CENTRAL α-ADRENOCEPTORS
AND
BEHAVIOUR

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Central Alpha Adrenoceptors and Behaviour

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Summary

A range of agonists and antagonists were found to show selectivity peripherally for α_1 and α_2 receptors *in vitro*. Further studies showed that drugs showing selective effects at α_2 receptors were able to modify the release of noradrenaline from central neurones. Behavioural studies were undertaken using all the drugs to determine whether central α_1 and α_2 receptors may mediate different effects.

Agonists selective for α_2 receptors clonidine, guanfacin and guanabenz, caused marked sedation, inhibited the pinna reflex, and were slightly analgesic. Clonidine also inhibited 5-HT-induced head twitches and haloperidol-induced catalepsy. In general, α_1 agonists such as methoxamine, had the opposite effect on observed behaviour, producing a syndrome of hyperalertness, hyperalgesia and hyperreactivity which resembled fear. However, high doses did inhibit the pinna reflex and 5-HT-induced head twitches. Both clonidine and methoxamine also inhibited motor activity alone, but potentiated apomorphine-induced activity in reserpinised mice.

Antagonists with selectivity for α_2 receptors, yohimbine and piperoxane, had behaviourally opposite effects to clonidine (although, like clonidine, these drugs again reduced activity) and in general were able to reverse these effects. Alpha$_1$ antagonists such as prazosin on the other hand, produced sedation and potentiated clonidine's actions. However α_1 antagonists did not inhibit the pinna reflex or produce analgesia alone.

The results suggest that central α_2 receptor stimulation leads to sedation, analgesia and inhibition of the pinna reflex, 5-HT head twitches, catalepsy and motor activity and also possibly to an anxiolytic-like effect. Stimulation of α_1 receptors, on the other hand, appears to result in hyperalertness, hyperreactivity, hyperalgesia, potentiation of catalepsy and fearfulness; and, when α_2 inhibitory effects are removed, increased activity and potentiation of 5-HT.

The drug selectivity found between α_1 and α_2 receptors *in vitro*, thus appears to be retained *in vivo* and may be manifested in the different behavioural effects produced by drugs which display such selectivity for one or the other type of receptor.

KEY WORDS

Alpha-adrenoceptors clonidine behaviour

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INTRODUCTION

1. Historical background - Noradrenaline (NA) as a transmitter.

Noradrenaline was first postulated to be the transmitter of peripheral sympathetic neurones by von Euler (1946), who suggested that it was present in nerve terminals from where it was released on stimulation to mediate the excitatory effects of such stimulation. The close correlation between the content of NA and the extent of sympathetic innervation of many tissues (Euler,1956) supports this hypothesis. Specific fluorescence histochemistry (Falck,1962) has since demonstrated that NA is indeed present in peripheral nerve terminals and its transmitter function is widely accepted.

It was not until 1954 that the suggestion was made that NA may also act as a transmitter in the CNS (Vogt, 1954), after the finding that the substance was unevenly distributed in the brain and may be affected by drugs which altered central control of adrenal gland secretion.

Criteria have since been established (Bradley, 1968; Bell et al.,1976) for the consideration of a substance as a transmitter in the brain. NA has, as far as is technically possible, been shown to satisfy all these criteria.

(i) The substance must be present in presynaptic terminals, together with the enzymes necessary for its synthesis.

Fluorescence histochemistry has established that NA is present in specific neurones (Dahlstrom & Fuxe,1964) and its presence in synaptosomes (Smith & Winkler,1972) indicates that it is located in nerve terminals. All the enzymes necessary for the synthesis of NA from tyrosine are present in brain i.e. tyrosine hydroxylase (TH) (Nagatsu et al.,1964), DOPA decarboxylase (Kuntzman et al.,1961) and dopamine-β-hydroxylase (DBH) (Undenfriend & Creveling,1959).

(ii) On stimulation of the presynaptic neurone, the substance should be released in adequate quantities.

This is difficult to demonstrate in the CNS, since it is impossible to measure release from only one neurone, However, NA has been shown to be released from slices of brain tissue *in vitro* (Baldessarini & Kopin,1967), while *in vivo* techniques employing histochemical and biochemical measurement of depletion of NA may be interpreted as showing that the substance is released on stimulation.

(iii) Its action on the postsynaptic membrane when applied directly should be the same as that of nerve stimulation.

The use of microiontophoresis to simultaneously apply substances and record discharges from CNS neurones has shown that NA has a similar action (usually inhibitory) to that of neuronal stimulation (Dillier et al.,1978).

(iv) There must be a mechanism for its removal or inactivation.

Both monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) are present in the brain, as is the high affinity uptake process found to be the main inactivation mechanism in peripheral neurones (Iversen,1967).

(v) Blocking agents should affect its normal action and the action on direct application in the same way.

Sotalol, a β antagonist, has been shown to prevent both the effect of stimulation of noradrenergic neurones and direct application of NA on cortical cell firing (Dillier et al.,1978).

Thus the suggestion that NA may act as a transmitter in the CNS is borne out by experiments and is now generally accepted. However, the effects which may be mediated by NA are much less clear, and the study of NA's involvement in behaviour is still in its infancy.

2. Distribution of noradrenergic neurones in the brain.

Following the introduction of a method of visualising catecholamine-containing neurones by fluorescence histochemistry (Falck,1962), Dahlstrom and Fuxe (1964) described the distribution of these neurones in the CNS. These workers designated the areas containing the cell bodies of such neurones A1 to A12, a nomenclature which is now widely used. They found that the cell bodies of monoamine (NA,DA and 5-HT) neurones were almost exclusively present in the lower brain stem, especially the midbrain; and suggested that most of the monoamine terminals, both in the brain and in the spinal cord, may derive from these cell groups. Ungerstedt (1971) combined the technique used by Dahlstrom and Fuxe with lesions to carry out investigations into the pathways originating from these nuclei.

Three separate noradrenergic systems were identified by Ungerstedt; descending systems to the spinal cord, ascending pathways from the lower brain stem and pathways originating in the locus coeruleus. The descending pathways which originate in the A1 and A2 regions of the medulla enter the spinal cord and terminate in the dorsal and ventral horns. Other neurones from these cell groups along with neurones from A5 and A7 regions ascend in the reticular formation, turn ventrally along the medial lemniscus and continue in the medial forebrain bundle. This system gives rise to nerve terminals in the lower brain stem, midbrain and diencephalon and innervates the whole hypothalamus.

Three pathways originate in the locus coeruleus (L.C.) or A6 cell group. A descending pathway innervates the lower brain stem nuclei, a lateral pathway innervates the cerebellum and an ascending pathway forms the dorsal noradrenergic bundle (also termed the dorsal tegmental bundle, since it forms part of the central tegmental tract). This pathway runs

dorsally to that previously described and forms a completely separated bundle of neurones in the midbrain. It then descends to join the other ascending noradrenergic neurones in the medial forebrain bundle at the level of the hypothalamus. This pathway innervates the hippocampus and cortex, possibly via the amygdala and hypothalamus, thus is able to influence many regions.

More sensitive fluorescence methods have confirmed the location of the pathways described, and have enabled more detailed studies of the pathways originating in the L.C. (Lindvall & Bjorklund,1974). Branches of noradrenergic neurones have thus been found to leave the MFB to innervate the thalamus, amygdala, some hypothalamic nuclei, the septum, striatum and olfactory lobe (Moore,1980). The preterminal fibres of such neurones branch into a highly collateralised network with regularly spaced round varicosities approximately 1 - 2μm in diameter.

Although the numbers of noradrenergic neurones is small compared to others in the brain, the projections of these neurones originating in the pons and medulla are able to influence many brain areas by the large numbers of terminal varicosities present on their branches. If such varicosities represent synapses en passant, then one axon may be able to innervate a large region along its entire length. The diffuse projection of noradrenergic neurones in the CNS suggests that, unlike, for example, dopaminergic neurones of the well-defined nigro-striatal pathway, which are specifically concerned in motor activity, NA is unlikely to be associated with any specific behavioural function. Alternatively, the widespread influence of noradrenergic neurones may affect many different types of behaviour.

3. Biochemical organisation of the noradrenergic synapse.

NA is synthesised in central neurones from tyrosine, which is taken up by an active process from the blood stream. The enzyme responsible for the conversion of tyrosine to di-hydroxyphenylalanine (DOPA) is TH, which is present in the nerve terminals. This conversion is the rate-limiting step in the formation of NA and blockade of the enzyme, which may be brought about by catecholamines, leads to depletion of NA (Levitt et al.,1965). DOPA is converted to dopamine (DA) by DOPA decarboxylase, which is also present in the nerve terminal. The enzyme responsible for converting DA to NA, which occurs only in noradrenergic neurones, is DBH. This enzyme may be localised in the membrane of storage granules. The use of drugs which block its action has proved useful in distinguishing between actions due to DA and NA in the brain.

Regulation of the amount of NA synthesis appears to be brought about by regulation of TH activity (Lin et al., 1969). This involves inhibition of the enzyme, possibly by competetive antagonism of cofactor binding, during periods of low neuronal activity, when the concentration of NA is high. During increased impulse flow, the amount of NA available is depleted, thus TH activity is increased. In addition, a short term mechanism for regulation of TH activity appears to exist and possibly also a trans-synaptic feedback which is effective after prolonged periods of neuronal activity.

Within the neurone, NA is located within subcellular particles termed storage granules, which also contain ATP and appear to have a dense core when viewed under the electron microscope (Smith & Winkler,1972). The mechanism by which NA is released from central neurones on stimulation is as yet unknown, although it seems probable that a similar mechanism to that seen peripherally may be involved. This involves

discharge of storage granules by fusion with the terminal membrane and is termed exocytosis (Baldessarini,1975). DBH and ATP are also released along with NA. Release is dependent on the presence of extracellular Ca^{2+} ions, and also on the frequency of stimulation (Baldessarini & Kopin,1967).

After release, NA may be inactivated by COMT or MAO (Musacchio,1975), which although usually assumed to be an intracellular enzyme, also occurs extracellularly. NA from central neurones is preferentially metabolised to 3-methoxy-4-hydroxy-phenylglycol, which is then excreted as a sulphate conjugate. However, the main inactivation mechanism is reuptake of the unmetabolised NA into the presynaptic neurone. This is an active process which is stereospecific and may be mediated by a saturable membrane transport mechanism (Iversen,1967). NA may also be taken up by other surrounding cells (ibid); although this is unlikely to be a major route of inactivation.

4. Adrenergic receptors.

The concept that drugs and neurotransmitters produce their effects by interaction with receptors has been in existence for many years. The theory suggests that drugs bind to the receptor substance and this combination can then lead to changes in the cell which bring about the biological response. The receptors are located on the surface of the effector cell. With the advent of radioactive substances of high specific activity which are able to bind to the receptors, many different types of receptor have been isolated and identified both from peripheral and central tissues. The use of ligand binding has enabled the identification of different receptors which specifically bind different classes of drugs and transmitters by a reversible, saturable process.

Early theories that the effects of sympathetic

nerve stimulation were mediated by NA (sympathin E) and adrenaline (sympathin I) were changed by Ahlqvist in 1948. He suggested that NA was responsible for all sympathetic effects and that the excitatory or inhibitory nature of the response of the effector cell depended on the type of receptor which it possessed. Excitatory receptors were designated α, and inhibitory receptors β; and it was found that NA activated maily α receptors, while adrenaline could stimulate both types. An exception to this general rule was that stimulation of receptors sensitive to isoprenaline, found to be a selective agonist of β receptors, lead to an increase in the rate and force of contraction in the heart.

