 100 mg/kg doses of disulfiram administered i.p. The 2 doses of disulfiram were separated by 12 hours. On the second and third days of treatment the cats received one dose only of disulfiram (100 mg/kg i.p.) Disulfiram produced no effect on the control resting blood pressures and heart rates of these 4 normotensive cats.

Dopamine (45 µg, icv) was administered 4 hours after the disulfiram dosage on the third day and was found to still induce hypertension and tachycardia in all 4 cats. The stimulant effects were slightly larger and of longer duration than the control responses obtained before disulfiram pretreatment. The blood pressure and heart rate increases did not develop into depressor and bradycardic effects as in control experiments. Thus, it would appear likely that the depressant effects observed after icv dopamine were due to central noradrenaline formation.

L-α-methyldopamine was administered icv, to one disulfiram pretreated cat, after termination of the central dopamine effects had been observed. As with dopamine, l-α-methyldopamine produced only increases in blood pressure and heart rate. L-α-methyldopamine (30, 45 µg, icv) has been previously shown to induce similar central effects to dopamine when given in the same cat. Thus, it would appear likely that the latter secondary hypotension and bradycardia observed in earlier control experiments (see part 2 of this chapter) are due to the central formation of l-α-methylnoradrenaline.

7. Effect of α-adrenoceptor agonist administration on the centrally mediated cardiovascular effects of isoprenaline

Clonidine was chosen for these experiments since it has already been shown to be the most potent centrally acting α-adrenoceptor agonist and it also produces very prolonged cardiovascular depressant effects. The effect of icv clonidine upon the icv isoprenaline response was investigated in 4 cats.

Initially, control responses were obtained to isoprenaline (30 μg, icv). After completion of the control response, 100 μl was infused icv containing isoprenaline (30 μg) and clonidine (10 μg). However, this procedure proved to be unsuccessful in producing a reduction in the icv isoprenaline response. As can be seen from previous figures the onset of the central isoprenaline response was very much more intense and immediate than observed for clonidine.

By infusing isoprenaline and clonidine together a rapid tachycardia was still observed. However, the duration of the response was shorter than seen with control isoprenaline responses.

The icv responses of isoprenaline (30 μg) were reduced in all 4 cats when isoprenaline was infused 30 minutes after icv clonidine (10 μg); 3 of the 4 cats used produced pressor effects with icv isoprenaline (30 μg). Fig. 39 illustrates icv isoprenaline (30 μg) before and 30 minutes after icv clonidine (10 μg). It can be seen that the control response to icv isoprenaline involved an 85 beat/minute

tachycardia and a $^{36}/_{21}$ mmHg hypertension. The response was completed within 30 minutes from the start of the icv infusion. When repeated 30 minutes after clonidine (10 µg icv) icv isoprenaline produced a smaller tachycardia of 55 beats/minute; only the systolic blood pressure was observed to rise (an increase of 18 mmHg was recorded). From trace B, the tachycardia was slower in onset than the control response and was of a 'flatter' nature. The heart rate and blood pressure response of the icv isoprenaline was slightly shorter in the presence of clonidine (10 µg, icv) than the control response.

Isoprenaline icv produced hypotension with associated tachycardia in the fourth cat. When administered 30 minutes after clonidine (10 µg, icv), isoprenaline produced a smaller tachycardia but the hypotension was relatively unchanged.

8. Effect of peripheral ganglion blockade on the centrally mediated responses to the β-adrenoceptor stimulants and dopamine.

In each of 6 cats, the pressor responses produced by icv administration of the 4 β-adrenoceptor stimulants and of dopamine were abolished after ganglion blockade with pempidine (5.0 - 7.5 mg/kg, i.v.) or hexamethonium (5.0 - 10.0 mg/kg, i.v.) The agonists were infused icv 30 to 60 minutes after the ganglion blocking agent. The exact time in each experiment was determined by assessing the degree of ganglion blockade by the reduction in the pressor responses to tetramethylammonium (25 - 100 µg/kg, i.v.)

Figs. 40 and 41 demonstrate the inhibition of the hypertension and tachycardias observed after icv isoprenaline (30 µg) and dopamine (45 µg) by pempidine (5.0 mg/kg, i.v.) and hexamethonium (10 mg/kg, i.v.) respectively.

In 3 newly cannulated cats, in which icv isoprenaline produced hypotension and tachycardia, these effects were also abolished by hexamethonium (5.0 - 10.0 mg/kg, i.v.)

Hexamethonium was preferred to pempidine in these experiments since it produced a quicker complete ganglion blockade than pempidine and also the recovery from the ganglion blockade was quicker for hexamethonium. The side effects such as relaxed nictitating membranes, mydriasis and cycloplegia were still observed 48 hours after i.v. pempidine, whereas these side effects were rarely seen 24 hours after hexamethonium administration.

9. Effect of peripheral and central administration of adrenergic neuron blocking agents on the centrally mediated responses of isoprenaline and dopamine.

This series of experiments was performed to evaluate the role of the peripheral sympathetic nervous system in mediating the central effects of icv isoprenaline and dopamine. The adrenergic neuron blocking agents bethanidine and guanethidine were used.

Bethanidine (5 mg/kg, i.v.) administered 2 - 5 hours before isoprenaline (30 µg, icv) completely abolished the centrally mediated effects of the β-adrenoceptor agonist.

Isoprenaline was administered centrally after abolition of the cardiovascular effects of McNeil A-343 (25 - 50 µg/kg i.v.) by bethanidine had been obtained. Both depressor and pressor effects observed in combination with tachycardia due to icv isoprenaline were inhibited by i.v. bethanidine.

The cardiovascular effects observed after dopamine (30, 45 µg) were abolished by i.v. bethanidine pretreatment. Fig. 42 shows an experiment in which the pressor effect and tachycardia observed after icv dopamine (45 µg) were completely inhibited by bethanidine (5.0 mg/kg, i.v.) administered 2 hours before the icv dopamine.

Guanethidine (10.0 mg/kg, i.v.) administered 6 hours before either isoprenaline (30 µg) or dopamine (45 µg) icv inhibited the centrally mediated effects of the two agonists.

The effect of icv bethanidine or guanethidine (0.5 mg) on the responses obtained to icv isoprenaline (30 µg) were investigated in 8 cats. In 4 of these cats icv isoprenaline produced pressor effects in association with tachycardias. 3 cats produced depressor effects with tachycardias after icv isoprenaline and in the remaining cat isoprenaline produced an initial short lasting hypertension followed by a depressor response.

Bethanidine and guanethidine (0.5 mg, icv) altered the pressor effect due to icv isoprenaline in all 4 cats. In one cat, the pressor effect of isoprenaline (30 µg, icv) was changed by icv bethanidine and guanethidine to an initial depressor effect followed by a rise in blood

pressure. In a second cat, the pressor response of icv isoprenaline was reduced by 75% and 80% by icv bethanidine and guanethidine respectively. In the third cat, the control pressor effect of icv isoprenaline consisted of a raised systolic blood pressure with the diastolic pressure remaining unchanged. Pretreatment with each adrenergic neuron blocking agent centrally caused isoprenaline to produce a decreased diastolic pressure with little effect on the systolic pressure. In the fourth cat, isoprenaline produced a large increase in systolic blood pressure (25 mmHg) in combination with a small decrease in the diastolic blood pressure (9mmHg) (See Fig. 43a). Bethanidine (0.5mg, icv) caused the systolic pressure to be decreased by 9mmHg and a larger decrease in the diastolic pressure (18mmHg) was observed after icv isoprenaline. Guanethidine induced the same changes in the icv isoprenaline response. As can be seen from Fig. 43a, pretreatment centrally with bethanidine did not alter the tachycardia due to icv isoprenaline. This was observed after icv isoprenaline in all the 8 cats pretreated with icv adrenergic neuron blockers.

In 2 of the 3 cats that responded to icv isoprenaline with depressor effects icv pretreatment with bethanidine or guanethidine caused the hypotension to be slightly enhanced while the tachycardias due to icv isoprenaline were unaltered. In the third cat, the blood pressure and heart rate effects due to icv isoprenaline were unchanged after icv administration of the adrenergic neuron blocking agents.

Isoprenaline, in the eighth cat used in this

series of experiments, produced an initial hypertension followed by a relatively prolonged fall in blood pressure. 60 minutes after either icv guanethidine or bethanidine (0.5 mg) the pressor component was totally absent but the hypotension remained unaltered (Fig. 43b).

The effect of icv guanethidine and bethanidine on the icv responses to dopamine was investigated in 2 cats; both of which reacted to icv dopamine with pressor effects associated with slight increases in heart rate. In one of these cats, icv isoprenaline induced a depressor and in the other a pressor effect. In the cat in which isoprenaline normally produced depressor effects, the pressor effects induced by icv dopamine remained relatively unaltered after icv guanethidine or bethanidine (0.5 mg). In the other cat the pressor effect observed after icv dopamine (45 µg) was potentiated both in magnitude and in duration by icv guanethidine (see Fig. 44) and bethanidine. Fig. 44 shows that dopamine icv produced a $^{21}/_{15}$ mmHg hypertension. A 25 beat/minute tachycardia was observed in combination with the hypertension. The response was completed within 30 minutes of the start of the icv infusion. Repeated icv dopamine administration 60 minutes after icv guanethidine (0.5 mg) produced a $^{40}/_{20}$ mmHg hypertension which returned to pretreatment levels after 80 minutes. At the time of the second icv dopamine administration the blood pressure was raised from $^{126}/_{90}$ to $^{205}/_{128}$ mmHg by icv guanethidine (0.5 mg).

Within a particular cat, guanethidine and bethanidine administered centrally produced similar changes in

blood pressure and heart rate. In 2 of the 8 cats treated with icv bethanidine or guanethidine, increases in blood pressure were observed associated with little or no changes in the heart rate (see Figs. 43aand 44). Pressor responses were observed in combination with tachycardias in a further 2 cats and in the remaining 4 cats, icv administration of the adrenergic neuron blocking agents produced variable cardiovascular effects. The variable responses obtained were a small hypertension associated with a very large tachycardia, a hypotension and bradycardia followed by a small rise in blood pressure and heart rate, a hypotension and bradycardia observed alone, and in only one cat did icv guanethidine or bethanidine produce no significant cardiovascular effects .

10. Effect of peripherally administered atropine methylnitrate on the centrally mediated cardiovascular responses of isoprenaline.

The effect of atropine methylnitrate (0.25 - 0.5 mg/kg, i.v.) was tested on 9 occasions with 4 cats, each of which regularly responded with pressor responses to icv isoprenaline (30 µg). 30 minutes after atropine methylnitrate, the icv isoprenaline mean tachycardia was increased from 80.3 $\pm$ 2.9 to 139.6 $\pm$ 4.3 beats/minute, the mean systolic blood pressure from 25.1 $\pm$ 1.2 to 32.3 $\pm$ 1.0 mmHg and the mean diastolic blood pressure from 14.2 $\pm$ 0.8 to 16.9 $\pm$ 1.1 mmHg.

Atropine methylnitrate was administered systemically to one cat in which icv isoprenaline had previously produced a tachycardia with little effect on blood pressure, This experiment is shown in Fig. 45. The control response

to icv isoprenaline consisted of a 95 beats/minute tachycardia. Isoprenaline (30 µg, icv) repeated 30 minutes after atropine methylnitrate (0.25 mg/kg, i.v.) produced a tachycardia of 125 beats/minute and a large hypertension.

The effect of peripheral atropinisation was tested on one occasion in each of 3 cats that produced regular hypotensive effects after icv isoprenaline (30 µg). In all 3 cats, the tachycardia due to icv isoprenaline was potentiated. However, in 2 of the 3 cats the falls in blood pressure due to icv isoprenaline remained unaltered after atropine methylnitrate (0.5 mg/kg, i.v.) The hypotension was potentiated in the presence of atropine in the third cat. The reduction in diastolic blood pressure was enhanced from -15 to -23 mmHg.

DISCUSSION

The results described in this chapter indicate that the central cardiovascular actions of β-adrenoceptor agonists are complicated. The blood pressure responses were variable within the population of 25 cats used. Variations consisted of pressor responses produced in 14 cats, depressor responses in 5 cats, biphasic responses in 4 cats and a lack of blood pressure effects observed in 2 cats. Under constant experimental conditions the type of blood pressure response due to icv β-adrenoceptor agonist administration was constant within a particular cat.

The pronounced tachycardia observed after icv isoprenaline, salbutamol, isoetharine and adrenaline (after icv phentolamine) was the only consistent response observed

in all 25 cats used. The tachycardia produced after icv isoprenaline in these experiments using conscious cats is in agreement with all the workers cited in the introduction with the exception of Schmitt (1970) and Schmitt & Fénard (1971). They found that the bradycardia due to icv isoprenaline was mainly accompanied by hypotension. Although hypotensive effects were observed, in the majority of conscious cats used a rise in blood pressure (mainly of systolic pressure) was observed. It is difficult to explain why these workers have not observed pressor effects after icv isoprenaline.

Poyser (personal communication) has occasionally observed pressor effects after icv isoprenaline in anaesthetised dogs. Toda et al.(1969) reported pressor effects after icv adrenaline in conscious rabbits but in anaesthetised rabbits it produced depressor effects. They did not attempt to investigate whether the responses observed after icv adrenaline were due to α-or β-adrenoceptor stimulation. The effects of species difference and anaesthesia do not appear to be responsible for the varying results obtained with icv β-adrenoceptor stimulants. These 2 parameters on the icv responses to icv β-agonists are discussed more fully in Chapters 3 and 4 of this section.

All the cardiovascular effects produced by central administration of β-adrenoceptor stimulants and dopamine are centrally mediated since they are inhibited by prior peripheral ganglionic blockade (with pempidine or hexamethonium) and by small doses of β-adrenoceptor antag-

onists administered centrally. Gagnon & Melville (1967) and Bhargava et al. (1972) abolished the effects of icv isoprenaline in anaesthetised cats and dogs by transection of the spinal cord at the C2 level, thus demonstrating that these responses are nervously mediated from the brain.

The centrally mediated effects (pressor, depressor effects and tachycardias) of icv isoprenaline and dopamine were mediated through the sympathetic nervous system as they were abolished by peripheral adrenergic neuron blockade with i.v. bethanidine or guanethidine. Unlike the bradycardia being partially produced by an increased efferent vagal tone after icv administrations of α-adrenoceptor agonists, the vagus appeared not to contribute to the tachycardia obtained after icv β-adrenoceptor agonists and dopamine as the tachycardia was completely absent after adrenergic neuron blockade.

Further indication of the involvement of the peripheral sympathetic nervous system in mediation of the central effects of β-adrenoceptor agonists is that systemic atropine methylnitrate greatly potentiated the pressor and tachycardic effects of icv isoprenaline. The depressor effects occasionally seen after icv isoprenaline were unchanged by peripheral atropinisation in 2 conscious cats and were potentiated in a third cat. Gagnon & Melville (1967) observed that the tachycardia induced by icv isoprenaline was significantly greater in vagotomised cats than in non-vagotomised cats, although in contrast, the depressor effect was smaller in the vagotomised cats than in the intact cats.

In the cats that responded to icv isoprenaline with pressor effects, it seems likely that the raised pressure was mainly due to an increased sympathetic outflow to the heart as the systolic pressures were raised to a greater extent than the diastolic pressures (see Table 5).

It is difficult to explain exactly how isoprenaline actually causes a centrally mediated hypotension. By using surgical procedures to interrupt efferent sympathetic nervous pathways, Bhargava et al. (1972) reported that the hypotension due to icv isoprenaline in the anaesthetised dog was unaffected by stellate ganglionectomy but was abolished by spinal section at C2. Using anaesthetised cats, Gagnon & Melville (1967) also abolished the hypotension due to icv isoprenaline by spinal C2 section. Bhargava et al. (1972) also observed that the hypotension due to central α-adrenoceptor stimulation with icv noradrenaline was absent after transection of the cervical cord. Thus, they suggested that the hypotension due to icv noradrenaline and isoprenaline was mediated by similar efferent pathways and that an inhibition of sympathetic vasomotor tone in the lower half of the body played a prominent role in the hypotension produced by these 2 compounds.

In the conscious cat, the icv isoprenaline induced hypotension was not produced as a result of a selective stimulation of sympathetic cholinergic vasodilator fibres as this procedure is usually accompanied with a rise in blood pressure and at some time a defence and

behavioural reaction normally occurs. However, behavioural effects were never seen at any time after icv isoprenaline. In addition, when icv isoprenaline was infused after i.v. atropine, the fall in blood pressure was unchanged in 2 cats and potentiated in another.

Hence, the hypotension observed occasionally in conscious cats after icv isoprenaline may be due to a direct selective stimulation of a central pathway leading to a decreased sympathetic activity to the vasculature. This mechanism is purely speculative and further experiments involving measurement of regional blood flow and electrical activity of several efferent sympathetic nerves after the central administration of isoprenaline, need to be performed to verify this.

The results obtained in the conscious cat are in conflict with the observations made by Conway & Lang (1974) after icv isoprenaline in conscious dogs. They found that pretreatment with hexamethonium reduced the tachycardia but potentiated the depressor effect observed after icv isoprenaline. Intravenous guanethidine also potentiated the fall in blood pressure whilst atropine methylnitrate failed to enhance the central effects of isoprenaline. The enhanced blood pressure effects of icv isoprenaline may be explained by leakage of the amine into the peripheral circulation (see demonstration of leakage of amines from the brain in Section 6).

Conway & Lang (1974) showed that an icv injection of propranolol (2.0 mg) in the dog totally inhibited the

effects of intravenous isoprenaline thus indicating that leakage of propranolol from the dog brain does occur. In the conscious cat, icv propranolol did not affect the responses to i.v. isoprenaline. Thus, the evidence from experiments in dogs suggests that propranolol leaks from the brain and it would therefore seem likely that isoprenaline does the same.

Within all the cats used in experiments for this thesis there appeared to be two definite opposing mechanisms influencing heart rate. Each is stimulated by opposing agonists, classified with respect to their peripheral actions as α- and β-adrenoceptor agonists. α-Adrenoceptor agonists administered centrally produce bradycardia due to a decreased sympathetic outflow and an increased vagal activity to the heart whilst β-adrenoceptor agonists produce tachycardia apparently by a direct increase of sympathetic outflow to the heart.

The centrally mediated blood pressure effects are more complicated. From the results described in Section 2 falls in blood pressure are mainly observed after icv administration of α-adrenoceptor stimulants. However, occasional rises in blood pressure were noticed after icv noradrenaline and adrenaline (for the latter the response was seen in the presence of icv dl-propranolol). The bradycardias and both types of blood pressure effects were abolished by icv phentolamine pretreatment, but were unaffected by icv β-adrenoceptor antagonists.

Icv isoprenaline, salbutamol, isoetharine and

adrenaline (in the presence of icv phentolamine) produced mainly increases in blood pressure but depressor effects were also noticed. However, the tachycardias in association with both types of blood pressure response were inhibited by icv β-adrenoceptor antagonist pretreatment and unaffected by icv phentolamine.

Rises in blood pressure could possibly be produced centrally by two mechanisms (a) stimulation of an excitatory pathway or (b) inhibition of an inhibitory pathway. Depressor effects could either be produced by (c) stimulation of an inhibitory pathway or (d) inhibition of an excitatory pathway. If, for example, central β-adrenoceptors were present in excitatory pathways and α-adrenoceptors were present in inhibitory pathways, then the rises in blood pressure due to icv α- and β-adrenoceptor agonists could be explained by predominent interactions with the appropriate pathways, (i.e. a and b). Conversely, the depressor effects observed after both types of agonist could be due to stimulation of pathways c and d.

Clonidine, although producing its hypotension via central α-adrenoceptor stimulation, failed to produce a centrally mediated pressor effect, unlike icv noradrenaline or adrenaline (after icv propranolol). The inability of clonidine to stimulate the α-adrenoceptor pressor pathway may indicate that clonidine is only specific for the depressor pathway and that two slightly different α-adrenoceptors are involved with the mediation of the pressor and depressor effects.

Peripherally, β-adrenoceptors have been classified into distinctly different groups designated β_1 and β_2 by Lands and his colleagues (Lands & Brown, 1964; Lands et al., 1967). Cardiac muscle and intestinal smooth muscle contain β_1-adrenoceptors and other β-innervated smooth muscles including bronchi, uterus, arteries and skeletal muscle contain β_2-adrenoceptors. Isoprenaline and adrenaline are potent stimulants of both β_1 and β_2-adrenoceptors. However, isoetharine and salbutamol are both very much more active on bronchial smooth muscle (β_2) than on cardiac muscle (β_1) (Lands et al., 1967; Brittain et al., 1968; Brittain, 1971). The classification of 2 types of peripheral β-adrenoceptor mainly rests upon the selectivity of synthetic β-stimulants to bronchial β-adrenoceptors.

With respect to the central effects of these compounds, each β-stimulant produced a similar type of response within a particular cat. Thus, it would appear that the central β-adrenoceptors responsible for either pressor or depressor effects were not selectively stimulated by these β-adrenoceptor agonists, which are known to be relatively selective peripherally (see Tables 5 and 6).

Of the 4 β-adrenoceptor agonists used none preferentially stimulated either heart rate or blood pressure resulting in an effect on one parameter only. It was also found impossible to inhibit any one of the centrally mediated cardiovascular effects leaving the other unaffected. None of the seven β-adrenoceptor antagonists

administered centrally, including practolol and ICI 66082 which are known to be selective β_1 adrenoceptor antagonists showed a preferential inhibition towards the tachycardia or the pressor and depressor effects observed after the β-agonists administered centrally. It was observed that the central effects of the β_2 stimulants, salbutamol and isoetharine, were reduced by icv pretreatment with the β_1 adrenoceptor blockers, practolol and ICI 66082.

Some degree of selectivity was observed with icv dopamine. Icv dopamine always produced hypertensive effects but the extent to which the heart rate was increased was always relatively small; of the 5 agonists used, icv dopamine produced the smallest tachycardias. Figs. 33,34, 36,37, 41 and 42 demonstrate relatively large increases in blood pressure associated with comparatively small tachycardias after icv dopamine.

The pressor effects and tachycardias produced by icv dopamine were similar to these effects produced by the 4 β-adrenoceptor stimulants in that they were prevented by peripheral ganglion blockade, peripheral adrenergic neuron blockade and central β-adrenoceptor blockade and unaffected by pretreatment with icv phentolamine and local anaesthetics.

However, there were 5 major differences between the icv responses of dopamine and the β-adrenoceptor stimulants. Firstly, in contrast to the usual pressor response observed after icv isoprenaline whereby the systolic pressure was increased to a greater extent than

the diastolic blood pressure and was seen in combination with a very large tachycardia, icv dopamine produced similar large rises in systolic and diastolic pressures and were associated with a relatively small tachycardia. These findings indicate that the pressor effects observed after icv dopamine were far less dependant upon the cardiac response, as seems the case for isoprenaline, but more probably upon centrally induced vascular changes.

Secondly, dopamine was able to produce hypertension in conscious cats that had previously responded to the other β-adrenoceptor agonists with a hypotension. However, both the hypertension of icv dopamine and hypotension of the other agonists were abolished by icv β-adrenoceptor blockade.

Thirdly, dopamine administered centrally produced hypotension and bradycardia in 4 cats as a secondary part of a biphasic cardiovascular response. However, unlike the hypotension produced by central administration of isoprenaline and salbutamol, the depressant effects of dopamine were inhibited by icv phentolamine indicating an α-adrenoceptor mechanism. The hypotension and bradycardia of icv dopamine were also inhibited by icv haloperidol and by pretreatment for 3 days with disulfiram indicating that these effects were due to central noradrenaline formation. The depressant effects of dopamine were unaffected by icv β-adrenoceptor antagonists and pimozide, thus confirming that the falls were due to central α-adrenoceptor stimulation produced by noradrenaline

formation and not to a direct or β-agonist action of dopamine.

Fourthly, the pressor effects and tachycardias of dopamine, were inhibited by central dopamine receptor blockade produced by haloperidol and pimozide, whilst this treatment proved ineffective against the central effects of isoprenaline.

Lastly, in 8 cats icv infusions of guanethidine or bethanidine appeared to generally depress the pressor effect of icv isoprenaline whilst not affecting the tachycardia or hypotensive responses of isoprenaline. This finding that the isoprenaline pressor effect was generally depressed by these compounds, whilst the tachycardia remained essentially unaltered, was slightly surprising as it was suggested, from previous results presented in this chapter, that the pressor effect due to icv isoprenaline appeared to be largely dependant upon the cardiac response, although some degree of peripheral vasoconstriction also probably occurred. However, in 2 cats central administration of the adrenergic neuron blockers appeared to potentiate the pressor effect due to icv dopamine (see Fig. 44). Doubts must be cast upon these experiments using icv adrenergic neuron blocking agents since it was not possible to determine whether or not complete central adrenergic neuron blockade had occurred. Confirmation of blockade using electrical stimulation of particular central neurons was not performed within the experimental conditions using conscious cats. Kaneko et al. (1961) and Bhargava,

Jaju & Tangri (1966) administered guanethidine into the lateral cerebral ventricles of anaesthetised dogs. They observed that the peak central adrenergic depression occurred 60 minutes after icv guanethidine. Hence, this time course was used in the conscious cat experiments and the doses used by these workers were accordingly reduced for use in the cat and 0.5 mg was decided upon.

It was extremely difficult to analyse and hence draw conclusions from the results obtained after giving icv dopamine or isoprenaline after central pretreatment with adrenergic neuron blocking agents. Isoprenaline is not taken up into adrenergic neurons (see Iversen, 1973) so the effects of isoprenaline were due to post-synaptic β-adrenoceptor stimulation. Hence, for the hypotension and tachycardia responses of icv isoprenaline not to be affected by these adrenergic neuron blockers might indicate that these effects were produced by isoprenaline stimulating post-synaptic receptors on the last central neuron of the pathways concerned. However, reduction or inhibition of the pressor effects of icv isoprenaline by these blockers given centrally might occur if these responses were due to stimulation, by isoprenaline, of an interconnecting neuron within this pathway and the latter part of the pathway was blocked by these compounds. In contrast, the hypertension due to icv dopamine was unaltered in one cat and potentiated in another by icv pretreatment with bethanidine or guanethidine. Thus, the pressor response of icv dopamine could have been either due to stimulation of a different pathway to that stimulated by isoprenaline which was unaffected by these

blockers or to stimulation by dopamine of post-synaptic receptors on the last neuron of the pressor pathway normally stimulated by isoprenaline. With respect to this latter suggestion, one should expect pimozide and haloperidol to inhibit the pressor effects of isoprenaline as well as dopamine. From the results described in part 5 of this chapter, icv isoprenaline was unaffected by pimozide or haloperidol whereas the pressor effects of icv dopamine were absent after pretreatment with the dopamine receptor antagonists. Therefore, there may possibly be 2 neural pathways, which when stimulated induce pressor effects; one of which may be a dopaminergic pathway. However, the pressor effects of dopamine were abolished by dopamine receptor blocking agents and by β-adrenoceptor antagonists. All the results presented in this chapter concerning the cardiovascular effects of the agonists are rather confusing and further experiments, involving discrete stimulation and blockade of several central neurons using microiontophoretic techniques, should be performed in an attempt to clarify this complicated picture.

An interesting observation was that α-methyldopamine acts in exactly the same manner as icv dopamine. As with dopamine, the initial stimulant effects of icv α-methyldopamine were inhibited by prior icv treatment with β-adrenoceptor antagonists and the secondary depressant effects appeared to be due to α-adrenoceptor stimulation induced by centrally formed α-methylnoradrenaline. From these findings, if certain areas or pathways are sensitive

to dopamine or α-methyldopamine resulting in hypertension and tachycardia, one might expect to observe these effects after administration of either l-dopa or α-methyldopa. Also, it was described in the previous section that icv administered noradrenaline produced pressor effects in 3 cats via a central α-adrenoceptor mechanism.

Because hypotension and bradycardia are the usual centrally mediated effects observed after l-dopa or α-methyldopa may indicate that the amino acids are preferentially taken up into central depressor neurons. If not, then the depressant effects on blood pressure and heart rate observed are resultant effects of stimulation of opposing mechanisms; the α-adrenoceptor stimulant effects of noradrenaline and α-methylnoradrenaline being much more pronounced or predominant than the β-effects of dopamine and its α-methylated analogue.

To date there has been no report in the literature describing the cardiovascular effects obtained after central administration of bethanidine. However, 4 reports have been made concerning the central cardiovascular actions of both bretylium and guanethidine. Kaneko et al. (1961) administered (0.2 - 10.0 mg) into the lateral ventricles and cisterna magna of anaesthetised dogs producing a hypotension, bradycardia and a marked blockade of the response to occlusion of both common carotid arteries. Bhargava et al.(1966) confirmed the findings of Kaneko et al.(1962), concerning icv guanethidine in dogs, and attributed the hypotension and bradycardia to the release of catecholamines by

guanethidine within the medullary region. Jaju et al.(1968) also produced hypotension and bradycardia after icv bretylium in anaesthetised dogs.

Cuparencu, Ticsa, Csutak, Safta & Mocan (1969) produced different results from the 2 previous groups of workers. They found that icv guanethidine (0.58 - 0.80 mg/kg), in hexobarbital anaesthetised dogs, produced a slight decrease in arterial blood pressure which was abolished or reversed to a pressor effect by icv pretreatment with propranolol. The previous groups of workers also used barbiturate anaesthesia in dogs. However, in chloralose anaesthetised dogs, Cuparencu et al. (1969) found that icv guanethidine, regardless of dose, elicited pronounced hypertensive effects. Icv bretylium also produced pressor effects. The pressor effects of icv guanethidine were abolished by pretreatment with dibenamine, 10 mg/kg, i.v. (an α-adrenoceptor antagonist). They suggested that the depressor effect of guanethidine was due to centrally released catecholamines causing the stimulation of central β-adrenoceptors, since the depressant effects were abolished by icv propranolol and that the degree of amine release and type of cardiovascular response depended upon the anaesthetic used. Variable cardiovascular effects were observed in the present experiments involving icv administration of guanethidine and bethanidine in conscious cats.

Boura & Green (1959) suggested that the pressor effects obtained after peripheral administration of bretylium were due to peripheral release of catecholamines from adrenergic tissues. This mechanism also explains the

sympathomimetic effects observed after peripherally administered guanethidine and bethanidine (see review of literature by Boura & Green, 1965). The adrenergic neuron blocking agents, bretylium and bethanidine, do not enter the central nervous system (Boura et al., 1960; Boura et al., 1962). However, direct administration of these compounds into the central nervous system would most probably cause release of catecholamine neurotransmitters from central adrenergic neurons by a similar mechanism as in peripheral neurons.

Thus, it would seem likely that the release of catecholamines within the central nervous system could lead to stimulation of both central α- and β-adrenoceptors. Hence, the type of response observed in the conscious cat after icv infusions of adrenergic neuron blockers may be due to a resultant effect of various actions of endogenously released catecholamines within different brain areas; the observed response would be due to the greatest amount of stimulation of either pressor or depressor receptors by the liberated catecholamines.

In the conscious cat, further experiments should be performed to establish whether the pressor and depressor effects and tachycardias and bradycardias were due to α- or β-adrenoceptor stimulation. Icv guanethidine or bethanidine should be administered after either icv phentolamine or propranolol.

It was observed that icv guanethidine and bethanidine appeared to selectively inhibit the pressor

effect of icv isoprenaline. Hence, the hypotension observed after these blockers administered centrally may be partly due to an inhibitory effect upon these pressor neurons leading to a decreased tone within this pathway. Further experiments must be performed to elucidate the exact mechanisms of action of this group of compounds within the brain.

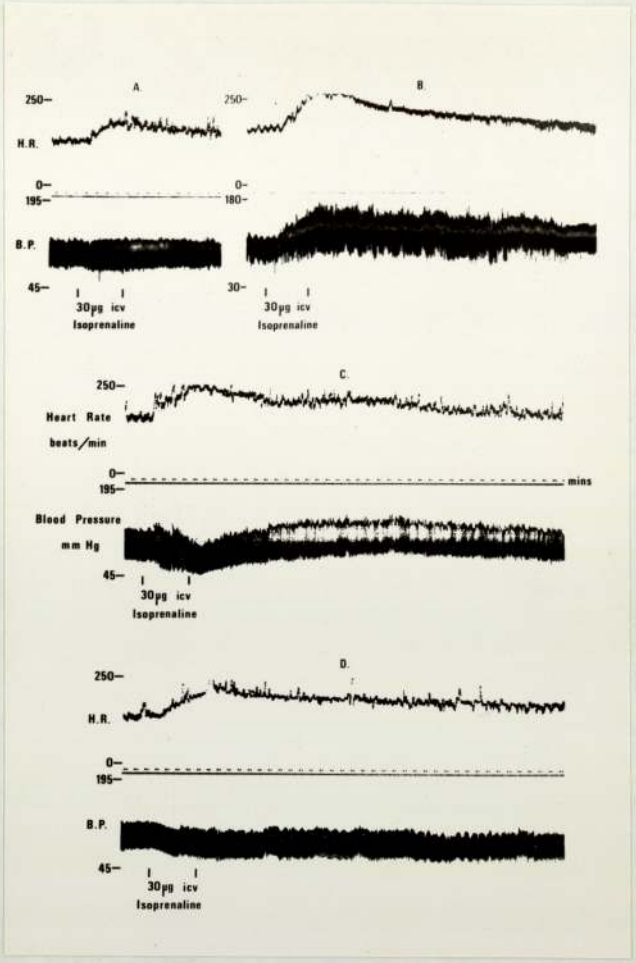


FIG. 29.

Conscious, normotensive, unrestrained cat. Illustration of 4 types of blood pressure responses observed after icv isoprenaline. Trace A shows a relatively short lasting tachycardia in combination with no blood pressure effect after isoprenaline (30 µg, icv). Trace B illustrates the most common type of response to icv isoprenaline (30 µg) whereby the large tachycardia is associated with a large hypertensive response. A biphasic response to isoprenaline (30 µg, icv) is shown in Trace C in which the blood pressure reduction precedes the pressure increase. Trace D demonstrates the hypotensive response occasionally obtained after icv isoprenaline (30 µg). All these blood pressure effects and heart rate responses were abolished by icv dl--propranolol pretreatment.

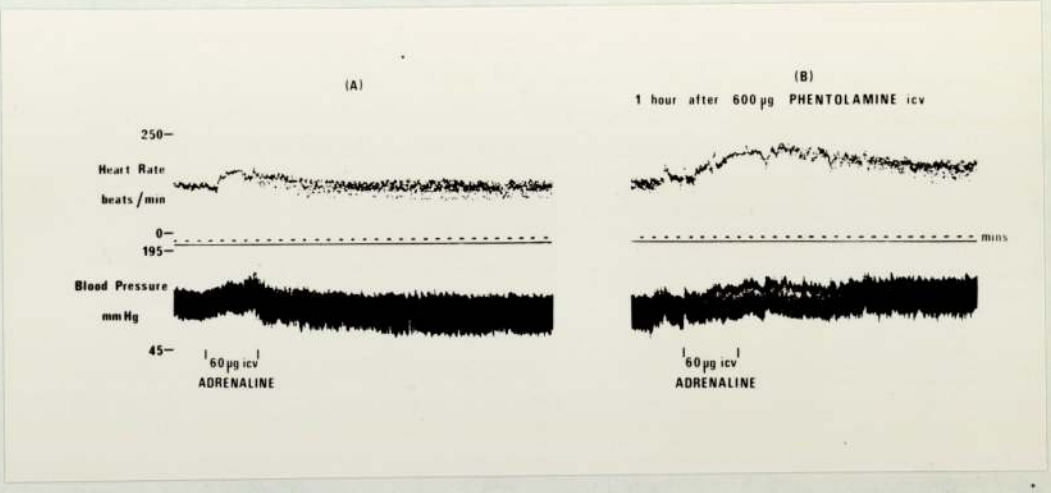


FIG. 30.