Further work showed that β receptors were not all identical, but could be divided into subtypes (Lands et al., 1967), which has lead to the development of drugs which stimulate only bronchial (β_2) or block only cardiac (β_1) type receptors. Recently, it has been suggested (Langer,1974) that α receptors should also be subdivided into α_1 and α_2 types, since many experiments have indicated the presence of an α receptor on the presynaptic nerve terminal, which differs in its sensitivity to drugs from those found on peripheral effector organs. These receptors have, in many cases, inhibitory pharmacological actions, but do not resemble β receptors in their sensitivity to drugs. Their pharmacological effects appear to be mediated indirectly by a reduction in the amount of transmitter released on stimulation; and their probable presynaptic location has lead to the concept of presynaptic receptor-mediated regulation of transmitter release.

Binding studies have shown that receptors which resemble pharmacologically α_1 and α_2 receptors found peripherally are present in brain tissue (U'Pritchard et al.,1978; Tanaka & Starke,1980), as are β receptors. In addition, inhibition of NA

release from isolated brain tissue may be brought about by α agonists (Baldessarini & Kopin,1967). Thus there is a strong possibility that NA released from central neurones may affect other neurones by a direct activation of α or β receptors or indirectly by activation of regulatory α receptors.

5. Presynaptic regulation of NA release.

5.1. Peripheral presynaptic α receptors.

The concept that NA release may be regulated by a presynaptic mechanism involving α receptors was introduced (Haggendal,1970) to explain discrepancies in the effects of certain drugs on NA overflow after stimulation of perfused tissues. The first report by Brown and Gillespie (1957) that phenoxybenzamine (PBZ), an α receptor blocking agent, increased the overflow of NA elicited by nerve stimulation was initially interpreted as a blockade of a site of loss of the transmitter.

This was first thought to be the α receptor itself. However, the effects of α blockers on NA overflow were found to bear no relationship to their α blocking activity (Starke,1977); and, in addition, reports that PBZ also increased stimulation-induced (SI) overflow of NA from tissues known to possess only β receptors could not be explained by this means. PBZ also blocks the neuronal and extraneuronal uptake of NA (Iversen,1967), which would be expected to lead to an increase in NA overflow. The effects of other drugs known to be more potent in blocking these uptake processes, however, suggests that this is not the means by which PBZ increases NA overflow (Kirkepar & Puig,1971).

An alternative explanation is that the drug actually increases the amount of NA released from the nerve terminal, possibly by blocking a local negative feedback mechanism which normally suppresses transmitter release

(Haggendal,1970). The report that PBZ increased the amount of DBH in perfusate (de Potter et al.,1971), an enzyme known to be released along with NA, supported this suggestion. Farnebo and Hamberger (1971) postulated that a neuronal negative feedback system was present in the brain which lead to a decrease in transmitter release. However, such a system is much less likely to be present on peripheral neurones and the suggestion that α receptors may mediate the negative feedback system (Kirkepar & Puig,1971) could also explain the results obtained by Farnebo and Hamberger using brain slices.

The location of these α receptors was thought to be on the presynaptic nerve terminal (Kirkepar & Puig,1971) and their function to regulate the amount of NA released on arrival of a nerve impulse, in order to control the response of the effector organ. Thus NA released on stimulation, once it reaches a threshold concentration in the synaptic gap, would activate these presynaptic α receptors, thus triggering a negative feedback mechanism and inhibiting further release. In support of this hypothesis, NA itself has been shown to decrease its own release from peripheral neurones when reuptake is inhibited (Langer,1974). Such a mechanism would not be expected to operate when the amount of transmitter released was low. The lack of effect of PBZ in affecting NA overflow when NA stores are depleted by reserpine (Enero & Langer,1973) supports the likelihood of a physiological role for the mechanism. Indeed, α blockers have been shown to increase plasma NA levels according to their ability to block regulatory type α receptors and the consequent increase in heart rate is also dependent on this ability (Grahame et al. ,1980).

Experiments using the cat spleen showed that a 30-fold increase in the concentration of PBZ over that needed to reduce responses to nerve stimulation was required to enhance

transmitter release (Dubocovich & Langer,1974). These workers also found exogenous NA to be more potent in reducing transmitter release than in stimulating postsynaptic receptors, thus showing that the greater effect of PBZ on these receptors was not due to greater receptor numbers. It was suggested, therefore, that the two α receptors present on the same synapse were not identical and that PBZ had a greater affinity for the postsynaptic, than for the presynaptic receptor.

Such a difference between the receptors has since been confirmed by the variation in their affinity for many drugs. Several workers have studied the effects of drugs on the two types of receptors in order to determine whether other drugs selectively act on one or the other type. Both agonists and antagonists have been found to display such selectivity, although in only very few cases is this absolute. Indeed, not all drugs do have a higher affinity for one type of receptor, thus a continuum of selectivity is shown by a range of drugs for both receptors.

The degree of selectivity shown by a drug has been found to be fairly constant over a range of peripheral tissues, although there are some exceptions. For example, of the agonists studied, clonidine has been found by Starke et al., (1974) to selectively stimulate the presynaptic type of receptor, but by Drew (1976) to be equipotent on both pre- and postsynaptic receptors. Methoxamine and phenylephrine were found by both workers to display strong selectivity for the postsynaptic receptors.

In addition to the report of the greater affinity of PBZ for postsynaptic receptors in the cat spleen (Dubocovich & Langer,1974), this α antagonist has been found to selectively block postsynaptic receptors in the rat cardiovascular system (Drew,1976) and vas deferens (Doxey et al.,1977). The latter

workers, in fact found it to be inactive on presynaptic receptors in this tissue. Prazosin is another α antagonist which has been shown to selectively block postsynaptic receptors with no presynaptic activity (Cambridge et al.,1977; Doxey et al., 1977), while phentolamine displays no selectivity at all (Drew, 1976; Doxey et al.,1977). Although both yohimbine (Starke et al., 1975a) and piperoxane (Drew,1976) have been found to selectively block presynaptic receptors, they also block postsynaptic receptors, thus do not have the same degree of selectivity as prazosin.

The general similarity in drug selectivity between the pre- and postsynaptically located α receptors suggests that the two types of receptors differ structurally. In view of this, Langer (1974) has suggested that the postsynaptic receptor be termed α_1 and regulatory presynaptic receptors, α_2. Such nomenclature has been widely accepted and will be used throughout this thesis. In addition, there appear to be slight differences between α_2 receptors in different tissues, since drug effects have been found to differ (see above). However, such differences may be due to variation in receptor accessibility or numbers between tissues. The wide range of selectivity which has been found to exist among both agonists and antagonists for the two receptors can be used to determine pharmacologically the type of receptor involved in many physiological effects.

Thus the terminals of all peripheral noradrenergic neurones studied appear to be endowed with α receptors, activation of which leads to a decrease in the amount of NA released on stimualtion. In addition, similar receptors may be present on the presynaptic terminals of peripheral cholinergic neurones, since α agonists are able to decrease release of acetylcholine (ACh) (Paton & Vizi,1969), an effect which is blocked by α antagonists.

5.2. Other peripheral presynaptic receptors.

In addition to modulation by presynaptic α receptors the release of NA from peripheral nerve endings has been shown to be influenced by many other substances, some of which appear to act on receptors also present on the presynaptic neurone.

DA, which is 10 to 100 times less potent than NA as an agonist at postsynaptic α receptors in most tissues, has been shown to be equipotent with NA in reducing release of transmitter from several tissues (Enero & Langer, 1975). This effect has been found to be reversed by pimozide, but not by phentolamine, hence specific DA receptors appear to be involved. Such receptors have not been found on all tissues studied, thus their physiological role is uncertain. Dopaminergic antagonists alone did not increase NA overflow in the cat nictitating membrane (Enero & Langer, 1975), hence it seems unlikely that presynaptic DA receptors normally mediate inhibition of NA release. However, Rand et al (1975) have postulated that the receptors may be of importance during periods of excessive stimulation, when stores of NA become depleted. Release of accumulated DA may then reduce the loss of storage vesicles and allow time for further NA synthesis.

ACh also affects the release of NA from nerve terminals. Activation of nicotinic receptors increases, while activation of muscarinic receptors decreases release. (Starke, 1977). There is no evidence that noradrenergic nerve endings contain stores of ACh, thus it seems unlikely that these receptors are evidence in favour of the cholinergic link hypothesis proposed by Burn and Rand (1959). A possible physiological role may exist, however, since many tissues receive both a noradrenergic and a cholinergic innervation and the terminals of both types of neurones have been shown to lie close together (Baldessarini, 1975). It may be possible, therefore, that ACh released

from cholinergic neurones may, in addition to antagonising the actions of NA on the postsynaptic effector cell, reduce its release by activation of a muscarinic receptor on the presynaptic noradrenergic nerve terminal. In support of this hypothesis, vagal stimulation has been shown to reduce the overflow of NA induced by symathetic stimulation in the isolated rabbit atria (Loffelholz & Muscholl,1970). The nicotinic effect of ACh on NA release requires much higher concentrations, thus may not be physiologically important. It is thus possible that noradnerergic and cholinergic neurones may exert mutually antagonistic effects both pre- and postsynaptically.

A further mechanism which may be important in the regulation of noradrenergic transmission involves prostaglandins. PGE_1 and PGE_2 have been found to decrease the amount of NA released on stimulation in many tissues (Starke,1977), probably by activating presynaptic PG receptors. The mechanism has been found to be independent of that involving presynaptic α receptors, since it is not reversed by α blocking drugs (Stjarne,1973). That noradrenergic transmission leads to the production of PG's in the surrounding tissue is well documented (Swedin,1971). It appears that these substances may then inhibit further release of NA. Substances which block the synthesis of PG's are able to prevent the inhibition of NA release. This mechanism of transmitter regulation has not been found to occur in all tissues and has been shown to be less effective than that mediated by presynaptic α receptors.

Morphine and other opiates are also able to reduce NA relase from peripheral nerves (Henderson et al.,1972). The effect is blocked by naloxone, but not phentolamine, thus specific opiate receptors are involved, and the mechanism is independent of α receptors. Naloxone has been found to increase release of ACh from guinea-pig ileum, hence may be reducing

inhibitory effects of endogenous opiates in this tissue. However, naloxone has no effect on NA release, hence the physiological role of the opiate receptor in modulating noradrenergic transmission is uncertain.

In addition to inhibitory presynaptic receptors which reduce transmitter release, facilitatory β receptors sensitive to low concentrations of NA have been postulated by Langer et al.(1975). Thus isoprenaline was found to increase NA release, an effect which was blocked by propranolol. The effect was most pronounced at low frequencies of stimulation. The significance of this effect as proposed by Langer et al., was that low frequencies of stimulation which release only small quantities of NA would result in activation of the presynaptic β receptor, leading to an increase in the amount of NA released; and that when synaptic levels of NA reach a sufficiently high concentration, presynaptic α receptors would be activated, thus reducing release. This may be possible if the higher sensitivity of β than α receptors to NA, which has been shown postsynaptically, also exists for presynaptic receptors. However, presynaptic α receptor-mediated negative feedback has been shown to operate at stimulation frequencies similar to those at which the facilitatory β receptors should be effective (Stjarne & Brundin, 1975), hence the mechanism may not be of major importance. In addition, the receptors have not been found on all tissues tested; and propranolol does not reduce NA release alone in certain tissues in which isoprenaline increased release (Langer et al.,1975). Thus the presynaptic β receptor may not operate a positive feedback under physiological conditions.

Angiotensin, an endogenous substance, formation of which is stimulated by the secretion of renin from the kidney, has also been shown to increase NA release (Zimmerman et al., 1972). This effect on release appears to be mediated by specific

receptors, as antagonists prevent it (Starke,1977). The mechanism may be of importance physiologically, since concentrations sufficient to affect release do occur in plasma; and may have a similar significance to that mediated by presynaptic β receptors, since the effect only operates at low stimulation frequencies (Hughes & Roth,1971). In common with all other mechanisms postulated to affect NA release, except presynaptic α receptors, angiotensin receptors have not been found to occur on all neurones tested.

5.3. Central regulatory α receptors.

The work of Farnebo and Hamberger (1971) using slices of rat cortex showed that a mechanism for regulating the amount of NA released from nerve endings was also present on central neurones. These workers used slices of cortex pre-incubated with ^{3}H-NA, which at the concentration used (10^{-7}M) is selectively taken up by noradrenergic neurones (Lidbrink et al.,1971). Thus the effects of drugs acting at α receptors which were able to modulate the amount of transmitter released were affecting only such neurones. Farnebo and Hamberger (1971) postulated that a negative feedback mechanism was present at the synapse to account for their results. Many other workers have since confirmed that α agonists are able to reduce, while antagonists increase the amount of NA released from nerve endings in the CNS.

Work with synaptosomal preparations has shown that the mechanism is located at the synapse (Raiteri et al., 1975). Drugs which have been shown to possess some selectivity for presynaptic receptors have been found to be potent in modifying central release also (Taube et al.,1977). In several cases, drugs which affect the amount of NA released have also been shown to modify the turnover of NA in whole brain. Thus

clonidine, which peripherally displays some selectivity for the presynaptic receptor (Starke et al.,1974), has been found to decrease NA release from brain slices (Farnebo & Hamberger,1971) and reduce NA turnover (Anden et al.,1970). Opposite effects have been shown for yohimbine, which selectively blocks presynaptic α receptor speripherally (Starke et al.,1975a). This drug increases NA release from cortical slices (Taube et al., 1977) and increases the turnover of NA in whole brain (Anden & Grabowska,1976). Whether the two effects are connected and possibly mediated by similar receptors is not proven, although it should be noted that the effect of clonidine on turnover is more effectively antagonised by drugs which selectively block peripheral presynaptic α receptors than by those which act on postsynaptic receptors (Anden & Strombom,1975).

Other central monoamine neurones have also been investigated for the presence of release-modulating α receptors. However, after incubation with ^{3}H-5-HT, the amount of tritium released from cortical slices was not affected by doses of clonidine or phentolamine which had been found to modify the release of ^{3}H-NA (Farnebo & Hamberger,1974a). Higher doses of both drugs (Starke & Montel,1973; Farnebo & Hamberger,1974a) were able to modify release of 5-HT, however, thus the possibility of a release-modulating α receptor which affects 5-HT neurones cannot be excluded. Any such system appears to be less sensitive to drugs than that which modifies NA release.

Similarly, slices taken from the striatum, which contains predominantly dopaminergic neurones, and incubated with ^{3}H-DA are also insensitive to modulation of release of tritium by drugs which act at α receptors (Farnebo & Hamberger, 1971).

5.4. Other central regulatory receptors.

An analogous situation appears to exist on central noradrenergic neurones to that found peripherally in that release of transmitter may be modulated by substances other than drugs acting at α receptors. Thus Taube et al.(1977) found morphine and other opiates reduced NA release from slices of rat occipital cortex after electrical stimulation or potassium stimulated depolarisation. This effect of morphine was found to be naloxone-reversible. These workers also showed that PGE_1 was able to reduce NA release. Both this and the effect of morphine were independent of α receptors as they were not reduced by phentolamine.

No inhibitory effect of ACh was found on NA release from slices of occipital cortex or hypothalamus, such as has been seen peripherally. However, a high concentration of nicotine (10^{-3}M) did increase NA release. This effect was not Ca^{2+}-dependent or reversed by hexamethonium and appeared to be due to increased overflow of metabolites. Westfall (1974) has, however, found ACh to reduce NA release in cerebellar slices, thus suggesting that there are regional differences in the presence of release-modulating receptors on noradrenergic neurones.

Gamma-amino-butyric acid (GABA) was found to slightly increase both the basal and SI overflow of NA from cortical slices (Taube et al.,1977), although Arbilla and Langer (1979) found that this only occurred when low stimulation parameters were used. This effect of GABA was not reversed by specific antagonists nor did muscimol, a GABA agonist, have any effect on release. Thus the presence of a GABA-sensitive release-modulating receptor is uncertain.

Other transmitters, DA and 5-HT, were shown to be ineffective in modulating SI overflow of NA, as were angiotensin and drugs acting at β-adrenoceptors. Farnebo and Hamberger

(1974b) also found no evidence of a facilitatory presynaptic β receptor in cerebral cortex slices. However, Adler-Graschinsky and Martinez (1978) did find propranolol to decrease NA release from cerebral cortex slices after stimulation by low concentrations of potassium. The lack of effect found by other workers may thus be due to excessive stimulation, especially since the positive feedback mediated by β receptors found peripherally has been shown to operate under conditions of low stimulation (Langer et al.,1975).

It seems likely that a similar situation may exist to that found peripherally i.e. that release-modulating α receptors are widespread and may be common to all noradrenergic neurones, but that other types of receptors may be present only on some neurones. The lack of effect of DA, ACh 5-HT and isoprenaline on NA release found by Taube et al.(1977) may be due to a lack of receptors sensitive to these substances on noradrenergic neurones of the part of the brain studied by these workers.

Indeed, in addition to the ability of transmitters to modulate their own release - which has been shown for NA (Taube et al.,1977), 5-HT (Cerrito & Raiteri,1979), GABA (Arbilla et al.,1979) and DA (Reiman et al.1979) - there does appear to be interaction of transmitters with other types of neurones. Thus, for example, NA reduces ACh release in the cortex (Beani et al.,1978), while DA reduces striatal ACh release (Bianchi et al.,1979). DA is able to increase the release of 5-HT in the anterior hypothalamus (Reubi et al.,1978) and of GABA in the substantia nigra (Reubi et al.,1977).

It therefore seems likely that other substances in the brain, may affect NA release in addition to those found to do so by Taube et al.,(1977); and that the lack of effect found by these workers may only hold for the brain areas they studied.

5.5. Possible mechanisms of α receptor-mediated regulation of NA release.

Certain possible mechanisms by which substances acting at presynaptically located receptors may influence release of transmitter may easily be eliminated. Thus local anaestheic or guanethidine-like actions are not involved, since complete blockade of release is not possible (Starke,1977). An effect on the storage of NA can also be discounted, as the inhibitory effects of α agonists are present when neuronal uptake is blocked. The reduction in release is not a secondary effect to reduction in transmitter synthesis, since exogenous NA, previously accumulated, is also affected (Baldessarini & Kopin,1967).

A likely mechanism by which these substances may act involves the influx of Ca^{2+} ions from the extracellular fluid into the presynaptic neurone via voltage-sensitive permeability channels. An increase in the intracellular Ca^{2+} concentration, which normally occurs upon depolarisation of the nerve membrane, is necessary to trigger transmitter release (Baldessarini,1975). Thus some means of restricting the Ca^{2+} influx would be expected to reduce release. The release of transmitter by mechanisms which appear to be Ca^{2+}-independent, such as by tyramine (Starke & Montel,1974) and DA release in the striatum (Arbilla & Langer,1978), are not affected by presynaptic modulation. The ability of α agonists to decrease NA relase has been found to be dependent on the Ca^{2+} concentration in the medium (Drew,1978a). Thus, the lack of effect of drugs at high stimulation frequencies, rather than being a consequence of increased synaptic NA concentrations, may be due to increased intraneuronal accumulation of Ca^{2+}. Oxymetazoline and phentolamine were still ineffective in modifying NA release induced by K^+ concentrations even when endogenous NA levels were reduced

by pretreatment with α-methyl-para-tyrosine (αmpt) (Dismukes et al.,1977). However, if the external Ca^{2+} concentration was lowered, oxymetazoline was effective in reducing release even when induced by high K^+ concentration. This lends support to the suggestion that the drugs may affect the influx of Ca^{2+} ions.

Further evidence has been provided by the work of Gothert and co-workers. These workers used Ca^{2+} ions to induce NA release, which is probably due to Ca^{2+} influx into the neurone and is not affected by tetrodotoxin , thus does not involve action potentials (Gothert,1979). Release by Ca^{2+} may only be elicited when the membrane is depolarised by high K^+ concentrations and is reduced by met-enkephalin and NA via presynaptic opiate and α receptors respectively (Gothert et al., 1979). NA is also able to reduce release induced by veratrine, which depolarises the membrane by opening Na^+ ion channels (De Langen & Mulder,1979). Both of these mechanisms of inducing release increase the conductance of the Ca^{2+} permeability channels in the neuronal membrane (ibid). High Mg^{2+} ion concentrations, which probably inhibit Ca^{2+} ion entry, enhance the inhibitory effect of NA on release under conditions of low Ca^{2+} concentration. Thus NA may reduce the release of transmitter either directly by inhibiting Ca^{2+} ion entry via the permeability channels or by inhibiting a subsequent step occurring after Ca^{2+} ion influx.

The use of a Ca ionophore A23187, which is able to induce release of NA independently of the Ca^{2+} channels, has helped to clarify the situation. This substance forms lipid-soluble complexes with Ca^{2+} ions which diffuse across the membrane, and releases the ions at the interior surface (Gothert, 1979). NA does not affect the release induced by A23187 (De Langen & Mulder,1979; Gothert et al.,1979). Thus the activation of presynaptic α receptors is unlikely to affect a step of

stimulus-secretion coupling subsequent to the influx of Ca^{2+} since such steps are likely to be identical for both methods of Ca^{2+}-induced stimulation. Under both conditions NA appears to be released by exocytosis, since DBH is also released (Gothert et al.,1979) and the proportion of NA in the total tritium overflow is also similar (De Langen & Mulder,1979). The suggestion that NA may reduce release by increasing the activity of the Na^{+}/K^{+}-dependent ATPase (Gilbert et al.,1975) thus seems unlikely since this would not involve Ca^{2+} influx.

The involvement of cyclic AMP (cAMP) in the mechanism of release modulation by drugs acting at presynaptic receptors has been suggested by several workers (Langer et al.1975; Fain & Garcia-Sainz,1980). The facilitatory effect of β stimulants found to occur in some tissues may indeed be mediated by cAMP, since β receptor activation does appear to stimulate adenyl cyclase, thus leading to an accumulation of cAMP (Mobley & Sulser,1979,Langer et al.,1975). Both cAMP analogues and inhibitors of the enzme which catalyses its breakdown (phosphodiesterase), but not cAMP itself, are able to increase the overflow of NA in several tissues (Langer et al.,1975). This is a reflection of increased NA release, since DBH is also released.

However,α receptor-modulated release is independent of effects on adenylate cyclase (Langer et al.,1975), although inhibition of this enzyme has been shown to be a property of α_2 agonists on several non-neuronal tissues (Fain & Garcia-Sainz,1980).This inhibition appears, however, to be indepentent of Ca^{2+}, thus the Ca^{2+} dependence of α receptor-mediated modulation of NA release argues against this as a possible mechanism of reducing stimulus-secretion coupling in neuronal systems.

5.6. Location of the peripheral regulatory α receptors.

The α receptor which modifies NA release from peripheral neurones has been assumed to be located on the presynaptic nerve terminal, as this seems the most logical position for release-modulating agents to act. The possibility of a receptor on the effector or other surrounding cells which then emit a second signal to the nerve endings should not be ruled out immediately, however. Initial suggestions that such a mechanism involving PG's may be responsible for the regulation of transmitter release (Hedqvist,1970) have been refuted by the finding that the two effects are independent (Stjarne,1973). No other substance has been suggested to act as a secondary messenger, however, thus it appears that the location of the α receptor responsible for modulating NA release from peripheral neurones is on the neurone itself. This would seem to be the optimal site for such a receptor, since it is only from the neurone that modulation of the amount of transmitter may eventually be effected.

Many of the in vitro studies undertaken to investigate release-modulating receptors have involved the use of preparations which lack nerve cell bodies or dendrites. Thus receptors appear to be present on the preterminal part of the axon or on the terminal varicosities. Release of transmitter induced by high K^+ concentrations is not dependent on the conduction of action potentials along axons i.e. results from depolarisation of the varicosities. Since drugs acting at α receptors are able to modulate this release, it appears that the receptors are present on these terminal varicosities. The concept that presynaptic terminals may be endowed with receptors which are capable of modulating release is not new. Such receptors bring about presynaptic inhibition in the spinal cord (Bell et al.,1976). Thus a similar location of the α receptors

involved in modulation of NA release seems a logical conclusion.