Conscious, normotensive, unrestrained cat. Trace A shows biphasic blood pressure and heart rate responses after icv adrenaline (60 µg). The arterial blood pressure and heart rate increased initially and developed into hypotension and bradycardia. Trace B illustrates that adrenaline (60 µg, icv) produced only prolonged rises in arterial blood pressure and heart rate when infused one hour after phentolamine (600 µg, icv).

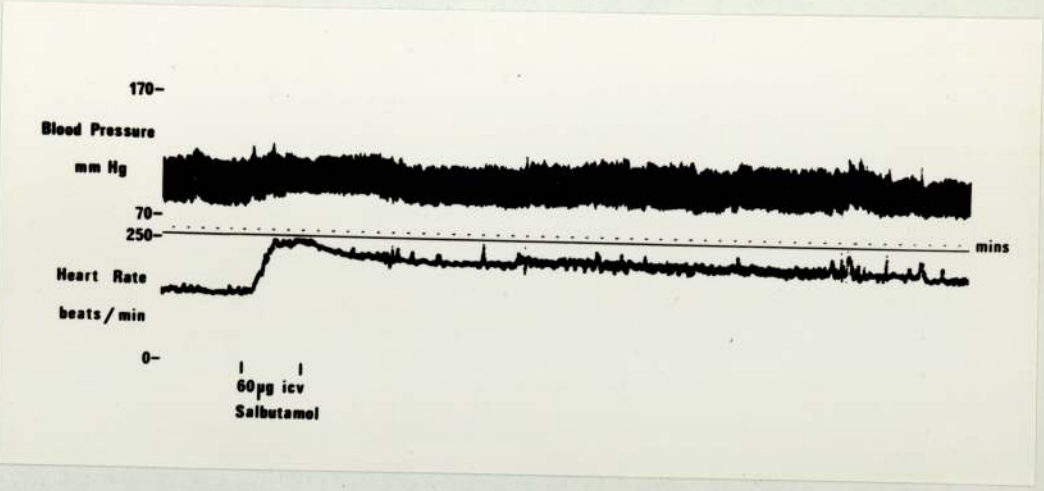


FIG. 31.

Conscious, normotensive, unrestrained cat. Trace shows a very prolonged tachycardia with relatively little effect on blood pressure after icv salbutamol (60 µg).

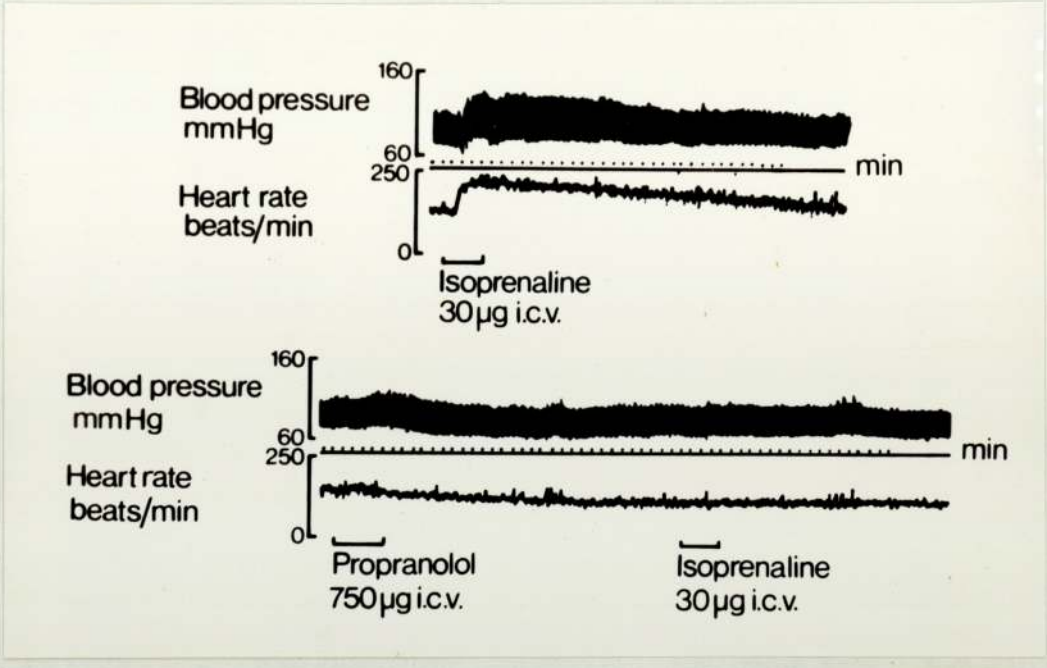


FIG. 32.

Conscious, normotensive, unrestrained cat. Upper trace demonstrates a typical hypertensive response and tachycardia after isoprenaline (30 µg, icv). Lower trace shows dl-propranolol (750 µg, icv) infusion 30 minutes before isoprenaline icv. The responses to icv isoprenaline were totally abolished by icv dl-propranolol.

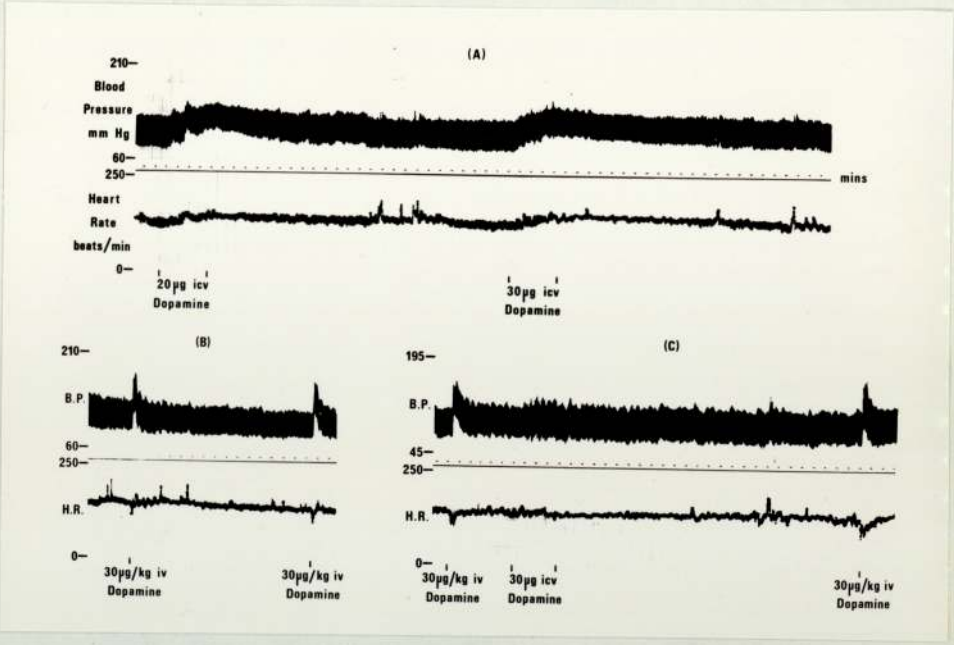


FIG. 33.

Conscious, normotensive, unrestrained cat. Trace A demonstrates control responses to icv dopamine (20 and 30 µg) Icv dopamine induced rises in arterial blood pressure and heart rate. Trace B shows control responses to dopamine (30 µg/kg, i.v.) Trace C shows the complete blockade of the responses to icv dopamine (30 µg) when given 30 minutes after dl-propranolol (0.75 mg, icv). The peripheral effects of dopamine (30 µg/kg, i.v.) were not blocked 25 and 60 minutes after icv dl-propranolol. The reflex bradycardia of i.v. dopamine appeared to be potentiated when given after icv dl-propranolol.

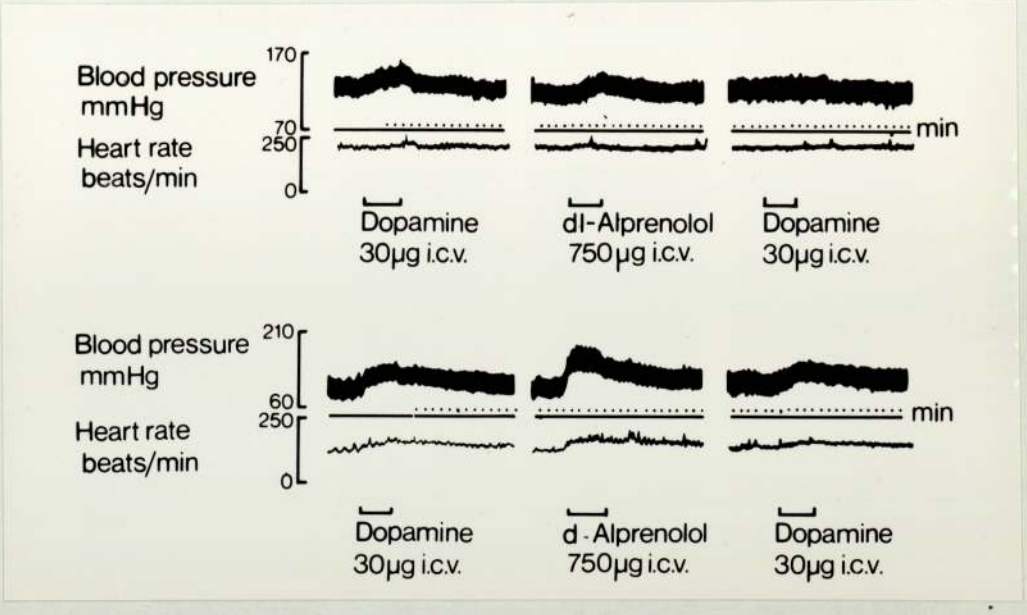


FIG. 34.

Conscious, normotensive, unrestrained cat. Upper traces show dopamine (30 µg, icv) given before and 60 minutes after dl-alprenolol (750 µg, icv). The pressor effect and slight trachycardia due to icv dopamine were abolished by icv dl- alprenolol. Lower traces show dopamine (30 µg, icv) given before and 60 minutes after d-alprenolol (750 µg, icv). The responses of icv dopamine were unaffected by icv d-alprenolol.

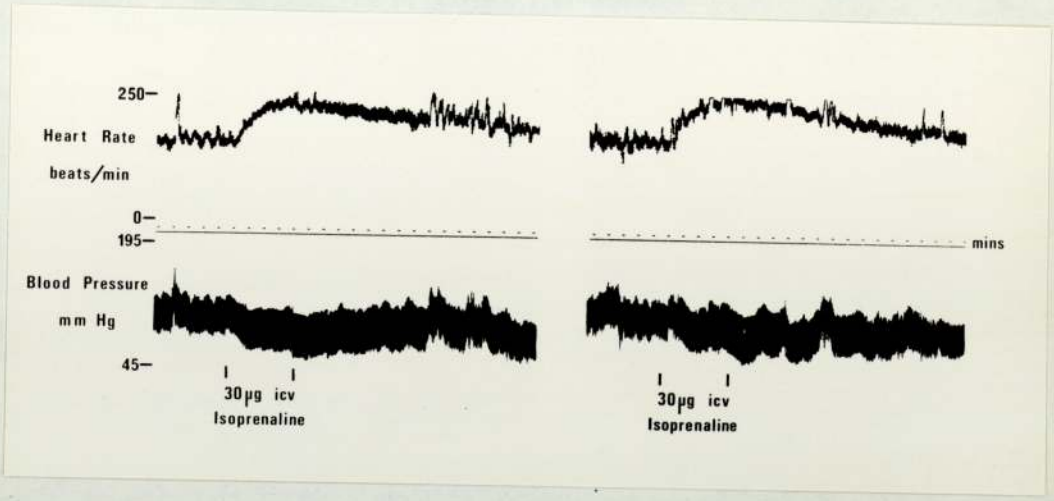


Fig. 35.

Conscious, normotensive, unrestrained cat. Trace A shows a control hypotensive effect with tachycardia after icv isoprenaline (30 µg). The second response (Trace B) demonstrates that phentolamine (0.75 mg, icv) given 60 minutes before isoprenaline (30 µg, icv) did not significantly alter the response of the β - adrenoceptor agonist .

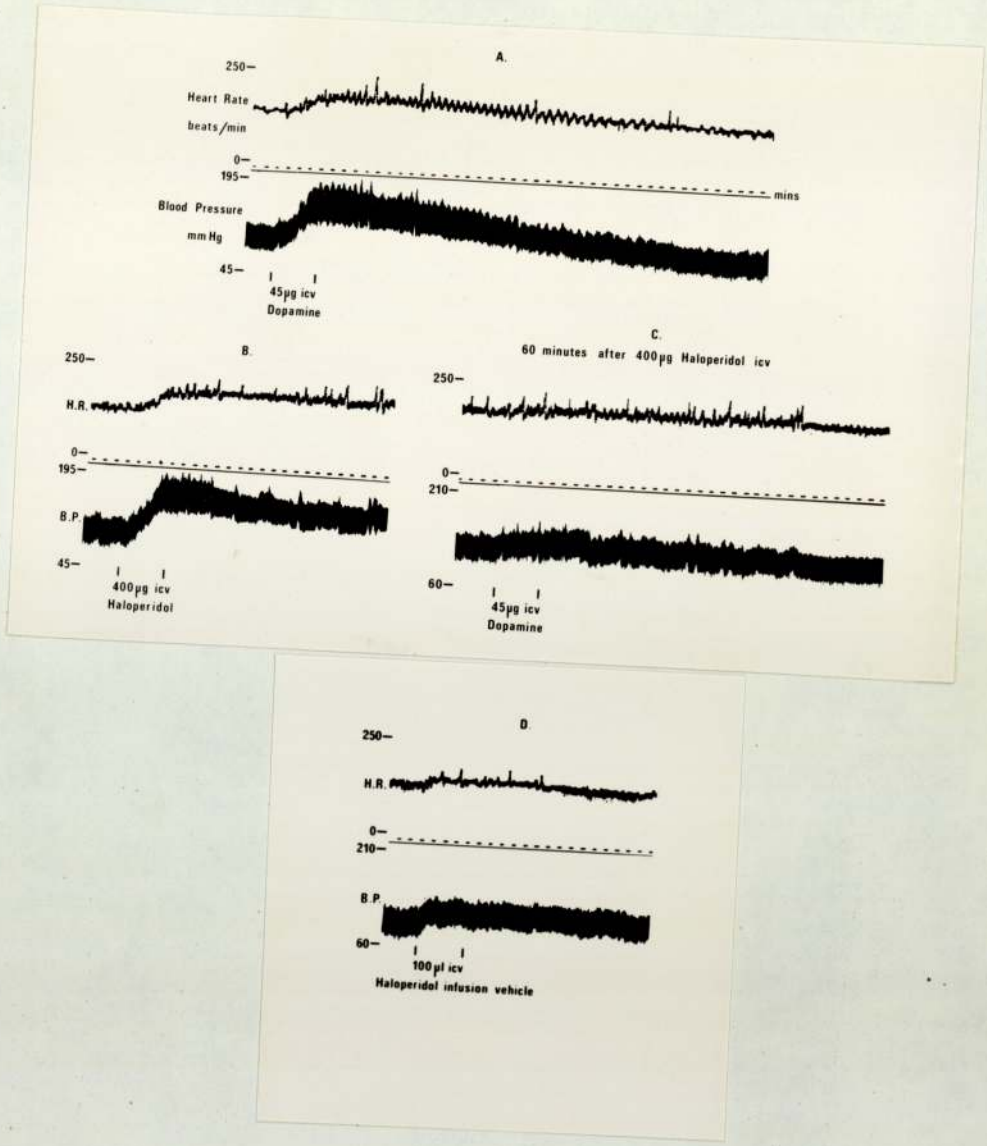


FIG. 36.

Conscious, normotensive, unrestrained cat. Trace A shows the control pressor response and tachycardia due to icv dopamine (45 µg). Trace C illustrates the almost total abolition of the icv dopamine responses 60 minutes after icv haloperidol (400 µg). Icv haloperidol itself produced large prolonged hypertension and tachycardia (Trace B). Trace D shows that icv infusion of 100 µl of the haloperidol infusion vehicle, which consisted of lactic acid adjusted to pH 4.8 with 0.1 N sodium hydroxide, produced only small blood pressure and heart rate rises.

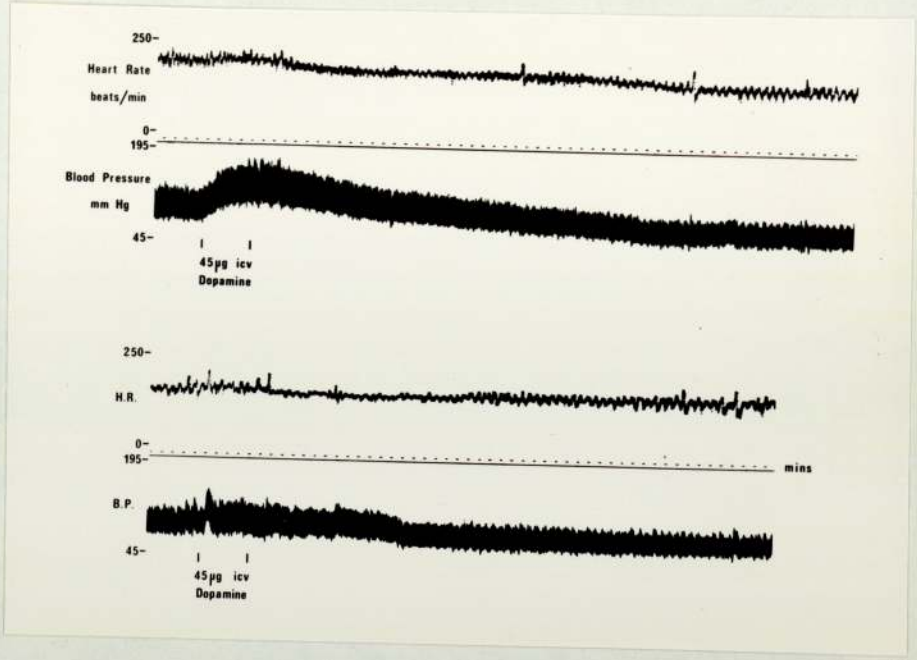


FIG. 37.

Conscious, normotensive, unrestrained cat. Upper trace shows control cardiovascular response to icv dopamine (4.5 µg). Dopamine produced an initial rise in blood pressure with only a very slight rise in heart rate. These initial responses developed into pronounced hypotension and bradycardia. Lower trace shows the effect of icv dopamine (45 µg) given 60 minutes after pimozide (1.0 mg/kg, i.v.) The initial stimulant effects of icv dopamine were greatly reduced whilst the cardiovascular depressant effects remained unaffected.

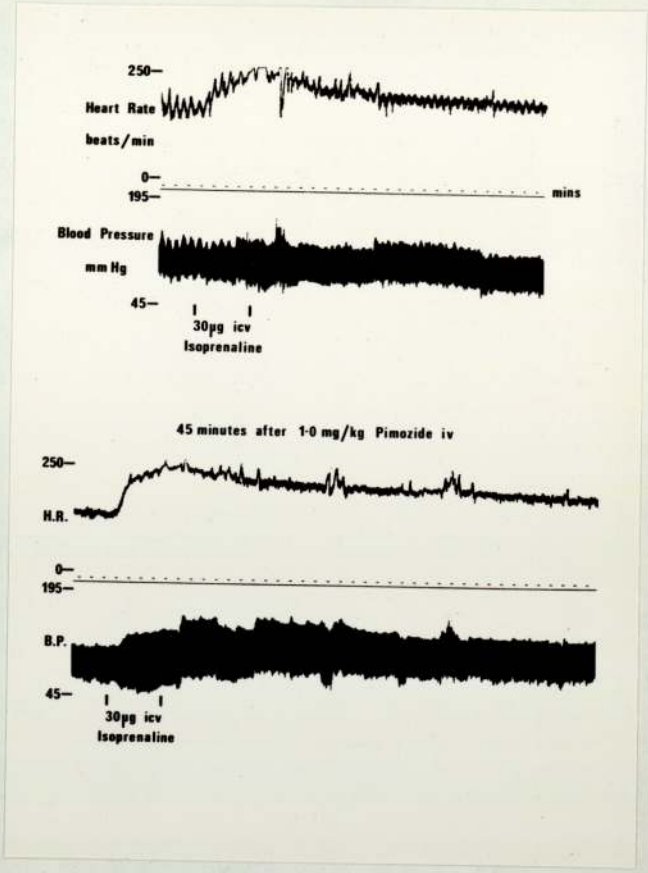


FIG. 38.

Conscious, normotensive, unrestrained cat. Upper trace illustrates a large tachycardia with little effect on blood pressure after icv isoprenaline (30 µg). Lower trace shows that isoprenaline (30 µg, icv) still produced tachycardia which was accompanied with a raised systolic and decreased diastolic blood pressure 45 minutes after pimozide (1.0 mg/kg, i.v.).

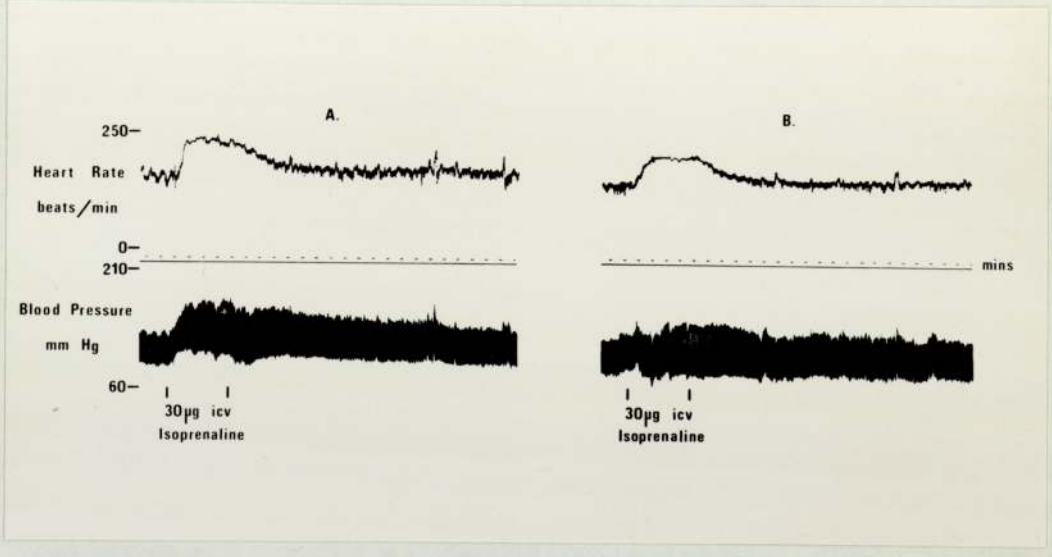


FIG. 39.

Conscious, normotensive, unrestrained cat. Trace A demonstrates the control hypertensive effect with tachycardia observed after icv isoprenaline (30 µg). Trace B shows that the response to icv isoprenaline was reduced when given 30 minutes after clonidine (10 µg, icv).

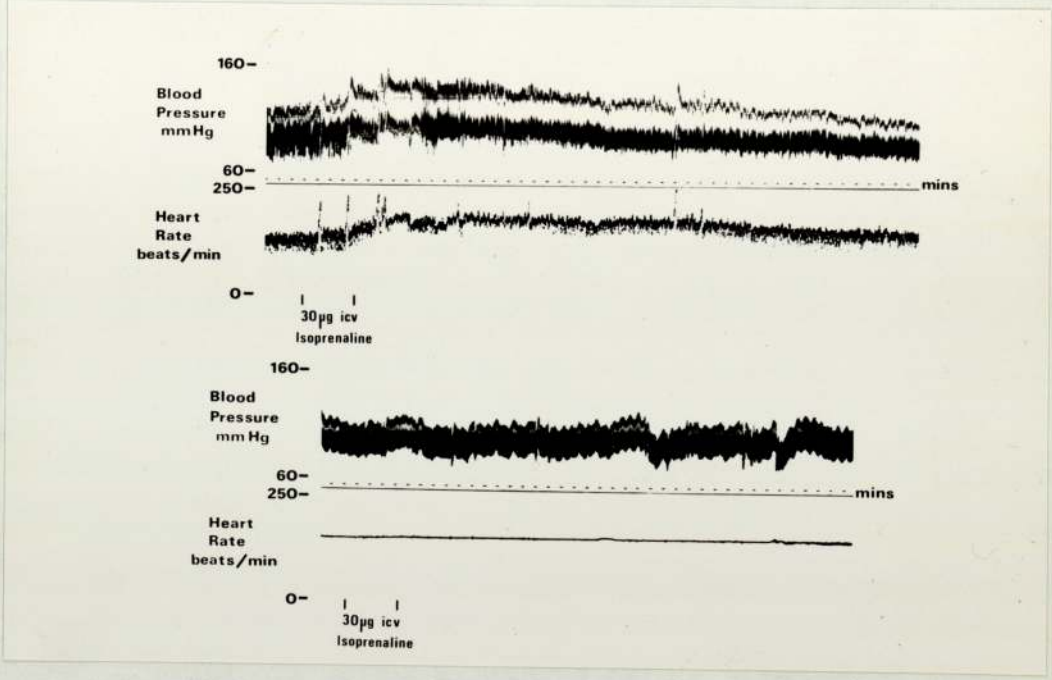


FIG. 40.

Conscious, normotensive, unrestrained cat. The upper trace shows the control hypertensive response with tachycardia after isoprenaline (30 µg, icv). The lower trace shows the complete abolition of the isoprenaline response 90 minutes after autonomic ganglion blockade with pemidine (5 mg/kg, i.v.).

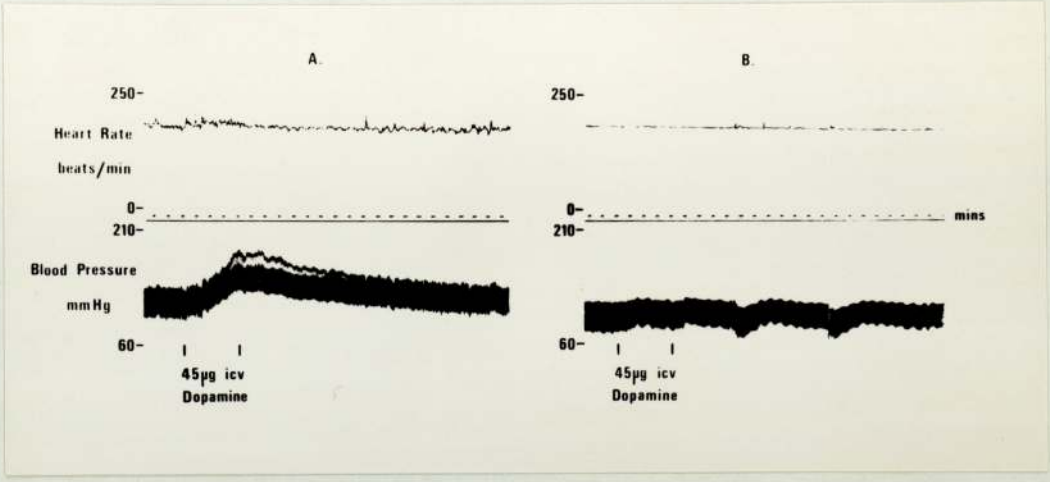


FIG. 41.

Conscious, normotensive, unrestrained cat. Trace A illustrates that dopamine (45 µg) infused icv produced a large hypertension with no accompanied heart rate effect in this particular cat. When dopamine (45 µg, icv) was repeated 45 minutes after hexamethonium (10 mg/kg, i.v.) the blood pressure increase was completely absent (Trace B). The second dopamine administration occurred at a time when the cardiovascular effects of tetramethylammonium (75 µg/kg, i.v.) were inhibited by hexamethonium.

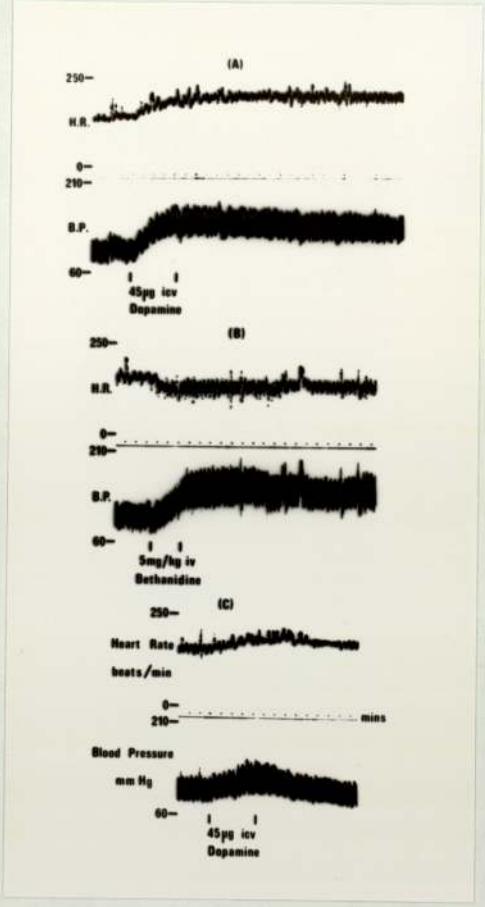


FIG. 42.

Conscious, normotensive, unrestrained cat. Trace A shows blood pressure and heart rate increases after dopamine (45 µg, icv). Trace B illustrates the initial pressor response with bradycardia to an intravenous infusion of bethanidine (5 mg/kg). Trace C shows a greatly reduced hypertension and tachycardia to icv dopamine (45 µg, icv) given 2 hours after i.v. bethanidine.

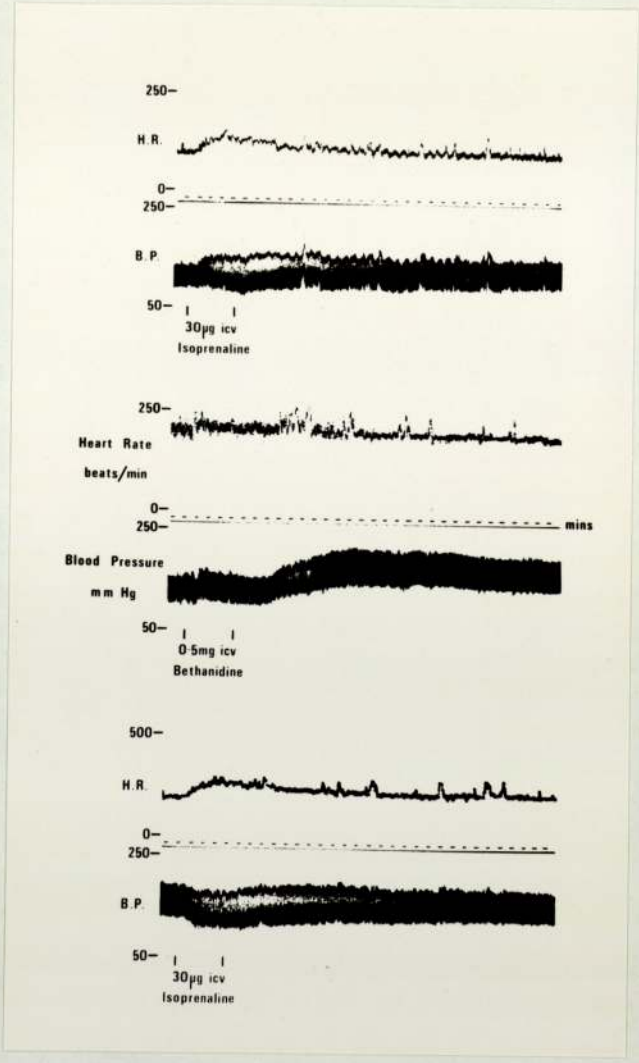


FIG. 43 a.

Conscious, normotensive, unrestrained cat. The upper trace illustrates the control response to icv isoprenaline (30 µg). The diastolic pressure fell slightly and was associated with a larger increase in systolic blood pressure. The middle trace shows the initial response observed after bethanidine (0.5 mg, icv) in this particular cat. The bottom trace demonstrates the central response of isoprenaline (30 µg, icv) administered 60 minutes after the icv bethanidine. Icv bethanidine did not alter the isoprenaline tachycardia but caused isoprenaline to reduce both the systolic and diastolic pressures.

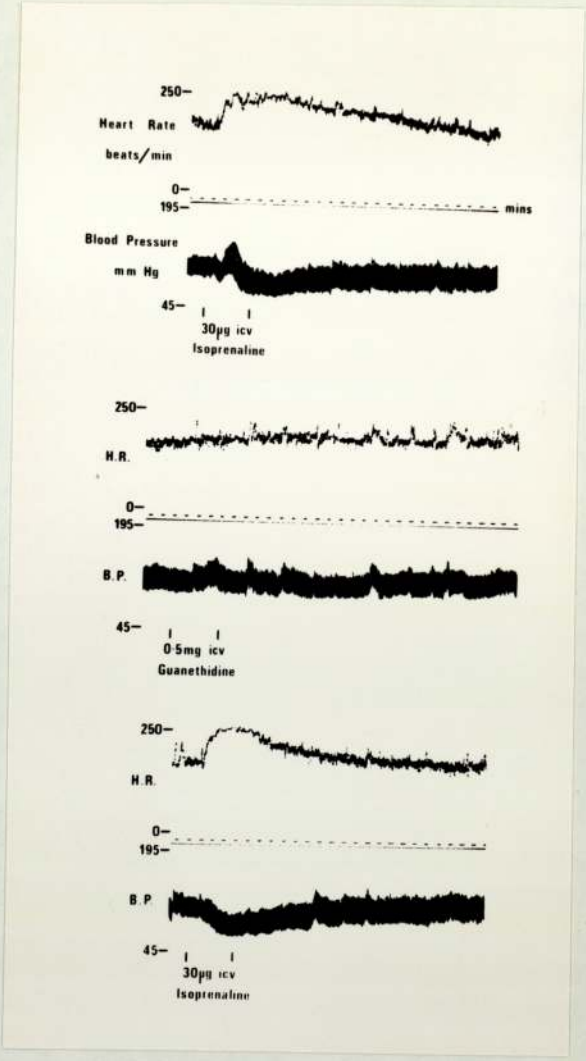


FIG. 43 b.

Conscious, normotensive, unrestrained cat. The upper trace illustrates the control response to isoprenaline (30 µg, icv). The large tachycardia was associated with an initial hypertension, of short duration, followed by a more prolonged hypotension. The transient hypertension was not due to movement of any kind as the cat remained completely still and was undisturbed throughout the icv infusion. The middle trace shows that icv guanethidine (0.5 mg) in this cat did not produce any cardiovascular effects. Isoprenaline (30 µg, icv) produced only hypotension with tachycardia when given 60 minutes after the icv guanethidine.

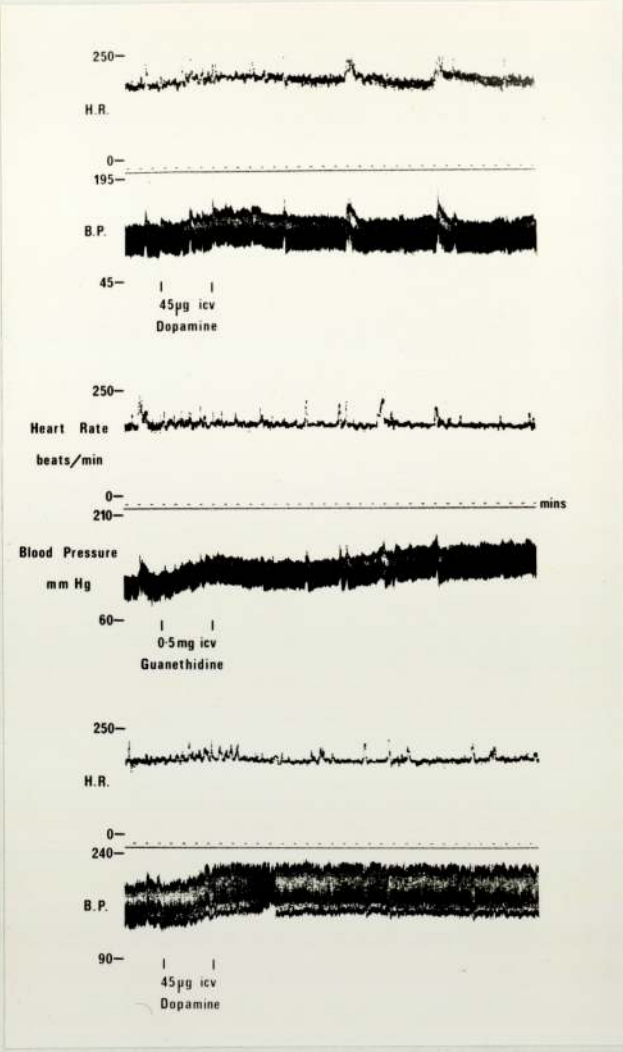


FIG. 44.