However, receptors displaying the pharmacological characteristics of regulatory α receptors have recently been found to occur postsynaptically to the noradrenergic neurone. Thus NA, clonidine and guanfacin have been found to stimulate a prazosin-insensitive postsynaptic receptor in the rat circulatory ststem (Drew & Whiting,1979; Timmermans & Van Zweiten,1980).The inhibitory effects of α agonists on ACh release in the guinea-pig ileum (Drew,1978b) are also mediated by an α receptor located postsynaptically to the noradrenergic neurone. Whether these receptors are also able to modify the release of NA is unknown. It seems likely, however, that such modulation would require the involvement of a second messenger or neuronal feedback loops. As this seems improbable, the use of the term α_2 to describe receptors with these pharmacological characteristics (Langer,1974) is more suitable than either presynaptic or regulatory.

5.7. Organisation of central noradrenergic synapses.

In the CNS, the location of α receptors which modulate NA release is more questionable than in the periphery. Studies using synaptosomes (Raiteri et al.,1975) have ruled out the possibility that drugs may act on the cell body or dendrites of interneurones, which may then synapse with the noradrenergic neurone. However, such studies do not preclude the possibility of effects on other nerve endings closely situated by noradrenergic varicosities, which may subsequently alter NA release. Indeed, the phenomenon of presynaptic inhibition is likely to be extremely widespread in the CNS. The effects of transmitters on the release of other transmitters found to occur by many workers is likely to involve such an arrangement i.e. one nerve terminal impinging on another.

The need for a notation other than pre- and post-synaptic is thus even more important in the CNS, since what is postsynaptic to one neurone may be presynaptic to another. The use of the terms α_1 to describe receptors sensitive to methoxamine, phenylephrine, prazosin and PBZ; and α_2 to decribe receptors sensitive to clonidine, yohimbine and piperoxane is thus essential when discussing central α receptors. As has been found peripherally, postsynaptic α_2 receptors have been demonstrated in the CNS. Thus the centrally-mediated antihypertensive effect of clonidine, which is known to involve α_2 receptors, is not prevented by reserpine (Haeusler, 1974).

In addition, binding studies have lead to the demonstration of two types of central α receptor, one which binds ^{3}H-clonidine and a second which binds ^{3}H-prazosin (U'Prichard et al., 1978). These two receptors appear to correspond to α_2 and α_1 receptors respectively (Tanaka & Starke, 1980). However, neither is reduced in number by destruction of noradrenergic neurones using the selective neurotoxin 6-hydroxy-DA (6-OHDA) (U'Pritchard & Snyder, 1979), thus both must be located postsynaptically. Such experiments do not preclude the possibility that presynaptic α_2 receptors are few in number in the CNS and that numbers of postsynaptic α_2 receptors were increased after 6-OHDA to restore these numbers.

The lack of discovery of any single substance other than those acting at α receptors which may act as a secondary conveyer of information to the noradrenergic terminal suggests that at least some α receptors able to modify NA release must be located here. The most economical explanation of the situation in the CNS is thus that α_2 receptors located presynaptically on the noradrenergic neurone may modulate release, while α_1, α_2 and β receptors may mediate the effects of NA postsynaptically.

6. Possible roles of NA in the CNS.

The behavioural effects of NA are likely to be diverse and multiple, due to the widespread influence which this transmitter is capable of exerting over many central areas. A suggestion as to what type of effects may be expected may be found by the observation of sufferers from familial dysautonomia (Riley-Day syndrome) (McGeer & McGeer,1980). This congenital disease involves a deficiency of DBH; major symptoms are motor inco-ordination, lack of sensitivity to pain, profuse sweating and emotional instability. The latter term may cover a whole range of emotional factors; and,indeed, NA has been variously implicated in reward and punishment (Stein et al.,1977), arousal (Delbarre & Schmitt,1971; Moore,1980), depression (Schildkraut,1965), anxiety (Schildkraut & Kety,1967) and learning and memory (Kety,1970). NA has also been shown to be important in feeding (Hoebel,1977), drinking (Setler,1977), body temperature regulation (Feldberg & Myers,1964), sensation of pain (Gardella et al.,1970), motor activity (Anden et al.,1970) and blood pressure regulation (Haeusler,1974).

6.1. Effects on reward and punishment.

It has been suggested that there are special sets of neurones in the brain which are involved in reward and punishment. Experiments in which animals will self-stimulate (SS) using electrodes placed in certain regions of their own brains (Crow et al.,1972) combined with pharmacological manipulations have implicated NA in reward systems. Drugs which release NA increase SS (Stein et al.,1977), while α-mpt (ibid) and clonidine (Franklin & Herberg,1977) inhibit it. This effect of clonidine has been shown to involve α_2 receptors. Early work showed that the L.C. was involved in SS (Crow et al.,1972), however, destruction of noradrenergic neurones by 6-OHDA does not abolish this effect (Clavier et al.,1976).

6.2. Effects on arousal.

The concept of a reticular activating system (RAS), which was thought to constitute the 'waking brain' was introduced by Morruzzi and Magoun in 1949. The notion that a particular system may induce activation of higher centres and maintain wakefulness, while simultaneously selecting only desired input to these centres is still in use today (Scheibel, 1980). The reticular formation encompasses several types of neurones and their collaterals originating in the brain stem. Thus noradrenergic neurones may play a part in the functions of the RAS.

The effects of administration of NA are varied. Experiments showing that the arousal induced by peripherally injected NA may be due to increased blood pressure, which then induced EEG arousal, suggested that the transmitter was not directly involved in arousal (see Jouvet,1977). However, very small doses of intracerebroventricular (i.c.v.) NA have a clear-cut activating effect (Segal & Mandell,1970), although larger doses may have a behaviourally depressant action (Mandell & Spooner,1969). Drugs which alter the availability of NA in central neurones have been studied in attempts to clarify the situation.

Increasing catecholaminergic activity by means of l-DOPA, amphetamine or inhibition of breakdown of the amines all lead to behavioural arousal (Jouvet,1977). However, none of these methods are specific to noradrenergic systems, thus do not provide further insight into arousal. Blockade of central α receptors by PBZ and dibenamine has a sedative effect (ibid), as does administration of clonidine (Delbarre & Schmitt,1971). This drug may, however, reduce noradrenergic activity, since it selectively stimulates α_2 receptors, or may act on inhibitory adrenergic neurones, reducing cortical neuronal activity (Fuxe

et al.,1974). Other means of decreasing the availability of NA have also been found to induce sedation (Jouvet,1977). Thus the involvement of NA in the sleep/waking cycle seems to be one of an increased availability of NA resulting in increased waking, while a decrease leads to sedation.

Jouvet (1969) proposed that the ascending catecholaminergic neurones from the reticular formation and ascending serotonergic neurones display a reciprocal interaction. Thus destruction of the 5-HT-containing cell bodies of the raphe nucleus leads to insomnia and also results in increased activity of noradrenergic neurones of the dorsal bundle, while destruction of the NA-containing locus coeruleus leads to increased serotonergic activity and hypersomnia. Other workers have postulated the involvement of ACh in arousal via the ascending cholinergic reticular system (Morgane & Sterne,1978). Again, this system may be modulated by NA, thus the sleep/waking cycle may depend on the balance between NA and other transmitters.

6.3. Effects on mood.

Effects on arousal and mood are probably closely interrelated. It is well known that depression leads to early morning wakening, but may also include lethargy and lack of interest among its symptoms. Stressful situations or anxiety, on the other hand, lead to increased arousal accompanied by symptoms of autonomic activity. The marked behaviourally depressant effect of reserpine, which depletes central catecholamine stores, includes reduction of activity, arousal and body temperature in its symptoms. This effect in animals has been likened to depression in humans and indeed reserpine has been shown to induce depression when used clinically (Muller et al.,1955). Many clinically antidepressant drugs are able to reverse the reserpine syndrome in animals, an effect presumed to occur by

potentiation of the effects of NA,DA and 5-HT at postsynaptic sites.

Tricyclic antidepressants are known to block the neuronal reuptake of NA and/or 5-HT in central neurones, while inhibition of MAO also has an antidepressant effect. For many years, the monamine hypothesis of affective disorders (Schildkraut,1965,Coppen,1972) has suggested that decreased monoamine levels are responsible for depressive syndromes, while an excess leads to mania. However, newer evidence has suggested that this view is too simplistic. Neither the degree of uptake blockade induced *in vitro* by tricyclic antidepressants (Ghose & Coppen, 1977), nor its consequent decrease in brain NA turnover (Rosloff & Davies,1978) correlate with clinical antidepressant potency. Other discrepancies which do not fit into the hypothesis are also apparent. Thus, cocaine, which is a potent inhibitor of NA reuptake (Iversen,1967), is not antidepressant, while some clinically proven antidepressants such as iprindole and mianserin are only weak uptake inhibitors. In addition, the biochemical effects of antidepressants occur soon after administration, while the therapeutic effect requires several weeks of continuous administration.

Recent studies have shown that the chronic effects of antidepressants may be due to receptor subsensitivity as a consequence of the increased transmitter levels in the synaptic cleft produced by these drugs. Some of the effects of NA in the CNS may be mediated through β receptors, stimulation of which results in increased cAMP levels (Mobley & Sulser,1979). Although antidepressants do not directly affect these β receptors (Tang & Seeman,1980), chronic administration may lead to subsensitivity of the receptors to NA. Thus Vetulani et al. (1976) have shown that chronically administered antidepressants reduce the ability of NA to stimulate adenyl cyclase and decrease the

density and sensitivity of β receptors in rat cortex. L.C. stimulation, which reduces activity of cells in the cingulate cortex via β receptors, is also inhibited by chronic desipramine (DMI) treatment (Olpe & Schellenberg,1980). In addition, other workers have reported chronic DMI to inhibit L.C. neuronal firing and reduce the sensitivity of these neurones to clonidine (McMillen et al.,1980), thus suggesting a subsensitivity of α_2 receptors to NA. 5-HT receptor sensitivity may also be reduced after chronic administration of some tricyclics (Maggi etal., 1980).

Thus antidepressants may still act by increasing synaptic levels of transmitter, although it is the long term consequences of this effect which may be important. Blockade of presynaptic α_2 receptors would also achieve a similar effect, thus may be important in the antidepressant effect of mianserin (Langer,1978). If so, drugs which selectively block such receptors may also possess antidepressant properties. Preliminary experiments by Puech et al.(1978) have suggested this to be the case. In addition, clonidine has been reported to cause depression clinically (Simpson,1973), thus indicating the possible involvement of noradrenergic systems, specifically α_2 receptors, in depression.

In addition to effects on depression, central noradrenergic systems, notably the dorsal bundle, have been implicated in anxiety (Davis et al.,1979; Redmond,1977; Corrodi et al.,1971). Stress has been shown to increase cortical NA turnover, an effect which arises from the L.C. (Korf et al., 1973) and may be reduced by benzodiazepine tranquilisers (Corrodi et al.,1971). Yohimbine and piperoxane, both of which increase the turnover and release of NA, have been reported to produce anxiety in humans (Holmberg & Gershon,1961), while clonidine has been postulated to possess anxiolytic effects (Redmond,1977). Whether these effects are centrally-mediated is

in doubt, since in man, peripherally administered adrenaline, which does not pass into the brain, appears to produce subjective feelings of anxiety and apprehension (Rothballer,1959). The effects on anxiety may be an extension of effects on arousal, as it seems likely that hyperalertness may contribute to 'fear'. However, the involvement of the dorsal noradrenergic bundle in such emotions is at present in dispute (Mason & Fibiger,1979a).