Conscious, normotensive, unrestrained cat. Dopamine (45 µg, icv) induced a rise in blood pressure associated with a small tachycardia, as illustrated in the upper trace. The middle trace shows that guanethidine (0.5 mg, icv) produced a hypertensive response, which was slow in onset, but did not alter the heart rate. The lower trace shows that the pressor effect of icv dopamine (45 µg) was potentiated both in magnitude and duration, when given 60 minutes after icv guanethidine (0.5 mg). The small tachycardia was of short duration and was similar to the control heart rate response of dopamine (45 µg, icv).

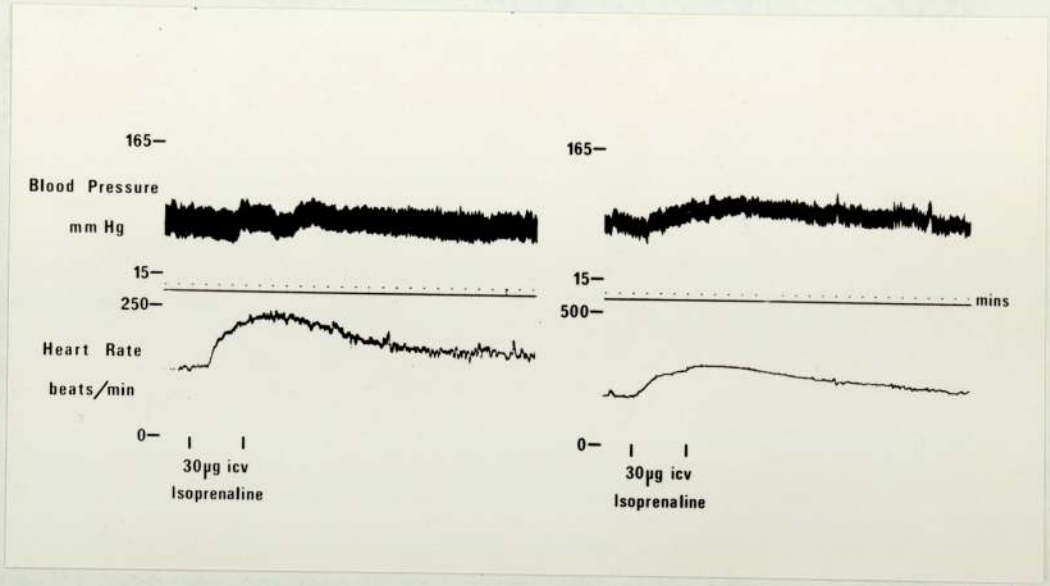


FIG. 45.

Conscious, normotensive, unrestrained cat. The left trace shows that isoprenaline (30 µg, icv) produced very little effect on blood pressure and a large tachycardia. When repeated 30 minutes after atropine methylnitrate (0.25 mg/kg, i.v.), isoprenaline (30 µg, icv) produced a larger tachycardia than the control response and was associated with a hypertensive effect (right trace).

β-adrenoceptor agonist	Total icv dose µg	Increase in Arterial Blood Pressure mmHg ± s.e.m.		Increase in Heart Rate Beats/minute ± s.e.m.	Total No. of observations	No. of cats used.
		SYSTOLIC	DIASTOLIC			
ISOPRENALINE	5	5.3 ± 1.9	4.8 ± 2.3	15.9 ± 2.4	16	14
	15	11.4 ± 2.1	6.8 ± 2.2	27.5 ± 3.0	28	
	30	23.7 ± 1.4	14.2 ± 1.5	75.9 ± 2.1	56	
	60	26.8 ± 2.9	15.7 ± 3.1	83.3 ± 3.4	26	
SALBUTAMOL	45	9.4 ± 2.6	8.3 ± 1.5	45.3 ± 3.2	10	5
	60	15.6 ± 1.8	12.3 ± 2.1	58.1 ± 2.8	12	
ISOETHARINE	75	10.0 ± 1.6	9.8 ± 1.5	24.4 ± 2.9	8	4
	150	16.6 ± 1.8	15.8 ± 1.3	45.6 ± 4.0	8	
ADRENALINE (1 hour after icv phentolamine)	60	15.6 ± 2.1	13.9 ± 2.0	38.7 ± 2.5	16	8
	120	25.0 ± 3.8	20.7 ± 3.1	49.3 ± 4.2	12	

TABLE 5. Maximal blood pressure and heart rate increases (mean ± s.e.m.) produced by icv isoprenaline, salbutamol, isoetharine and adrenaline. Adrenaline was administered 1 hour after icv phentolamine (0.5 - 0.75 mg). The responses produced after icv salbutamol, isoetharine and adrenaline were obtained in cats already found to respond with pressor effects to icv isoprenaline. All responses were obtained in conscious normotensive unrestrained cats.

β-ADRENOCEPTOR AGONIST	TOTAL icv dose µg	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s.e.m.		INCREASE IN HEART RATE beats/min. ± s.e.m.	TOTAL No. of observations	No. of cats used.
		SYSTOLIC	DIASTOLIC			
ISOPRENALINE	15	- 9.4 ± 2.2	- 8.9 ± 2.7	+24.9 ± 3.9	10	5
	30	-16.3 ± 3.0	-18.2 ± 2.5	+76.3 ± 4.0	28	
	60	-22.9 ± 3.3	-25.0 ± 3.5	+84.9 ± 4.2	12	
SALBUTAMOL	45	-10.1 ± 2.0	-10.6 ± 2.3	+42.1 ± 3.3	6	2
	60	-14.6 ± 2.1	-15.3 ± 2.9	+56.4 ± 2.9	5	

TABLE 6 Maximal blood pressure decreases and heart rate increases (mean ± s.e.m.) observed after icv isoprenaline and salbutamol in conscious, normotensive, unrestrained cats. The responses observed after icv salbutamol were obtained in 2 cats previously found to produce hypotension with icv isoprenaline.

	TOTAL icv dose µg	INCREASE IN ARTERIAL BLOOD PRESSURE mmHg ± s.e.m.		INCREASE IN HEART RATE (beats/min. ± s.e.m.)	TOTAL No. of observations	No. of cats used.
		SYSTOLIC	DIASTOLIC			
DOPAMINE	30	27.6 ± 3.1	25.4 ± 3.2	15.1 ± 3.6	18	6
	45	36.8 ± 4.0	30.7 ± 4.1.	20.9 ± 3.8	12	

TABLE 7. Maximal increases in blood pressure and heart rate (mean ± s.e.m.) produced by icv dopamine in conscious, normotensive unrestrained cats.

	TOTAL icv dose µg	INCREASE IN ARTERIAL BLOOD PRESSURE mmHg ± sem		INCREASE IN HEART RATE beats/min. ± sem	DECREASE IN ARTERIAL BLOOD PRESSURE mmHg ± sem.		DECREASE IN HEART RATE beats/min ± sem	TOTAL No. of Responses	No. of cats used.
		SYST:	DIAST:		SYST:	DIAST:			
DOPAMINE	30	+23.1±2.9	+22.0±3.1	+14.2±3.8	-12.2±2.6	-12.7±2.7	-16.6±3.0	12	4
	45	+30.8±3.2	+29.5±3.3	+19.9±3.9	-18.6±2.9	-18.9±3.0	-23.2±3.2	12	

TABLE 8. Maximal increases and decreases in arterial blood pressure and heart rate observed after icv dopamine in conscious, normotensive, unrestrained cats.

CHAPTER 2. The cardiovascular effects of isoprenaline and dopamine administered into the cisterna magna of conscious cats.

No reports were found in the literature concerning the cardiovascular effects of isoprenaline, or other β-adrenoceptor agonists, administered into the cisterna magna.

The groups of workers previously cited in Chapter 1 of this section administered isoprenaline into the lateral or third cerebral ventricles of various species.

Two cats, that had previously been cannulated for drug administration into the left lateral cerebral ventricles, were cannulated allowing drug administration into the cisterna magna. A comparison of blood pressure and heart rate effects was made after administrations of isoprenaline and dopamine into the lateral ventricles and cisterna magnas of these cats.

Results.

Isoprenaline (30 µg) administered into the lateral ventricle of each cat produced a tachycardia with an associated fall in blood pressure.

Administration of isoprenaline (30 - 45 µg) into the cisterna magna (icm) in each cat produced tachycardia and hypotension. The icm infusion volume containing the isoprenaline was reduced to 50 µl in an attempt to localise the effects of isoprenaline to the areas surrounding the cisterna magna.

Fig. 46 compares the cardiovascular effects produced by icv and icm isoprenaline (30 µg) in the same cat. The upper trace is the effect observed after icm isoprenaline and the lower trace of icv isoprenaline.

The blood pressure and heart rate responses after icv isoprenaline were quick in onset. The tachycardia and hypotension began during the icv infusion of isoprenaline (see also Fig. 29 which demonstrates the different types of icv isoprenaline responses). This was also the case after icm isoprenaline. The start of the response usually occurred before completion of the icm infusion. There appeared to be little difference in the magnitude or duration of responses to isoprenaline by icv and icm routes (Fig. 46).

The tachycardia and hypotension observed after icm isoprenaline (30 µg) in both cats were reduced or abolished with doses of β-adrenoceptor antagonists administered via the same route. The icm doses of dl - and l-propranolol, dl-alprenolol, practolol, pindolol and ICI 66082 needed to inhibit icm isoprenaline were the same as used for icv administration. The blood pressure and heart rate effects of these blockers are described in Section 4.

D-propranolol (0.5 mg, icm) was ineffective in reducing the cardiovascular effects observed after icm isoprenaline (30 µg).

Doses of 45 µg isoprenaline infused icm often induced emesis, whilst higher doses almost always did.

In all cases, vomiting due to isoprenaline (45 µg,

icm) was absent after reduction or abolition of the cardiovascular effects of isoprenaline by icm pretreatment with β-adrenoceptor antagonists.

Dopamine (75 µg) was administered icm to each cat on one occasion. In both cats, dopamine (75 µg, icm) produced an initial tachycardia and rise in blood pressure. This icm dose of dopamine induced emesis in both cats. Vomiting occurred within 2 minutes of completion of the icm infusion. The initial rises in blood pressure and heart rate after icm dopamine did not appear as large as observed after smaller doses of dopamine administered icv. A large bradycardia and hypotension then developed from the initial stimulant effects. The maximum falls in blood pressure and heart rate occurred 30 - 45 minutes after the icm dopamine infusion.

Icm phentolamine (0.5 mg) in each cat, produced little effect on blood pressure and heart rate. 60 minutes after icm phentolamine, dopamine (75 µg, icm) produced an initial rise in blood pressure and heart rate but prevented the hypotension and bradycardia. Dopamine (75 µg, icm) given 60 minutes after phentolamine induced vomiting in only one cat. Fig. 47 demonstrates the abolition of the depressant effects of icm dopamine by icm phentolamine (0.5 mg). Dopamine produced only a pressor effect and tachycardia and in this cat vomiting did not occur with the second dopamine administration.

Icm pretreatment with dl-propranolol (0.5 mg) given 1.5 hours before icm dopamine, abolished the initial

stimulant effects of dopamine but the hypotension and bradycardia were still present. Dopamine icm did not induce emesis in either cat when given after icm dl-propranolol. The cardiovascular effects produced by icm dl-propranolol, in both cats, are described in Section 4, Chapter 3.

Dopamine (30,45 µg, icv) produced a rise in both blood pressure and heart rate in one cat. In the other cat the initial stimulant effects of icv dopamine were followed by prolonged hypotension and bradycardia.

Fig. 47 also demonstrates that 50 µl sterile 0.9% $^{W}/_{v}$ sodium chloride solution produced no effects on blood pressure and heart rate after icm infusion. Also the icm infusion of normal saline did not produce emesis, indicating that emesis was induced by the drugs administered and not as a result of the icm infusion.

DISCUSSION

Isoprenaline (30,45 µg) administered icv and icm produced tachycardia and hypotension in two cats. The magnitude and duration of the responses to isoprenaline were similar by either route of administration. Thus, it would appear that the tachycardias and hypotension observed after isoprenaline are produced by stimulation of brain areas quickly perfused by both routes.

Infusion of isoprenaline into the left lateral ventricle allows passage of isoprenaline, by virtue of the flow of the cerebrospinal fluid (c.s.f) into the third ventricle via the foramen of Monro. From the third

ventricle isoprenaline may pass retrogradely into the right lateral ventricle or pass through the aqueduct into the fourth ventricle. The flow of c.s.f. may then transport the isoprenaline through the lateral foramena of Luschka into the subarachnoid spaces and into the cisterna magna, that portion of the subarachnoid cavity between the cerebellum and the medulla. From the cisterna magna c.s.f. passes down the spinal canal within the subarachnoid space where it circulates around and upwards and finally enters the venous circulation. Fluid from the cisterna magna also bathes all parts of the brain.

In the previous chapter it was demonstrated that the cardiovascular effects of isoprenaline, after administration into the lateral ventricle were due to simulation of brain structures situated near the cerebro-ventricular lumen or near the surface of the brain stem and not due to leakage of isoprenaline into the peripheral blood stream (compare results of previous Chapter with those of Section 6.)

As the response to icv isoprenaline was rapid in onset, the start of the response occurring before the end of the infusion, the initial part of the response at least might be expected to be due to stimulation of structures within the diencephalon (e.g. the hypothalamus which forms part of the lateral walls and floor of the third ventricle) rather than areas within the hind brain or brain stem.

After infusion of isoprenaline into the cisterna

magna the area of stimulation was most likely to be the brain stem (i.e. the medullary region). The volume of the icm infusion was reduced to 50 µl, compared to the 100 µl volume used for icv infusions, in an attempt to localise the isoprenaline effect. It seems unlikely that the responses to icm isoprenaline, in these two cats, were due to leakage of isoprenaline into the venous circulation because they were substantially reduced or completely abolished by relatively small icm doses of β-adrenoceptor antagonists.

Perfusion of the medulla oblongata by icm infusions is further demonstrated by the frequent observations of emesis after isoprenaline, dopamine and β-adrenoceptor antagonists administered by this route. Borison & Brizzee (1951) demonstrated that the emetic chemoreceptor trigger zone of the cat was situated in the medulla oblongata. The zone is essentially a non-neural zone situated between the ala cinerea and the vestibular nucleus complex and is in the proximity of the area postrema which overlies the ala cinerea.

Emesis was never observed as a result of isoprenaline and dopamine administered into the lateral ventricles which suggests that the concentration of isoprenaline after icv administration, at the cisterna magna did not reach sufficient levels to produce emesis. However, from Section 2, clonidine icv frequently produced vomiting. Clonidine-induced vomiting occurred within 5 minutes of the completion of the icv infusion. This also suggests that compounds infused into the lateral ventricles quickly reach hind brain areas.

Although little indication was provided from the isoprenaline results of icm and icv administration, as to the exact central site of action of isoprenaline, the results concerning icv and icm dopamine were more revealing. By comparing the responses to icv and icm dopamine in 2 cats, it was observed that larger pressor effects were obtained with smaller doses of icv dopamine than observed after dopamine icm. Also it was observed that the depressor effects obtained after icm dopamine were slightly larger than those obtained with icv administration and that their onset was quicker after icm dopamine than icv dopamine.

Pressor effects were present after icv dopamine in both cats. Hypotension and bradycardia followed the initial stimulant effects of icv dopamine in only one cat. However, cardiovascular depressant effects were observed after icm dopamine in both cats.

Thus, the pressor effects and small associated tachycardias observed after icv dopamine may be due to a direct dopamine action via β-adrenoceptors within diencephalic areas whilst the hypotension and bradycardia observed after dopamine administered centrally may be mainly due to formation of noradrenaline within the medullary region stimulating central α-adrenoceptors. It may also be possible that dopamine is able to partially stimulate these central α-adrenoceptors directly.

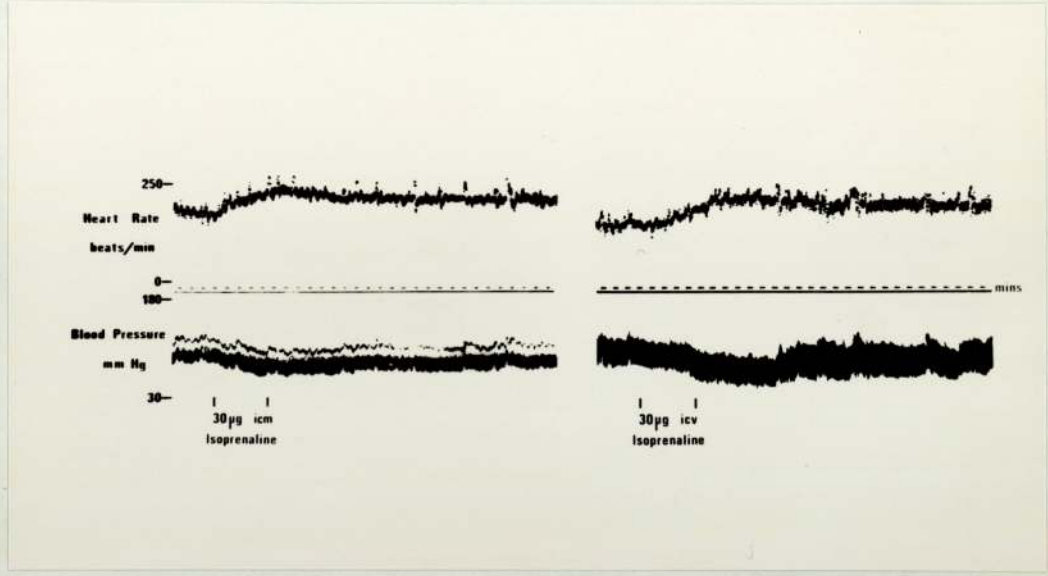


FIG. 46.

Conscious, normotensive, unrestrained cat. Comparison of the central responses to isoprenaline given icv or into the cisterna magna (icm) of the same cat. The left trace demonstrates the tachycardia and hypotension obtained after icm isoprenaline (30 µg). The response of isoprenaline (30 µg) given icv is shown in the right trace. Isoprenaline given by both routes in the same cat produced similar effects. The icm and icv isoprenaline infusions were made in volumes of 50 and 100 µl respectively.

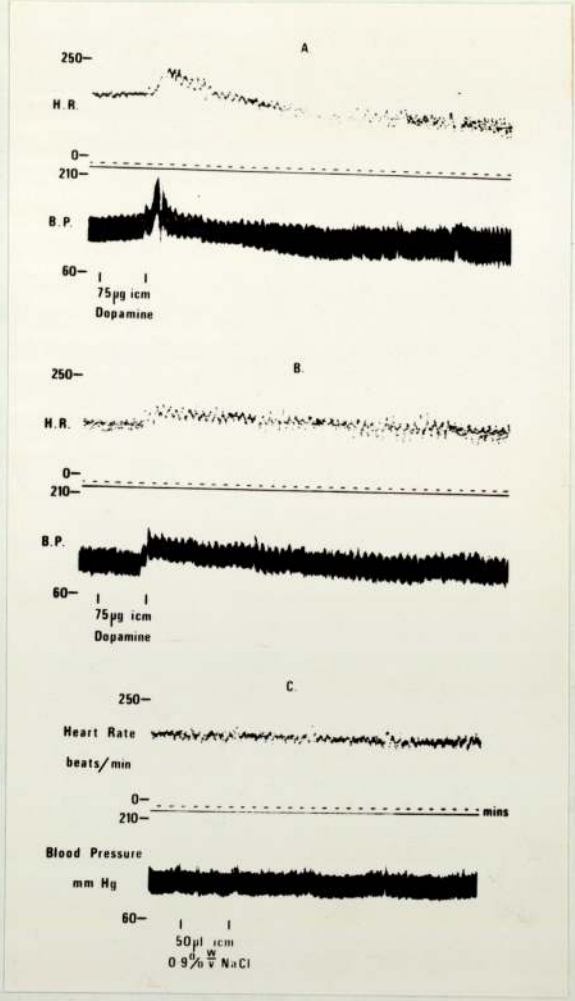


FIG. 47.

Conscious, normotensive, unrestrained cat. The upper trace demonstrates a biphasic cardiovascular response to dopamine (75 µg, icm) given alone. Emesis occurred 2 minutes after the end of the icm infusion. Hypotension and bradycardia developed from the initial short lasting hypertension and tachycardia. The middle trace shows that only the pressor effect with tachycardia were present after icm dopamine (75 µg) infused 60 minutes after phentolamine (0.5 mg, icv); the hypotension and bradycardia of icm dopamine were absent. Icm phentolamine failed to produce any cardiovascular effects. The lower trace shows that 50 µl icm infusion of 0.9% w/v sodium chloride did not alter blood pressure or heart rate.

CHAPTER 3. The effect of anaesthesia upon the centrally mediated cardiovascular effects of icv isoprenaline and adrenaline in the cat.

With the exception of Day & Roach (1972,1973) Dollery et al. (1973) and Conway and Lang (1974), who used conscious cats, rabbits and dogs respectively, all the published evidence concerning the cardiovascular effects of central α- and β-adrenoceptor stimulation has been obtained with anaesthetised animals.

Dollery et al. (1973) and Conway & Lang (1974) produced differing results from those of Day & Roach (1972, 1973) in that the latter workers observed regular pressor effects after icv isoprenaline in the conscious cat whilst in the conscious rabbit and dog it produced only hypotension.

Bolme, Ngai, Uvnas & Wallenberg (1967) demonstrated that pressor effects obtained after electrical stimulation of hypothalamic and mesencephalic areas were much more common in conscious than in anaesthetised dogs.

An attempt to explain the differences between the depressant nature of icv isoprenaline on blood pressure observed in most anaesthetised animals and the pressor effects observed after icv isoprenaline in several conscious cats was made in this series of experiments by anaesthetising previously cannulated cats used for conscious experiments with icv isoprenaline. A comparison was made between the type of blood pressure response obtained after icv isoprenaline and adrenaline in 6 cats before and after the influence of anaesthesia.

Results

3 cats, which had previously responded to icv isoprenaline (30,45 µg) in the conscious state with hypertension and tachycardia, were anaesthetised with a gaseous mixture of halothane, nitrous oxide and oxygen. Isoprenaline was administered icv after the blood pressure and heart rate had remained at a steady level for at least 10 minutes. Halothane caused the blood pressure and heart rate to fall slightly in all 3 cats.

It was found that under halothane anaesthesia icv isoprenaline produced similar responses to those obtained in the conscious state. Fig. 48 illustrates a pressor effect (mainly of systolic blood pressure) accompanied by a tachycardia obtained after icv isoprenaline under halothane anaesthesia.

The magnitude of the pressor effect and tachycardia appeared to be reduced by the anaesthetic but the type of response and its time course remained relatively unaltered. It was observed in all 3 cats that pretreatment with dl-propranolol (0.5 - 1.0 mg, icv) 60 minutes before a second icv isoprenaline dose, completely abolished the central pressor effects and tachycardias due to icv isoprenaline.

After completion of these experiments using halothane anaesthesia the cats were allowed to recover. The effects of icv adrenaline were not investigated under the influence of halothane anaesthesia.

Of the 3 remaining cats used in this series of

experiments, 2 cats were found to respond to icv isoprenaline with pressor effects and tachycardia in the conscious state and in the other cat depressor effects and tachycardia were produced.

In all 3 chloralose anaesthetised cats, icv isoprenaline produced similar types of blood pressure responses as observed in previous experiments using these cats in the conscious state. As with the halothane anaesthesia, chloralose caused the pressor effects of icv isoprenaline in 2 cats to be reduced. In one of these cats the pressor effect of icv isoprenaline was only 25% of that observed in this cat when used in the conscious state. The size of the depressor effect observed after icv isoprenaline in the other cat was virtually unchanged by chloralose anaesthesia.

Adrenaline (120 µg, icv) was administered to one cat anaesthetised with chloralose that normally produced hypertension in response to icv isoprenaline or adrenaline (the latter in the presence of icv phentolamine) when conscious. Fig. 49 demonstrates the experiment in this cat in which adrenaline (120 µg, icv) was administered alone (trace A), 30 minutes after 0.5 mg icv phentolamine (trace B) and 30 minutes after 0.5 mg, icv dl-propranolol (trace C).

Adrenaline alone (120 µg, icv) produced a small biphasic pressor effect with little effect on heart rate (trace A). When administered 30 minutes after icv phentolamine (0.5 mg), it produced a large pressor effect and

tachycardia (trace B). This response was similar to that observed in this cat in the conscious state but the duration was much shorter under anaesthesia. The size of the pressor effect was also reduced by the anaesthetic. Trace C demonstrates almost complete inhibition of the icv adrenaline response when administered 30 minutes after dl-propranolol (0.5 mg, icv).

Unlike halothane, chloralose produced a reduction in the duration of the central responses to icv isoprenaline and adrenaline as well as a reduction of the magnitude of the induced pressor responses.

Cats anaesthetised with chloralose were not allowed to recover.

DISCUSSION

Isoprenaline (30, 45 µg, icv) normally produced rises in blood pressure and heart rate in 5 of the 6 conscious cats used. In the remaining conscious cat icv isoprenaline caused hypotension with a tachycardia.

Halothane and chloralose anaesthesia did not alter the type of blood pressure and heart rate responses in any of the 6 cats, although the sizes of the pressor effects to icv isoprenaline and adrenaline were reduced. Little change was observed in the size of the depressor effect of icv isoprenaline in one chloralose anaesthetised cat although the duration of response was reduced when compared to the normal icv isoprenaline response observed in this cat in the conscious state.

The effect of anaesthesia upon central cardiovascular responses is varied. McCubbin et al. (1960) demonstrated that the central cardiovascular effects mediated after catecholamines administered icv were essentially similar in anaesthetised and conscious dogs.

Icv isoprenaline was reported to produce hypotension and tachycardia in anaesthetised and unanesthetised dogs (Bhargava et al., 1972; Conway & Lang, 1974). However, since some doubt exists as to whether the cardiovascular effects of icv isoprenaline observed by Conway & Lang (1974) in conscious dogs are centrally mediated, these results cannot be convincingly compared to those of Bhargava et al. (1972) in the anaesthetised dog. The latter workers demonstrated that the icv effects of isoprenaline in the anaesthetised dog were nervously mediated from the brain.

Stimulation of hypothalamic and mesencephalic brain areas produced pressor effects in conscious dogs. During chloralose anaesthesia the pressor effect was less pronounced (Bolme et al., 1967). Thus, these workers observed that the pressor responses were more common in conscious animals after central stimulation since anaesthesia abolished or reduced the responses.

Icv nicotine, in chloralose anaesthetised cats produced a hypotensive response but was observed to produce a rise followed by a fall in blood pressure in unanaesthetised cats. Hence, it appeared that the rise in pressure due to icv nicotine was abolished by subsequent anaesthesia with chloralose (Armitage & Hall, 1967a).

Pentobarbital anaesthesia was also observed to alter the response to icv adrenaline in rabbits (Toda et al., 1969). In the conscious rabbit, icv adrenaline produced hypertension and tachycardia followed by hypotension and bradycardia. During anaesthesia the initial rises in blood pressure and heart rate were absent.

Isoprenaline icv in anaesthetised rabbits produced hypotension and tachycardia (Toda et al., 1969). As described in Chapter 4 of this section, hypotension has been observed in combination with tachycardia after icv isoprenaline in conscious rabbits. However, these observations were made in a minority of animals used. Hypertension was observed in the majority of conscious rabbits treated with icv isoprenaline. In contrast, Dollery et al. (1973) have never reported pressor effects after icv isoprenaline in conscious rabbits.

In chloralose anaesthetised cats, Gagnon & Melville (1967) failed to observe pressor effects with icv isoprenaline. However, in contrast, hypertension in combination with tachycardia were common observations after icv isoprenaline and other β-adrenoceptor agonists in the conscious cats used in this project. Results in this Chapter indicate that the same type of responses can be obtained in halothane and chloralose anaesthetised cats as in conscious cats after icv isoprenaline or adrenaline, although the responses were reduced in magnitude. Chloralose anaesthesia failed to reduce the hypotension observed after icv isoprenaline in one cat.

Thus the nature of the response obtained after icv isoprenaline in cats and rabbits appears to be due directly to the technique or method of icv administration. The method by which drugs are administered icv determines the exact location of stimulation within the brain. For example, different icv volumes and speed of administration might be expected to produce differing diffusion of isoprenaline within the brain and thus the type of response obtained might depend upon which brain areas were preferentially stimulated.

Variable results obtained by a particular group of workers using a constant technique may be accounted for by slight anatomical and physiological differences between different animals.

In conclusion, it would appear that if icv isoprenaline produced hypotension then anaesthesia did not alter this response. However, when isoprenaline produced pressor effects then anaesthesia reduced them. This may account for the fact that pressor responses have never been reported in anaesthetised animals after icv isoprenaline.

Although complete abolition of the pressor responses to icv isoprenaline was never achieved, the depth of anaesthesia may not have been sufficient in the 6 cats used to completely inhibit the responses.

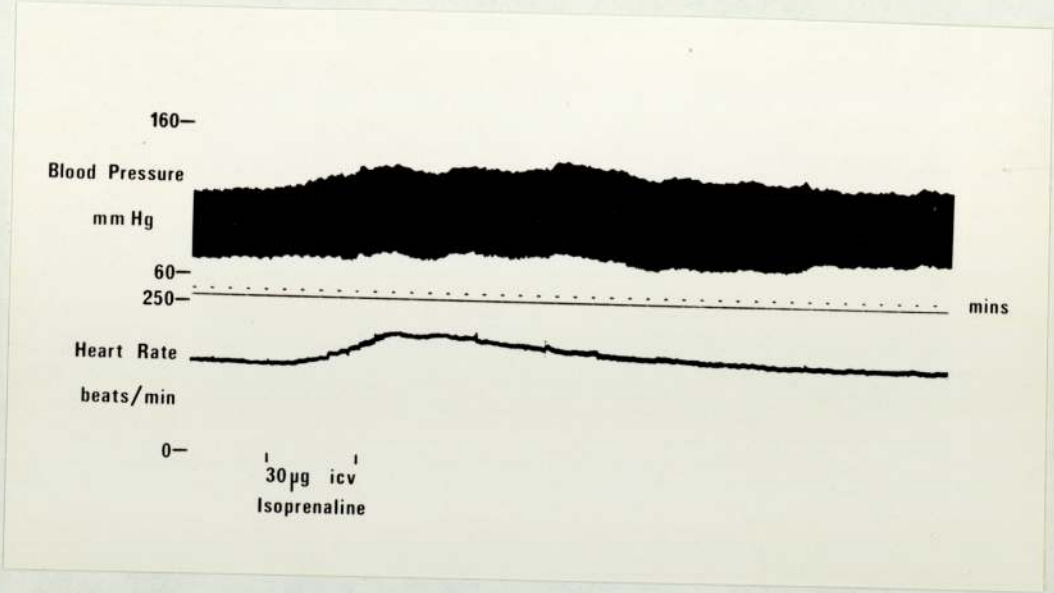


FIG. 48.

Halothane anaesthetised, normotensive cat. During halothane anaesthesia, icv isoprenaline (30 µg) produced hypertension (mainly of the systolic blood pressure) associated with tachycardia. This response obtained during anaesthesia can be compared to the response to isoprenaline (30 µg, icv), seen in Fig. 40, recorded in the same cat, but under normal conscious and unrestrained conditions.

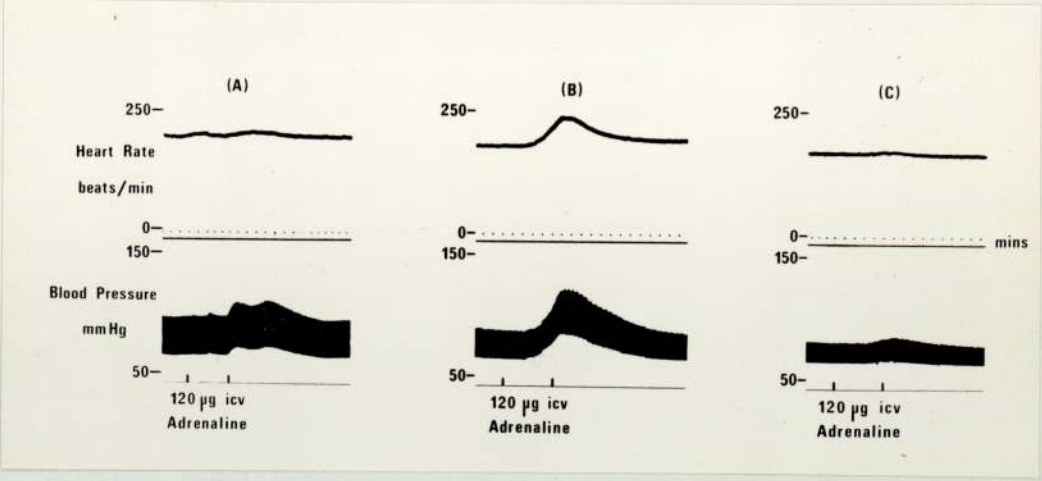


FIG. 49.

Chloralose (80 mg/kg, i.v.) anaesthetised, normotensive cat. Trace A demonstrates the response to adrenaline (120 µg, icv) given alone; a small tachycardia and biphasic pressor effect resulted. Trace B illustrates the response to adrenaline (120 µg, icv) given 30 minutes after phentolamine (0.5 mg, icv). A large pressor effect and tachycardia occurred. Trace C shows the almost complete abolition of the adrenaline response when the agonist was infused 30 minutes after dl-propranolol (0.5 mg, icv).

CHAPTER 4. Cardiovascular effects observed after icv isoprenaline in conscious rabbits and rats.

Toda et al. (1969) and Dollery et al. (1973) observed, in anaesthetised and conscious rabbits respectively that icv isoprenaline (50 - 200 µg) produced falls in mean arterial blood pressure associated with tachycardia.

In anaesthetised rats, Lavy & Stern (1970) observed tachycardia after injection of isoprenaline (20 µg) into the third ventricle. Propranolol via this route produced a pronounced bradycardia. They did not record blood pressure effects.

Although Ito & Schanberg (1974) did not record the cardiovascular response to isoprenaline administered alone into the cisterna magna of anaesthetised rats, they did report its effect after icm α- and β-adrenoceptor blocking agents. Phentolamine and phenoxybenzamine injected (in volumes of 10 - 25 µl) icm produced depressor effects with bradycardias. Noradrenaline and phenylephrine reversed the depressor effect without altering the bradycardia. However, isoprenaline icm caused the blood pressure to fall further and the bradycardia remained unaltered.

Icm propranolol in low doses caused a significant increase in blood pressure whilst larger doses produced significant falls in pressure. All doses of icm propranolol produced bradycardia. The increase in mean arterial blood pressure after low doses of propranolol icm was further increased by noradrenaline and reversed by isoprenaline. After the large doses of icm propranolol, noradrenaline

produced an increase in blood pressure and isoprenaline decreased the pressure already at reduced levels by icm propranolol. Heart rate, although reduced by icm propranolol, was not altered by subsequent icm noradrenaline but was slightly increased by isoprenaline.

Thus, generally, these workers suggested that noradrenaline stimulated different receptors in the medullary region of the rat to produce opposing blood pressure effects. The heart rate effects could not be explained with respect to opposing α- and β-receptors.

A series of experiments was performed to observe the blood pressure and heart rate effects produced by infusion of isoprenaline into the lateral cerebral ventricles of conscious rabbits and rats.

Results.

1. Conscious rabbits.

Isoprenaline (150, 300 µg icv) was infused into 13 rabbits and produced tachycardias in all of them. In 8 of these rabbits icv isoprenaline produced pressor effects associated with the tachycardias whilst in 2 others it produced very small pressor effects. The mean blood pressure and heart rate changes produced in these 10 rabbits by icv isoprenaline (150, 300 µg) are summarised in Table 9.

150 µg isoprenaline icv appeared to increase the mean systolic pressure by a slightly smaller extent than the diastolic pressure whilst after 300 µg the converse was found.

Fig. 50 demonstrates a hypertensive response and tachycardia after icv isoprenaline (150 µg). The hypertension and tachycardia observed after icv isoprenaline (150 µg) was of similar duration to that obtained with icv isoprenaline in conscious cats. Dl-alprenolol (1.0mg, icv) produced an initial pressor effect followed by a large hypotension and bradycardia. 60 minutes after icv dl-alprenolol, isoprenaline (150 µg, icv) failed to produce any change in blood pressure and heart rate. The pressor effects and tachycardias observed in 10 conscious rabbits after icv isoprenaline (150, 300 µg) were totally inhibited by icv pretreatment with dl-alprenolol or dl-propranolol (1.0mg).

In the 3 remaining rabbits, icv isoprenaline (150, 300 µg) produced small hypotensive effects associated with tachycardia. The diastolic blood pressure decreases in these rabbits to the 150 µg icv dose were 6, 10 and 12 mmHg and to the 300 µg dose of isoprenaline 9,15 and 16 mmHg. The diastolic pressures were observed to be decreased by a larger amount than the systolic pressures.

The hypotensive responses in these 3 rabbits induced by icv isoprenaline were prevented by icv pretreatment with dl-alprenolol or dl-propranolol (1.0mg).

The 150 µg isoprenaline dose was infused in a volume of 50 µl over a period of 4 minutes. Isoprenaline (300 µg, icv) was administered in a volume of 100 µl and infused in a period of 4 minutes also.

0.9% $^{w}/_{v}$ sterile sodium chloride solution (50µl&100µl

infused over 4 minutes) did not produce any changes in blood pressure and heart rate.

Conscious rats

Isoprenaline (0.3, 0.5 µg) was administered icv to 12 conscious rats. Both doses of isoprenaline given icv produced tachycardias in all 12 rats. In 7 of these animals the tachycardias were associated with increases in blood pressure. In a further 2 rats icv isoprenaline did not significantly increase the blood pressure and in the remaining 3 rats hypotension was recorded. The mean values of the blood pressure increases and tachycardias obtained with icv isoprenaline (0.3, 0.5 µg) summarised in Table 9 are taken from experiments using the first 9 rats (i.e. the 7 rats that responded with rises in blood pressure and the 2 rats that produced little blood pressure effects.)

Fig. 51 shows two pressor responses in combination with tachycardias after icv isoprenaline (0.3 µg). It can be seen that responses to icv isoprenaline are short lasting, unlike those observed in the cat and rabbit.

Hypotension with tachycardia was obtained after icv isoprenaline (0.3, 0.5 µg) in 3 rats. The hypotension and tachycardia were of short duration. The blood pressure and heart rate effects in all rats had usually completely terminated within 10 minutes of the icv isoprenaline administration. The falls in diastolic blood pressure in each of the 3 rats after the 0.3 µg dose of isoprenaline were 6, 8 and 11 mmHg and after the 0.5 µg dose, 9,14 and 13 mmHg.

The cardiovascular effects due to icv isoprenaline in all 12 rats were inhibited by pretreatment, 45 minutes before, with dl-alprenolol and dl-propranolol (250 µg, icv). Icv administration of either of the β-adrenoceptor antagonists produced an initial small rise in blood pressure followed by a prolonged and marked fall in blood pressure. The initial stimulant effect on blood pressure was occasionally accompanied by a small tachycardia, although in the majority of cases a bradycardia occurred from the onset after icv administration of the β-adrenoceptor blockers. The bradycardia was the most pronounced effect observed after icv dl-alprenolol or dl-propranolol. The resting heart rate usually fell from approximately 250 beats/minute to around 150-175 beats/minute.

The short lasting cardiovascular effects seen after icv isoprenaline were due to central actions of isoprenaline and not to artifacts of the icv administration. The 10 µl volume containing the dose of isoprenaline or β-blocker was slowly injected at a rate of 1.0 µl/second In initial experiments, in which the 10 µl volumes were administered as a quick injection, the rats appeared disturbed by the injection. This procedure often made the rats move around the cage thus disturbing the blood pressure and heart rate records. It was found that by injecting the drugs at a much slower rate, the rats were not disturbed and remained still thus allowing the responses to be clearly recorded. In Fig. 51 the rat remained completely still during the period recorded.

Slow icv injection of 10 µl of 0.9% W/v sterile sodium chloride in the conscious rat produced no changes in resting blood pressure and heart rate.

Experiments were not performed using either i.v. pempidine or bethanidine to confirm that leakage of isoprenaline did not occur. The responses to icv isoprenaline were most probably centrally mediated as they were inhibited by low icv doses of dl-propranolol or dl-alprenolol.

DISCUSSION

Icv isoprenaline produced either pressor or depressor effects in conscious rabbits and rats in combination with tachycardias. These results were similar to those observed in conscious cats. In the majority of conscious animals used icv isoprenaline produced pressor effects in combination with tachycardia.

The difference in the majority of results described in this Chapter to those in anaesthetised rabbits and rats (Toda et al., 1969; Ito & Schanberg, 1974) may be explained mainly by the use of anaesthetics by the latter two groups of workers. However, anaesthesia cannot account for the differences, obtained in conscious rabbits in this series of experiments, with the results of Dollery et al. (1973). The rabbits used in this project and by Dollery and co-workers are of the New Zealand white variety. Dollery et al. (1973) produced hypotensive effects in their experiments with 50 µg isoprenaline icv. It is interesting to note that this dose of isoprenaline icv in rabbits used for this project produced no change in blood pressure

and heart rate. The icv dose of isoprenaline had to be increased to 150 µg to produce clear measurable cardiovascular responses. The conscious rabbits used by Dollery and colleagues appeared to be sensitive to similar doses of dl-propranolol (1.0mg) to those used in this project. Similar cardiovascular responses were obtained in these experiments to those reported by Dollery et al.(1973) with icv dl-propranolol. Thus, the only explanation accounting for the differences in the type of central isoprenaline response obtained in this project and by Dollery et al. (1973) may lie in the use of slightly different experimental techniques.

Hence, from experimental results described in this section, isoprenaline is able to stimulate areas within the brains of cats, rabbits and rats leading to similar centrally mediated cardiovascular effects. These effects would appear to be produced by the same central β-adrenoceptor mechanism in the cat, rabbit and rat.

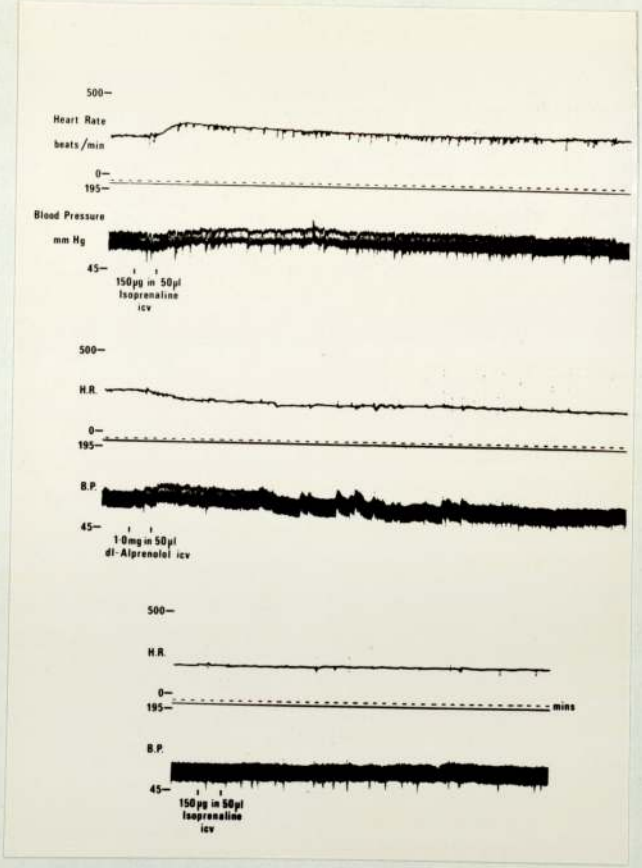


FIG. 50.

Conscious, normotensive, unrestrained rabbit. The upper trace shows the control hypertensive effect and tachycardia observed after isoprenaline (150 µg, icv). The middle trace illustrates the cardiovascular effect of dl-alprenolol (1.0 mg, icv). Dl-alprenolol icv induced an initial pressor effect which developed into hypotension. Bradycardia resulted directly from the icv infusion of the β-blocker. The lower trace shows the complete blockade of the isoprenaline (150 µg, icv) response when given 60 minutes after the icv infusion of dl-alprenolol (1.0 mg) All icv infusions in the rabbit were given in 50 µl volumes.

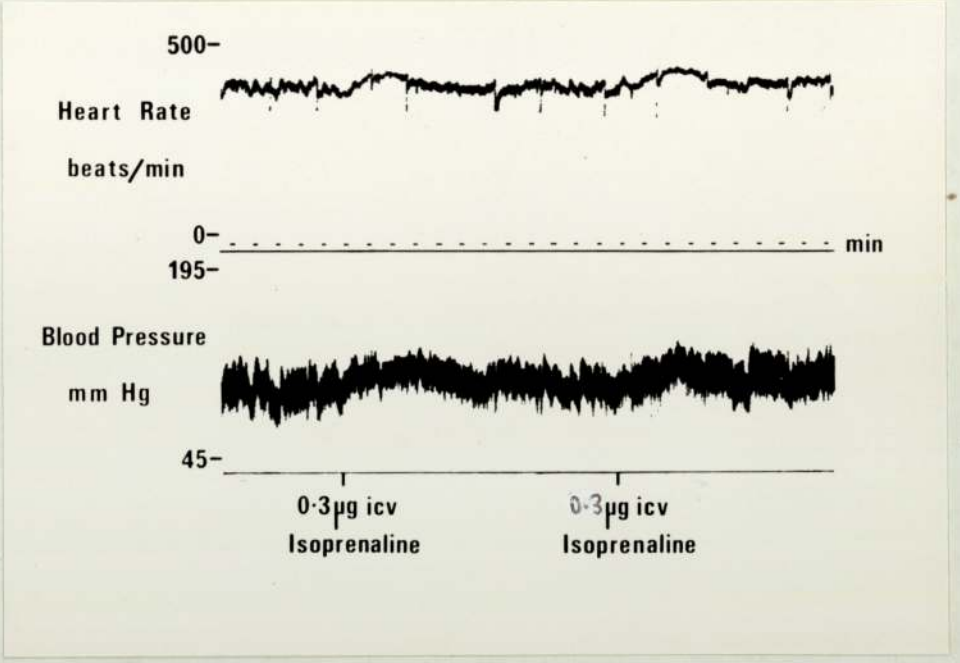


FIG. 51.

Conscious, normotensive, unrestrained rat. Trace shows blood pressure and heart rate increases to two doses of 0·3 µg isoprenaline infused icv in volumes of 10 µl over periods of 10 seconds.

SPECIES		RABBIT (10)		RAT (9)	
ISOPRENALINE. TOTAL icv dose µg.		150	300	0.3	0.5
Increase in arterial blood pressure mmHg ± s.e.m.	SYSTOLIC	10.2 ± 3.7	19.6 ± 4.6	20.6 ± 5.0	25.3 ± 4.9
	DIASTOLIC	11.1 ± 4.1	18.9 ± 4.3	19.3 ± 3.9	22.9 ± 4.4
Increase in Heart Rate beats/minute ± s.e.m.		35.6 ± 6.3	50.4 ± 5.9	49.7 ± 6.8	58.6 ± 7.1

TABLE 9. Maximal blood pressure and heart rate increases (mean ± s.e.m.) produced by icv isoprenaline in conscious, unrestrained, normotensive rabbits and rats. The number of animals used are in parenthesis after the species used.

SECTION 4. Analysis of the cardiovascular effects of seven β-adrenoceptor antagonists administered centrally in conscious normotensive cats.

CHAPTER 1. Effects on blood pressure and heart rate of icv β-adrenoceptor antagonists in conscious cats.

Since the initial observation of Prichard & Gillam (1974) that systemically administered propranolol caused a reduction of arterial blood pressure in hypertensive patients, several workers have confirmed this finding with propranolol and also with several other β-adrenoceptor antagonists. The mechanism of action of the antihypertensive activity of β-adrenoceptor antagonists is not clear (see Historical Introduction for references) but recent evidence suggests that a central mode of action may be important.

The antihypertensive effects of systemically administered β-adrenoceptor blockers have been difficult to demonstrate in animal studies (Farmer & Levy, 1968; Menard et al., 1973). In hypertensive rats, falls in blood pressure have been reported after systemic administration of relatively large doses of β-adrenoceptor antagonists (Roba et al., 1972; Vavra et al., 1973; Karppanen, 1974) and more recently after chronic low dose treatment with propranolol (Dusting & Rand, 1974).

Gagnon & Melville (1967) when investigating the central activity of isoprenaline in anaesthetised cats, observed that icv pronethalol occasionally produced hypotension and bradycardia.

Pronounced falls in blood pressure in the anaesthetised normotensive dog, after central administration of dl-propranolol, have been reported by several groups of workers. Hypotension and bradycardia occurred after dl-propranolol administration into the carotid (Stern et al., 1971) or vertebral (Stern et al., 1971; Carter et al., 1974). arteries or into the lateral or fourth cerebral ventricles (Srivastava et al., 1973; Carter et al., 1974) or after injections into the cisterna magna (Carter et al., 1974). Ito & Schanberg (1974) found that icm dl-propranolol in anaesthetised rats produced pronounced hypotension and bradycardia.

In conscious cats and rabbits (Day & Roach, 1972, 1973; Dollery et al., 1973) prolonged hypotension and bradycardia were observed after dl-propranolol administered icv. However, in conscious dogs, Conway & Lang (1974) failed to report any long-lasting cardiovascular depressant effects after icv dl-propranolol.

The mechanism by which the centrally mediated hypotension and bradycardia is produced after centrally administered dl-propranolol is still not clear. Kelliher & Buckley (1970) cast doubt as to whether the hypotension and bradycardia produced by icv dl-propranolol in anaesthetised cats is due to central β-adrenoceptor blockade since they found that icv d-propranolol, the optical isomer that possesses practically no β-blocking activity, induced a centrally mediated hypotension. In contrast to this observation, Dollery et al. (1973) using conscious rabbits found that the l-isomer but not the d-isomer of propranolol

was active in reducing the central actions of isoprenaline and producing hypotension and bradycardia itself.

More recently, Offerhaus & van Zwieten (1974) observed an insignificant difference in the hypotension after dl-propranolol was administered into the vertebral artery and femoral vein of anaesthetised cats. However, dl-, d- and l-alprenolol produced significant falls in blood pressure when infused into the vertebral arteries but were relatively ineffective after administration into the femoral vein. Thus, these workers suggested that propranolol probably lacked significant central hypotensive properties, whereas alprenolol most likely caused a centrally mediated hypotension but through a mechanism other than central β-adrenoceptor blockade.

In the dog and rabbit, hypotension and bradycardia were observed to follow initial short lasting hypertension and tachycardia after icv dl-propranolol (Srivastava et al., 1973; Dollery et al., 1973).

The present series of experiments were undertaken to establish the central cardiovascular effects of 7 β-adrenoceptor antagonists after icv administration to conscious cats. It was also hoped to investigate the mechanisms by which β-adrenoceptor blockers produce their central cardiovascular effects in the cat.

Results.

1. Short and long-term cardiovascular changes observed after icv β-adrenoceptor antagonists.

The centrally mediated blood pressure and heart

rate responses of 7 β-adrenoceptor blockers were observed in 25 conscious cats. The antagonists administered icv were propranolol, alprenolol, pindolol, practolol, oxprenolol, sotalol and ICI 66082. The blood pressures and heart rates of the conscious cats were monitored for at least 2 hours before and 4 - 6 hours after the icv administration of the β-blockers.

The cardiovascular response to each blocker, with the exception of ICI 66082, was biphasic. Initially, rises in blood pressure and heart rate were observed after icv infusions of the blockers. The maximum rises occurred 5 - 15 minutes from the end of the icv infusion. The initial cardiovascular stimulant effects were followed by a prolonged hypotension and bradycardia. The maximum falls in blood pressure and heart rate were observed 0.75 - 1.5 hours after icv infusions of the antagonists. Control resting levels of blood pressure and heart rate were usually reached within 3 - 4 hours after central administration of the β-adrenoceptor antagonists.

The mean maximum initial cardiovascular stimulant and prolonged depressant effects observed after the 7 β-adrenoceptor antagonists administered icv are summarised in Tables 10a,b. Two dose levels of 5 β-adrenoceptor blockers were given icv. From Tables 10a,b, both parts of the biphasic response after icv dl-propranolol, dl-alprenolol. pindolol, practolol and ICI 66082 are dose-dependant.

With the exception of ICI 66082, all the β-adrenoceptor blockers given icv produced pronounced initial

stimulant effects. ICI 66082 produced no cardiovascular stimulant effect at the 1.0 mg dose level and after 2.5 mg icv produced a small rise in blood pressure without affecting the heart rate. Fig. 52 illustrates a typical record of an experiment involving icv administration of ICI 66082 (2.5 mg icv) to a conscious cat. In this particular cat ICI 66082 (2.5 mg,icv) produced no initial stimulant effects on blood pressure and heart rate and an immediate hypotension and bradycardia resulted from the icv infusion.

Oxprenolol icv produced the most marked stimulant effects. Oxprenolol (0.5mg) was administered icv on one occasion only to 6 cats. The mean increase in systolic pressure was 89.1 $\pm$ 13.6 mmHg and heart rate 37.5 $\pm$ 6.5 beats/minute. The large standard errors on the mean blood pressure increases are due to a large variation in the initial blood pressure stimulation produced by oxprenolol. For example, Fig. 53 demonstrates an experiment in which oxprenolol (0.5 mg, icv) produced very potent central effects. Before the icv infusion of oxprenolol the resting blood pressure and heart rate were $^{135}/_{90}$ mmHg and 175 beats/minute respectively. At the end of the 4 minute icv infusion the blood pressure and heart rate had risen sharply to $^{250}/_{172}$ mmHg and 215 beats/minute, respectively. This large increase remained almost constant for approximately 2 minutes, after which the blood pressure and heart rate slowly decreased. Approximately 30 minutes after the end of the icv infusion, the blood pressure and heart rate had fallen below the pre-dosage resting levels to $^{120}/_{75}$ mmHg and 135 beats/minute.

In 2 cats, pronounced respiratory stimulation

occurred after icv oxprenolol with increases to 220 and 260 in the respiration rate from normal levels of approximately 30 breaths/minute. In both cases, the tachypnoea was associated with panting. Tachypnoea and panting occurred during the experiment shown in Fig. 53. The time of onset of panting is indicated by the fluctuating heart rate observed approximately 2 minutes from the end of the icv oxprenolol infusion. Normal respiration rates were observed approximately 30 minutes after the oxprenolol administration.

Piloerection was observed in the 2 cats that responded with increases in respiratory rate. However, piloerection was also observed in 2 other cats after icv oxprenolol. Mydriasis was observed in all 6 cats after icv oxprenolol administration. Because of the severity of the initial stimulant effects obtained after icv oxprenolol the dose was not increased above 0.5 mg.

After oxprenolol, the order of stimulant potency was pindolol > alprenolol > propranolol > sotalol > practolol > ICI 66082.

The d-isomers of propranolol and alprenolol produced initial rises in blood pressure and heart rate. Local anaesthetics, procaine and lignocaine, also produced initial stimulant effects after icv administrations (See Table 10a).

Table 10a demonstrates that very similar mean increases in blood pressure and heart rate occurred with dl-, d- and l- propranolol (0.5 mg, icv). Similar stimulant

effects of equal doses of d- and l- propranolol (0.5mg, icv) are clearly shown in Fig. 54. Each isomer of propranolol was administered icv on a separate day to a cat that was particularly sensitive to icv propranolol (Unfortunately, the blood pressure scales of the two records are not the same). In the upper trace d-propranolol (0.5 mg icv) produced an initial increase in systolic blood pressure and heart rate of 58 mmHg and 45 beats/minute respectively. The same dose of l-propranolol icv produced rises in the systolic blood pressure and heart rate of 54 mmHg and 40 beats/minute respectively.

Piloerection was frequently observed in combination with the initial cardiovascular stimulant effects after the β-adrenoceptor antagonists with the exception of sotalol, practolol and ICI 66082. The d-isomers of propranolol and alprenolol and the local anaesthetics, procaine and lignocaine, administered icv also produced piloerection. Pupil dilatation was also a common observation with these substances.

A small increase in respiratory rate was occasionally noticed after icv pindolol, alprenolol and propranolol. Panting was never observed after any β-adrenoceptor antagonist other than oxprenolol.

Emesis did not occur after icv administrations of β-adrenoceptor antagonists, the d-isomers of propranolol and alprenolol and the local anaesthetics, lignocaine and procaine (compare these findings with those observed after icm β-adrenoceptor blockers).

Defaecation occurred in one cat after icv oxprenolol during the initial cardiovascular stimulant effects. However, this was the only occurrence of defaecation after icv administration of these compounds. As with vomiting, defaecation was much more frequent after icm administration of β-adrenoceptor blocking agents.

All 7 β-adrenoceptor antagonists used icv produced hypotension and bradycardia (see Table 10b). Propranolol was the most active compound, on a weight basis, followed by alprenolol.

It is difficult to list the order of potency of the β-adrenoceptor antagonists with respect to their depressant effects on blood pressure and heart rate, since the β-blockers icv appeared to produce differing depressant actions on blood pressure and heart rate. For example, dl-alprenolol, pindolol and ICI 66082 (1.0 mg, icv) produced mean reductions in the diastolic blood pressures of 26.5, 22.9 and 22.6 mmHg respectively. However, the bradycardias observed after these 3 blockers were 33.9, 26.9 and 38.4 beats/minute respectively. Thus, although icv ICI 66082 produced the smallest hypotension it induced the largest bradycardia of these 3 compounds on a weight basis.

Practolol and sotalol (2.0mg, icv) produced similar hypotension and bradycardia. Oxprenolol (0.5mg, icv) produced larger hypotensive and bradycardic effects than observed after pindolol (0.75mg), practolol (2.0mg) and sotalol (2.0mg). Examples of hypotension and bradycardia

by dl- and l-propranolol, ICI 66082 and oxprenolol are shown in Figs. 32, 54, 52 and 53 respectively.

The differences between the depressant effects observed after icv l-propranolol (0.5mg) and dl-propranolol (1.0mg) on systolic blood pressure ($p > 0.20$), diastolic blood pressure ($p > 0.15$) and heart rate ($p > 0.25$) were not significant.

The d-isomers of both propranolol and alprenolol (0.5 and 0.75mg respectively, icv) failed to reduce the blood pressure and heart rate after the initial stimulant effects had subsided. Icv administrations of lignocaine and procaine also failed to produce hypotension and bradycardia (see Table 10b). Fig. 54 illustrates the lack of hypotensive and bradycardic activity of d-propranolol icv in comparison to the active l-isomer of propranolol.

Table 10b lists the mean percentage inhibition of the icv isoprenaline-induced tachycardia at each dose level of all the compounds used in this series of experiments In initial experiments, it was observed that the maximum inhibition of the tachycardia to icv isoprenaline occurred during the peaks of the sustained depressor and bradycardia responses of the β-adrenoceptor blockers. Peak depressant effects of the β-adrenoceptor antagonists usually occurred between 0.75 - 1.5 hours after the icv infusion of the antagonist. Subsequent experiments in which the percentage inhibition of the icv isoprenaline tachycardia was measured, were performed with isoprenaline administered icv 60 minutes after the β-adrenoceptor antagonist centrally.

The progression of the inhibition of the icv isoprenaline tachycardia paralleled the time course of the falls in blood pressure and heart rate produced by the β-blockers administered centrally. The d-isomers of propranolol and alprenolol failed to inhibit the central effects of isoprenaline, as did the local anaesthetics, lignocaine and procaine.

At the time of the maximum cardiovascular depressant effects produced by icv β-adrenoceptor antagonists the responses to systemically-administered isoprenaline (200 - 400 ng/kg, i.v.) remained unaltered.

2. Cardiovascular effects produced by a combination of dl-propranolol and clonidine administered icv.

In section 2, Chapter 4, part 3, it was reported that icv dl-propranolol failed to inhibit the centrally mediated cardiovascular depressant actions of clonidine in 3 cats. Fig. 55 demonstrates the cardiovascular effects of a combination of dl-propranolol and clonidine given icv. A large hypotension and bradycardia was obtained using a smaller dose than usual of each substance.

Lowering the dose of clonidine to 5.0 µg reduced the usually observed side effects. Vomiting was absent and the sedative effects were not apparent. Reduction of the dl-propranolol dose to 0.5mg reduced the magnitude of the initial cardiovascular stimulatory effect.

Dl-propranolol (0.5 mg, icv) was administered 60 minutes before clonidine (5.0µg, icv). At the time of the clonidine icv infusion the blood pressure and heart

rate had fallen from $^{140}/_{102}$ to $^{125}/_{87}$ mmHg and 165 to 140 beats/minute. Icv clonidine (5 µg) further reduced the blood pressure and heart rate to $^{108}/_{65}$ mmHg and 100 beats/minute. The normal resting levels of blood pressure and heart rate were reached approximately 3.5 hours after the icv clonidine administration.

DISCUSSION

Several mechanisms have been proposed to account for the antihypertensive activity of β-adrenoceptor blocking agents. These are discussed in the Historical Introduction However, none of these peripheral mechanisms are wholly satisfactory.

Many clinically used β-blockers are known to enter the central nervous system. Several reports have shown that centrally administered dl-propranolol lowers blood pressure and heart rate in anaesthetised normotensive dogs, cats and rats and in conscious cats and rabbits (see introduction of this Chapter for references.)

The results presented in this Chapter demonstrate that icv administration in conscious normotensive cats, of 6 other clinically active β-adrenoceptor blocking agents, as well as dl-propranolol, are able to produce centrally mediated hypotension and bradycardia.

Four main points indicate that the centrally mediated cardiovascular depressant effects, produced after icv β-adrenoceptor antagonists are due to central β-adrenoceptor blockade and not to other properties of these compounds.

Firstly, the d-isomers of propranolol and alprenolol, which are devoid of β-adrenoceptor blocking activity but possess all the other properties of propranolol and alprenolol (Fitzgerald, 1969), administered icv did not lower blood pressure and heart rate. This observation is in contrast to the findings of Kelliher & Buckley (1970) and Offerhaus & van Zwieten (1974) but is consistent with the results obtained in conscious rabbits by Dollery et al. (1973). Similarly, the local anaesthetics lignocaine and procaine were found not to reduce blood pressure and heart rate after icv administration in conscious cats. Dollery et al. (1973) also observed icv procaine to be inactive in the conscious rabbit.

Carter et al. (personal communication) have produced, in anaesthetised dogs, falls in blood pressure and heart rate after central administration of d-propranolol and local anaesthetics. All the groups of workers that have reported cardiovascular depressant effects after central administration of these compounds possessing local anaesthetic activity have used anaesthetised animals. Anaesthesia, therefore, may alter the responses to centrally administered local anaesthetics. However, icv procaine and lignocaine were observed to produce only pressor effects in both conscious and anaesthetised dogs (Haranath, Begum & Sitaramayya, 1965; Haranath & Bhatt, 1968). They did not observe depressor effects at any time after icv administration of procaine and lignocaine.

Secondly, ICI 66082, a relatively selective

β_1-adrenoceptor blocker without local anaesthetic or intrinsic β-agonist properties (Barrett, Carter, Fitzgerald Hull and Le Count, 1973; Harry, Knapp & Linden, 1973) caused marked falls in blood pressure and heart rate.

Thirdly, the maximum falls in blood pressure and heart rate coincided with the maximum inhibitory effect of the β-adrenoceptor antagonists on the centrally mediated tachycardia induced by icv isoprenaline.

Several workers have shown that propranolol possesses an adrenergic neuron blocking action (see Historical Introduction for references). However, the hypotension and bradycardia observed in these experiments after icv β-adrenoceptor antagonists cannot be explained in terms of a guanethidine or bethanidine type adrenergic neuron blockade. As described earlier (Section 3, Chapter 1, part 8), icv guanethidine and bethanidine in conscious cats frequently produced prolonged hypertension and tachycardia. Hypotension and bradycardia were only observed in 2 of 8 cats treated with icv adrenergic neuron blockers.

The effects of β-adrenoceptor antagonists in the conscious cat were confined to the central nervous system since the cardiovascular responses to intravenous isoprenaline were unaffected, unlike the observations in the conscious dog (Conway & Lang, 1974).

Spinal transection (at the level of C_2) completely abolished the hypotension and bradycardia induced by icv propranolol indicating that the depressant effects are nervously mediated from the central nervous system (Srivastava

et al., 1973). They also demonstrated that bilateral vagotomy did not significantly alter the icv effects of dl-propranolol indicating that, unlike clonidine, propranolol does not produce part of its cardiovascular depressant effects by inducing an increased peripheral parasympathetic tone. The prolonged hypotension due to icv propranolol remained after the procedures of bilateral adrenalectomy vagotomy and stellate ganglionectomy (thus removing neural and humoral influences on the heart) indicating that the fall in blood pressure after icv propranolol is not as a result of the bradycardia but directly due to a reduced sympathetic tone to the vasculature.

The initial stimulant effects on the cardiovascular system after icv β-adrenoceptor antagonists, reported in both anaesthetised and conscious animals, appear to be associated with either local anaesthetic or partial β-agonist properties or a combination of these effects. The mechanism of the initial stimulant responses is investigated and discussed in Chapter 2 of this Section.

Reports in the literature indicate that it is difficult to demonstrate antihypertensive effects after systemic treatment with β-adrenoceptor antagonists. The workers that successfully demonstrated hypotensive activity used relatively large doses, with the exception of Dusting & Rand (1974). Results described in this Chapter show that 7 clinically active antihypertensive β-adrenoceptor blocking agents effectively lowered blood pressure and heart rate after central administration into conscious cats. These findings suggest that the conscious normotensive cat may

be a suitable model for screening the antihypertensive activity of new β-adrenoceptor antagonists.

In clinical practice, drug combinations are now commonly used to treat hypertension. Additive effects are often obtained by drugs acting by different mechanisms to lower the blood pressure. The major advantage in using combination therapy is that lower doses of each compound are used and thus the chances of side effects are reduced. It was demonstrated in these experiments that centrally administered dl-propranolol and clonidine (given in smaller doses) produced a large combined hypotension and bradycardia. Thus, it may be that an effective combination of lower doses of dl-propranolol (or other effective β-adrenoceptor antagonists) and clonidine may prove clinically useful in the treatment of hypertension.

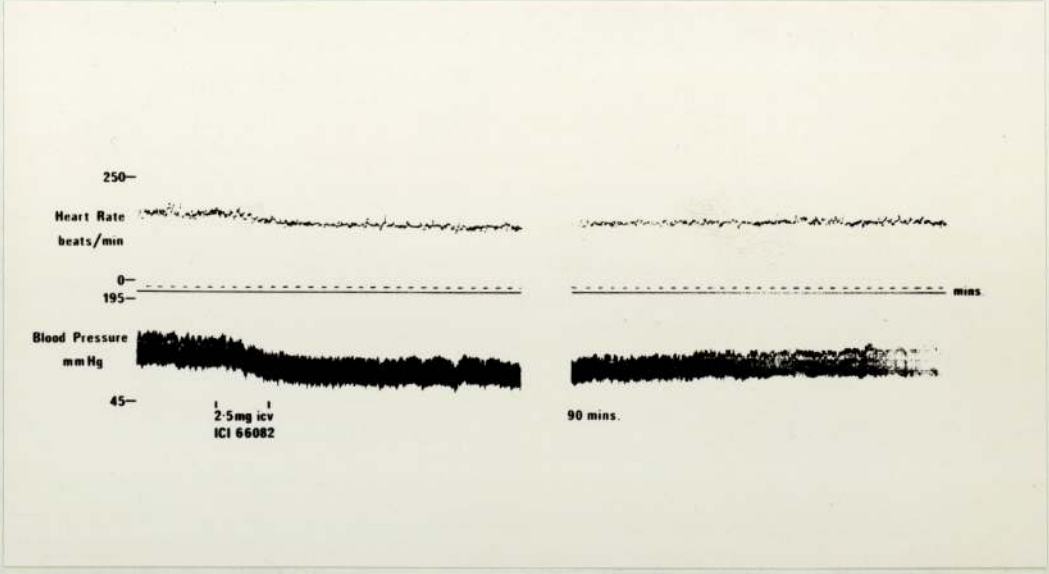


FIG. 52.

Conscious, normotensive, unrestrained cat. Illustration of a typical response to icv ICI 66082 (tenormin) (2.5 mg). It can be seen that no initial stimulant effects occurred and the blood pressure and heart rate fell immediately during and after the icv infusion. The gradual return of the hypotension and bradycardia to control resting levels can be seen to occur after 90 minutes.

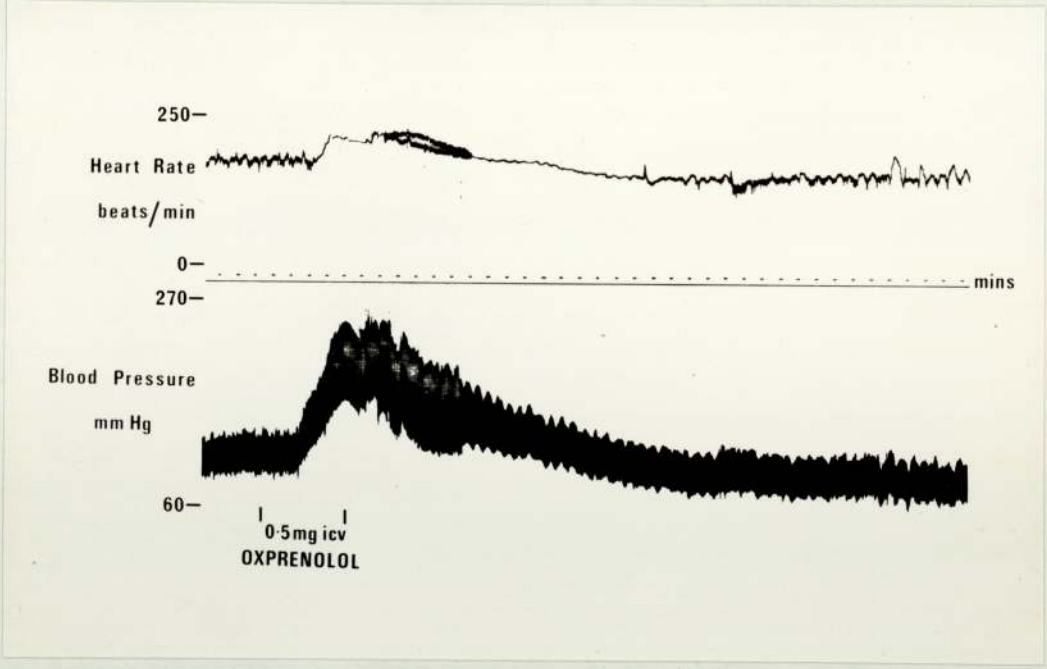


FIG. 53.