6.4. Effects on learning.

The involvement of catecholamines in learning was first suggested by Kety (1970), since the diffuse projections of noradrenergic neurones seemed ideally suited to reinforce information at individual synapses. Many studies have been made on the effects of NA on learning and memory. In general, drugs which facilitate catecholaminergic activity may facilitate acquisition and retention, and also the consolidation of recently acquired information (Hunter et al.,1977; Dunn,1980). Drugs which reduce catecholaminergic activity appear to reduce performance of well-learned responses, an effect which may involve both NA and DA (Hunter et al.,1977). However, DA has been shown to be of greater importance in disrupting conditioned avoidance (Fibiger et al.,1974).

Another learned response, that of running for food in an L-shaped runway, has been shown to be impaired by L.C. lesions (Anzelark et al.,1973). However, other workers have shown that such lesions do not impair other responses (Mason & Fibiger,1979a;Sessions et al.,1976), thus it is possible that other noradrenergic pathways must be destroyed before learning is reduced. Inhibition of conditioned avoidance by clonidine has been shown to be due to an effect on central α_2 receptors (Hawkins & Monti,1979), thus it is possible that this drug may reduce noradrenergic transmission in pathways other

than the dorsal bundle to bring about this effect.

Other studies have shown that NA may be involved in memory formation, since inhibition of DBH appears to reduce learning, an effect which may be reversed by i.c.v. NA (Hunter et al.,1977).

6.5. Effects on feeding.

There are at present two theories involving feeding behaviour, reviewed by Hoebel (1977). One involves an α receptor-mediated feeding response, possibly located in the hypothalamus, combined with a β receptor-mediated satiety response. However, a second theory suggests that NA produces satiety by α receptors, thus reducing feeding, and that β receptor activation also reduces feeding, but by inducing taste aversion.

6.6. Effects on drinking.

Both β stimulants and α receptor blockers appear to induce drinking, which may be due to increase renin release from the kidney (Setler,1977) and is probably a peripheral response. However, i,c,v. NA has been shown to inhibit drinking (ibid), although the importance of this effect is uncertain.

6.7. Effects on body temperature.

When injected i.c.v., NA reduces body temperature in several species (Feldberg & Myers,1964; Brittain & Handley, 1967), while 5-HT increases it (Feldberg & Myers,1964). These workers proposed that body temperature was regulated by release of these amines in the hypothalamus. Further work suggested that 5-HT stimulated a cholinergic heat production pathway in the hypothalamus, while NA could inhibit this pathway (Myers & Yaksh,1969), thus the balance between transmitters again appears to be important.

6.8. Effects on pain sensation.

The involvement of NA in opiate analgesia is in general found to be facilitatory (for review, see Blasig,1978). In addition, both NA and clonidine have been shown to possess analgesic activity (Gardella et al.,1970; Paalzow & Paalzow, 1976). The lack of competition for receptors (Farsang & Kunos, 1979), however, suggests that the analgesia produced by opiates and noradrenergic stimulants are independent of each other. Naloxone does not reverse clonidine analgesia (Paalzow & Paalzow, 1976), although α antagonists are capable of doing so. Thus it appears that a noradrenergic mechanism of analgesia may exist which is independent of opiate receptors.

6.9. Effects on motor activity.

Drugs which deplete the brain of NA, such as reserpine or αmpt, markedly reduce motor activity (Corrodi et al.,1970). In view of the sedative effects of these drugs, however, it is not clear whether this is a specific effect on activity. Experiments by Anden and Strombom (1974) have shown that apomorphine stimulates activity in reserpnised animals, and that this effect may be potentiated by clonidine. It thus appears that a specific noradrenergic system may be involved in the control of motor activity *per se*, rather than effects on arousal mediating changes in activity. In addition, clonidine has been found to have marked sedative effects alone, both in animals (Delbarre & Schmitt,1971) and in man (Holman et al.,1971). This effect of clonidine has been postulated to involve central α_2 type receptors (Delbarre & Schmitt,1973), while its stimulatory effect may involve α_1 receptors (Anden & Strombom,1975).

6.10. Effects on blood pressure regulation.

The peripheral involvement of NA in the control

of blood pressure is well known. However, studies on the mechanism of the hypotensive effect of clonidine and α-methyl-DOPA have shown that a central effect also involving NA and α receptors is probably more important (Anden & Strombom,1975; Van Zweiten,1973). It was suggested by van Zweiten that clonidine activated an inhibitory neurone, possibly the bulbo-spinal sympathetic neurone, to decrease peripheral sympathetic nerve stimulation. It is also possible that clonidine and α-methyl-NA, formed from α-methyl-DOPA, may stimulate central α_2 receptors, which may then decrease NA release. Such an action could also lead to inhibition of peripheral symathetic tone. However, the antihypertensive action, although possibly mediated by α_2 receptors (Van Zweiten,1973), appears to be postsynaptic (Draper et al.,1977).

7. Aims of the project.

7.1. To study the possible involvement of both α_1 and α_2 receptors in the behavioural effects of NA. A range of agonists differing in their selectivity between α_1 and α_2 receptors peripherally may be used to determine the relative importance of the two types of receptor in a particular behavioural effect, while the use of a similar range of antagonists may add further conformation.

7.2. To determine by means of such behavioural experiments, two behavioural effects, one involving α_1 and one α_2 receptors, which may then be used to obtain a ratio of drug selectivity between the two types of receptor *in vivo*. Such a ratio may then be compared to a similar ratio obtained from *in vitro* experiments on peripheral tissues to assess whether the selectivity of drugs was similar under both conditions. This comparison may thus be useful in comparing the characteristics of peripheral and central α receptors.

7.3. To investigate the possibility of developing a behavioural test which may be used to study the relative potencies of α agonists on central α receptors *in vivo*. Such a test may be used for the screening of potential antihypertensive agents and must therefore be simple, preferably not involve other drugs and easily quantifiable. In addition, methods which may be used to study the sedative side effects of such drugs will be investigated.

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

1. Animals, animal husbandry and laboratory conditions.

1.1. Rats.

All experiments on rats were carried out at the research laboratories of Reckitt and Colman, Hull, using male Sprague-Dawley strain animals (Bantin & Kingman) weighing 250 to 300g. Animals were housed in the animal house under normal lighting conditions in metal cages in groups of 6 prior to use and were fed rat cubes and allowed tap water ad libitum. Immediately before use, animals were transfered to the experimental room and killed by cervical dislocation.

1.2. Mice.

All the behavioural experiments were performed on male albino T.O. mice bred at Aston, weighing between 20 and 30g. All animals, including breeding stock, were housed in the animal house under a 12 hour light/dark cycle, which was set for part of the duration of the research to change at 0430 and 1630 hours and for the remainder of the time at 0600 and 1800 hours. After weaning, animals were housed in polypropylene cages in groups of 40 to 50, fed on 41B cubes (Pilsbury's, Birmingham) and allowed tap water ad libitum. Animals were transfered to the experimental room at least 3 days prior to use. This was a small, enclosed inner room in the laboratory which was maintained on the same light cycle as the animal house. Experiments in this room were performed in quiet conditions, between 0830 and 1800 hours. Locomotor activity experiments were performed in a separate room under similar light conditions. Animals were transfered to this room 3 days prior to use.

2. Injection technique.

2.1. Subcutaneous (s.c.) injections.

Injections were made into the loose skin at the back of the neck in mice. Where animals received more than one s.c. injection, the second injection was made by inserting the

needle under the skin in the flank of the animal. The injection volume was 10 ml/kg.

2.2. Intraperitoneal (i.p.) injections.

Injections were made by inserting the needle into the abdominal wall towards the diaphragm, Care was taken not to penetrate too deeply thereby damaging the internal organs. Where more than one injection was made by this route in the same animal, care was taken not to use the same injection site. The injection volume was again 10 ml/kg.

2.3. Intracerebroventricular (i.c.v.) injections.

The method used was that described by Brittain and Handley (1967). A 0.25ml glass tuberculin syringe was used, fitted with a 27 gauge needle which was 3mm in length. This needle length had been found to be optimum for administration of drugs into the ventricular ststem in A_2G mice (Handley,1970). The injection volume was 20µl.

The mouse was immobilised by holding the head firmly on a flat surface by the scruff skin at either side of the head. The needle was placed vertically on the mid-line of the skull and drawn backwards until a depression was felt. This depression is the junction between the two parietal and the interparietal bones, an area which is not ossified in the mouse. The skull was the penetrated and the solution delivered. After injection animals were immediately placed in a cage and the lid firmly closed as many mice tended to run around and try to jump out. This behaviour subsided after several minutes, after which time, mice were generally indistinguishable from controls.

After experimentation, all mice were killed by cervical dislocation and examined for the location of the hole in the skull due to needle insertion. Theresults from any mice which were incorrectly injected were then discarded. Similarly, results from mice seen to exhibit circling after injection, were also discarded.

Previous investigation (Handley,1970) has shown that injection by this method delivers the injected fluid into the 4th ventricle, from where it passes to the 3rd and lateral ventricles. Studies using ^{3}H-NA indicated that after i.c.v. injection, the amine was taken up by the hypothalamus, medulla, cerebellum and, to a lesser extent, the cerebral cortex. The large percentage of injected volume which was lost from the brain (85 to90%) immediately after injection (Handley,1970) indicates that the relative potency of drugs administered by this route is much higher than would be assumed from the doses used. However, this also indicates that in addition to leakage around the injection site, a large proportion of the drug may leak into the periphery via the subarachnoid space (Shaw,1974). Hence caution must be exercised when interpreting results from drugs injected by this method.

3. Peripheral in vitro experiments.

3.1. Isolated rat vas deferens.

Male rats were killed and the vasa deferentia removed. These were suspended in 2ml organ baths and perfused with Krebs solution of composition in mMol/lt: NaCl 18, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 0.6, $NaHCO_3$ 25, dextrose 11.1; which was gassed with 95% O_2, 5% CO_2 and maintained at 27°C. Field stimulation was provided by an SRI square-wave stimulator at 25 to 40V, pulse width 3msec and frequency 0.1Hz. The resultant twitch responses of the tissue were recorded using a Stratham gold cell transducer and a Smiths pen recorder.

Agonist potency was assessed by the addition of increasing doses of drug to the perfusing medium and measurement of the inhibition of the twitch response thus produced. Where compounds were found to have considerable postsynaptic activity, which caused contractions of the tissue, prazosin 10ng/ml was also added to the perfusing fluid, to prevent inhibitory actions from being obscured. At the end of each experiment, phentolamine

3μg/ml was perfused over the tissue to ensure the inhibition of the twitch had been due to α stimulation.

Antagonists were tested in the presence of propranolol 10^{-7}M and corticosterone 4×10^{-5}M to ensure that β receptors and extraneuronal uptake were blocked. Inhibition of the twitch response was achieved by the addition of cocaine 3μg/ml or clonidine 3ng/ml to the perfusing medium. When maximal inhibition was attained, increasing concentrations of α antagonists or antidepressants were also added. The percentage reversal of the twitch response which these drugs produced was used as a measure of presynaptic α antagonist activity.

3.2. Isolated rat anococcygeus muscle.

The anococcygeus muscles were removed from rats and either suspended in a 5ml organ bath at 37°C or surrounded by a water bath and superfused with gassed Krebs solution. Contractions of the tissues were recorded isotonically on a pen recorder.

Agonist activity was measured using superfused preparations as the percentage of the maximum contraction obtained, Phentolamine was again used to demonstrate α stimulation.

Antagonist potency was measured against NA using cumulative dose response curves obtained with NA alone and after 10 minutes incubation with the antagonist. pA_2, pD_2 and pA'_2 values were calculated by the method of Van Rossum(1963).

4. Biochemical studies.

Rat brains were carefully removed from the skull and the cerebellum, striatum and hippocampus dissected away while maintaining the tissue at 0°C. Slices of 320 or 600 μm thickness were obtained from the remaining cortex using a McIlwain tissue chopper. The slices were transferred to Starke's solution of composition in mMol/lt: NaCl 18, KCl 4.7, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, dextrose 10 and ascorbic acid 0.1; contain-

ing 10^{-7}M 7-^{3}H-l-noradrenaline of specific activity 5 Ci/mMol and incubated for 30 minutes in an atmosphere of 95% O_2,5%CO_2. The solution was then decanted and the slices washed with 3 x 20ml of Starke's solution. at room temperature to remove excess radioactive NA. Four slices were placed in a small (volume 1ml) stimulation chamber between platinum electrodes and superfused with gassed Starke's solution at 37°C at various flow rates.

Superfusate was collected using an LKB Ultrorac fraction collector over four minute periods. The radioactivity in 0.5 ml samples of each fraction was determined by liquid scintillation spectrometry using 5 ml of either PCS (Amersham Corporation) or NE 260 (Nuclear Enterprises) scintillation fluor. The samples were counted for 2 minutes each in a Packard Tri-Carb 2450 scintillation spectrometer. The counts per minute obtained using the tritium detector were used for calculations as the counting efficiency of the instrument was regularly checked and found to be constant by the staff of the drug metabolism department. The brain slices were solubilised in 0.5 ml hyamine hydroxide and counted similarly.

Slices were stimulated 40 minutes after the start of superfusion at 2msec pulse width, 20V and 5 or 10Hz using a square wave pulse stimulator.and superfusion continued for a further 30 minutes.A slice thickness of 320µm, flow rate of 0.7 ml/min and stimulation frequency of 10HZ were found to be optimum for a large increase in outflow of ^{3}H-NA on stimulation. However, considerable variation was found in the increase of tritium between experiments. Each set of slices was therefore stimulated twice, enabling it to act as its own control. There was no significant difference between the amounts of tritium overflowing on successive stimulations of the same set of slices ('t' test for paired samples).

Drugs were added to the superfusing fluid so that

the slices were in contact with the drug for 20 minutes before the second period of stimulation. The stimulation-induced (SI) release of tritium was calculated using the formula:

$$\frac{\text{total c.p.m. - estimated c.p.m.}}{\text{estimated c.p.m.}} \times 100\%$$

The counts per minute were plotted for each fraction and the spontaneous overflow which would have occurred in the absence of stimulation estimated. The results were also expressed as the ratio of the percentage overflowing on the second stimulation over that on the first stimulation. The differences in overflow of tritium induced by the drugs studied from control experiments were calculated by means of a paired 't' test.

5. Behavioural experiments.

5.1. Open field assessment.

The behavioural effects of drugs were studied in mice using a modified form of the scoring scheme described by Irwin (1968). The observation area consisted of a metal box 17cm deep with the front side reduced to a height of only 4 cm. This box was divided into two equal compartments 30 cm square; and both sides were fully lined with absorbent paper. Three mice were placed in each compartment for 10 minutes to allow for initial exploration of the novel environment. One group then received saline s.c. and the other group the drug under test.

Behavioural ratings were made over 10 minute periods, beginning 5 minutes after injection and subsequently every 20 minutes until there was no difference between the groups. The method of scoring was standardised by using a 0 - 6 scale, a score of 0 being allocated for absence of effect, 6 for maximal effect, 2 or 4 for progressively increasing effect and odd numbers where the degree of effect could not be allocated otherwise. Each test mouse was compared to the mean of the control mice. The ratings were made using a standardised procedure,

observations on unrestrained mice being rated first, followed by testing which involved approaching the mice and observations which required some degree of handling, as follows:

a. observations on unrestrained animals

The following were scored by degree when present:- straubtail, exophthalmos/ptosis, head twitches, convulsions, writhing, tremor, abnormal gait, limb splay, piloerection, lachrymation, salivation, vasodilatation/cyanosis, vocalisation and diarrhoea. Increases in total motor activity, respiration rate and depth, grooming and stereotyped activity, the nature of which was also noted, and the intensity and incidence of abnormal posturing were also scored.

b. observations which required mimimal handling.

The following were scored as increases or decreases over controls:

fearfulness - the freezing or withdrawal on approach of a pen towards the mouse's head

alertness - assessed simultaneously as amount of interest

startle response - response to a puff of air directed at the mouse's head

touch response - escape response to application of pressure to the flanks with the finger and thumb

tail pinch response - reaction to application of pressure to a point 1cm from the base of the tail with forceps

flexor reflex - limb withdrawal on pinching the toe of the hind limb with forceps

pinna reflex- degree of twitch responseto stimulation of either external auditory meatus with a fine wire

c. observations which required removal of the mice from the box

The degree of pupil dilation and hypothermia were assessed when holding the animal in a scruff grip. The following were scored as increases or decreases over controls:

passivity - point at which the mouse struggled to escape when

held first by the scruff, then the hind limb, then the fore limb

grip strength — ability to hang from a wire grid

body position — the height of the ventral abdomen above a raised bar. It was noted that this sometimes differed from the same perameter in the box, as did ptosis, thus this was also assessed on the bar.

The animals were also assessed for catalepsy by placing the fore-paws on a 4cm high bar; and for loss of righting reflex when flipped over to land in the box from a height of 20cm.

5.2. Measurement of chloral hydrate sleeping time.

The method of Delbarre and Schmitt (1971) was used with the modification of an increase in the dose of chloral hydrate from 250 mg/kg, found by these workers to be sub-hypnotic, to 300 mg/kg. This was to enable the detection of possible decreases in sleeping time which may occur with drugs found to produce an increase in arousal.

Agonists or antagonists were administered i.p. 10 minutes before chloral hydrate 300 mg/kg i.p. and the duration of loss of righting reflex measured by turning the mice onto their backs repeatedly until such an effect occurred. A control group, receiving saline and chloral hydrate, was included in each experiment, since there was found to be considerable day to day variation in the duration of righting reflex loss. In addition, experiments were performed so that injection of the hypnotic occurred at the same time each day to avoid diurnal variations in sleeping time.

Experiments to study the effect of α antagonists on sleeping time potentiated by α agonists were carried out using an injection programme as follows:

Time	Group 1	Group 2	Group 3	Group 4
0	saline	saline	antagonist	antagonist
10 mins	saline	agonist	saline	agonist
20 mins	chloral hydrate	chloral hydrate	chloral hydrate	chloral hydrate

All experiments of this nature involving clonidine were performed so that injection of chloral hydrate was given at 1000 hours, while experiments using guanabenz were commenced 1 hour later. All results were compared to saline-pretreated controls of the appropriate day by Student's 't' test. The effects of α agonists were also expressed as percentage increase over controls to allow comparison between drugs to be made.

5.3. Measurement of sedation using an accelerating Rotarod.

The Rotarod (Ugo Basile Ltd.) consisted of a roughened horizontal rod divided into 5 compartments by vertical circular plates. The apparatus was set up to rotate, accelerating from 0 to 50 r.p.m. over 5 minutes. After placing the mice on the rod, the time at which they fell off was recorded by the triggering of a trip switch to stop the timer, which was started automatically on commencement of acceleration. Since previous work has shown that some training effect does occur on repeated testing, mice, in groups of 5, were tested 3 times before drug administration. Antagonists when given, were administered 10 minutes after the last test, and agonists or saline after a further 10 minutes. Animals were then retested on the Rotarod at 20,40,60,90 and 120 minutes after injection. All results were compared to saline-treated controls by Student's 't' test.

5.4. Assessment of sedation by observation.

A modification of the method of Drew et al.(1979) was used, whereby the effect of drugs on the following behaviour was assessed in groups of 3 mice after s.c. injection.

spontaneous activity - the amount of activity in the box, scored

0 - 4, multiplied by the speed of this activity, scored 0 - 2.

Startle response - scored 0 - 4, assessed as in Section 5.1.

Body position - assessed in the box and on transfer to a new cage, 0 - 4: 0 - lying down, 2 - sitting upright, 4 - jumping and rearing.

Ptosis - assessed in box and on transfer, 0 - 4: 0 - eyes closed, 4 - eyes wide open.

Transfer arousal - animals transfered to a new cage lined with sawdust and appearance observed, scored 0 - 4 : 0 - no movement, indifferent to cage, 2 - some sniffing, 4 - sniffing, locomotion, 6 - sniffing, locomotion, rearing, animal interested in cage, 8 - hyperalert, jumping and rearing.

Muscle tone - abdominal, assessed by pressing abdomen with fore-finger, scored 0 - 4: 0 - finger leaves a slight depression on removal, 4 - abdomen resistant to pressure.

Visual placing - distance at which mouse reaches for grid when held by tail and distance at which it sees object passed to and fro in its visual field, scored 0 - 4.

The average score for each item was calculated and also the total of these means.

5.5. Locomotor activity.

The apparatus used to measure activity was an Animex activity meter type SE (LKB Farad) which consists of 6 inductance coils forming part of a resonant circuit. Movement over any of the coils results in a change in current within the circuit, which is then amplified and registered. Each movement was thus registered by means of an Animex counter type I-X, which was set to print out cumulative counts over 10 or 20 minute periods.

Experiments were performed on groups of 3 mice placed in a polypropylene cage (42 x 28 x 15 cm) lined with a thin (90g) layer of sawdust. Such a cage just fitted over the 6 coils of the Animex.

Experiments on exploratory activity were initially carried out in an illuminated cupboard (34 x 60 x 50 cm) within the laboratory, with a constant background noise provided by a ventilator system. Animals were all injected at 0925 hours and placed in the exploratory cage which contained unusual objects such as an ice-cube tray, on the Animex at 0930 hours.

Subsequent experiments were performed in a separate room used solely for this purpose, using a cage devoid of novel objects. Injections were performed at 1400 hours and mice placed in the new cage on the Animex at 1430 hours.

Experiments on activity in the dark were also performed in this room. Animals were injected at 1830 hours and placed on the Animex in their home cage. Recording of activity began at 1900 hours when the light was extinguished. In all the above experiments, the Animex was tuned to 40 μA and the sensitivity was set at 25 μA. This sensitivity has previously been shown to detect locomotion, but not small movements, such as grooming or tremor (Thomas,1975).

Studies on reserpinised animals were performed with the Animex set at a sensitivity of 30 μA, to detect finer movements in addition to locomotion. Animals were placed in separate cages in groups of 3 and treated with reserpine 5 mg/kg 24 hours before the measurement of activity commenced. Apomorphine was injected s.c. 5 minutes before; and the α agonists i.p. 20 minutes before placing on the Animex. Alpha antagonists, when given, were injected s.c. 10 minutes before the α agonist. Three experiments were performed per day, at 0930, 1130 and 1300 hours. All results were compared to the appropriate saline

controls by Student's 't' test.

5.6. Analgesia.

Initial experiments were carried out using the tail clip test as described by Hoffner (1929). However, large variability was found to occur between mice, many 'freezing' on application of the clip to the tail. This method was therefore discontinued.

The hot plate method of Woolfe and MacDonald (1944) was subsequently used. A round copper container was placed over a water bath such that the base was submerged in the water, which was maintained at 61 ± 0.5°C. Preliminary experiments had shown that this temperature produced less variable results than the temperature of 56°C normally used.

Mice were placed on the copper base and the time taken for the animal to lick the front or hind paws noted, unless such a reaction did not occur within 60 seconds, when the mouse wss removed. This reaction has been found to be characteristic of mice placed on a hot plate (Handley,1970). However, a small proportion of drug-treated animals did not exhibit this reaction, but jumped out of the container instead. In such cases, this was taken as the end-point. Animals were tested only once on the hot plate, as it was found that subsequent testing caused the majority of animals, including controls, to jump out. Since this was thought to be an inferior end-point, as it depended on the degree of motor co-ordination, only one test per animal was performed. The time course of analgesia was therefore studied using a separate group of animals for each time after injection.