Conscious, normotensive, unrestrained cat. The trace demonstrates the extremely large pressor effect and tachycardia observed after oxprenolol (0.5 mg, icv). Panting occurred during the initial stimulatory effects of oxprenolol and is shown by the thickened heart rate record during the tachycardia. It can be seen that the initial pressor effect and tachycardia were relatively short lasting and potent hypotension and bradycardia developed.

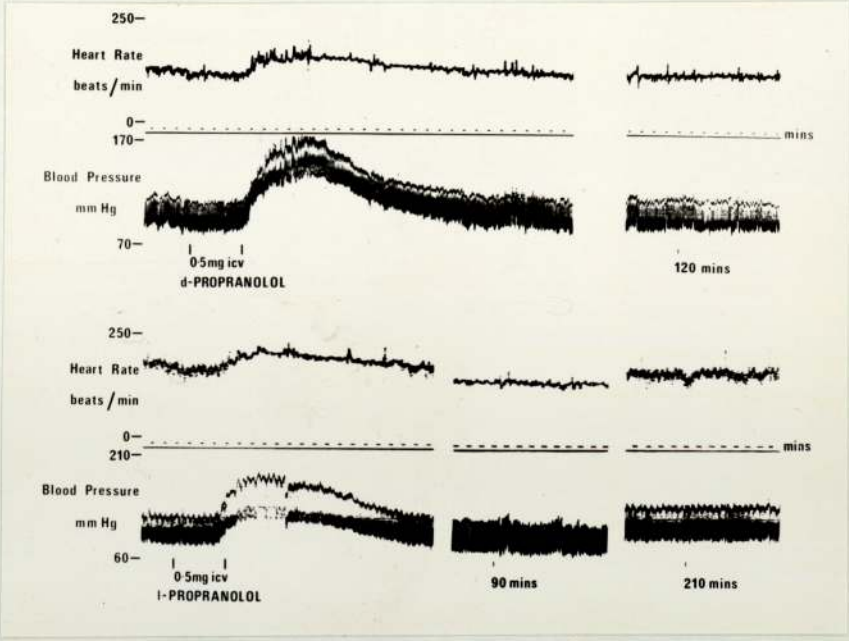


FIG. 54.

Conscious, normotensive, unrestrained cat. Comparison of the central cardiovascular effects to l- and d-propranolol (0.5 mg, icv) in the same cat. The upper traces show the initial pressor effect and tachycardia of d-propranolol (0.5 mg, icv) after the icv infusion and at 120 minutes the blood pressure and heart rate were still at normotensive resting levels; thus, hypotension and bradycardia did not occur. The lower traces demonstrate that l-propranolol (0.5 mg, icv) produced a very similar pressor effect and tachycardia to its d-isomer. However, at 90 minutes the blood pressure and heart rate were reduced below resting levels and the hypotension and bradycardia had returned to normotensive levels within 210 minutes of the icv infusion.

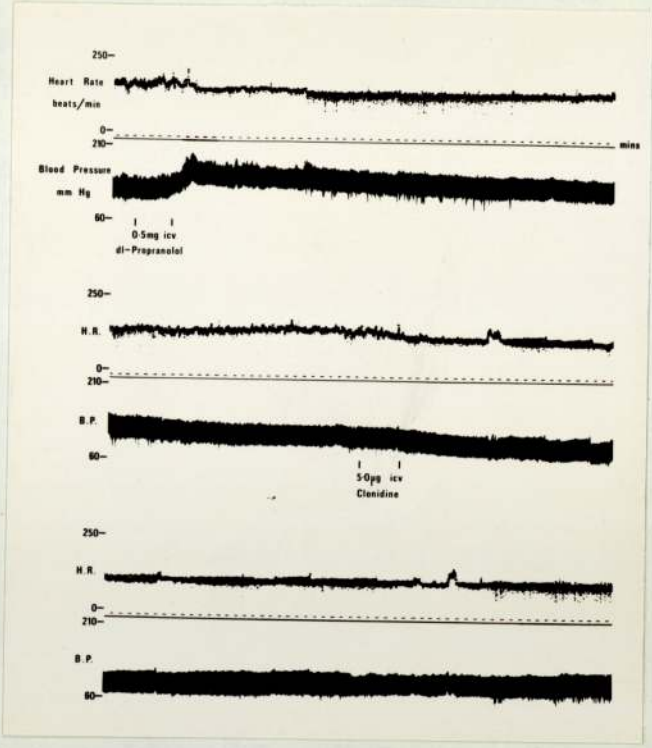


FIG. 55.

Conscious, normotensive, unrestrained cat. This figure shows 3 continuous traces of an experiment in which clonidine (5.0 µg, icv) was given 80 minutes after dl-propranolol (0.5 mg, icv). The upper trace illustrates the initial pressor effect of dl-propranolol. The development of bradycardia and hypotension after dl-propranolol is seen in the upper and middle traces. The icv infusion of clonidine (5.0 µg) further induced the blood pressure and heart rate to fall.

	TOTAL icv dose mg	INITIAL RISE Blood Pressure mmHg		HEART RATE beats / minute	No. of cats used
		Systolic	Diastolic		
dl-Propranolol	0.5	16.7 ± 3.2	16.2 ± 3.6	10.1 ± 2.2	12
	1.0	25.4 ± 2.8	24.1 ± 3.2	22.3 ± 3.6	10
d-Propranolol	0.5	18.4 ± 3.6	17.7 ± 3.8	12.4 ± 3.9	5
l-Propranolol	0.5	15.3 ± 3.3	15.1 ± 3.4	9.8 ± 3.5	5
dl-Alprenolol	0.5	19.9 ± 2.4	20.1 ± 2.5	12.4 ± 2.1	12
	1.0	28.4 ± 2.6	27.6 ± 2.3	25.7 ± 2.5	10
d-Alprenolol	0.75	21.7 ± 3.9	20.2 ± 3.8	15.4 ± 4.2	5
Pindolol	0.75	27.8 ± 6.8	18.2 ± 3.0	35.0 ± 6.1	5
	1.0	45.6 ± 9.4	27.0 ± 5.4	41.7 ± 8.0	5
Practolol	2.0	10.3 ± 3.8	9.6 ± 3.7	7.3 ± 2.4	5
	4.0	18.9 ± 4.1	15.9 ± 3.9	14.0 ± 2.4	4
ICI 66082	1.0	NIL	NIL	NIL	6
	2.5	5.1 ± 2.1	4.8 ± 2.3	NIL	5
Sotalol	2.0	16.8 ± 4.3	15.9 ± 4.1	8.4 ± 3.9	5
Oxprenolol	0.5	89.1 ± 13.6	62.0 ± 7.9	37.5 ± 6.5	6
Procaine	1.5	28.4 ± 4.6	26.1 ± 4.4	26.0 ± 5.1	4
Lignocaine	0.75	30.1 ± 2.3	29.3 ± 3.5	26.3 ± 3.6	5

TABLE 10a. Maximal initial blood pressure and heart rate increases observed after icv administration of β-adrenoceptor blockers and local anaesthetics in conscious unrestrained normotensive cats. Initial rise is the peak stimulant effect usually reached 5 - 15 minutes after the end of the icv infusion.

	TOTAL icv dose mg	PROLONGED FALL Blood Pressure mmHg		HEART RATE beats/minute	% Inhibition of tachycardia to icv isoprenaline	No. of cats used.
		Systolic	Diastolic			
dl-Propranolol	0.5	24.7 ± 2.9	24.9 ± 2.8	30.7 ± 3.2	87	12
	1.0	29.9 ± 3.1	30.3 ± 2.9	41.5 ± 3.6	100	10
d-Propranolol	0.5	NIL	NIL	NIL	NIL	5
l-Propranolol	0.5	25.8 ± 4.0	26.0 ± 3.8	38.6 ± 3.7	100	5
dl-Alprenolol	0.5	20.3 ± 3.1	20.5 ± 3.2	24.5 ± 2.8	81	12
	1.0	26.4 ± 2.6	26.5 ± 2.7	33.9 ± 3.0	100	10
d-Alprenolol	0.75	NIL	NIL	NIL	NIL	5
Pindolol	0.75	14.3 ± 1.2	16.0 ± 1.5	17.8 ± 2.8	92	5
	1.0	22.1 ± 2.9	22.9 ± 3.2	26.9 ± 3.1	100	5
Practolol	2.0	12.6 ± 2.2	12.6 ± 2.4	19.7 ± 3.7	66	5
	4.0	20.3 ± 3.0	20.9 ± 3.2	29.4 ± 3.8	85	4
ICI 66082	1.0	22.4 ± 3.1	22.6 ± 3.1	38.4 ± 4.6	71	6
	2.5	32.8 ± 3.7	34.2 ± 3.6	47.6 ± 5.0	88	5
Sotalol	2.0	15.1 ± 3.8	15.9 ± 3.9	18.2 ± 3.5	67	5
Oxprenolol	0.5	14.7 ± 2.6	14.3 ± 2.1	25.0 ± 2.7	79	6
Procaine	1.5	NIL	NIL	NIL	NIL	4
Lignocaine	0.75	NIL	NIL	NIL	NIL	5

TABLE 10b. Maximal prolonged blood pressure and heart rate decreases observed after icv administration of β-adrenoceptor blockers and local anaesthetics in conscious unrestrained normotensive cats. The prolonged fall refers to the maximal depressor effect recorded 60 - 75 minutes after the end of the icv infusion. The inhibition of the icv isoprenaline induced tachycardia was measured 60 minutes after the administration of each blocking agent.

CHAPTER 2. Investigation into the initial cardiovascular stimulant effects of centrally administered β-adrenoceptor antagonists.

Srivastava et al. (1973) and Dollery, Lewis, Myers & Reid (1974) have investigated the pressor effects and tachycardias produced by centrally administered propranolol in the anaesthetised dog and conscious rabbit.

Srivastava et al. (1973) observed that the pressor effect and tachycardia were observed after administration of propranolol into the lateral ventricles, but these effects were absent on introduction of the β-blocker into the fourth ventricle; thus indicating that the stimulatory effect of propranolol must arise from suprapontine structures, probably the hypothalamus. These workers suggested that propranolol produced stimulation of the hypothalamus leading to a centrally mediated stimulation of catecholamine release from the adrenal medulla. The pressor effect and tachycardia was abolished after bilateral adrenalectomy and by peripheral α-adrenoceptor blockade with yohimbine. The rise in blood pressure and heart rate was associated with an increase in the total catecholamine output in the adrenal vein.

With respect to the central mechanism of propranolol, Dollery et al. (1974) observed that icv pretreatment with yohimbine significantly diminished the pressor effect of icv propranolol in the conscious rabbit. Srivastava et al. (1973) observed different results in the dog. They

found that central pretreatment with phenoxybenzamine did not alter the initial stimulant effects of propranolol whilst central treatment with either propranolol or INPEA abolished the initial effects of icv propranolol.

Both Dollery et al. (1973,1974) and Srivastava et al.(1973) implicated the importance of central adrenergic neurons in the mediation of the initial stimulant effects of propranolol. Both groups reduced or abolished the hypertension and tachycardia of icv propranolol by central destruction of central catechclamine containing neurons with 6-hydroxydopamine or by depletion of catecholamines from central neurons by reserpine pretreatment.

Dollery et al. (1974) reduced the hypertension and tachycardia seen after icv propranolol by icv pretreatment with desmethylimipramine, a tricyclic compound which is known to inhibit the uptake of noradrenaline in adrenergic neurons (see Iversen, 1967). They hypothesised that the propranolol is taken up into central noradrenergic neurons via uptake$_1$ and thus causes the release of endogenously stored noradrenaline. This in turn produces a pressor effect and tachycardia, due to central α-adrenoceptor stimulation.

Experiments were performed using conscious cats to investigate the possibility that the initial stimulation produced by icv β-adrenoceptor antagonists is caused by β-agonist activity or local anaesthetic activity or by a combination of these properties.

RESULTS

(a) Mechanism of stimulant effects within the brain.

The pressor effects produced by dl-propranolol, practolol and lignocaine were investigated. These 3 compounds were used because propranolol possesses membrane stabilising activity, practolol possesses only β-agonist activity and lignocaine is a potent local anaesthetic without intrinsic β-agonist activity.

1. Effect of icv phentolamine pretreatment.

Pretreatment with icv phentolamine (0.6mg) failed to alter the initial stimulant effects of icv dl-propranolol (1.0mg), practolol (2.0mg) and lignocaine (0.75mg) in each of 3 cats. This central dosage of phentolamine was sufficient to inhibit the centrally mediated effects of noradrenaline (30 µg, icv) administered 60 minutes after the α-adrenoceptor blocking agent.

2. Effect of icv dl-propranolol pretreatment

Using the same 3 cats, as in 1 above, icv administrations of 1.0 mg of dl-propranolol infused 60 minutes before icv dl-propranolol (1.0mg), practolol (2.0 mg) or lignocaine (0.75 mg) completely blocked the initial effects of practolol and lignocaine and reduced the stimulation produced by propranolol. In all 3 cats propranolol pretreatment totally inhibited the effects of practolol and lignocaine but did not fully reduce the effects of propranolol.

Fig. 56 illustrates the stimulatory effects of dl-propranolol, practolol and lignocaine. These control

responses were each obtained on the day before the experiments involving dl-propranolol in combination with the 3 compounds above. The second responses to dl-propranolol, practolol and lignocaine were obtained 60 minutes after icv dl-propranolol (1.0mg). It was shown in previous experiments that this time coincides with the maximum central β-blocking action of icv dl-propranolol.

Control experiments were performed in 3 cats to ensure that the reduction in the stimulant effects of the 3 compounds after icv dl-propranolol was not due to the local anaesthetic action of propranolol. Lignocaine (0.75 mg, icv) administered 60 minutes before dl-propranolol (1.0mg), practolol (2.0mg) or lignocaine (0.75 mg) had no effect on the stimulant effects of the 3 compounds.

3. Effect of icv desmethylimipramine pretreatment.

1.0mg desmethylimipramine was infused icv 60 minutes before 1.0mg dl-propranolol icv in 4 cats. In all cats, icv desmethylimipramine produced rises in blood pressure and heart rate. In 2 cats, the blood pressure was still elevated 60 minutes after the icv infusion, the time of the icv dl-propranolol administration. In the remaining 2 cats, the hypertensions and tachycardias were of relatively short duration (lasting normally for approximately 30 minutes). In these cats the blood pressure and heart rate were slightly below control levels 60 minutes after the desmethylimipramine.

The initial stimulatory effects of dl-propranolol (1.0mg, icv) were completely absent in 2 cats. At the time of the icv dl-propranolol infusions the blood pressure

and heart rate due to icv desmethylimipramine was still raised in one cat and slightly depressed in the other. The hypotension and bradycardia due to icv dl-propranolol were unaffected by icv desmethylimipramine and were observed soon after the icv infusion of dl-propranolol.

Dl-propranolol (1.0mg, icv) still produced pressor effects, although reduced, in the remaining 2 cats pretreated with icv desmethylimipramine. The maximum increases in systolic blood pressure were 15 and 18 mmHg compared to control responses of 35 and 45 mmHg.

The pressor effects induced by icv dl-propranolol in the presence of central desmethylimipramine were not of the short duration (15 - 30 minutes) normally observed after dl-propranolol administered icv alone, but remained elevated for approximately 60 minutes, after which time the pressure gradually returned to control levels. The blood pressure was observed to progress below normal levels. The hypotensive effects of dl-propranolol after icv desmethylimipramine were accompanied by bradycardias. These depressant effects were observed to be similar to the control effects of icv dl-propranolol.

(b) Peripheral mechanism of stimulant effects.

1. Peripheral ganglion blockade.

Pempidine (7.5mg/kg, iv) or hexamethonium (10 mg/kg, iv) administered 30 - 60 minutes before icv dl-propranolol (1.0 mg) or lignocaine (0.75mg) abolished the initial hypertension and tachycardia normally produced by these compounds in each of 3 cats. The complete abolition of the

pressor effect of icv dl-propranolol (1.0mg) by hexamethonium (10mg/kg, i.v.) is demonstrated in Fig. 57. It can also be seen from Fig. 57 that the resulting hypotension and bradycardia regularly observed after icv dl-propranolol were also absent. The absence of the hypotension and bradycardia after ganglion blockade was observed in all 3 cats.

Hexamethonium (10mg/kg, i.v.) raised the heart rate in all cases by approximately 35-40 beats/minute and caused slight falls in blood pressure of about 10-15 mmHg.

2. Peripheral adrenergic neuron blockade.

The effect of bethanidine (7.5mg/kg, i.v.) on the response to icv dl-propranolol (1.0mg) was tested in 3 cats. A rise in blood pressure after icv dl-propranolol was observed in 2 cats but was absent in the third cat. During adrenergic blockade by bethanidine, a large pressor effect occurred (an increase in systolic blood pressure of 60mmHg), beginning 5 minutes after the end of the icv propranolol infusion. A bradycardia of 30 beats/minute accompanied the pressor effect. The blood pressure returned to control levels after approximately 20 minutes.

In the second cat, a 30mmHg increase in systolic blood pressure was observed in combination with a 15 beat/minute bradycardia. In both cats the bradycardia subsided as the hypertension returned to control levels. Hence the usual tachycardia observed after icv dl-propranolol, under control conditions, was not present after i.v. bethanidine pretreatment.

Dl-propranolol (1.0mg, icv) failed to produce

hypotension and bradycardia, when administered after i.v. bethanidine in all 3 cats tested.

Bethanidine (7.5mg/kg, iv) administered slowly always produced large hypertensive effects combined with reflex bradycardia. As the pressor effects returned to normal control levels the heart rate remained lowered producing bradycardias of 30,40 and 30 beats/minutes respectively in 3 cats. Only in the cat with the 40 beat/minute bradycardia did the blood pressure fall below control resting levels causing a hypotension of 24 mmHg. In the other cats, the blood pressure remained at normotensive levels.

DISCUSSION

Dollery et al. (1974) observed, in the conscious rabbit, that the initial stimulatory effects of icv dl-propranolol were probably due to central α-adrenoceptor stimulation as they were abolished by icv yohimbine, an α-adrenoceptor antagonist. However, Srivastava et al. (1973) observed that icv phenoxybenzamine did not affect the initial stimulant effect of icv dl-propranolol whilst icv pretreatment with propranolol or INPEA abolished or reduced these effects.

The mechanism by which propranolol produced initial stimulant effects in the conscious cat appeared to be the same as in the dog, since central α-adrenoceptor blockade with icv phentolamine did not alter the response whilst central β-adrenoceptor blockade reduced it.

The initial stimulant effects of practolol appear to be due to its intrinsic β-adrenoceptor agonist activity

since the compound possesses little or no local anaesthetic activity (Fitzgerald, 1969) and the stimulant effects were abolished by icv dl-propranolol.

The stimulant effects of icv lignocaine, a potent local anaesthetic, were completely abolished by icv pretreatment with dl-propranolol. The initial effects of dl-propranolol itself, were probably not due to an intrinsic β-agonist action but due to its local anaesthetic activity. Practolol but not dl-propranolol appears to be able to stimulate post-synaptic β-adrenoceptors leading to initial pressor effects and tachycardias.

Lignocaine may produce its pressor effect and tachycardia by releasing noradrenaline or dopamine from central neurons, consequently producing β-adrenoceptor stimulation. Propranolol also possesses pre-synaptic actions.

The reduction in the stimulant effects by dl-propranolol was due to central β-adrenoceptor blockade and not to prolonged local anaesthesia as icv pretreatment with lignocaine failed to alter the intial responses to icv dl-propranolol, practolol or lignocaine.

With respect to the initial stimulant effects of dl-propranolol the rabbit may be different to cats and dogs. If the stimulant effects of icv dl-propranolol were produced mainly as a result of an indirect mechanism, i.e. the release of endogenously stored amines, the rises in blood pressure and heart rate may be due to α-adrenoceptor stimulation in the rabbit and β-adrenoceptor stimulation in the cat and dog.

Although the type of central adrenoceptor stimulation appears to be different in the cat and rabbit, essentially similar results were obtained in the cat to those reported by Dollery et al. (1974) in the rabbit, concerning the effect of icv pretreatment of desmethylimipramine on the initial stimulant effects of icv dl-propranolol. Dollery et al. (1974) found that icv desmethylimipramine greatly reduced the pressor effects and tachycardia produced by d-propranolol indicating that a large part of the response was due to stimulation of catecholamine release from central neurons.

In 2 of 4 cats treated with icv desmethylimipramine, the stimulant effects of icv dl-propranolol were completely abolished which suggests that, in these 2 cats, the stimulant effects were probably dependant upon endogenous catecholamine release. In the remaining 2 cats, the pressor effects of icv propranolol were reduced by approximately 60% of control values by icv desmethylimipramine. The residual effects are accountable either by stimulation of post-synaptic β-adrenoceptors or because the uptake mechanism was not fully inhibited by icv desmethylimipramine.

Since Dollery et al. (1974) found that central α-adrenoceptor blockade inhibited the stimulant effects of dl-propranolol, it is possible that the icv dose of desmethylimipramine (1.5mg) may have reduced the stimulant properties of dl-propranolol to some degree by α-adrenoceptor blockade. In the conscious cat the icv dose of desmethylimipramine was reduced to 1·0 mg, which is comparable to the dose of desmethylimipramine infused into the vertebral arteries of anaesthetised cats by van Spanning & van Zwieten (1973). It is

unlikely that this dose of desmethylimipramine icv affected the initial response of icv dl-propranolol by α-adrenoceptor blockade as phentolamine, a potent α-adrenoceptor blocking agent, was found to be ineffective.

Therefore, it would appear likely from these experiments using conscious cats, that dl-propranolol produced its initial stimulant effects mainly by being taken up into catecholaminergic neurons and releasing endogenous amines; these in turn produced stimulation of central β-adrenoceptors. As propranolol does not possess intrinsic sympathomimetic activity its membrane stabilising action may account for the indirect sympathomimetic action of propranolol.

Unfortunately, experiments to show the effect of desmethylimipramine on the central actions of icv practolol and lignocaine were not performed. Hence it is not known whether compounds such as practolol and pindolol which possess intrinsic β-activity but are devoid of local anaesthetic activity and in contrast to propranolol which only possesses membrane activity (Fitzgerald, 1969), produce their effects by releasing endogenous amines or by direct stimulation of post-synaptic β-adrenoceptors.

It was observed in the experiments using icv desmethylimipramine that the uptake inhibitor did not affect the hypotension and bradycardia of icv dl-propranolol. Therefore, it would seem that the central cardiovascular depressant effects of dl-propranolol are due to an inhibition of central post-synaptic β-adrenoceptors, receptors which

may be able to produce pressor effects and tachycardias when stimulated, leading to a decreased sympathetic tone to the periphery.

The dual effects of icv dl-propranolol are both nervously mediated from the central nervous system as shown by Srivastava et al. (1973). These workers abolished the initial effects of icv dl-propranolol by bilateral adrenalectomy and the following bradycardia by bilateral stellate ganglionectomy and vagotomy. All central effects due to icv dl-propranolol, including the prolonged hypotension were abolished by spinal cord transection at C_2.

Complete ganglion blockade obtained by pempidine (7.5mg/kg) or hexamethonium (10mg/kg) abolished all the cardiovascular effects produced by icv dl-propranolol in the conscious cat. The prolonged hypotension and bradycardia normally produced after icv propranolol were also completely absent in the 3 cats pretreated with bethanidine, indicating that the cardiovascular depressant effects of propranolol were probably mediated by a reduced sympathetic tone to the vasculature and heart.

The initial tachycardia observed after icv propranolol was also absent after i.v. bethanidine; the pressor effect regularly observed with icv dl-propranolol was still present in 2 of the 3 cats tested but with an associated bradycardia. Boura et al. (1962) demonstrated that bethanidine possessed little affinity for the adrenal gland and would thus be relatively inactive in inhibiting catecholamine release from the adrenal medulla. Thus, it is

possible, as suggested by Srivastava et al. (1973), that propranolol acts centrally to produce release of catecholamines from the adrenal medulla. However, in one cat the icv stimulant effects of dl-propranolol were abolished by i.v. bethanidine and thus it may be possible that part of the initial icv dl-propranolol response (or possibly all of the response in this particular cat) is due to central β-adrenoceptor stimulation leading to a general increased sympathetic tone to the vasculature and heart.

Srivastava et al. (1973) suggested that the probable area of stimulation within the brain producing stimulant cardiovascular effects after icv dl-propranolol was in the hypothalamus. Electrical stimulation of the posterior hypothalamus has been shown to release catecholamines from the adrenal medulla (von Brucke, Kaindl & Mayer, 1952; Folkow & von Euler, 1954). Aconitine administered icv has been shown to release amines from the adrenal medulla by a mechanism which is inhibited by prior central β-adrenoceptor blockade. (Bhargava & Srivastava, 1972).

Although the blood pressure increases and tachycardias due to icv dl-propranolol and isoprenaline were both

inhibited by prior central β-adrenoceptor blockade, the pressor effects and tachycardias due to icv isoprenaline were completely abolished by i.v. bethanidine. This is in contrast to the results obtained in 2 out of 3 cats using icv dl-propranolol after i.v. bethanidine. The centrally mediated pressor effects and tachycardias produced by icv isoprenaline and dl-propranolol seem to be produced by different mechanisms. Dl-propranolol may induce part of its initial stimulant effect via a central β-adrenoceptor mechanism causing a release of catecholamines from the adrenal medulla whereas isoprenaline, by stimulating central β-adrenoceptors, produces an increased sympathetic output to the heart and vasculature. However, the mechanism/s by which the initial stimulant effects of propranolol are mediated to the periphery in the cat are still unclear and further experiments using adrenalectomised cats should be performed.

It is interesting to note that, in conscious rabbits and cats, icv desmethylimipramine produced pressor effects and tachycardias. This is in contrast to the findings of van Spanning and van Zwieten (1973). They observed transient hypotension and bradycardia after vertebral artery infusions of desmethylimipramine and explained the results in terms of central α-adrenoceptor stimulation in the rhombencephalon produced by the inhibition of noradrenaline re-uptake into central adrenergic neurons. The small delayed hypotensive and bradycardic effects observed in 2 of the 4 cats after icv desmethylimipramine may be attributed to central α-adrenergic stimulation by excess noradrenaline

present in the medullary region of the rhombencephalon.

The pressor responses obtained after icv desmethylimipramine and also icv guanethidine and bethanidine may be produced within the same brain areas by a similar mechanism to that of dl-propranolol. Desmethylimipramine, by virtue of its inhibition of the re-uptake processes, would produce catecholamine build up at the receptor area. Guanethidine and bethanidine, known to possess indirectly acting sympathomimetic properties (see Boura & Green, 1965) could produce direct release from central adrenergic neurons in a similar mechanism proposed for dl-propranolol.

However, experiments should be carried out to determine the type of central adrenoceptor stimulation produced by these compounds inducing pressor and tachycardia effects.

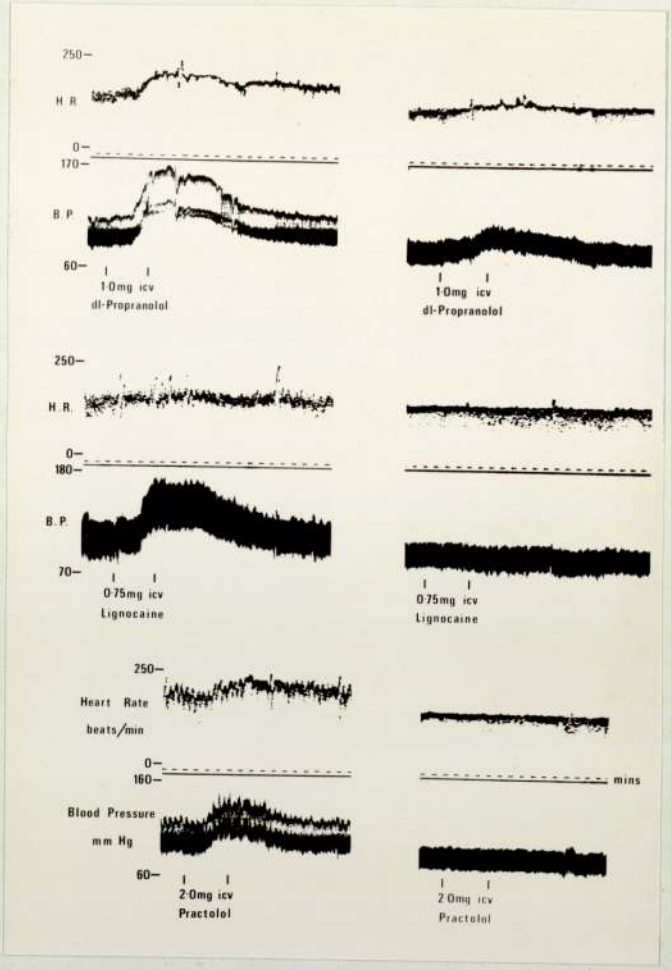


FIG. 56.

Conscious, normotensive, unrestrained cat. The traces on the left of the Fig. illustrate the respective control rises in blood pressure and heart rate to icv dl-propranolol (1.0 mg), lignocaine (0.75mg) and practolol (2.0 mg). These control responses were obtained on the day prior to the experiments involving the administration of dl-propranolol (1.0 mg, icv) in combination with the 3 compounds above. The traces on the right show the responses of icv dl-propranolol, lignocaine and practolol given 60 minutes after dl-propranolol (1.0 mg, icv). It can be seen that the response to dl-propranolol was reduced and those of lignocaine and practolol totally abolished by central β-blockade.

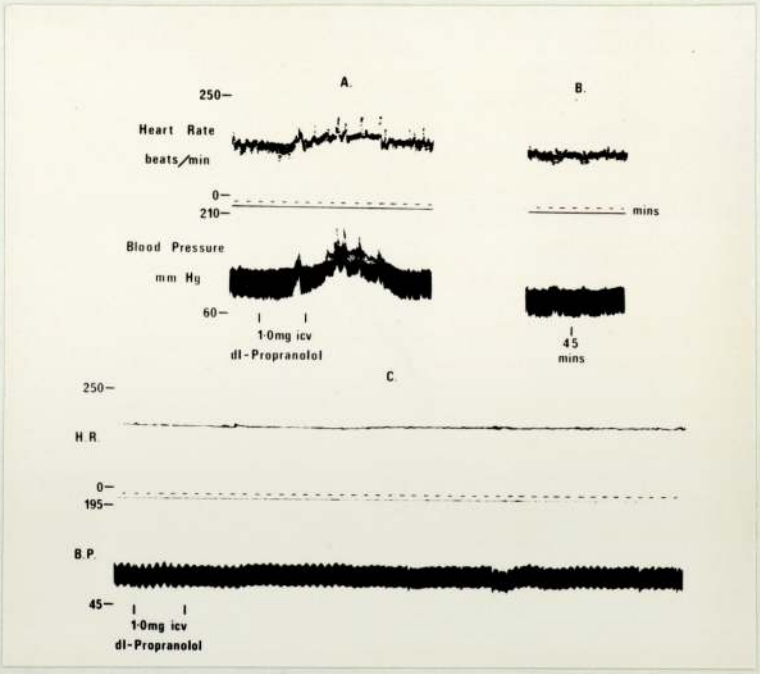


FIG. 57.

Conscious, normotensive, unrestrained cat. Trace A illustrates the initial pressor effect and tachycardia recorded after dl-propranolol (1.0mg, icv). Trace B demonstrates the peak hypotensive effect and bradycardia obtained 75 minutes after the dl-propranolol (1.0 mg, icv) infusion. This control response was obtained on the day prior to the experiment involving the administration of propranolol after i.v. hexamethomium. Trace C shows the infusion of dl-propranolol (1.0 mg, icv) 60 minutes after hexamethonium (10 mg/kg, i.v.). It can be seen that both the initial hypertension and tachycardia were absent and the cardiovascular depressant effects of dl-propranolol did not develop.

CHAPTER 3. Cardiovascular effects resulting from the administration of various β-adrenoceptor antagonists into the cisterna magna of conscious cats.

To date the blood pressure and heart rate effects of centrally administered propranolol have been mainly recorded after administrations into the carotid and vertebral arteries and into the lateral and fourth cerebral ventricles (for references see Chapter 1 of this Section). However, Carter et al.(1974) measured cardiovascular parameters after propranolol administration into the cisterna magna of anaesthetised dogs. They observed falls in heart rate, blood pressure, cardiac output and total peripheral resistance after icm propranolol. These workers did not report the presence of initial stimulant effects after icm propranolol.

Using 2 cats chronically cannulated for icm drug administrations an attempt was made to compare the responses obtained after icm dl-, l-propranolol, oxprenolol, pindolol, practolol and ICI 66082 with the responses obtained after these compounds administered icv to conscious cats.

RESULTS

The blood pressure and heart rate changes observed after icm dl- and l-propranolol, oxprenolol, pindolol, practolol and ICI 66082 were essentially similar to the responses produced by these compounds administered into the lateral cerebral ventricles.

Generally, each β-adrenoceptor antagonist, with the exception of ICI 66082, produced initial increases in blood pressure and heart rate after icm administration into both cats. All compounds administered icm produced prolonged hypotension and bradycardia. The initial increases in systolic blood pressure and heart rate and the subsequent decrease in diastolic blood pressure and heart rate observed after the β-adrenoceptor antagonists icv are summarised in Table 11.

ICI 66082 was administered icm to both cats at 2 dose levels. 2.0 mg ICI 66082 (icm) produced no initial cardiovascular stimulant effects in either cat. Small pressor effects and tachycardias were observed after 5.0 mg ICI 66082 icm. The increases in systolic pressure and heart rate were 10 and 13 mmHg and 15 and 15 beats/minute after ICI 66082 (5.0mg, icm) in each cat respectively. As observed after icv administration, ICI 66082 (2.0mg, icm) produced pronounced hypotension and bradycardia occurring immediately after the icm infusion. The peak effects were seen 30 - 45 minutes after the infusion. The maximum hypotension (fall in diastolic pressure) and bradycardia in each cat, were 22 and 25 mmHg and 30 and 35 beats/minute.

The maximum depressant effects on blood pressure and heart rate after 5.0 mg ICI 66082 (icm) were observed later than with the 2.0 mg dose because the large dose produced initial stimulant effects. The maximum hypotension and bradycardia, being 28 and 30 mmHg and 40 and 35 beats/minute, were observed approximately 45 - 60 minutes after the icm infusion.

Practolol (1.0 mg, icm) produced intitial pressor effects and tachycardias which lasted for approximately 15 minutes after the completion of the icm infusion. The increases in blood pressure and heart rate due to icm practolol were slightly larger than those seen after ICI 66082 (5.0mg, icm). Vomiting occurred in one cat after the icm infusion. Emesis occurred 5 minutes after the infusion, at a time when the blood pressure and heart rate increases had already reached a peak. The initial hypertension and tachycardia developed into hypotension and bradycardia in both cats. The peak cardiovascular depressant effects were observed 45 - 60 minutes after administration. Although the pressor effects of practolol (1.0 mg, icm) were larger than those to ICI 66082 (5.0 mg, icm), the depressant effects were smaller . The peak falls in diastolic pressure and heart rate, in each cat, were 10 and 12 mmHg and 20 and 25 beats/minute, respectively.

From Table 11, l-propranolol (0.25 mg), dl-propranolol (0.5mg), pindolol (0.5mg) and oxprenolol (0.5mg) administered icm produced very large prolonged hypertensive effects initially accompanied by pronounced tachycardias. Variable side effects were observed in combination with the cardiovascular stimulant effects, after icm administration of these 4 compounds.

As after icv administration, oxprenolol, (0.5 mg, icm) produced the largest increases in blood pressure and heart rate. Because of the disturbing effects observed after this icm dose of oxprenolol, it was only administered to

one cat. The systolic blood pressure was increased by 105 mmHg and was accompanied by a 60 beat/minute tachycardia. These maximum increases were observed 2.5 minutes after completion of the icm infusion of oxprenolol.