Mean values for each group of animals were calculated and compared to appropriate controls by Student's 't' test However, standard errors of means which include cut off values are not statistically valid, thus, where more than one animal in a group had a time of over 60 seconds, standard errors were not calculated.

5.7. Measurement of pinna reflex.

Animals were given saline or various doses of the drug under test and tested for the presence of pinna reflex at 20,40,60,90,120,150 and 180 minutes after injection. The reflex was said to be present if a twitch or laying back of either ear occured on stimulation of both ears with a fine wire. Reversal of the inhibition of the twitch by α agonists was achieved by administration of α antagonists s.c. 15 minutes before administration of the agonist. Ten animals were used for each dose of each drug; and ED_{50} values with confidence limits were obtined by the method of Litchfield and Wilcoxon (1949).

5.8. 5-HT head twitch.

These experiments were performed under direction by Miss F. Dewshi. Animals, in cages of 4, were kept in a quiet room used solely for this purpose for at least 3 days prior to use. 5-HT was administered at a dose of 50 μg i.c.v. to all mice. Two groups of 3 animals were required for each dose of each drug studied, therefore allowing for the possibility of convulsion or circling induced by the injection of 5-HT. Any animals exhibiting such behaviour were removed.

The number of twitches was counted over alternate 2 minute periods by saline- or drug-treated animals from 4 to 28 minutes after injection of 5-HT. The effect of each dose of each drug was calculated as a percentage change in the total number of twitches in control animals over this period. ED_{50} values were calculated where appropriate by the method of Litchfield and Wilcoxon (1949).

5.9. Assessment of catalepsy.

Animals were tested for catalepsy by gently placing the mouse on the bench and lifting by the scruff so that the forepaws rested on a 4cm high bar. Care was taken not to touch the tail throughout this procedure, as pressure on

the tail has been shown to increase catalepsy (Ariyanayagam & Handley,1975). The time taken for the mouse to remove its fore-paws or jump onto the bar was taken as catalepsy intensity. Animals which had not moved by 120 seconds were removed and allocated a score of 121 seconds. Each animal was replaced on the bar only once at each test if it did not maintain the position. Failure to remain on this second trial resulted in a score of 0.

Drugs were administered s.c. 15 minutes before haloperidol 0.2 mg/kg or i.c.v. 45 minutes after haloperidol. Catalepsy testing was carried out at 60,90,120,150 and 180 minutes after the neuroleptic. In the case of experiments in which haloperidol was not given, catalepsy testing was carried out at 15,30,45,60,90,120,150 and 180 minutes after injection of the drug under study.

Statistical procedures were carried out using the Mann Whitney 'U'test (Seigel,1965). This was because the data were found not to be normally distributed (Sign test, Documenta Geigy,1962) , hence parametric statistics could not be used. In addition, problems caused by the use of a cut-off time were eliminated by the use of this statistical test. Mean values were used, however, as a large proportion of mice had a value of 0 on the first few trials, thus decreases in catalepsy (a larger proportion of animals scoring 0) could not be shown graphically using median values.

5.10. Measurement of plate-crossing activity.

The method of Marriott and Smith (1972) was used to measure the effect of drugs on exploratory activity. The measurement of ambulation in a novel environment as a means of studying the emotionality of animals is based on an approach-avoidance conflict (Montgomery,1955). The apparatus used was a box 22cm square with sides 15cm high. The floor consisted of 4

metal plates 10cm square, which were separated from each other and from the sides of the box by 5mm gaps and were also raised 5mm from the bench. These gaps were used to exaggerate the avoidance part of the conflict.

Animals used in this study were placed in small cages in groups of 5 or 6 24 hours prior to use. All experiments were carried out between 0945 and 1100 hours. Animals were treated with saline or the drug under test and placed individually in the box at exactly 30 minutes after injection, always in the same position, facing the same wall. Where two drugs were given, the first was injected 15 minutes before the second and animals placed in the box 30 minutes later.

The times at which animals crossed from one plate to another was noted for a period of 90 seconds. In addition, the presence of autonomic signs and unusual behaviour was recorded. The effects of drug treatments on four parameters was studied - the latency to the first and fifth crossings and the total number of crossings in both 60 and 90 seconds. Statistical evaluation was done by means of the Mann Whitney 'U' test, because of the small numbers of animals used and the fairly large range.

7. Drugs and vehicles used.

The drugs used were obtained from the following sources:

amitriptyline HCl	Squibb
d-amphetamine sulphate	Sigma
amylobarbitone	Lilly
apomorphine HCl	Macfarlan Smith
chlordiazepoxide	Roche
clonidine HCl	Boehringer
cocaine HCl	Macfarlan Smith
corticosterone	Sigma
desipramine	Geigy
diazepam	Roche
diethyldithiocarbamate	British Drug Houses
guanabenz	Wyeth
guanfacin	Sandoz
haloperidol	Searle
5-hydroxytryptamine creatinine sulphate complex	Sigma
isoprenaline sulphate	Sigma
methoxamine HCl	Burroughs Wellcome
α-methyl - noradrenaline HCl	Hoechst
mianserin HCl	Organon
morphine HCl	British Drug Houses
naloxone HCl	Endo Labs.
*l-noradrenaline bitartrate	Koch Light
l-noradrenaline HCl	Sigma
nortriptyline HCl	Lilly
oxymetazoline HCl	E.Merck Ltd.
phentolamine mesylate	CIBA
l-phenylephrine HCl	Koch Light
piperoxane HCl	May & Baker

prazosin HCl	Pfizer
d-propranolol	I.C.I.
dl-propranolol	I.C.I.
reserpine	British Drug Houses
sotalol	Mead Johnson
viloxazine HCl	I.C.I.
yohimbine HCl	Sigma

* noradrenaline bitartrate was used in <u>in vitro</u> experiments, and noradrenaline HCl in behavioural experiments.

All drugs were dissolved or suspended in normal saline, apart from the following:

corticosterone	- dissolved in ethylene glycol;
diazepam	- obtained in injectable form and diluted with distilled water;
haloperidol	- dissolved in the minimum amount of ethanol and made to volume with distilled water;
5-hydroxy-tryptamine	- moistened with distilled water, dissolved in 0.1M HCl, then returned to pH 7 with 0.1M NaOH and made to volume with water;
reserpine	- moistened with ethanol, dissolved in the minimum amount of lactic acid and made to volume with distilled water.

Ascorbic acid was added to solutions of noradrenaline and isoprenaline to reduce oxidation. All drugs were made up as stock solutions of 1.0 mg/ml, apart from yohimbine, which was made up as a stock of 0.5 mg/ml, and diluted immediately before use.

CHAPTER 1

EFFECTS OF α AGONISTS AND ANTAGONISTS AND ANTIDEPRESSANTS ON PRE- AND POSTSYNAPTIC α RECEPTORS *IN VITRO*

CHAPTER 1.

INTRODUCTION

Since the introduction of the concept of the presence of α-adrenoceptors on presynaptic nerve terminals modifying transmitter release, much work has shown that these and classical postsynaptic α-adrenoceptors differ in their sensitivity to drugs (e.g. Doxey et al.,1977; Drew,1976). It is thus possible to ascribe certain effects of drugs to actions on either classical (α_1) or 'presynaptic' (α_2) receptors, according to their previously shown selectivity for one or the other type of receptor. A range of drugs may be used, some of which show selectivity between the two types of receptor, to determine the involvement of both types in the response under study. In order to use this method for the determination of receptor types involved in the central actions of drugs, a wide range of both agonists and antagonists is required. A simple method of assessing drug activity on both types of receptor is therefore necessary. No method of assessing this centrally was available at the start of this project. Since then, however, receptor binding studies have been carried out on rat brain homogenates using ^{3}H-WB4101, ^{3}H-prazosin and ^{3}H- clonidine which have shown that drugs may show selectivity between α_1 and α_2 receptors centrally (U'Pritchard et al,1978).

Peripheral methods are available for assessing drug potency using both *in vitro* and *in vivo* techniques. Many involve the use of stimulation-induced release of ^{3}H-NA from isolated tissues, which is a difficult and time-consuming method (e.g. Dubocovich & Langer,1974; Starke et al.,1975 a,b). Methods utilising cardiovascular tissues are common, probably since clonidine, which is frequently studied, is an antihypertensive. When investigating antihypertensive actions it is necessary to use *in vivo* techniques because of reflexes which

may be important; however, *in vitro* techniques are much simpler to perform and effects on other neuronal systems and reflexes may be eliminated. Since the method required here is a simple one which need not involve the cardiovascular system, the method used by Doxey et al.,(1977) was chosen. This method uses inhibition of the twitch response of the isolated rat vas deferens as a model for 'presynaptic' agonists and contractions of the isolated rat anococcygeus muscle as a model for 'post-synaptic agonists. Qualitatively similar results may be obtained *in vivo* (Doxey,1979 and personal communication), hence the method seems to be highly suitable.

Drugs which stimulate presynaptic or α_2 receptors in the vas deferens have been found to decrease the amount of transmitter released (Marshall et al.,1977 ; Vizi et al.,1973) thus decreasing the twitch response. Cocaine has also been shown to decrease the twitch response by blockade of neuronal reuptake of transmitter, which results in stimulation of α_2 receptors by endogenous NA (Marshall et al.,1978). Inhibition of the twitch response by either α_2 agonists or cocaine is reversible by drugs which block α_2 adrenoceptors. The method may thus be used to determine the potency of both agonists and antagonists on α_2 adrenoceptors. In addition, drugs such as tricyclic antidepressants may also be studied for their effects on α_2 receptors in the presence of cocaine without the problem of interference from blockade of neuronal reuptake. The rat anococcygeus muscle is an ideal preparation for the study of α_1-adrenoceptors, since the muscle cells are arranged in a thin layer, thus minimising problems of drug access and it has a dense noradrenergic but no cholinergic innervation.

A range of drugs known to act on α-adrenoceptors were studied using this method to compare their effects on α_1

and α_2 receptors peripherally, thus extending the available data and gaining first hand experience of the properties of these drugs. This knowledge may then be applied to any behavioural effects which the drugs may have, to enable these effects to be ascribed to actions at α_1 or α_2 receptors.

Six antidepressant drugs were also studied using this method, to determine their potencies as antagonists of α_1 or α_2 receptors. This was because it was thought possible that blockade of α_2 receptors centrally may contribute to antidepressant activity (Langer,1978), whilst blockade of α_1 receptors may mediate sedative effects of these drugs (U'Pritchard et al.,1978).

1. Effects of αagonists, antagonists and antidepressants on the isolated rat vas deferens

1.1. Agonists

Field stimulation of the vas deferens as described in Methods produced a twitch response which after approximately 15 minutes became constant in size. Five of the seven α agonists studied were able to inhibit this response in a dose-dependent manner. This effect was due to stimulation of α receptors, since it was reversed by phentolamine (Fig 1.1.). Table 1.1. shows the ED_{50} concentrations of the agonists for inhibition of the twitch response. Clonidine was the most potent in this respect and hence in stimulating α_2 receptors. Guanfacin and diethylclonidine were slightly less potent. Methoxamine, phenylephrine, NA and α-methyl-NA were all tested in the presence of prazosin 10ng/ml, as postsynaptic stimulation by these drugs, resulting in an increase in baseline, had previously been found to be a problem. NA and α-methyl-NA had similar potency in inhibiting the twich response, though were much less potent than clonidine, whilst methoxamine and phenylephrine were the least active. Methoxamine had no effect on five out of nine preparations studied, while on four preparations, inhibition was seen at fairly high concentrations. Phenylephrine did produce inhibition at high concentrations, although considerable postsynaptic stimulation prevented the calculation of an ED_{50}. The order of potency for stimulation of α_2-adrenoceptors was thus found to be:

clonidine > guanfacin > diethylclonidine > α-methyl-NA > NA > methoxamine > phenylephrine;

with potency ratios:

1 : 2.36 : 2.92 : 1466 : 1940 : 2315 : > 7000.