At the peak of the pressor effect the cat vomited. The blood pressure returned to the elevated level within one minute. 2 minutes later the blood pressure and heart rate began to slowly return to normal levels. At this time the respiration rate was observed to be greatly increased and the cat started to pant. The cat also defaecated and profuse salivation occurred.

The blood pressure and heart rate reached normal resting levels 30 minutes after the icm infusion of oxprenolol. Panting had ceased by this time but the respiration rate was still elevated. The hypertension and tachycardia, due to icm oxprenolol, gradually developed into a significant hypotension and bradycardia. The peak effects were observed 60 minutes after the icm infusion. The diastolic blood pressure was reduced by 18 mmHg and was associated with a 35 beat/minute bradycardia . Normal resting blood pressure and heart rate levels were observed 3.5 hours after icm oxprenolol administration.

Dl-propranolol and pindolol (0.5mg, icm) produced very similar blood pressure and heart rate effects. From Table 11, it can be seen that the magnitude of the initial stimulant effects and the subsequent depressant effects of both compounds administered icm were very similar.

Pindolol icm produced more marked side effects,

in combination with the initial hypertension and tachycardia, than dl-propranolol. After icm pindolol each cat vomited twice, their respiration rate increased dramatically and panting occurred. Both cats were observed to salivate and in 1 cat as it stood upright its body swayed from side to side. As it walked, it appeared to be ataxic. None of these side effects occurred until after the pressor effects had reached maximum levels and had started to return to contol levels. The systolic pressure and heart rate increases of 80 and 65 mmHg and 55 and 45 beats/minute were observed 3 and 4 minutes after the icm infusion, in each cat respectively.

Dl-propranolol produced similar cardiovascular effects as did pindolol icm. However, after icm dl-propranolol both cats vomited on one occasion only and although their respiration rates were increased, panting was not observed. Salivation occurred in only one of the 2 cats and neither cat appeared to become ataxic.

The hypertension due to icm pindolol and dl-propranolol (0.5 mg) was longer lasting than observed after oxprenolol (0.5 mg).

The elevated blood pressures returned to control levels after 45 - 60 minutes. However, in both cats, after icm pindolol and dl-propranolol the tachycardias were of shorter duration than the hypertensive effects. The tachycardias were observed to be terminated after approximately 30 minutes, after which time the heart rate progressively decreased below control rates. The maximum

bradycardias were seen at the time the initial elevated blood pressures had reached normotensive levels, The bradycardias remained constant while the hypotensive effects developed. The maximum hypotensive effects due to icm pindolol and dl-propranolol were recorded between 1.5 and 2.25 hours after the icm infusions of the β-blockers.

L-propranolol (0.25 mg, icm) produced smaller stimulant effects than dl-propranolol, pindolol and oxprenolol (0.5 mg, icm) but these were larger than the initial responses after practolol (1.0mg) and ICI 66082 (5.0mg). L-propranolol (0.25mg, icm) induced maximum rises in systolic blood pressure and heart rate of 45 and 35 mmHg and 30 and 30 beats/minute, respectively. The maximum cardiovascular stimulant effects were observed between 4 and 7 minutes after the icm infusions. As in the observations of icm dl-propranolol, the hypertensions due to icm l-propranolol were apparent for twice as long as the induced tachycardias. The hypertensive effects lasted for approximately 30 minutes and the tachycardias for 15 minutes.

L-propranolol induced emesis in both cats. Emesis occurred approximately 10 minutes after the end of the icm infusions. Some degree of respiratory stimulation occurred but panting was not observed in either cat. Salivation and ataxia were also absent.

The bradycardias, observed after icm l-propranolol were long lasting and although the maximum falls in heart rate were observed during the development of the hypotensive responses, the bradycardias were observed to return to

control levels parallel with the reversal of the hypotensions. Normal blood pressure and heart rate levels were obtained between 2 and 2.5 hours after icm administration of l-propranolol. The maximum falls in diastolic pressure, due to icm l-propranolol (see Table 11) were observed after 60 minutes.

D-propranolol (0.5 mg) was administered icm to both cats. Initial blood pressure and heart rate increases were observed. The systolic blood pressure and heart rate were increased by 59 and 64 mmHg and 45 and 45 beats/minute respectively. The hypertension and tachycardias reached their maximum effects approximately 5 to 7 minutes after the icm administration but as with icm dl- and l-propranolol the hypertension lasted longer than the tachycardias.

As in the cases of icm dl- and l-propranolol, icm d-propranolol induced vomiting in both cats. The respiration rates were increased in both cats and panting occurred in one cat only. Salivation and ataxia were not observed in either cat.

However, unlike icv administration of d-propranolol when cardivascular depressant effects were not seen, icm d-propranolol produced very small reductions in the diastolic blood pressures (4 and 6 mmHg) and heart rates (5 and 7.5 beats/minute) in both cats, after the initial stimulant effects.

Mydriasis (dilatation of the pupils) was observed in both cats during the initial stimulant effects

produced by icm l-propranolol, d-propranolol, dl-propranolol and pindolol. Oxprenolol also induced mydriasis in the cat given this blocker.

All cardiovascular effects and side effects produced after these compounds icm were not produced as a result of the infusion itself. Icm infusions of 50 µl 0.9% $^{w}/_{v}$ sterile sodium chloride solution, adjusted to a pH range of 4.5 - 7.5, produced no behavioural or cardiovascular effects.

DISCUSSION

The magnitude of the hypotension and bradycardia observed after the icm administration of the β-adrenoceptor blocking agents, used in the above experiments, generally appear to be similar to those obtained after icv administration (compare Tables 10b and 11). It can also be seen from these tables that icv administration of d-propranolol failed to induce hypotension and bradycardia in 5 cats, whereas after icm administration in 2 cats, d-propranolol produced small hypotensive and bradycardic effects. However, in comparison with the hypotension and bradycardia obtained after the β-adrenoceptor antagonists, icm, the effects of d-propranolol were relatively small.

The initial stimulatory effects, produced after icm β-adrenoceptor blockers and d-propranolol, appeared to be larger and of longer duration than the stimulant effects obtained after icv administration of the same compounds.

Severe side effects such as emesis, salivation,

ataxia, defaecation, respiratory stimulation and panting were common after icm administration of these compounds. This is in complete contrast to the observations after icv administrations.

Carter et al. (1974) did not report initial cardiovascular stimulation after icm dl-propranolol (0.3, 1.0 mg) in anaesthetised dogs. Although Srivastava et al. (1973) observed pressor effects and tachycardias, after icv dl-propranolol they found that injection of dl-propranolol into the fourth ventricle elicited only a depressor response and bradycardia, in anaesthetised dogs. These findings led these workers to suggest that the pressor response to icv propranolol occurs after activation of central β-adrenoceptors probably in the hypothalamus and the depressant effect originates from the medullary region.

Electrical stimulation of the medullary reticular formation produces hypertension (Wang & Ranson, 1939a). Hence it is possible that the pressor effects and tachycardias observed after icm β-adrenoceptor antagonists and d-propranolol may result from stimulation of these areas.

However, the factor of anaesthesia does not explain the absence of pressor effects after dl-propranolol administered into the medullary region of dogs as Srivastava et al.(1973) could still elicit pressor effects due to medullary stimulation in the anaesthetised dog. The differences may be accounted for by the use of cats in this project compared to experimentation in dogs by Srivastava et al.(1973) and Carter et al. (1974). In the cat

β-adrenoceptor antagonists and d-propranolol may be able to stimulate these medullary pressor sites.

In the previous Chapter, it was suggested that β-adrenoceptor antagonists and local anaesthetics probably produced their hypertensive and tachycardic effects, after icv administration, by releasing central endogenous catecholamines, thus activating the central hypothalamic β-adrenoceptors and also partly causing direct stimulation of β-adrenoceptors by the compounds themselves. These mechanisms may also explain the pressor effects and tachycardias originating from the medullary region of cats. β-Adrenoceptor stimulation occurring after icm administration of dopamine produced pressor effects and tachycardias (see Section 3, Chapter 2).

That initial medullary stimulation was observed after icm β-adrenoceptor antagonists and d-propranolol was further substantiated by the findings that vomiting, tachypnoea and panting regularly occurred in combination with the induced hypertension and tachycardia. The side effects were not usually observed until the peak of the hypertensive or tachycardic effects had been obtained. As the size of the initial stimulant effects increased with particular blockers, see Table 11, so the associated side effects appeared to be more intense and severe. Hence, the possibility that these pressor effects and tachycarias are induced by the production of disturbing and severe side effects cannot be ignored.

The most probable mechanism accounting for the hypotension and bradycardia observed after icv and icm

β-adrenoceptor antagonists is central β-adrenoceptor inhibition leading to depression of the medullary vasopressor areas. Reduction of the central β-adrenoceptor pressor tone in the medullary reticular formation could lead to decreased efferent sympathetic outflow to the periphery.

	Total icm dose mg	Initial increase		Later decrease		Respective cat used.
		Systolic Blood Pressure mmHg	Heart Rate Beats/ minute	Diastolic Blood Pressure mmHg	Heart Rate Beats/ minute	
ICI 66082	2.0	NIL	NIL	22	30	A
	2.0	NIL	NIL	25	35	B
	5.0	10	15	28	40	A
	5.0	13	15	30	35	B
Practolol	1.0	15	20	10	20	A
	1.0	12	25	12	25	B
L-Propranolol	0.25	45	30	15	25	A
	0.25	35	30	20	35	B
D-Propranolol	0.5	59	45	4	5	A
	0.5	64	45	6	75	B
Dl-Propranolol	0.5	68	45	18	35	A
	0.5	74	50	24	40	B
Pindolol	0.5	80	55	16	30	A
	0.5	65	45	20	40	B
Oxprenolol	0.5	105	60	18	35	A

TABLE 11.

Maximal initial increases in systolic blood pressure and heart rate and maximal decreases in diastolic blood pressure and heart rate observed after icm administration of β-adrenoceptor blockers and d-propranolol in 2 conscious normotensive cats. The responses to each compound are compared within each cat, designated A and B respectively. The responses are a result of one administration in each cat. Oxprenolol was only administered icm to one cat.

SECTION 5. Are the cardiovascular effects obtained after icv parasympathomimetic agents mediated through central adrenoceptors?

Chapter 1.

Cardiovascular effects of centrally administered parasympathomimetic agents in conscious cats.

The role of acetylcholine as a central transmitter is now established. The evidence is based upon the presence and distribution of acetylcholine, choline acetylase and choline esterase within the brain (Curtis, Ryall & Watkins, 1965). Ryall (1963) found that acetylcholine is contained within synaptic endings in the central nervous system.

A large number of workers have observed behavioural effects after centrally administered cholinergic and anti-cholinergic compounds (see Feldberg & Sherwood, 1954). Similarly, numerous studies have been undertaken to investigate the central cardiovascular actions of cholinomimetic and respective blocking agents, in both conscious and anaesthetised animals.

Hypertension was obtained in anaesthetised cats and dogs after administration of acetylcholine into the cisterna magna and onto the floor of the fourth ventricle (Suh, Wang & Lim, 1935). Bhawe (1958) observed both pressor and depressor responses with large doses of acetylcholine injected into the lateral ventricles of anaesthetised cats and dogs. Pressor effects after icv

injections of acetylcholine and carbachol in the anaesthetised dog were also reported by Dhawan, Gupta, Dixit & Chandra (1965) and Sinha, Dhawan, Chandra & Gupta (1967). Scrimal, Jaju, Sinha, Dixit & Bhargava (1969) observed an initial rise followed by a prolonged fall in arterial blood pressure after icv choline in the anaesthetised dog.

More recently, Lang & Rush (1973) produced pressor effects and tachycardias after icv acetylcholine and methacholine in conscious dogs. The cardiovascular effects of icv acetylcholine and methacholine were abolished by icv atropine but were unaffected by icv mecamylamine indicating that the effects of the 2 agonists were a result of stimulation of central muscarinic receptors.

Brezenoff and colleagues have investigated the effects of various cholinergic agents administered into the rat hypothalamus. Varying results were obtained after microinjections of carbachol into the posterior hypothalamus. Carbachol produced mainly falls in blood pressure but occasionally a delayed increase in blood pressure or an immediate fall followed by a rise in blood pressure was observed (Brezenoff & Jenden, 1969). Brezenoff & Jenden (1969) and Brezenoff & Wirecki (1970) found that muscarinic agents such as muscarine, methacholine and oxotremorine produced only falls in blood pressure after intrahypothalamic administration. Brezenoff & Jenden (1970) produced biphasic blood pressure effects following injections of carbachol onto the floor of the fourth ventricle. The depressor effects were abolished by atropine and the pressor effects

by hexamethonium pretreatment. Hence, these workers suggested that in the rat central muscarinic receptors were responsible for producing hypotension whilst stimulation of nicotinic receptors resulted in hypertension.

Armitage & Hall (1967b) observed pronounced hypotension and bradycardia after icv carbachol in anaesthetised cats. Generally, these falls were inhibited by atropine or hyoscine. However, occasionally in the presence of hyoscine carbachol icv still produced hypotension which was found to be abolished by icv hexamethonium. These workers found that acetylcholine injected into the lateral ventricles of anaesthetised cats never caused more than transient falls in blood pressure, even after doses as high as 1 mg. They suggested that the site of action of carbachol was the ventral brain stem.

Guertzenstein (1973) produced hypotension in anaesthetised cats after application of carbachol onto the ventral surface of the brain stem. The hypotension was reversed by topical application of atropine methylnitrate. Guertzenstein (1973) did not observe a nicotinic action of carbachol in anaesthetised cats.

Armitage & Hall (1967a) injected nicotine into the lateral ventricles of anaesthetised cats and also into 2 conscious cats. They observed variable blood pressure effects after icv nicotine (20 - 80 µg) in anaesthetised cats but found the most consistent response to be a fall in blood pressure. Nicotine (10 - 40 µg, icv) was administered to 2 conscious cats. In one a rise in blood

pressure was followed by a fall. After anaesthesia, the pressor effect due to icv nicotine in this cat was absent. In the other conscious cat icv nicotine produced a hypotension. The blood pressure effects of icv nicotine in the anaesthetised cat were abolished by peripheral or central pretreatment with hexamethonium.

Pradhan, Bhattacharya & Atkinson (1967) using anaesthetised cats, found that icv nicotine (25 - 50 µg) evoked pressor and depressor responses. The pressor effects observed by these workers, after icv nicotine, were more common than reported by Armitage & Hall (1967a) and the pressor effects were directly related to the particular type and depth of anaesthesia used.

In the conscious dog, icv nicotine produced regular biphasic increases in blood pressure and heart rate (Lang & Rush, 1973). An initial transient pressor effect and tachycardia was followed by a secondary rise in blood pressure and heart rate, which was both greater in magnitude and duration than the initial response. Unlike the responses obtained after icv acetylcholine and methacholine, these workers could not affect the central cardiovascular effects of nicotine by icv atropine but abolished them with icv mecamylamine pretreatment.

In this Chapter, results are presented describing the central cardiovascular responses obtained after icv acetylcholine, tetramethylammonium, nicotine and carbachol in the conscious cat.

Results.

1. Blood pressure and heart rate responses after icv acetylchcline, tetramethylammonium, nicotine and carbachol.

Acetylchcline (50, 75 µg, icv) failed to produce any blood pressure or heart rate effects in 4 cats. However, in each of these cats acetylcholine (150 µg, icv) produced small transitory increases in blood pressure and heart rate. The peak increases were observed approximately 5 minutes after the end of the icv infusion and normal control levels of blood pressure and heart rate were reached after 10 - 15 minutes. The mean increase of the systolic pressure and heart rate after acetylcholine (150 µg, icv) in the 4 cats was 12 mmHg and 15 beats/minute. None of the behavioural effects observed by Feldberg & Sherwood (1954) after acetycholine, in conscious cats, were seen.

Tetramethylammonium was administered icv to the same 4 cats treated with icv acetylcholine. Tetramethylammonium (100µg, icv) failed to produce any cardiovascular effects in any of the 4 cats. In 1 cat, tetramethylammonium (200 µg, icv) produced a 20 beat/minute bradycardia without affecting the blood pressure. The bradycardia was observed for approximately 45 minutes after which time resting heart rate levels were reached. In the remaining 3 cats tetramethylammonium (200 µg, icv) produced small hypertensive effects with associated tachycardias. The mean increases in systolic blood pressure and heart rate were 16 mmHg and 22.5 beats/minute. The maximum increases in blood

pressure and heart rate were observed approximately 15 minutes after the icv infusions and the responses had usually terminated after 60 minutes.

Nicotine was administered icv to 5 cats. Doses up to 50 µg were found to be ineffective in all cats. Nicotine (75 µg, icv) produced, in 3 cats, pressor effects associated with tachycardias. The maximum effect on blood pressure and heart rate was reached between 10 - 15 minutes after the end of the icv administration. The average increase of systolic pressure and heart rate, in these 3 cats to icv nicotine (75 µg) was 18mmHg and 20 beats/minute. The cardiovascular effects due to icv nicotine were absent within 30 - 45 minutes of the icv infusion.

In the remaining 2 cats, a small pressor effect and tachycardia was followed by a small hypotension and bradycardia. The initial pressor effect and tachycardia (average increases of 8 mmHg and 10 beats/minute) lasted for approximately 10 minutes after which time hypotension and bradycardia developed. The cardiovascular depressant effects lasted for 20 minutes. Bradycardia of 15 and 20 beats/minute accompanied by falls in blood pressure of 12 and 10 mmHg were observed in each cat respectively.

As described by Armitage, Milton & Morrison (1966) and by Hall & Reit (1966), nicotine (75 µg, icv) in these experiments produced some degree of activation of the pinna reflex. Twitching of the ears was rarely observed in doses of nicotine below 50 µg. However, nicotine (75 µg, icv) produced twitching of the ears in all

5 cats. Twitching was not rapid and was observed almost immediately after the icv infusions and persisted for approximately 10 minutes after. However, nicotine (75 µg icv) did not induce salivation, licking or vomiting in any of the cats used in this series of experiments in contrast to the report of Armitage et al. (1966).

Unlike the previous cardiovascular responses, in which the conscious cats used appeared to be relatively insensitive to icv acetylcholine, tetramethylammonium and nicotine, icv carbachol induced very marked behavioural and cardiovascular changes. Carbachol was administered to a total of 10 conscious cats. Initially, carbachol (15 - 30 µg, icv) was administered to 6 of the 10 cats. At this dose range it produced very large prolonged tachycardias and hypertensions. These cardiovascular effects were always observed in conjunction with severe rage reactions which limited the possible pharmacological analysis of the cardiovascular changes. The rage reaction appeared to vary from a very positive aggression shown towards the experimenter to a definite frightened appearance whereby the cat cowered in the back of its cage and occasionally hissed or cried when approached. The rage reactions and cardiovascular effects usually lasted for 3 - 5 hours after the icv carbachol.

On reduction of the icv carbachol dose to 2.5 - 7.5 µg, the rage response could be very much reduced or abolished leaving an undisturbed cardiovascular response Thus, carbachol (2.5 - 7.5 µg) produced hypertension and tachycardia when administered icv to 10 cats.

The hypertensive effects and tachycardias due to icv carbachol (15 - 30 µg) were observed to be much larger and were of longer duration than the cardiovascular effects observed after icv carbachol (2.5 - 7.5 µg). The mean systolic and diastolic blood pressure and heart rate increases seen after a single dose of 30 µg carbachol, icv in 6 cats, were 51.6 ± 4.2 and 39.9 ± 5.3 mmHg and 86.1 ± 7.6 beats/minute. The onset of the responses was seen immediately after the icv infusion.

The mean systolic and diastolic blood pressure and heart rate increases observed after a single icv carbachol (7.5µg) dose in 10 cats were 30.0 ± 3.9 and 19.9 ± 4.3 mmHg and 48.2 ± 4.9 beats/minute respectively. The onset of the responses occurred usually 5 - 10 minutes after the infusion and were completed 1.5 - 2.5 hours after the icv carbachol administration.

2. Effect of icv hexamethonium and atropine on the central cardiovascular effects of carbachol.

It was decided to further investigate the central actions of carbachol because its effects were observed to be potent, and consistent within a particular cat and from cat to cat.

The experiments involving icv hexamethonium and atropine methylnitrate in combination with icv carbachol were carried out in the following manner. On day one a control response was obtained with icv carbachol. On the following day the antagonist was infused icv 45-60 minutes before the second icv dose of carbachol. The cardiovascular

effects of carbachol before and after the antagonists were compared.

Within a particular cat, very similar cardiovascular responses were obtained after icv carbachol on two consecutive days.

The effect of hexamethonium (200 µg, icv) on the central cardiovascular actions of carbachol (7.5 µg, icv) was tested in 4 cats. Hexamethonium was administered icv 45 minutes before carbachol in each cat. Hexamethonium (200 µg, icv) produced no behavioural or cardiovascular effects in each of the 4 cats used. The central hypertension and tachycardia due to icv carbachol were absent after icv pretreatment with hexamethonium in all cats tested. A typical experiment is shown in Fig. 58. Record A demonstrates the control cardiovascular response to icv carbachol (7.5 µg). Record B shows the lack of central cardiovascular activity of hexamethonium (200 µg). The inhibition of the icv carbachol response by hexamethonium is shown in Record C.

Icv atropine methylnitrate (200 µg) also inhibited the hypertension and tachycardia due to icv carbachol (7.5 µg) in the same 4 cats. Atropine methylnitrate was administered icv 45 minutes before the icv carbachol. In 2 cats icv atropine produced short lasting tachycardias (40 and 35 beats/minute) without affecting blood pressure. Atropine (200 µg, icv) in the other 2 cats produced pronounced tachycardias (100 and 90 beats/minute) with associated pressor effects. Atropine icv

induced larger increases in the diastolic than systolic pressures, thus resulting in a decreased pulse pressure. This reponse is shown in Fig. 59. Trace A shows the control response to icv carbachol. Atropine methylnitrate (200 µg, icv) produced a prolonged tachycardia of 100 beats/minute. The systolic and diastolic pressure rose by 12 and 18 mmHg respectively. The blood pressure and heart rate increases reached a peak 7 minutes from the end of the atropine infusion. The systolic blood pressure was observed to return to normal levels within 45 minutes. However, the heart rate and diastolic blood pressure were still elevated after 45 minutes, at which time carbachol was infused icv. The effects of icv.atropine are shown in Record B. Trace C shows the complete abolition of the central actions of icv carbachol (7.5 µg).

DISCUSSION

The doses of cholinergic stimulants administered icv to produce centrally mediated cardiovascular effects, in this study using conscious cats, are anomalous compared to the doses used by other workers using conscious and anaesthetised animals. In the conscious dog, Lang & Rush (1973) produced transient pressor effects after icv acetylcholine (10 - 20 µg). In this study, doses of acetylcholine up to 75 µg did not induce any cardiovascular responses after icv administrations. Transient pressor effects were observed in the conscious cat after icv acetylcholine (150 µg).

Armitage & Hall (1967a) observed cardiovascular

effects after nicotine (10 - 40 µg, icv) in the conscious cat. Nicotine (20 - 80 µg, icv) was found to be effective in anaesthetised cats (Armitage & Hall, 1967a; Pradhan et al. 1967). In the conscious dog, Lang & Rush used icv doses of nicotine of 20 - 60 µg. However, in the experiments described in this Chapter using conscious cats, doses of icv nicotine up to 50 µg were ineffective. Cardiovascular reponses were seen after nicotine (75 µg, icv).

The results obtained using icv carbachol administered to conscious cats were in contrast to those observed after icv acetylcholine, tetramethylammonium and nicotine. Very small doses of carbachol icv produced marked changes in behaviour and blood pressure and heart rate. The behavicural effects observed after icv carbachol in conscious cats were eliminated by reducing the icv doses from 15 - 30 µg to 2.5 - 7.5 µg. With these reduced doses only cardiovascular responses were obtained. In the anaesthetised cat, Armitage & Hall (1967b) produced effects after 40 µg carbachol icv.

The central cardiovascular effects of cholinomimetic substances are complex. Pressor effects were usually observed after central administration of acetylcholine in anaesthetised cats and dogs. In the conscious dog transient pressor effects and tachycardias were observed after icv acetylcholine (Lang & Rush, 1973). Similar responses were obtained in this study using conscious cats. However, Bhawe (1958) observed both pressor and depressor responses due to icv acetylcholine in anaesthetised cats and dogs. Scrimal et al.(1969) also observed biphasic effects after

icv choline in the anaesthetised dog.

No reports were found in the literature describing effects due to icv tetramethylammonium. However, in conscious cat experiments, tetramethylammonium produced a pronounced bradycardia without altering blood pressure in one cat and in the other 3 cats produced pressor effects and tachycardias. Tetramethylammonium is mainly a stimulant of nicotinic receptors but does possess some muscarinic stimulant activity.

The blood pressure and heart rate effects produced by icv nicotine are varied. Armitage & Hall (1967a) observed both pressor and depressor effects after icv nicotine in anaesthetised and conscious cats, although the depressor effects were more consistent. Pradhan et al. (1967) also observed depressor and pressor effects in the anaesthetised cat. Injections of nicotine into the vertebral artery of anaesthetised cats produced a small pressor response followed by a prolonged hypotension (Schaeppi, 1967).

Nicotine was administered icv to 5 conscious cats. In 3 cats, only pressor effects with associated tachycardias were produced. However, in the remaining 2 cats, nicotine induced small pressor effects with tachycardias followed by hypotension and bradycardia. In the conscious dog, nicotine icv produced only biphasic pressor and tachycardic effects (Lang & Rush, 1973).

The responses due to icv nicotine, observed by Armitage & Hall (1967a) and Lang & Rush (1973) were

unaffected by icv atropine but were abolished by hexamethonium or mecamylamine.

The central responses of centrally administered carbachol are also not consistent. Dhawan et al.(1965) observed pressor effects due to icv carbachol in anaesthetised dogs. Brezenoff (1972) produced specific pressor and depressor effects after micro-application of carbachol to specific hypothalamic nuclei of anaesthetised rats. The pressor effects and tachycardias were abolished by inhibitors of nicotinic receptors and conversely the depressor effects and bradycardias were blocked by atropine, an inhibitor of muscarinic receptors.

Armitage & Hall (1967b) observed in anaesthetised cats that icv carbachol produced hypotension and bradycardia. The depressant effects of carbachol were found to possess 2 components, one of which was inhibited by atropine or hyoscine and the other by hexamethonium. Application of carbachol to the ventral surface of the brain stem produced hypotension which was reversed by atropine (Guertzenstein, 1973).

However, in conscious cats, depressant effects were never observed after icv carbachol; pronounced hypertension and tachycardias were always obtained. The stimulant effects were inhibited by icv pretreatment with atropine and hexamethonium.

A large number of the differences in the particular type of response obtained to a certain cholinergic compound can be explained by the use of anaesthesia in some cases.

Armitage & Hall (1967a) demonstrated that the pressor component of a biphasic blood pressure response obtained in a conscious cat after icv nicotine was completely absent when icv nicotine was repeated in this same cat anaesthetised. Pradhan et al. (1967) also observed that the type of response obtained after icv nicotine was dependant upon the type and depth of anaesthesia used.

Hence, the use of conscious dogs by Lang & Rush (1973) and conscious cats in this study may account for the predominance of pressor effects and tachycardias observed after cholinergic compounds administered centrally.

With the exception of the work of Brezenoff and co-workers, who describe opposing muscarinic and nicotinic actions within certain hypothalamic nuclei of the rat, opposite and separate cardiovascular responses cannot be attributed to specific nicotinic and muscarinic actions within the brains of cats and dogs. Armitage & Hall (1967b) obtained nicotinic and muscarinic actions of carbachol, both inducing depressor and bradycardic effects. In the conscious cat, the pressor effects and tachycardias observed after icv carbachol were inhibited by icv atropine and icv hexamethonium. Lang & Rush (1973) produced pressor effects and tachycardias after icv acetylcholine and metacholine which were inhibited by icv atropine and unaffected by icv mecamylamine. Conversely, the hypertension and tachycardias produced after icv nicotine were inhibited by icv mecamylamine but unaffected by icv atropine. However, if central

cholinergic pathways are similar to the peripheral parasympathetic nervous system one might expect to observe similar responses after central administrations of nicotinic and muscarinic stimulants.

It is difficult to conclude from these results that there are depressor and pressor pathways containing both nicotinic and muscarinic receptors within the pathways or that the cholinergic receptors are of a non-specific type. Various types of responses would be expected after administration of these stimulants into the cerebroventricular spaces as several pathways within different areas must be stimulated. The relative effects are dependant upon the degree of stimulation produced within certain areas.

Further work must be carried out in more animal species involving greater control of drug administration Drug application by microinjections into discrete brain areas proved relatively successful in separating muscarinic and nicotinic actions within the rat hypothalamus (Brezenoff, 1972).

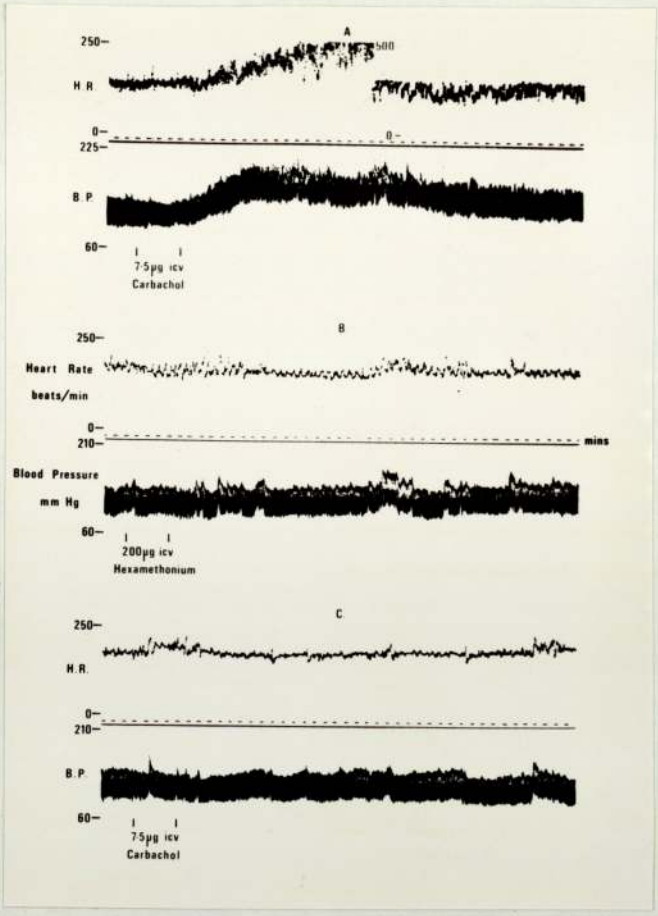


FIG. 58.

Conscious, normotensive, unrestrained cat. The upper trace illustrates the control hypertensive response with tachycardia after carbachol (7.5 µg, icv). The middle trace shows that hexamethonium (200 µg, icv) failed to produce any central cardiovascular effects. The lower trace shows the complete blockade of carbachol (7.5 µg, icv) when given 90 minutes after icv hexamethonium. The control response to icv carbachol (Trace A) was obtained on the day before the experiment involving icv administration of carbachol after hexamethonium. (Traces B and C).

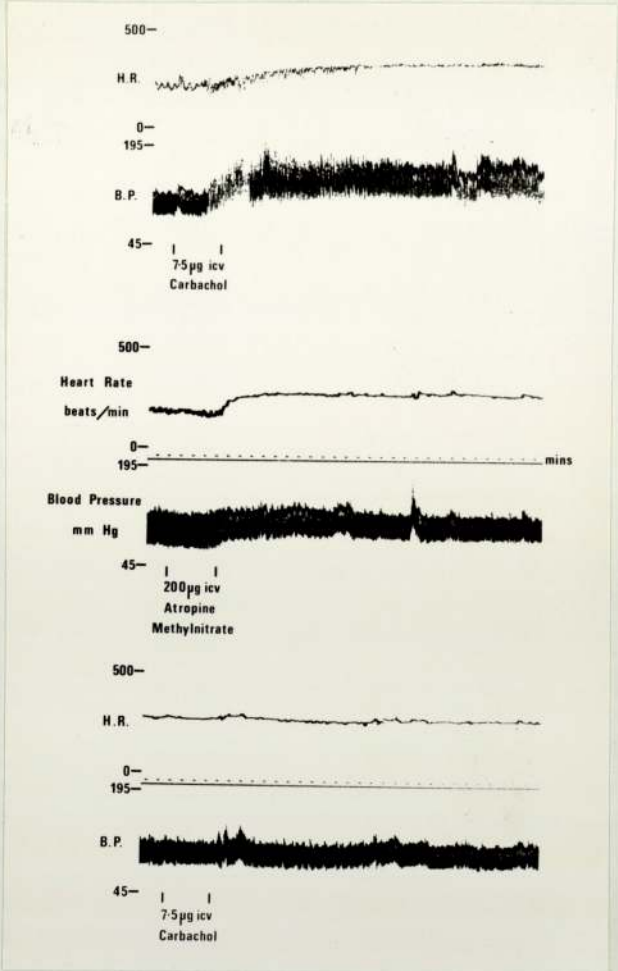


FIG. 59.

Conscious, normotensive, unrestrained cat. The upper trace illustrates the control hypertensive response with tachycardia after carbachol (7.5 µg, icv). The middle trace shows that atropine methylnitrate (200 µg, icv) also induced tachycardia and hypertension. The lower trace shows the complete blockade of carbachol (7.5 µg, icv), when given 45 minutes after icv atropine methylnitrate. As in Fig. 58 the control response to icv carbachol was obtained on the day before the experiment involving icv administration of carbachol after atropine.

CHAPTER 2. The effect of sympathetic blocking agents on the cardiovascular effects of icv carbachol.

Three groups of workers have investigated whether the central cardiovascular effects of cholinomimetic agents are linked to central 'sympathetic' effects. Dhawan et al. (1965) suggested that the central response to tyramine in anaesthetised dogs may be due to release of acetylcholine as well as of noradrenaline. They reported that 100 µg. carbachol and 10 µg tyramine icv produced similar pressor effects. Icv atropine reversed the pressor effects of tyramine to a depressor response. Thus, these workers suggested that the pressor effect of tyramine was normally due to acetylcholine release and after blockade with atropine the effects of released noradrenaline were predominant, causing hypotension.

Scrimal et al. (1969) abolished the initial pressor effects of icv choline in the anaesthetised dog with icv atropine. The prolonged fall in blood pressure which followed the initial rise was inhibited by icv β-adrenoceptor antagonists, icv reserpine and i.v. tetrabenazine. Thus, they suggested that the initial hypertension due to icv choline was due to central cholinergic stimulation causing secretion of catecholamines from the adrenal medullae as the pressor effects were absent in adrenalectomised cats, whilst the subsequent hypotension was produced as a result of central β-adrenoceptor stimulation; propranolol administered icv abolished the hypotension due

to icv choline.

Philippu & Przuntek (1967) reported that calcium and acetylcholine accelerated the release of noradrenaline from isolated hypothalamic vesicles. Perfusion of the third ventricles of cats with acetylcholine enhanced the release of noradrenaline and its metabolites from the hypothalamus (Philippu et al., 1970). However, the effect of acetylcholine was dependant on the presence of calcium ions. Philippu et al. (1974) observed that superfusion of the posterior hypothalamus with carbachol or DMPP (a potent nicotinic stimulant) enhanced the pressor responses to hypothalamic stimulation. The pressor response to hypothalamic stimulation had previously been shown by Przuntek et al. (1971) to be mediated by central adrenergic neurons. Hexamethonium did not affect the rise of blood pressure to hypothalamic stimulation but abolished the enhancing effects of carbachol and DMPP. Hence Philippu et al. (1974) concluded that nicotinic agents enhance the effects of hypothalamic stimulation by an action on nicotinic receptors and that more than one neurotransmitter seems to be involved in the central modulation of blood pressure.

In this Chapter, a series of experiments is described in which blood pressure and heart rate effects of icv carbachol were recorded before and after centrally and peripherally administered sympathetic blocking drugs.

Results.

1. Effect of icv bethanidine and guanethidine.

The effects of icv pretreatment with either bethanidine or guanethidine on the central actions of carbachol (7.5 µg) were observed in 4 cats. 2 cats were pretreated with icv bethanidine (0.75mg) and the other 2 cats with icv guanethidine (0.75mg). In Section 3, Chapter 1, part 9, bethanidine and guanethidine were administered icv to conscious cats in a dose of 0.5 mg. Since the cardiovascular actions of icv carbachol were so pronounced and prolonged icv bethanidine and guanethidine (0.75 mg) were used.

The adrenergic neuron blockers were administered icv 60 minutes before the carbachol. The control response to icv carbachol (7.5 µg) in each cat, was obtained on the day prior to the experiment in which the blocking agents were used.

Guanethidine (0.75 µg, icv) produced tachycardia with a small hypertension in both cats. The tachycardia and hypertension to icv carbachol were totally absent in both cats after icv guanethidine pretreatment. Bethanidine also prevented the cardiovascular effects of icv carbachol. Fig. 60 demonstrates the lack of activity of icv carbachol administered 60 minutes after icv bethanidine (0.75 mg). Bethanidine icv produced a 40 beats/minute tachycardia without significantly altering the blood pressure.

2. Effect of icv dl-propranolol.

The effect of dl-propranolol (1.0 mg, icv) on the central actions of carbachol (7.5 µg, icv) were

examined in 9 cats. In 7 of these, dl-propranolol greatly reduced or abolished the effect of icv carbachol. However, in the remaining 2 cats dl-propranolol did not alter the icv response of carbachol.

3. Effect of icv local anaesthetics.

Bethanidine, guanethidine and dl-propranolol each possess potent local anaesthetic properties (see Boura & Green, 1965 and Fitzgerald, 1969). Therefore the effects of lignocaine (1.0 mg) and procaine (2.0 mg) were tested on the icv carbachol responses. The experiments were performed in 4 cats. Icv bethanidine, guanethidine and dl-propranolol were shown previously to inhibit the central actions of carbachol in all 4 cats. Lignocaine (1.0 mg, icv) and procaine (2.0 mg, icv) were each tested in 2 cats. The local anaesthetics were administered 45 minutes prior to icv carbachol. Lignocaine in one cat and procaine in another did not affect the response to icv carbachol. However, in each of the other cats tested with lignocaine and procaine, the effects of carbachol were reduced by 40% and 35% respectively.

4. Effect of i.v. bethanidine.

Bethanidine (7.5 mg/kg, i.v.) was administered 4 hours before icv carbachol (7.5 µg) in 3 cats. In all cats, i.v. bethanidine completely prevented the centrally induced hypertension and tachycardia produced by icv carbachol.

DISCUSSION.

Scrimal et al. (1969) noticed that the prolonged

fall in blood pressure observed in the anaesthetised dog after a large dose of choline administered into the lateral ventricle, was abolished after central depletion of catecholamines by reserpine or tetrabenazine and also by central pretreatment with β-adrenoceptor antagonists. Thus, they demonstrated that central cholinergic stimulation led to stimulation of a central β-adrenoceptor pathway.

This observation has now been repeated in the conscious cat. Pretreatment with dl-propranolol (1.0 mg, icv) greatly reduced or completely inhibited the blood pressure and heart rate increases normally seen after icv carbachol. The inhibition of icv carbachol by icv dl-propranolol was seen in 7 of the 9 cats used. Hence, carbachol may stimulate central cholinergic pathways, either by direct stimulation of nicotinic receptors by carbachol itself, or by releasing acetylcholine (Renshaw, Green & Ziff, 1938; Koelle, 1970a), leading to an activation of central β-adrenergic neurons, influencing peripheral cardiovascular parameters.

In Section 3, Chapter 1, part 9, icv guanethidine and bethanidine were shown to inhibit the central β-adrenoceptor pressor pathway stimulated by icv isoprenaline, which is responsible for producing hypertensive responses. Since the effects of icv carbachol were blocked by icv bethanidine and guanethidine, it seems very likely that icv carbachol in turn causes activiation of the same pressor pathways stimulated by isoprenaline.
The tachycardias due to icv carbachol like those for icv isoprenaline were generally

inhibited by icv dl-propranolol pretreatment. However, all the cardiovascular effects of icv carbachol were blocked by icv bethanidine or guanethidine unlike the experiments involving icv isoprenaline whereby only the pressor effects of icv isoprenaline were depressed by this treatment; the tachycardias were unaffected.

It is impossible at this stage to describe the link between the observed central cholinergic and adrenergic responses. For example, the situation centrally may be similar to that found in the peripheral sympathetic nervous system, i.e. acetylcholine is the ganglionic transmitter with noradrenaline being released from the endings of the postganglionic neuron. Since 1931, it has been known that stimulation of peripheral sympathetic fibres sometimes leads to release of acetylcholine in addition to releasing the main transmitter noradrenaline (von Euler & Gaddum, 1931; for review of literature see Burn, 1965). Hence central stimulation may lead to release of acetylcholine as well as the adrenergic transmitter.

The Burn-Rand hypothesis, which states that acetylcholine is released from the adrenergic nerve endings and subsequently mediates the release of noradrenaline (Burn & Rand, 1959), may also explain the central cholinergic/adrenergic link. Philippu et al. (1970) hypothesised that acetylcholine, the transmitter of cholinergic neurons, may influence the release of catecholamines from hypothalamic noradrenergic nerve terminals.

Nicotine is known to release noradrenaline from

peripheral adrenergic neurons (Lindmar, Loffelhotz & Muscholl, 1968; Su & Bevan, 1970). There is also evidence that similar effects may be achieved in the central nervous system. Nicotine administered systemically reduced the noradrenaline content in the brains of cats (Vogt, 1954). Westfall, Fleming, Fudger & Clark (1967) showed that nicotine and related analogues lowered the noradrenaline content of the rat diencephalon and mouse whole brain. Chronic administration of nicotine was shown to increase the turnover of noradrenaline in the rat brain (Bhagat, 1969). Intravenous nicotine increases the efflux of ^{3}H-noradrenaline into effluent of the perfused third ventricle of the cat. Also, the efflux of ^{3}H-noradrenaline from superfused slices of rat hypothalamus is increased after addition of nicotine to the superfusion medium (Hall & Turner, 1972).

More recently, Westfall (1974) has shown that nicotine produced a significant increase in the release of ^{3}H-noradrenaline from incubated slices of rat hypothalamus, cortex and cerebellum; the largest effect being in the hypothalamus. Nicotine also induced a similar release of ^{3}H-dopamine from rat striatal slices. The release of the ^{3}H-catecholamines by nicotine was reduced by acetylcholine and by hexamethonium added to the superfusion medium. Phenoxybenzamine produced a significant increase in the release of ^{3}H-dopamine induced by nicotine. Thus, Westfall (1974) suggested that the release of amines from central neurons is produced by activation of nicotinic receptors and in addition acetylcholine may modulate the release of

dopamine and noradrenaline by influencing a muscarinic inhibitory mechanism, which has previously been suggested for peripheral adrenergic neurons (Loffelholz, 1967; Lindmar et al., 1968; Haeusler, Thoenen, Haefely & Huerlimann, 1968; Malik & Ling, 1969; Rand & Varma, 1970).

Kaul & Grewal (1968) and Brezenoff (1973) showed that the anticholinesterase agent physostigmine released catecholamines from the adrenal medulla via stimulation of central muscarinic receptors. Scrimal et al. (1969) suggested that the initial pressor effect obtained after icv choline was due to central muscarinic stimulation producing adrenal catecholamine release.

The hypertension and tachycardia induced by icv carbachol in the absence of behavioural effects were similar to those after icv isoprenaline in that the systolic blood pressure was usually increased to a greater extent than the diastolic blood pressure. As with the responses to icv isoprenaline the cardiovascular stimulant effects of icv carbachol were abolished by intravenous administration of bethanidine. Thus the pressor effects and tachycardias to icv carbachol are apparently mediated via the peripheral sympathetic nervous system and are unlikely to be mediated by catecholamine release from the adrenal gland, since bethanidine has been shown previously to be relatively ineffective in blocking the release of catecholamines from the adrenal medulla (Boura et al., 1962).

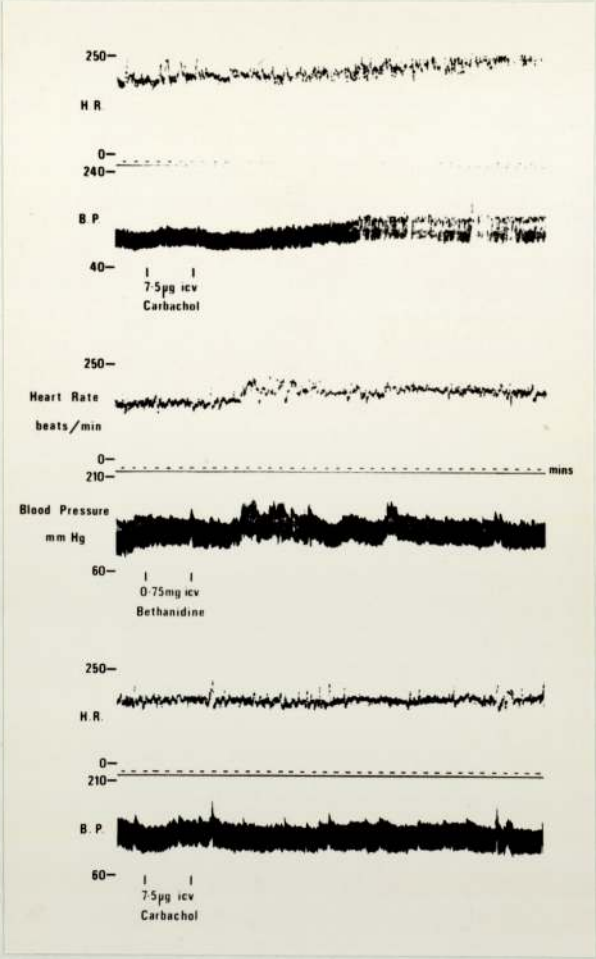


FIG. 60.

Conscious normotensive, unrestrained cat. The upper trace illustrates the control hypertensive response with tachycardia after carbachol (7.5 µg, icv). The middle trace shows that bethanidine (0.75 mg, icv) produced tachycardia with a small hypertension. The lower trace shows the blockade of carbachol (7.5 µg, icv) when given 60 minutes after icv bethanidine. As in Figs. 58 and 59 the control response to carbachol was obtained on the day before the experiment involving icv administration of carbachol after bethanidine.

SECTION 6. The problem of drug leakage from the brain to the peripheral circulation after intracerebroventricular injection.

CHAPTER 1. Demonstration of leakage of catecholamines after icv administration from the cat brain.

Numerous authors have reported that absorption into the blood stream takes place after icv injections of histamine and adrenaline. This absorption has been attributed to the circulation of the injected material into the subarachnoid space and its absorption by blood vessels or the arachnoid villi (Bedford, 1953; Bhawe, 1958; Draskoci, Feldberg, Fleischhauer & Haranath, 1960). The pars tuberalis (part of adenohypophysis of pituitary gland) has been suggested as a specific site for adrenaline absorption (Sproull, 1963). Da Silva & Sproull (1964) demonstrated in the cat that absorption following icv injections of adrenaline is slow and only a small proportion of the injectate ever reaches the blood stream.

After injection of 30 µl fluid into the subcortical tissue of mice, only 2 - 8% of the injected material is recovered in the brain (Cairns, 1950). Mims (1960) suggested that the high injection pressure associated with injections made into the tissue results in rupture of the arachnoid villi allowing passage of material into the sagittal sinus. Thus, it would seem likely that losses of material after icv injection into mice and rats could be explained in a similar manner, since the injection

volumes used for mice and rats (10 - 20 µl) are very large in relation to the total volume of cerebrospinal fluid normally found in the ventricular spaces of such small animals. Large sudden pressure changes must occur after icv injections in mice and rats. Shaw (1974) observed that the amount of putrescine escaping from the brains of mice after icv administration was reduced by decreasing the icv injection volume of putrescine from 10 µl to 5 µl.

The volumes administered icv to the rabbit, cat and dog are very small when compared to the volumes of the ventricular spaces of these animals. Icv injections or infusions of such volumes (50 - 100 µl) into the brains of rabbits, cats and dogs would not produce such pressure changes and thus would not be so likely to cause internal brain damage.

After acute icv injections of drugs into mice in which the injection needle is pushed through the skull, leakage occurred as injected material was seen in the subcutaneous tissue overlying the skull (Shaw, 1974). This is probably the route by which most of the leaked material travels since it is the shortest route to the subarachnoid space. Refluxing of material up the needle track, as seen after icv injections in mice, would be a much smaller problem in animals with chronically indwelling cannulae implanted 7 days or more before the icv administration.

It was observed in the experiments described in this thesis that after several months the blood pressure

and heart rate responses to icv catecholamines changed in some cats. At this time, experiments were performed to elucidate whether or not the changes were due to leakage or absorption of the amines into the peripheral blood stream.

Results

In several cannulated cats the blood pressure and heart rate effects observed after icv infusions of α- or β- adrenoceptor stimulants were different to those when these compounds were administered peripherally. For example, icv isoprenaline, dopamine or adrenaline produced tachycardia with combined hypertensive effects. Conversely, noradrenaline and α-methylnoradrenaline produced bradycardias with associated hypotensive effects. All these responses were centrally mediated since they were inhibited by peripheral ganglion blockade or by adrenergic neuron blockade or by low doses of the respective adrenoceptor antagonists administered centrally.

In the remaining cats used in experiments described in the 5 previous sections, the responses to icv α and β- adrenoceptor agonists were similar to the effects observed after peripheral administration. However, these cardiovascular effects were also centrally mediated since they too were inhibited by the drug treatment mentioned above.

Occasionally, in some cats that produced differing effects after peripheral and central administrations of drugs, the responses to icv isoprenaline and noradrenaline

appeared to alter with time. After at least 4 - 6 months of regular experimental usage (which included 2 or 3 experiments per week involving icv administrations) the central effects due to icv isoprenaline and noradrenaline gradually changed. For example, the pressor effect observed after icv isoprenaline gradually diminished in magnitude until finally a depressor response was observed in combination with the tachycardia. This reversal of responses was a gradual change and was observed to occur over a period of 3 - 6 weeks. The changes in the icv noradrenaline and isoprenaline responses occurred within a particular cat at approximately the same time.

In the second group of cats in which responses to both i.v. and icv administered catecholamines were of the same type, occasional changes were observed in the icv responses to isoprenaline and noradrenaline. It was found in 3 cats that the blood pressure response after icv isoprenaline and noradrenaline appeared much quicker in onset than normally seen after the icv infusions and the size of the hypotension after isoprenaline and hypertension after noradrenaline gradually increased. The duration of the responses after icv isoprenaline and noradrenaline were often reduced. The changes in the icv responses gradually occurred after 4 - 6 months.

At this stage, central pretreatment with dl-propranolol did not significantly reduce the blood pressure and heart rate effects of icv isoprenaline (30 µg) in these cats. Icv phentolamine also failed to alter the hypertension

and bradycardia observed after icv noradrenaline. Fig. 61 shows responses after icv noradrenaline before and after icv phentolamine (0.6 mg). It can be seen that icv phentolamine was completely ineffective in reducing the response to icv noradrenaline. Compare this result with the inhibition of the pressor effect obtained after icv noradrenaline by icv phentolamine demonstrated in Fig. 22.

In Section 3, experiments were performed demonstrating the complete abolition of the cardiovascular effects of icv isoprenaline in all cats by i.v. pempidine or hexamethonium or by i.v. guanethidine or bethanidine. However, in all cats, in which changes in the icv response to isoprenaline were observed, a large potentiation of the blood pressure response was obtained after icv isoprenaline in the presence of ganglion blockade or adrenergic neuron blockade. Fig. 62 demonstrates the potentiation of the icv isoprenaline response by i.v. hexamethonium. The control response to icv isoprenaline (20 µg) was a 100 beat/minute tachycardia seen with a $^{19}/_{23}$ mmHg hypotension. 60 minutes after i.v. hexamethonium (7.5 mg/kg), icv isoprenaline (20 µg) produced a 110 beat/minute tachycardia with a $^{50}/_{58}$ mmHg hypotension. The diastolic blood pressure was reduced to below 40 mmHg by the second icv dose of isoprenaline. The initial icv isoprenaline response shown in Fig. 62 demonstrates the response obtained after icv isoprenaline (30 µg), in the same cat 4 weeks after implantation of the icv cannula.

A similar potentiation of the icv noradrenaline

response was observed after i.v. pempidine (5 mg/kg). The response to noradrenaline (30 µg, icv) 60 minutes before and 90 minutes after i.v. pempidine is shown in Fig. 63. The systolic blood pressure was increased by 80 mmHg after icv noradrenaline. After pempidine, the systolic blood pressure rose by 165 mmHg in response to icv noradrenaline.

Fig. 64 demonstrates a large potentiation of the response to icv isoprenaline (20 µg) when given 2 hours after i.v. bethanidine (5 mg/kg). Icv isoprenaline (20 µg) produced a 95 beat/minute tachycardia and a $^{24}/_{28}$ mmHg hypotension. However, although the blood pressure was still elevated due to i.v. bethanidine, icv isoprenaline administered 2 hours later produced a 115 beat/minute tachycardia and a $^{67}/_{68}$ mmHg hypotension.

The responses observed in Figs. 62, 63 and 64 were obtained from the same conscious cat.

DISCUSSION

Ganglion blocking agents (Haas & Goldblatt, 1960) and adrenergic neuron blocking agents (Boura & Green, 1959; 1963; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960) potentiate the effects of catecholamines. Thus, the potentiation of the responses to icv noradrenaline and isoprenaline after intravenous hexamethonium, pempidine or bethanidine indicates leakage from the brain of these two amines, especially as the central effects were originally inhibited by these peripheral blockers.

The failure to inhibit the cardiovascular effects observed after icv isoprenaline and noradrenaline in these

cats, with icv dl-propranolol or phentolamine, respectively can be explained in terms of the central doses used of each compound. For example, if 50% of the isoprenaline (30 µg, icv) dose quickly escaped or leaked from the brain then this would be equivalent to a 5 µg/kg dose of isoprenaline in a 3 kg cat given systemically. Thus a relatively small degree of leakage of the amine from the brain could lead to a relatively high peripheral blood level of the amine. However, if the same proportion of the dl-propranolol (1.0 mg, icv) dose leaked from the brain the blood level of the blocker would be small compared to the systemic dose of 1 - 2 mg/kg of propranolol normally used. 50% leakage of 1.0 mg dose of propranolol icv in a 3 kg cat would only lead to an equivalent systemic dose of 170 µg/kg. Hence if relatively the same proportion of blocker leaked from the brain as with the agonist, the blood level of the antagonist would not be sufficient to significantly reduce the peripheral effects of the escaped agonist.

It is difficult to explain why after a long period of time compounds leak from the brains of some but not all cats used for this project. The techniques for icv cannula implantation and for drug administrations were common for all cats used. Experimental conditions were kept constant throughout. No cat was used more frequently than any other and all cats were involved in 2 or 3 icv experiments per week. It is unlikely that physical damage occurred within the brain due to icv administrations.

The icv infusion volume, 100 µl, is approximately 1/20th of the total cerebroventricular volume of c.s.f. within the cat brain. As the 100 µl volume was infused slowly at the approximate rate of c.s.f. production from the choroid plexus (20 µl/minute), the induced pressure increase within the brain during and after the icv drug administration would not be as great as if the total 100 µl volume was injected quickly.

One might expect damage to occur, after drug administrations, if the indwelling cannula was situated incorrectly and the drug solutions were infused directly into the brain tissue and not into the ventricular spaces. However, icv dye infusions always indicated that the cannulae were correctly placed in the lateral ventricles. In cats, in which leakage of drugs from the brain occurred, staining was observed, after icv pontamine sky blue, on the outside of the brain within the subarachnoid spaces. This was not usually seen in normal cats. Thus, it would seem probable that compounds are rapidly transported from the subarachnoid spaces into the peripheral blood stream.

Studies on the metabolism of adrenaline and noradrenaline have shown that little, if any, of the catecholamines traverse the blood brain barrier from the systemic circulation (Weil-Malherbe, Axelrod & Tomchick, 1959; De Schaepdryver & Kirshner, 1961). Thus it might be expected that little of the active amine administered centrally would escape to the systemic circulation under normal conditions.

Enzymes chiefly responsible for the degradation and inactivation of catecholamines in peripheral tissues, monoamine oxidase (M A O) and catechol-o-methyl transferase (C O M T) have been shown to be present in the brain (Blaschko, Richter & Schlossman, 1937; Bhagvat, Blaschko & Richter, 1949; Axelrod, Albers & Clemente, 1959). Mannarino, Kirshner & Nashold (1963) found that in the brain COMT mainly deactivated ^{14}C-noradrenaline after administration into the lateral ventricles of cats. The noradrenaline was metabolised mainly to normetanephrine and 3-methoxy-4-hydroxy-phenylglycol in the cerebrum and cerebellum. They recorded the amount of radioactivity that appeared in the blood and urine after icv ^{14}C-noradrenaline and found that the metabolites were responsible for the radioactivity detected in the periphery. Thus noradrenaline is quickly metabolised within the brain and the metabolite 'excreted' from the central nervous system.

Leakage of the active amines into the periphery from the brains of some cats may be a result of a build up of a large concentration of the amine within the brain due to an alteration or reduction of central metabolic enzymes. A transport mechanism may be facilitated or induced forcing the exogenously administered catecholamine from the brain into the periphery.

As soon as changes in the cardiovascular responses to icv noradrenaline or isoprenaline were observed and after experiments using peripheral ganglion blockade and adrenergic neuron blockade were performed, the cats were no longer used experimentally.

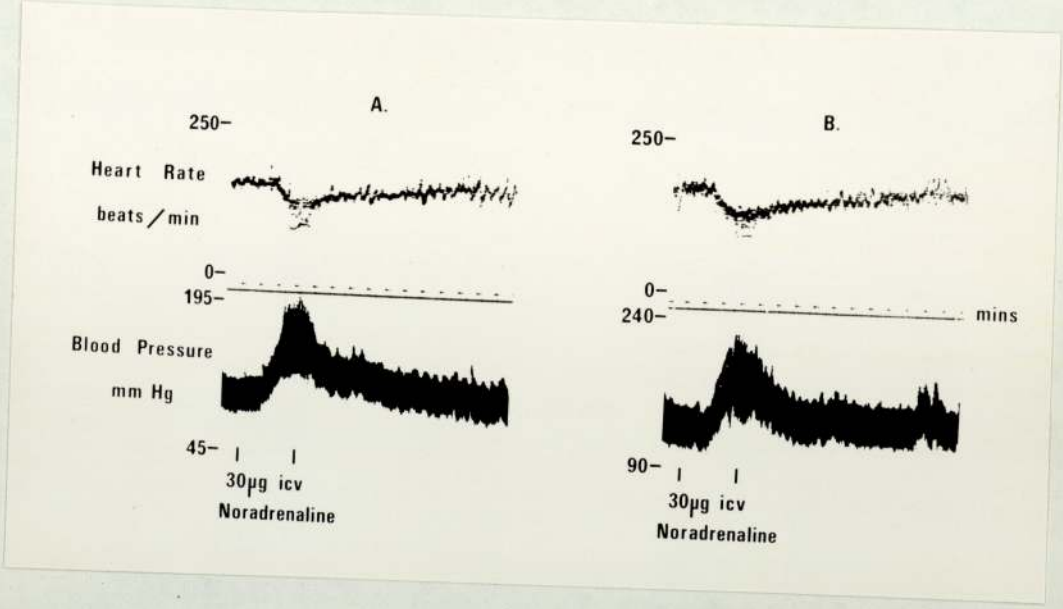


FIG. 61.

Conscious, normotensive, unrestrained cat. Comparison of the effects of noradrenaline (30 µg, icv) before and 45 minutes after phentolamine (0.6mg, icv). As can be seen from trace B, icv administration of the α-adrenoceptor blocking agent had no effect on the response to noradrenaline (30 ug, icv). Phentolamine (0.6 mg, icv) induced slight rises in blood pressure and heart rate. At the time of the second noradrenaline infusion the blood pressure was raised due to icv phentolamine. Compare these traces to those of Fig. 22 in which icv phentolamine abolished the hypertensive effect of icv noradrenaline.

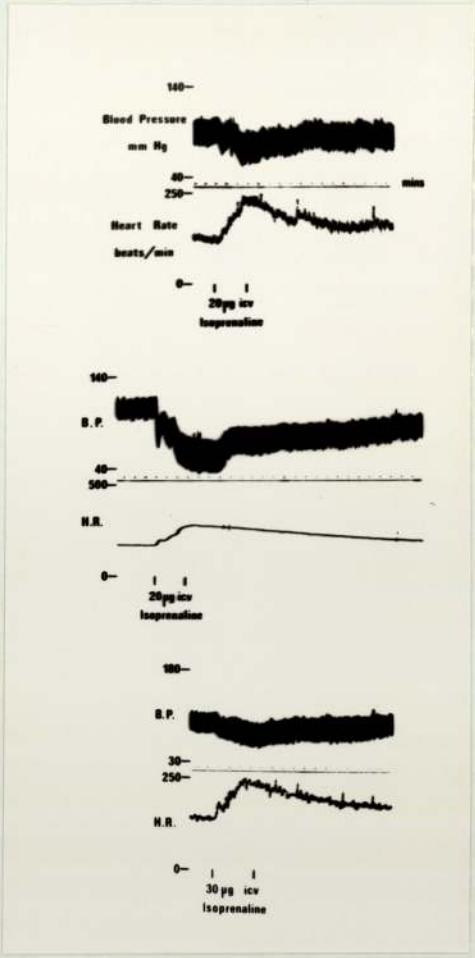


FIG. 62.

Conscious, normotensive, unrestrained cat. The upper trace illustrates a response to icv isoprenaline (20 µg) 20 weeks after being originally cannulated. Isoprenaline induced a large tachycardia with a large hypotension which was rapid in onset and of a relatively short duration. The middle trace shows the response to icv isoprenaline (20 µg) given 60 minutes after hexamethonium (7.5 mg/kg, i.v.); a large tachycardia accompanied with a large and prolonged hypotension resulted. The lower trace demonstrates the response to icv isoprenaline (30 µg) recorded in the same cat 4 weeks after cannulation. It can be seen that the hypotensive response is smaller, not rapid in onset and persists for a longer duration than the response in the upper trace.

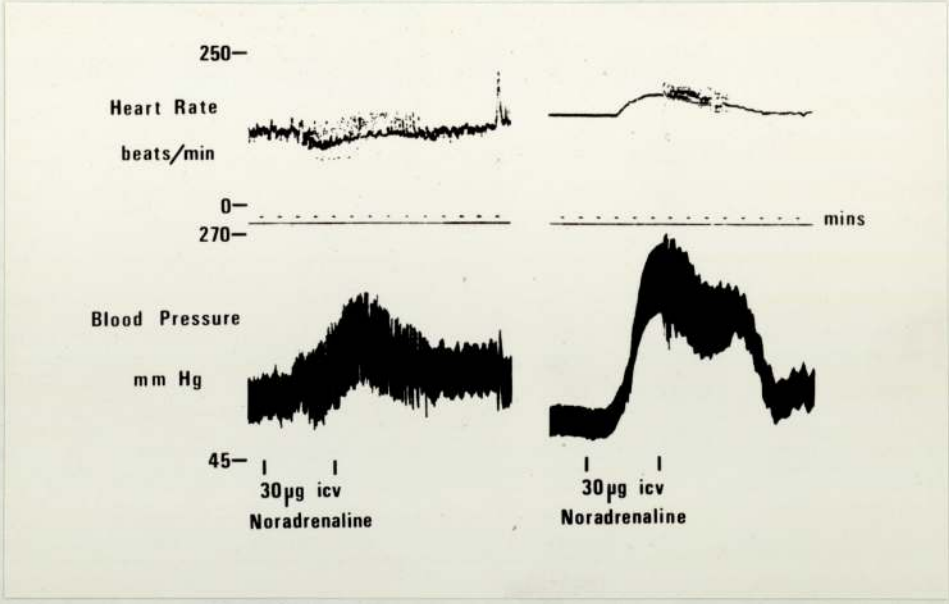


FIG. 63.

Conscious, normotensive, unrestrained cat. The left trace shows a pressor effect with bradycardia after noradrenaline (30 µg, icv), when given alone 60 minutes before pempidine (5 mg/kg, i.v.) The right trace illustrates the response to icv noradrenaline (30 µg) 90 minutes after pempidine; a large hypertension with tachycardia occurred with icv noradrenaline during peripheral autonomic ganglion blockade.

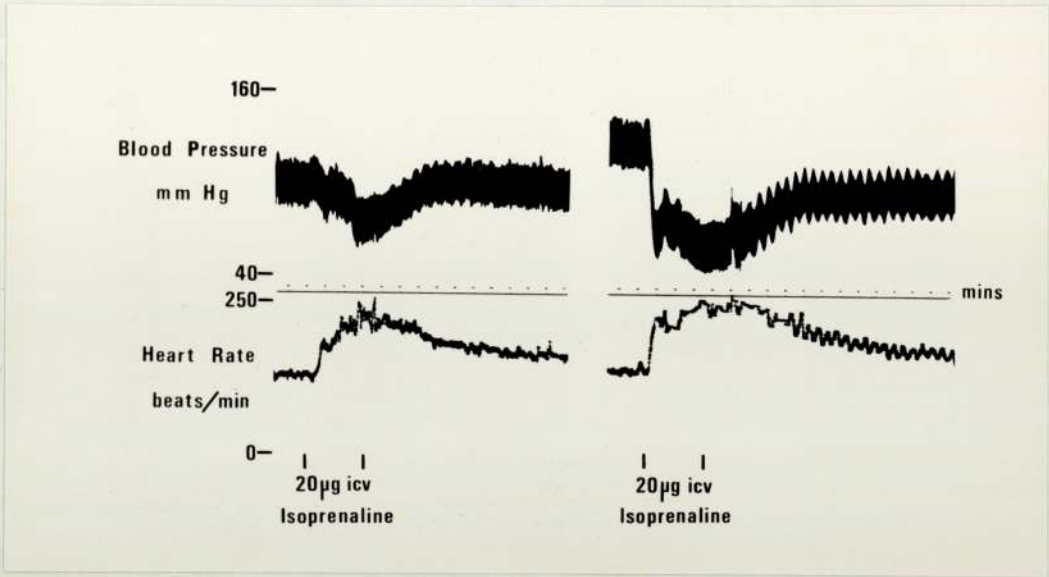


FIG. 64.

Conscious, normotensive, unrestrained cat. The left trace shows the hypotensive effect with tachycardia due to icv isoprenaline (20 µg) given alone. The hypotension was quick in onset and of short duration. The right trace illustrates the effect of isoprenaline (20 µg, icv) given 2 hours after bethanidine (5 mg/kg, i.v.). Isoprenaline produced a potentiated hypotension and tachycardia which were seen immediately at the beginning of the infusion. Figs. 62, 63 and 64 were obtained from the same cat.

GENERAL DISCUSSION

GENERAL DISCUSSION

Central α-Adrenoceptor Mechanisms.

In the experiments using DOCA/saline hypertensive rats, it was observed that the antihypertensive effect of systemically administered α-methyldopa was absent after inhibition of both peripheral and central dopa decarboxylase enzymes, with a large dose regimen of Ro4-4602, or dopamine β-hydroxylase enzymes with disulfiram, DDC or U-14,624. A smaller dose regimen of Ro4-4602, which was reported to inhibit only peripheral dopa decarboxylase enzymes, did not affect the antihypertensive action of α-methyldopa. These results indicate that α-methylnoradrenaline must be formed within central neurons in order to mediate the antihypertensive response observed after α-methyldopa. α-Methyldopa given icv to hypertensive rats in a dose one hundred times less than normally given peripherally produced pronounced antihypertensive effects, which were only inhibited by the large dose regimen of Ro4-4602. A low dose regimen of Ro4-4602 given icv almost completely abolished the antihypertensive effect of i.p. α-methyldopa. These findings are in agreement with those of Henning (1969a) and Henning & Rubenson (1971) and have recently been confirmed by Cohen et al. (1974) and Nijkamp et al. (1975) and demonstrate that the central production of α-methylnoradrenaline is necessary for the mediation of the antihypertensive action of the amino acid in rats. Torchiana et al. (1973) also inhibited the hypotensive response to icv α-methyldopa by blocking central dopa decarboxylase or dopamine β-hydroxylase enzymes in cats.

A comparison was made between the central cardiovascular effects of α-methylnoradrenaline and noradrenaline in conscious cats. Both compounds produced hypotension and bradycardia of similar magnitude. However, the depressant effects of icv α-methylnoradrenaline were more prolonged than those observed for noradrenaline. Day and Rand (1963a) postulated that the antihypertensive effect of α-methyldopa was due to the formation of α-methylnoradrenaline, which in turn acted as a weaker 'false' transmitter than the naturally occurring noradrenaline, in peripheral sympathetic nerves. Hence, from the results in conscious cats and anaesthetised cats (Heise & Kroneberg, 1973), it would appear that the antihypertensive or hypotensive effects of α-methyldopa are produced by the formation of a 'false' transmitter (α-methylnoradrenaline) in central neurons which is at least as potent as noradrenaline but has a more prolonged action than the natural transmitter, most probably due to its immunity to monoamine oxidase enzymes. This is further substantiated by the finding that a similar dose of metaraminol, which is knownto produce a weaker 'false' transmitter in peripheral sympathetic neurons by the synthetic metabolism of α-methylmetatyrosine, given icv to conscious cats, did not produce hypotension or bradycardia.

The hypotension and bradycardia observed after icv noradrenaline, α-methylnoradrenaline or α-methyldopa and α-methyldopa given orally in conscious cats were inhibited by prior central pretreatment with the α-adrenoceptor blocking agent phentolamine. The effects of oral or icv α-methyldopa were also inhibited by i.v. phentolamine. It was also found

that the hypotensive effects and bradycardia of i.v. or icv clonidine were inhibited by phentolamine either administered centrally or systemically. The absence of the cardiovascular depressant effects of α-methyldopa or clonidine after systemically administered phentolamine was not due to the depressant effects of phentolamine masking the responses of the former compounds.

If only peripheral α-adrenoceptors were inhibited by i.v. phentolamine then at least some degree of bradycardia should have occurred after clonidine, as the reduced heart rate due to clonidine is produced as a result of a simultaneous reduced sympathetic and increased vagal tone to the heart. Hence, sufficient phentolamine may enter the brain to inhibit the central effects of α-methyldopa and clonidine.

In the conscious cat, the hypotensive and bradycardic effects of icv noradrenaline, α-methylnoradrenaline, α-methyldopa, clonidine, adrenaline, phenylephrine and methoxamine are abolished by central pretreatment with phentolamine and thus appear to be due to stimulation of central α-adrenoceptors. That a central α-adrenoceptor mechanism is important in the mediation of the central cardiovascular depressant effects of this type of compound is now generally accepted (van Zwieten, 1973).

A large body of evidence has been presented by several groups of workers indicating that clonidine is able to stimulate α-adrenoceptors in both the medulla oblongata and the anterior hypothalamus producing hypotension and bradycardia (see Historical Introduction for references). After infusions of pontamine sky blue into the lateral ventricles of conscious cats, it can be seen that the dye, and therefore probably the

active drugs, pass throughout the cerebroventricular system and reach the fourth ventricle with ease (see Figs. 9b and c). Hence, the hypotension and bradycardia responses observed in the present experiments using conscious cats could result from the stimulation of either or both depressor sites.

Noradrenaline and adrenaline, the latter being given after central dl-propranolol, infused icv in the same doses that normally produced hypotension and bradycardia induced hypertension with associated bradycardia in 3 cats. As with the depressant effects, the hypertension was abolished by small icv doses of phentolamine and was unaffected by icv dl-propranolol. The pressor responses were nervously mediated and not due to leakage into the periphery, as they were absent after peripheral ganglion blockade.

Gagnon & Melville (1966) reported that small doses (0.01 - 0.1 μg) of noradrenaline given icv to cats produced hypertension and tachycardia. Both responses were abolished by central β-adrenoceptor blockade. These small doses of noradrenaline infused icv to conscious cats failed to produce any cardiovascular effects.

Pressor effects have been reported by Philippu and colleagues (see Historical Introduction for references), after administration of noradrenaline into the posterior hypothalamus of cats. Both α- and β-adrenoceptors appear to be important in the mediation of the hypertension as the respective blockers of these receptors reduce or abolish the

effects of noradrenaline.

Pressor effects have been induced by stimulation of medullary pressor loci (Wang & Ranson, 1939a; Alexander, 1946; for other references see Introduction). The central mechanism involved in mediating the medullary pressor effect is uncertain. Singh, Scrimal & Dhawan (1973) inhibited the excitability of hypothalamic as well as medullary vasomotor loci by central administration of α-methylnoradrenaline. Dhawan, Johri, Singh, Scrimal & Viswesaram (1975) also reduced the pressor responses to stimulation of hypothalamic and medullary vasomotor loci by central administration of clonidine. Srivastava et al. (1973) reduced the hypertensive effects to medullary stimulation by centrally administered dl-propranolol. Abraham, Tikare & Ahmed (1973) observed in cats that icv phentolamine abolished the pressor effect to electrical stimulation of the nucleus obivaris superioris lateralis in the medulla but did not affect the pressor effect to posterior hypothalamic stimulation.

Since pressor responses can be elicited from the medulla oblongata or the hypothalamus, both areas could provide sites of action for noradrenaline and adrenaline after infusions into the lateral cerebral ventricles of conscious cats, although from the findings of Abraham et al. (1973) the medulla may appear to be the more likely site of action. It may be that by virtue of the perfusion of these compounds throughout the ventricular systems of these 3 cats, preferential stimulation of pressor α-adrenoceptors occurred or significantly more pressor receptors were stimulated than depressor ones resulting in the production of a pressor effect.

In all but 2 cats used in the project icv phentolamine induced hypertension and tachycardias. It was probable that the cardiovascular stimulant effects of phentolamine were due to an inhibition of the central depressant α-adrenoceptor tone. In the 2 cats that produced hypotension and bradycardia to icv phentolamine, it was observed that icv noradrenaline and α-methylnoradrenaline also produced hypotension and bradycardia. Additionally, in the 3 cats that responded with pressor effects after icv noradrenaline icv phentolamine produced increases in blood pressure and heart rate. In all cases, icv phentolamine prevented the respective central effects of the agonists given centrally.

If the depressor or pressor responses to icv noradrenaline were resultant effects produced by stimulation of a majority of the respective receptors caused by slight differences in the distribution of noradrenaline within the brains, then perfusion with phentolamine might be expected to produce opposing responses to noradrenaline within the particular cat concerned. Hence, results have been presented which indicate that noradrenaline and phentolamine may not diffuse to the same brain areas within a particular cat.

In the majority of conscious cats, cardiovascular responses were obtained with icv phentolamine, which were contrary to the findings of Hilliard et al. (1972), Kleinrok et al. (1972) and Ito & Schanberg (1974). They reported that centrally administered phentolamine induced cardiovascular depressant effects. These results appear to be in accordance with the report of Abraham et al.(1973) in which they described that the pressor responses to carotid occlusion

and medullary stimulation were antagonised by icv phentolamine in cats.

However, Bergman & Ramu (1968), although failing to observe cardiovascular responses to icv doses of phentolamine up to 0.8 mg in anaesthetised cats, reported that icv phentolamine reversed the hypotension, due to vagal stimulation, to a pressor effect. No significant effect was observed by icv phentolamine on the rise in blood pressure due to sciatic nerve stimulation. After large doses of pentobarbitone, stimulation of the sciatic nerve produced falls in pressure but these effects were not affected by icv phentolamine. Bergman & Ramu (1968) suggested that the effect of phentolamine was selective within the central nervous system and if part of the depressor vagal reflex arc passed near the floor of the fourth ventricle then phentolamine and maybe other drugs could come in close contact with susceptible synapses. It may be possible in the conscious cat that the pressor effects and tachycardias due to icv phentolamine may be due to an inhibition of central depressor neurons, thus leading to a generalised raised sympathetic outflow to the periphery.

In contrast to the results obtained with icv noradrenaline, icv clonidine was observed never to produce centrally mediated pressor effects in the conscious cat. When administered to 2 of the 3 cats that normally responded to icv noradrenaline with pressor responses, clonidine given icv induced hypotension and bradycardia. As with the stimulant effects of icv noradrenaline, the depressant effects of clonidine were abolished by icv phentolamine pretreatment;

both types of blood pressure response appear to be attributed to stimulation of central α-adrenoceptors, as classified with respect to the peripheral sympathetic nervous system. However, the inability of clonidine to induce pressor effects may indicate that clonidine possesses a specificity for the depressor receptors and that these receptors may be slightly different to the pressor receptors.

Schmitt & Schmitt (1970) failed to antagonise the centrally mediated depressant effects of clonidine in dogs and suggested that the central receptors may differ from the peripheral receptors and can still be activated by α-adrenoceptor agonists and inhibited by some α-adrenoceptor antagonists. However, this report by these workers is the only one in which icv phentolamine has failed to be effective against the central actions of clonidine (see Introduction and Section 2, Chapter 4 of the Experimental Results for references).

In the conscious cat, it was confirmed that the bradycardia observed after icv clonidine is mediated by a decreased sympathetic tone and an increased vagal tone to the heart; bradycardia was still observed after pretreatment with i.v. dl-propranolol, bethanidine or atropine methylnitrate.

Central β-Adrenoceptor Mechanisms

In approximately 60% of the conscious cats used for recording responses to icv infusions of β-adrenoceptor stimulants, pressor effects associated with tachycardia were observed. The results obtained in these cats were contrary

to all previous reports describing the central effects of this class of compounds in both conscious and anaesthetised animals (see Introduction and Section 3, Chapter 1 of the Results for references). However, Poyser (personal communication) using anaesthetised dogs and Cooling and Sempik (from these laboratories) using conscious cats have also observed occasional pressor effects after icv isoprenaline. In these cats, icv isoprenaline, salbutamol, isoetharine and adrenaline (the latter given after icv phentolamine), generally produced a greater rise in the systolic blood pressure than the diastolic blood pressure. Both the increased blood pressure and heart rate were potentiated by i.v. atropine methylnitrate; a treatment which inhibited the reflex vagal inhibitory influence to the heart. The hypertension and tachycardia due to icv β-adrenoceptor agonists were unaffected by phentolamine or the local anaesthetics, lignocaine and procaine administered icv, but were abolished by icv propranolol and other β-adrenoceptor antagonists and by peripheral adrenergic neuron blockade or ganglion blockade. Since the responses to icv isoprenaline were absent after i.v. bethanidine , catecholamine release from the adrenal glands appeared not to contribute to the responses but these were due to central β-adrenoceptor stimulation leading to a raised sympathetic efferent outflow mainly to the heart. It was observed that pretreatment with clonidine, a compound which produced a pronounced reduction in efferent sympathetic outflow along with an increased efferent vagal outflow to the heart resulting in marked hypotension and bradycardia, reduced both the pressor effects

and tachycardias due to icv isoprenaline. Hence, central α-adrenoceptor stimulation can directly antagonise the central β-adrenoceptor actions of isoprenaline in the cat.

In 5 of the 25 conscious cats given icv isoprenaline depressor effects were observed in combination with tachycardia. In a further 4 cats, biphasic pressor and depressor responses were observed and in a further 2 cats blood pressure responses were absent. The depressor effects of icv isoprenaline were abolished by prior central β-adrenoceptor blockade; these findings being in agreement with most reports in the literature involving icv isoprenaline. In contrast to the results obtained with α-adrenoceptor stimulants given icv in which bradycardia was always obtained, icv infusions of β-adrenoceptor agonists always produced tachycardia.

In addition to the cat, pressor effects could be obtained after icv isoprenaline in both the conscious rabbit and rat. In the minority of rabbits and rats used, isoprenaline given icv produced hypotension and tachycardia. After anaesthetising cats, that responded in the conscious state to icv isoprenaline with hypertension, with halothane or chloralose similar types of cardiovascular responses were obtained. Pressor effects to icv isoprenaline and adrenaline under respective halothane and chloralose anaesthetised cats are shown in Figs. 48 and 49. Therefore, the use of variable animal species and anaesthesia does not appear to explain why only depressor responses have been reported after icv administered isoprenaline.

Peripheral β-adrenoceptors have been classified

into β_1 and β_2 types; the former describing cardiac and the latter bronchial and vascular β-adrenoceptors. Isoprenaline and adrenaline are potent stimulants of both receptors whilst isoetharine and salbutamol are more potent stimulants of β_2 receptors. From Table 5, it can be seen that all compounds produced similar centrally mediated cardiovascular stimulant effects. In 3 cats that responded to icv isoprenaline with depressor effects, icv salbutamol produced falls in blood pressure in 2 cats and in the third cat induced a pressor effect associated with tachycardia. With respect to the use of these compounds icv, there does not appear to be 2 types of β-adrenoceptors within the brain, and both the depressant and stimulant blood pressure effects and tachycardias appear to be due to stimulation of similar β-adrenoceptors. The cardioselective β_1-adrenoceptor antagonists practolol and ICI 66082 reduced the effects of the β_2-stimulants salbutamol and isoetharine as well as those of isoprenaline and adrenaline.

Icv dopamine always produced pressor effects in combination with relatively small tachycardias in 10 cats. In 6 of these cats the stimulant effects were observed alone but in the remaining 4, hypotension and bradycardia followed the initial rises in heart rate and blood pressure. The pressor effects and tachycardias of icv dopamine were abolished by central β-adrenoceptor blockade or central dopamine receptor blockade and were unaffected by icv phentolamine. α-Methyldopamine produced similar centrally mediated cardiovascular responses to dopamine when administered

to the same cat. The stimulant effects of α-methyldopamine were also blocked by central β-adrenoceptor blockade. The effects of dopamine receptor blockers on the effects of icv α-methyldopamine were not investigated.

Treatment for 3 days with disulfiram, in order to inhibit central dopamine β-hydroxylase enzymes, was found not to affect the stimulant effects of icv dopamine or α-methyldopamine but inhibited the resulting hypotension and bradycardia. It was also observed that icv phentolamine antagonised the cardiovascular depressant effects of icv dopamine. It was concluded from these results in the conscious cat that the stimulant effects of icv dopamine were due to either direct stimulation of dopamine or β-adrenoceptors and the following depressant effects were produced by α-adrenoceptor stimulation brought about by the central formation of noradrenaline (and α-methylnoradrenaline in the case of α-methyldopamine).

Although the pressor effects and tachycardias of icv isoprenaline and dopamine are similarly blocked by icv dl-propranolol and unaffected by phentolamine or local anaesthetics some differences between the responses were observed. Dopamine produced greater blood pressure effects than isoprenaline; dopamine being more potent in raising the diastolic blood pressure than isoprenaline. In comparison with isoprenaline, dopamine produced smaller tachycardias. The effects of isoprenaline were unaltered by dopamine receptor blockade whilst this treatment abolished the responses to icv dopamine. The administration of icv

bethanidine or guanethidine tended to preferentially depress the pressor effects of icv isoprenaline; the hypotensive effects and tachycardia remaining unchanged. However, icv pretreatment with either adrenergic neuron blocking agent produced little effect on the centrally mediated effects of dopamine.

If the pressor effects of dopamine and isoprenaline were mediated by the same central pathway then neurons within this pathway may possess both β-adrenoceptors and dopamine receptors; the β-receptors being on the post-synaptic membrane of a later neuron than the dopaminergic neuron. This situation could account for the findings that β-adrenoceptor antagonists inhibit both the central effects of dopamine and isoprenaline whereas pimozide and haloperidol, dopamine receptor blockers, inhibited only the responses to dopamine. From these results, it appears unlikely that the effects of dopamine are due to direct stimulation of the same receptors stimulated by isoprenaline. However, if the dopaminergic neuron preceded the neuron containing the β-adrenoceptors then the pressor responses to icv dopamine might be expected to be depressed by icv guanethidine or bethanidine, as were the pressor effects of isoprenaline.

There may be 2 pathways present within the brain which produce pressor effects when stimulated; one of which could be a dopaminergic pathway and the other a β-adrenergic pathway. β-Adrenoceptor antagonists may directly affect the dopamine pathway, by an action other than local anaesthesia as lignocaine and procaine were ineffective, or if both

pathways joined to form a common efferent pathway blockade may occur if β-adrenoceptors are present in the pathway after this junction.

Isoprenaline produces its effect in the periphery by stimulation of post-synaptic β-adrenoceptors and is not taken up into adrenergic nerve terminals. This is also probably the case in the central nervous system. Hence, in order for icv bethanidine or guanethidine to depress the pressor response to icv isoprenaline, the adrenergic neuron blockers must inhibit later neurons than the ones which are stimulated by isoprenaline. Therefore, it may be postulated that these blocking agents preferentially inhibit the adrenergic pathway stimulated by isoprenaline at a point above the junction with the dopaminergic pathway.

Both explanations of these results are not fully satisfactory and further experiments must be performed. Cardiovascular responses should be monitored after discrete microiontophoretic applications of the pharmacological agents, mentioned above, in an attempt to localise these proposed pathways.

Responses obtained with peripherally classified α- and β-adrenoceptor agonists and antagonists given centrally, indicate that 2 mechanisms are present within the brain which exert opposing effects on the heart. Stimulation of central α-adrenoceptors decreased efferent sympathetic tone (and increased efferent vagal tone) resulting in bradycardia whilst stimulation of β-adrenoceptors always produced a raised efferent sympathetic tone leading to

tachycardia.

However, blood pressure responses to these agonists given icv were more complex. Pressor and depressor responses were observed after both α- and β-adrenoceptor agonists given icv. If the peripherally recorded responses were produced by either a decreased or an increased sympathetic tone then it may be possible that there are 4 pathways innervating the efferent sympathetic neurons to the vasculature. At the present time it is not known which of the central α- or β-adrenoceptors are excitatory or inhibitory. If, for example, the β-adrenoceptors represented excitatory receptors and the α-adrenoceptors inhibitory ones and there also existed an excitatory and an inhibitory pathway innervating the final sympathetic outflow then the pressor effects could be produced by the β-adrenoceptor agonists stimulating the excitatory pathway or the α-agonists inhibiting the inhibitory pathway and conversely the depressor responses could be produced by the β-agonists stimulating an inhibitory pathway or the α-agonists inhibiting an excitatory pathway.

Effects of possible central neurotransmitters on blood pressure and heart rate.

In the peripheral sympathetic nervous system, the neurotransmitter noradrenaline stimulates both α- and β-adrenoceptors. However, from the experiments using icv administrations of possible transmitters in the conscious cat, at no time was a β-adrenoceptor response observed after icv noradrenaline; all responses to icv noradrenaline were

abolished by the α-adrenoceptor blocker phentolamine. This is in contrast to the results of Gagnon & Melville (1966) who observed that pressor effects and tachycardias after low doses of noradrenaline given icv were inhibited by icv pronethalol. Hence it would appear extremely likely that noradrenaline is the transmitter involved in the α-adrenoceptor pathways.

Icv adrenaline in the presence of propranolol produced potent α-adrenoceptor responses (including both pressor and depressor effects with bradycardia). When given after icv phentolamine, adrenaline produced large rises in blood pressure and heart rate due to β-adrenoceptor stimulation. Unfortunately, adrenaline was never administered to cats in which icv isoprenaline induced depressor effects, hence it it not know whether adrenaline is able to stimulate β-adrenoceptors involved in mediating depressor effects.

Although both pure α- and β-adrenoceptor responses were obtained with adrenaline it is impossible to indicate whether or not adrenaline is physiologically involved in either central α- or β-adrenoceptor regulatory mechanisms. Hökfelt et al. (1973, 1974) suggested that adrenaline containing neurons were stimulated by clonidine via an α-adrenergic mechanism to induce its cardiovascular depressant effects in the rat. Further experiments should be performed in the cat to identify and map adrenaline containing neurons, if they are present.

Icv dopamine produced potent blood pressure increases which were abolished by β-adrenoceptor antagonists or

dopamine receptor blockers. Icv dopamine never produced depressor effects via a β-adrenoceptor mechanism and in cats that responded to icv isoprenaline with falls in blood pressure, icv dopamine still induced pressor effects. Since dopaminergic neurons have been identified within the central nervous system (see Historical Introduction) and as dopamine produced pronounced pressor effects when administered directly into the brain, it is likely that dopamine is a central neurotransmitter involved with central cardiovascular control. Central dopaminergic neurons may help to maintain a vasoconstrictor tone to the periphery. Bolme & Fuxe (1971) reported falls in blood pressure and heart rate after pimozide and spiroperidol administrations to anaesthetised cats and suggested that these responses were due to removal of the central dopaminergic regulatory mechanism. They also proposed that these dopaminergic pathways interacted with depressant noradrenergic neurons.

In the conscious cat, icv pimozide or haloperidol mainly caused small pressor effects after which the blood pressures returned to and remained at normotensive levels. It may be possible that the initial pressor effects were due to release of dopamine from the dopaminergic neurons.

A possible link between the central effects of parasympathetic and sympathetic agents, as suggested by Dhawan et al. (1965), Scrimal et al. (1969) and Philippu et al. (1970, 1974) was investigated in the conscious cat. Attempts were made to record the centrally mediated cardiovascular effects of acetylcholine, nicotine, tetramethylammonium and carbachol infused icv in the conscious cat.

Only icv carbachol produced potent and consistent responses. Icv doses of carbachol above 15 µg produced marked blood pressure and heart rate increases but were associated with severe rage reactions. It was observed that by reducing the dose of icv carbachol to 2.5 - 7.5 µg blood pressure and heart rate increases were still obtained but the behavioural effects were absent. The cardiovascular responses to icv carbachol were very similar to those observed after icv isoprenaline in that the systolic blood pressure was usually raised to a greater extent than the diastolic pressure and were associated with a large tachycardia.

Experiments performed to determine the type of cholinergic receptor involved in the mediation of the icv carbachol response were inconclusive as icv pretreatment with either the nicotinic blocker, hexamethonium or the muscarinic blocker, atropine methylnitrate, inhibited the responses to icv carbachol. Within the periphery, carbachol possèsses both nicotinic and muscarinic actions (Koelle, 1970b) and it also appears that its centrally mediated effects are mediated by both types of receptors. In the dog, Lang & Rush (1973) observed that stimulation of both central nicotinic and muscarinic receptors produced increases in blood pressure and heart rate.

Icv guanethidine and bethanidine prevented the cardiovascular effects of icv carbachol. In addition, icv propranolol inhibited the effects of carbachol in 7 of 9 cats tested. That these sympathetic blocking agents did not inhibit carbachol by local anaesthetic actions was

confirmed when icv lignocaine and procaine produced little effect on the carbachol response. The pressor effect and tachycardia of icv carbachol appeared to be mediated via the sympathetic outflow to the heart and vasculature as they were inhibited by i.v. bethanidine treatment.

The results describing the effects of peripherally and centrally administered sympathetic blocking drugs on the icv carbachol response are relatively similar to those described for icv isoprenaline with one exception. Icv bethanidine or guanethidine tended to depress only the pressor effect of icv isoprenaline leaving the tachycardia unaltered; they also did not affect the depressor actions of icv isoprenaline. However, icv bethanidine or guanethidine blocked both the blood pressure and heart rate increases of icv carbachol.

Although icv carbachol never produced hypotension the centrally mediated stimulant responses of icv isoprenaline and carbachol were somewhat similar and could possibly have been mediated through the same efferent pathways to the periphery. However, a small increase in the dose of carbachol resulted in the emergence of violent behavioural effects, whereas at no time were any behavioural effects observed after isoprenaline, even after very large doses given icv or icm. Thus, carbachol appears to be able to stimulate the defence reaction; large doses producing the behavioural effects and low doses of carbachol probably inducing preparatory responses. Hence, from the results described in Section 5, cholinergic and sympathetic

mechanisms are implicated in the mediation of defence reactions in the cat.

I.v. bethanidine abolished the centrally mediated cardiovascular effects of icv carbachol (2.5 - 7.5 µg) as with icv isoprenaline. Since Boura et al. (1962) showed that bethanidine was relatively ineffective in blocking the release of catecholamines from the adrenal medulla, it appears that this mechanism plays little part in mediating the pressor responses and tachycardias of both icv carbachol and isoprenaline. This is in contrast to the findings of Kaul & Grewal (1968) and Brezenoff (1973) who showed that physostigmine released adrenal catecholamines by a central mechanism in rats and Scrimal et al. (1969) suggested that adrenal catecholamine release accounted for the initial pressor effect to icv choline in dogs.

Although a link between central cholinergic and sympathetic mechanisms has been demonstrated in the conscious cat, the exact nature of this link cannot be described at the present time. For example, the important central neural pathway or pathways influencing the cardiovascular system may be similar to the situation in the peripheral sympathetic nervous system whereby acetylcholine is released from the ganglion and noradrenaline is released from the post-ganglionic neuron to react with post-synaptic receptors. A situation may also exist in which acetylcholine is important in mediating the release of noradrenaline from nerve endings as postulated for the peripheral sympathetic nervous system by Burn & Rand (1959). They suggested that the action

potential within the sympathetic fibres results first in the release of acetylcholine which, in turn, causes the release of noradrenaline. Experiments should be carried out to further investigate this problem.

The role of possible central neurotransmitters in cardiovascular control has only been examined briefly and it should be remembered that the situation is even more complicated as serotoninergic mechanisms are also probably involved.

Possible Areas of Stimulation.

In 2 conscious cats, the depressor effects and tachycardias observed after icv and icm isoprenaline were compared. Both responses were extremely rapid in onset indicating that isoprenaline may be able to produce depressor effects and tachycardias by stimulating the relevant pathways in both the mid and hind brain areas. The responses produced after isoprenaline given via both routes appear to be due to the same mechanism, as both were inhibited by β-adrenoceptor blockers. However, since only 2 cats were cannulated icm few conclusions can be drawn from these results and experiments should now be performed in more cats that produce depressor effects to confirm these initial findings. Additionally, cats should also be cannulated icm that respond to icv isoprenaline with pressor responses to assess whether or not isoprenaline can stimulate medullary pathways to produce hypertension. de Jong et al. (1975) observed pronounced falls in blood pressure and heart rate after α-methylnoradrenaline was applied directly to the nucleus

tractus solitarius of the medulla oblongata of the rat. However, after phentolamine injected into this area, α-methylnoradrenaline produced hypertension and tachycardia Thus, these latter responses may be due to β-adrenoceptor stimulation by α-methylnoradrenaline. Experiments could be performed to verify this by investigating the effect of dl-propranolol on the α-methylnoradrenaline response after phentolamine and also whether injections of β-adrenoceptor agonists into the nucleus tractus solitarius actually cause hypertension and tachycardia.

Dopamine given icm to both cats produced initial pressor effects and tachycardia followed by prolonged falls in blood pressure and heart rate. Depressant effects to icv dopamine were only observed in one cat. The initial rises in blood pressure and heart rate after icm dopamine appeared to be smaller than the responses obtained after icv dopamine and conversely the depressant effects observed after icm dopamine appeared slightly larger than the responses obtained in one cat after icv dopamine. Therefore, the depressant effects observed after icv dopamine were probably due to an action primarily within the medulla oblongata (hind brain); the stimulant effects seen after icv dopamine were probably mainly produced by stimulation of higher brain areas, although stimulation of medullary pathways (obtained with icm dopamine) produced stimulant effects to some degree.

The icv cannulae were implanted in such a position that the opening at the base of the cannula shaft faced towards the formen of Monro in order to allow quick passage

of compounds into the third ventricles. However, from dye studies, it was observed that the icv infusion allowed the dye to stain the whole of the surface of the left lateral ventricle (see coronal sections A - D of Figs. 9b and c). Areas such as the caudate and amygdaloid nuclei, which are innervated by central dopaminergic cell bodies (Ungerstedt, 1971, a, b ; Bolme et al. 1972; Livett, 1973), form a large part of the wall of the lateral ventricles. Thus, it may be possible that dopamine stimulates these areas surrounding the lateral ventricles to produce pressor effects and tachycardias in the cat.

However, the nigro-striatal dopaminergic pathway, which innervates these basal ganglia areas is believed to be involved in a number of behaviour patterns such as feeding, drinking and sexual behaviour, various locomotor activities and self-stimulation (Bolme et al., 1972). They defined this pathway as "a specific locomotion system facilitating the induction of a large number of various behaviours". After icv administration of dopamine to conscious cats behavioural or locomotor effects were not observed suggesting that the central cardiovascular effects of dopamine were specific responses and did not occur as a result of behavioural or locomotor responses. Further experiments should be carried out to determine the area or areas involved in mediating the pressor effect and tachycardia of dopamine.

The exact central site of action of noradrenaline in mediating both depressant and stimulant cardiovascular effects was not determined from the experiments performed in

conscious cats. However, the hypotensive and bradycardic effects were probably produced by stimulation of α-adrenoceptors within the nucleus tractus solitarius of the medulla oblongata thus inducing activation of efferent baroreceptor pathways (de Jong et al.,1975; Struyker Boudier et al.,1975). Hypothalamic sites may also have contributed to the depressant effects of icv noradrenaline in the cat, as Toivola & Gale (1970) and Struyker Boudier et al.(1975) observed that noradrenaline administered into the anterior hypothalamus of baboons and rats respectively produced falls in blood pressure and heart rate. From the work reported by Philippu and colleagues (see Historical Introduction for references) it is very likely that the pressor effects obtained after icv noradrenaline in 3 cats were due to predominant stimulation of posterior hypothalamic areas.

Central actions of β-adrenoceptor antagonists.

7 clinically active antihypertensive β-adrenoceptor blocking agents produced hypotension and bradycardia when administered icv or icm to conscous normotensive cats. These effects appeared to be due to central β-adrenoceptor blockade as the maximum falls in blood pressure and heart rate corresponded to the greatest inhibitory effect on the central cardiovascular actions of β-agonists. Also, it was found that the d-isomers of propranolol and alprenolol, isomers which do not possess β-blocking properties (Fitzgerald, 1969) and the local anaesthetics, procaine and lignocaine, given icv or icm failed to induce significant falls in pressure

or heart rate.

All the β-adrenoceptor blockers and local anaesthetics administered centrally, with the exception of ICI 66082, which possesses no intrinsic sympathomimetic or local anaesthetic activity (Barrett et al.,1973; Harry et al.,1973) induced transient initial rises in heart rate and blood pressure. The size of the initial stimulant effects generally appeared to be proportional to the degree of local anaesthetic or intrinsic properties of the blockers tested. Practolol and sotalol produced smaller stimulant effects than either propranolol, alprenolol, pindolol or oxprenolol. The pressor effects and tachycardias were produced by compounds with only local anaesthetic effects (e.g. propranolol and lignocaine) and intrinsic sympathomimetic activity (e.g. practolol and pindolol). Oxprenolol and alprenolol possess both properties.

In the anaesthetised dog, Srivastava et al. (1973) abolished the initial pressor effect and tachycardia due to icv propranolol by prior central β-adrenoceptor blockade. However, Dollery et al. (1973, 1974) found that the initial pressor effects of icv propranolol in conscious rabbits were inhibited by icv pretreatment of α-adrenoceptor antagonists. In the present experiments using conscious cats, the initial stimulant effects of propranolol, practolol and lignocaine were completely abolished and propranolol greatly reduced by prior central β-adrenoceptor blockade with propranolol. The reduction of the initial stimulant responses of these compounds by propranolol was not due to a local anaesthetic effect as icv lignocaine proved to be ineffective.

Dollery et al.(1974) showed that in rabbits propranolol had to be taken up into central noradrenergic neurons in order to produce its intial stimulant effects as they were absent when propranolol was given icv after central desmethylimipramine. In the cat, desmethylimipramine given icv in a dose lower than used in the rabbit by Dollery et al. (1974), tended to reduce the initial stimulant responses of icv propranolol. Thus it may be in the cat, that the initial responses seen after central administration of propranolol were produced by central β-adrenoceptor stimulation probably caused to some extent by endogenously released catecholamines from central adrenergic neurons.

Since the stimulant effects of icv propranolol were accompanied with raised catecholamine levels in the adrenal vein and were absent after injection of the blocker into the fourth ventricle of dogs, Srivastava et al. (1973) proposed that these responses were produced by β-adrenoceptor stimulation of the hypothalamus leading to the release of catecholamines from the adrenal glands. Experiments were performed in the conscious cat to investigate this proposed mechanism. The pressor effects of dl-propranolol in 3 cats were abolished by either i.v. hexamethonium or pempidine indicating that these responses, like those of icv isoprenaline, were nervously mediated from the central nervous system. In the same 3 cats pretreated with i.v. bethanidine the rises in pressure after icv propranolol were present in 2 cats and absent in the third. The pressor responses observed in the presence of bethanidine were accompanied with bradycardia. The bradycardia appeared not to be reflex in nature

as propranolol was administered icv at a time when the pressor effects and bradycardia of McNeil A-343 were inhibited. Hence, the reduced heart rate may have been induced by an increased efferent vagal tone to the heart. If the pressor effects of propranolol were due to adrenal catecholamine secretion, then an increased heart rate should have been expected in the presence of bethanidine. The situation therefore, is still unclear and these experiments must be repeated in adrenalectomised and intact cats.

With respect to the areas of the cat brain responsible for mediating the initial stimulant and prolonged depressant effects of the β-adrenoceptor blockers, both types of response were obtained after icv and icm administrations of these compounds. By comparing Tables 10a and b and 11, it can be seen that the depressant effects of each β-adrenoceptor blocker given icv and icm were generally similar. However, in contrast to the results of Srivastava et al. (1973) using dogs, stimulant effects could be obtained after direct administration of the blockers to the medullary region. The rises in pressure and heart rate observed after icm infusions appeared to be larger and of longer duration than the responses seen for the same compounds given icv. Hence, the pressor effects due to icm infusions of β-blockers and d-propranolol appeared to be due to β-adrenoceptor stimulation of medullary pathways, although hypothalamic stimulation may contribute to the effects seen after these compounds given icv.

The central mode of action by which clonidine lowers

blood pressure and heart rate was discussed earlier. However, Kobinger & Walland (1972a, b) reported that clonidine facilitated the reflex bradycardia, induced by i.v. administrations of pressor substances, by stimulation of central α-adrenoceptors. From Fig. 33, it was observed that the centrally mediated pressor effect of dopamine was inhibited by icv propranolol and at the time of the maximum depressant effect seen after icv propranolol the reflex bradycardia due to i.v. dopamine (30 μg/kg) was potentiated compared to the control dopamine response. Since, icv dl-propranolol produced potent, centrally mediated hypotension and bradycardia and also appeared to be able to potentiate reflex bradycardia, it may be possible that the central effects of propranolol and other β-blockers are mediated via the same efferent pathways as clonidine, not as a result of direct stimulation of the central depressant pathways, as with clonidine, but by inhibiting an opposing β-adrenoceptor mechanism.

However, unlike the centrally mediated bradycardia of clonidine, which was partially due to an increased efferent vagal tone to the heart, the reduced heart rate observed after icv dl-propranolol was most probably due entirely to a reduced efferent sympathetic outflow to the periphery as both the hypotension and bradycardia of icv propranolol were absent after i.v. bethanidine. That a vagal component is not present in the central propranolol response can be confirmed by infusing propranolol icv after complete cardiac β-blockade with i.v. propranolol or practolol; bradycardia induced after this treatment would indicate a

vagal component. In the dog, Srivastava et al. (1973) observed that the falls in blood pressure and heart rate after icv propranolol were unaltered by bilateral vagotomy.

Results obtained in the conscious normotensive cat demonstrate that 7 β-adrenoceptor blocking agents once introduced into the central nervous system were capable of inducing centrally mediated falls in blood pressure and heart rate. Hence, for the lipid soluble blockers, which easily pass the blood brain barrier, the central nervous system provides a potent site of action. However, the water soluble compounds (e.g. practolol and ICI 66082) do not readily enter the brain and thus the brain provides a less accessible site of action for these compounds. Scales & Cosgrove (1970) observed that only very small amounts of practolol enter the brains of cats, dogs and monkeys and at the present time it is not known whether or not the small concentration of practolol that reaches the brain is sufficient to produce significant cardiovascular depressant effects.

In man, it is very probable that several mechanisms are involved in mediating the antihypertensive effects of β-adrenoceptor blocking agents, (see Historical Introduction for references of proposed mechanisms), with a central component playing an important part in the response. However, in experimental animal studies antihypertensive effects of β-adrenoceptor blockers have been difficult to demonstrate. Therefore, direct administration of β-blockers into the brains of conscious normotensive or hypertensive cats may

provide a suitable experimental model to screen for active antihypertensive β-adrenoceptor blocking agents.

This project has demonstrated in the cat that 3 types of compounds are able to lower blood pressure and heart rate through central mechanisms. These are α-methyldopa, which needs to be metabolised to α-methylnoradrenaline in order to stimulate α-adrenoceptors within the brain, clonidine, which directly stimulates central α-adrenoceptors and β-adrenoceptor blocking agents. Thus, it would appear that the central nervous system may be a fruitful area for future research into new and better antihypertensive agents. Compounds are needed that specifically lower blood pressure and heart rate without causing other central side effects such as sedation.

As more compounds are being found to lower blood pressure and heart rate through central mechanisms extensive research is now needed to investigate whether pathogenic occurrences within the brain contribute to the onset and pathogenesis of hypertension in man. Work using the spontaneous hypertensive rat has led to speculation that an apparent decrease in catecholamine levels, turnover and synthesis rates in the brain stem and hypothalamus may be related to the pathogenesis of the genetic hypertension (Yamori et al. 1970; Yamabe, de Jong & Lovenberg, 1973). However, in a recent paper de Jong et al. (1975) reported the absence of low noradrenaline levels in the hypothalamus and pons-medulla of recent generations of spontaneous hypertensive rats. Hence, further work is needed to clarify this situation.

In this project, it has been shown that the conscious cat is a good experimental model to record the cardiovascular effects of centrally administered drugs. It was also demonstrated that if the cats were used conservatively then leakage of catecholamines and other substances did not occur for several months, if at all. The cats were easily trained which permitted easy recording of steady resting cardiovascular parameters and also centrally mediated responses With the use of a relatively unspecific method of central drug administration, responses were recorded to α- and β-adrenoceptor agonists and antagonists in the attempt to identify opposing mechanisms, if present, within the brain which affect the peripheral cardiovascular system. The use of infusions into the lateral ventricles allowed passage of drugs throughout the brain, thus the responses obtained were probably a resultant response of stimulation or blockade of several areas. Unfortunately, only 2 cats were cannulated icm allowing fairly discrete application to the medulla.

Attempts should now be made to identify the pathways and receptors involved in mediating the various cardiovascular responses observed in this project by administering these drugs into very discrete brain areas by using microiontophoretic or push-pull cannulae in the conscious cat. It may also be possible to record efferent nerve activity in the conscious cat as Schmitt, Schmitt & Fénard (1974) have developed a technique for recording sympathetic nerve activity in unanaesthetised dogs. Measurement of blood flow in various vascular beds would also provide useful information concerning the peripheral mechanism by which these central responses are mediated.

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