1.2. Antagonists.

The addition of cocaine 3μg/ml to the Kreb's solution caused marked inhibition of the twitch response. Table 1.2. shows the potency of four α receptor antagonists in reversing this inhibition. Of the antagonists used, phentolamine, piperoxane and yohimbine completely reversed the effect of cocaine (Fig 1.2.) . Prazosin at a concentration of 3μg/ml produced only 35% reversal of the cocaine inhibition, higher concentrations having no further effect (Fig 1.3.). The potency order for blockade of α_2-adrenoceptors was thus:

piperoxane > phentolamine > yohimbine > prazosin;

with potency ratios:

1 : 1.85 : 2.92 : 1200.

Piperoxane was found to be the most potent antagonist of cocaine inhibition, hence was also tested against inhibition due to clonidine. The drug was found to be 10 times less potent against clonidine than against cocaine-induced inhibition (Fig 1.4.).

1.3. Antidepressants.

The use of the cocaine-inhibited twitch response was found to be suitable for determining α_2 receptor blocking activity of antidepressants, since effects due to blockade of neuronal reuptake did not interfere with the results. The potency of six antidepressants in reversing the inhibition are shown in Table 1.2. Mianserin and trazodone completely reversed the effect of cocaine, being less potent than piperoxane, phentolamine and yohimbine, but more potent than prazosin. Viloxazine and desipramine were found to have no α_2 blocking activity in concentrations up to 30μg/ml. Amitriptyline showed a dose-dependent

increase in the antagonism of cocaine in high concentrations, up to 10μg/ml. Further increases in concentration, however, resulted in complete blockade of the twitch response (Fig 1.5.). This was also seen with nortriptyline; 3μg/ml producing a maximum increase in twitch height and higher concentrations eliminating the response. For these reasons, ED_{20}, rather than ED_{50}, concentrations were calculated(see Fig 1.6.).

2. Effects of α agonists, antagonists and antidepressants on the isolated rat anococcygeus muscle.

2.1. Agonists.

All the agonists studied produced dose-dependent contractions of the anococcygeus muscle, enabling ED_{50} concentrations to be obtained (Table 1.1.). Clonidine and diethylclonidine were the most potent α_1 agonists, while phenylephrine, guanfacin and methoxamine were 7 - 18 times less potent than clonidine. NA and α-methyl-NA again had similar potencies, being 70 - 80 times less potent than clonidine.

2.2. Antagonists.

All the receptor antagonists produced parallel shifts to the right in the dose-response curve to NA on the anococcygeus muscle. pA_2 values are shown in Table 1.2. Prazosin was the most potent antagonist at α_1 receptors, being twice as potent as phentolamine and over a hundred times more potent than yohimbine or piperoxane.

2.3. Antidepressants.

Four of the antidepressants were found to have competitive blocking activity at α_1 receptors, as they produced parallel shifts to the right in the dose-response curve to NA. pA_2 values were calculated for these drugs and

are shown in Table 1.2. Viloxazine had no α_1 blocking activity in concentrations up to 10μg/ml. Desipramine caused non-competitive blockade of the α_1 receptor as shown by a dose-dependent decrease in the maximum contraction (Fig 1.8.), with a pD_2' value of 5.21. Nortriptyline was weak as a competitive antagonist, being 425 times less potent than prazosin, but also showed non-competetive antagonism at 1 and 10μg/ml.

3. Selectivity of α agonists, antagonists and antidepressants for α_1 or α_2 receptors.

Despite being the most potent agonist at α_1 receptors, clonidine showed a marked selectivity for α_2 receptors, being almost 19 times more potent as an agonist at α_2 than at α_1 receptors (Table 1.1.). Guanfacin showed much less activity on α_1 receptors than clonidine, but was almost equipotent as an agonist of α_2 receptors, hence had a greater selectivity for α_2 receptors. NA,α-methyl-NA and diethylclonidine did not display any selectivity between the two receptors, whilst methoxamine and phenylephrine were more potent agonists of α_1 than α_2 receptors.

Of the antagonists studied, prazosin was found to be a highly selective antagonist of α_1 receptors, while both yohimbine and piperoxane displayed selectivity for α_2 receptors. This selectivity was not as marked as that of prazosin for α_1 receptors, however. Phentolamine was found to be approximately equipotent as an antagonist of both α_1 and α_2 receptors.

None of the antidepressants investigated displayed selectivity for α_2 receptors (Table 1.2.); and viloxazine was found to have no α blocking activity at all. Amitriptyline appeared to be selective for α_1 receptors, as did nortriptyline although at high concentrations, the effect of the latter drug on α_1 receptors became non-competitive.

DISCUSSION

The isolated rat vas deferens is a simple preparation, consisting of longitudinal and circular smooth muscle with an internal endothelium and connective tissue externally, which was removed before use. It receives a sympathetic innervation from the inferior seminal nerve, but is fairly insensitive to sympathomimetics. Such drugs are required in high concentration to produce a contraction, and low doses may inhibit the twitch response to nerve stimulation. It is possible, therefore, that the nerve terminals lie in folds of the muscle cell membranes, making very close contact, thus allowing drugs only poor access to the postsynaptic receptor (Swedin,1971).Evidence is accumulating for the existence of two differing types of postsynaptic α receptor, one of which may be similar to the α_2 receptor previously only found to occur on noradrenergic neurones presynaptically (Docherty et al.,1979; Drew & Whiting, 1979). However, the postsynaptic receptor in the rat vas deferens appears to be of the α_1 type, hence there is no interference with the actions of drugs on presynaptic α_2 receptors in this tissue (Docherty et al.,1979).

Farnebo and Malmfors (1971) found NA to decrease and phenoxybenzamine(PBZ) to increase the stimulation-induced release of NA from isolated mouse vasa deferentia; and postulated a trans-synaptic regulatory mechanism for transmitter release. Many other workers since then have proposed a presynaptic mechanism of regulation involving α receptors (e.g. Starke,1972), the presence of which has since been demonstrated in the rat vas deferens (Vizi et al.,1973). Clonidine stimulates these presynaptic α receptors, thereby decreasing the amount of transmitter released. This is a calcium-dependent effect (Drew,1978a),the inhibitory effect of clonidine increasing with decreasing calcium concentration. The drug is thought to

act by reducing the accumulation of intraneuronal free Ca^{2+} and thus preventing electrosecretory coupling (Stjarne,1973). Other mechanisms of decreasing transmitter release have also been found to occur in this tissue. The involvement of prostaglandins E_1 and E_2 in the regulation of sympathetic transmission was proposed by Hedqvist for the guinea pig vas deferens and other tissues; however, such a mechanism does not appear to exist in the rat (Iles et al.,1973) and may in any case be independent of the regulation mediated by α-adrenoceptors (Stjarne,1973). Regulation of transmitter release may also be mediated by dopamine receptors. Tayo (1977) was able to reverse inhibition of the twitch due to DA with pimozide and haloperidol. However, studies in our laboratories indicate that DA receptors may only play a minor role, since haloperidol and chlorpromazine were only able to block the effect of DA in the presence of phentolamine. An opiate receptor may also be present on the presynaptic neurone of the rat vas deferens (Lemaire et al., 1978), but unlike that of the mouse, is insensitive to opiate analgesics, only enkephalins and β endorphins being able to inhibit the twitch.

In contrast to the work of Langer et al., (1975), which suggests there may be a positive feedback system mediated by presynaptic β receptors in certain tissues, β agonists inhibit the twitch response of the rat vas deferens (Hedqvist & Von Euler,1976; Jenkins et al.,1977; Doxey, personal communication), which may involve both β and α components. Despite the presence of several other types of inhibitory receptor in the rat vas deferens, the presynaptic α_2 receptor appears to be the most important for regulation of transmitter release. The preparation is therefore suitable for investigation of drugs acting on this receptor due to the inaccessibility of the postsynaptic receptor.

The anococcygeus muscle in the rat consists of smooth muscle cells organised in parallel bundles which permit easy access of drugs to the receptors. It has a dense noradrenergic but no cholinergic innervation, thus making it an ideal preparation for the study of drugs acting on adrenoceptors. The tissue contains postsynaptic receptors of the α_1 type, which have a similar sensitivity to drugs as those found in other tissues (Doggrell,1979). Presynaptic α_2 receptors have also been shown to be present in this tissue (Idowu & Zar,1978), hence it may be used to determine drug potency at both α_1 and α_2 receptors (Doxey,1979).

The agonists studied presented a wide range of selectivity between the two receptors. Clonidine and guanfacin were shown to act preferentially at presynaptic α_2 receptors, guanfacin being much more selective than clonidine in this respect. Methoxamine and phenylephrine acted preferentially on postsynaptic α_1 receptors, while NA, α-methyl-NA and diethylclonidine were equipotent on both types of receptor. These results are in agreement with most of the work which has been published on drug selectivity (Doxey,1979; Doxey et al.,1977; Drew,1976; Starke et al.,1974,1975b). However, Drew (1976) found clonidine to be non-selective using the rat cardiovascular system; and Starke et al.,(1975b) found α-methyl-NA to act preferentially on presynaptic receptors. These differences may be due to slight differences in either receptor numbers or affinity both between species and between organs in the same species (Doxey,1977; Pacha et al.,1975).

Of the α blocking drugs studied, yohimbine and piperoxane acted preferentially on presynaptic receptors, while prazosin was selective for postsynaptic receptors and phentolamine was approximately equipotent on both types. A similar spectrum of selectivity has previously been shown for yohim-

bine (Borowski et al.,1976; Doxey et al.,1977; Drew,1976), prazosin (Cambridge et al.,1977,1978; Cavero et al.,1977) and phentolamine (Drew,1976). Other workers have found piperoxane to be non-selective (Drew,1977; Borowski et al.,1976), however Blakely and Summers (1978) found it to block presynaptic α receptors in the cat spleen and it has been used by several workers in combination with yohimbine and phentolamine to indicate the involvement of α_2 receptors in a particular response (e.g.Drew,1978b; Drew et al.,1979; Davis et al.,1979). Piperoxane was found to be ten times less potent as an antagonist of clonidine inhibition than of cocaine inhibition, hence would appear to be less selective against clonidine. It may also block DA receptors in the rat vas deferens (Doxey, personal communication); hence, if cocaine also blocks DA reuptake, then this action may explain the increased activity against cocaine. However, the dopaminergic blocking activity was weak and unlikely to be sufficient to account for a ten-fold increase in potency.

A range of α agonists and antagonists have thus been chosen which vary in selectivity between presynaptic α_2 and postsynaptic α_1 receptors. These drugs will be used to determine which type of receptor may be involved in certain central behavioural responses.

The α blocking properties of six antidepressants were also investigated using this model. It has been suggested (Langer,1978) that presynaptic α receptor blockade centrally may have an antidepressant effect by increasing the concentration of NA present in the synaptic cleft. This end result would thus be the same as that achieved by blockade of neuronal reuptake. The actions of both existing and newer antidepressants on α_2 receptors was therefore examined. The two newer drugs,

mianserin and trazodone, did show α_2 blocking activity, although they were much less potent than phentolamine. Mianserin has also been shown to block α_2 receptors peripherally *in vivo* (Doxey et al.,1978) and centrally *in vitro* (Baumann & Maitre,1976; Maggi et al,1980) and *in vivo* (Clinschmidt et al.,1979; Gower & Marriott,1979). It does not, however, display any selectivity for these recptors either *in vitro* or *in vivo* (Clineschmidt et al.,1979; Doxey et al.,1978; Maggi et al.,1980). Mianserin also blocks NA reuptake, although it is much less potent than amitriptyline (Goodlet et al.,1977), has a slight effect on 5-HT reuptake (Baumann & Maitre,1977) and is a 5-HT receptor antagonist (Maj et al.,1978a). Hence the particular property or properties which mediate its antidepressant action are unclear, although α_2 receptor blockade may be involved.

Trazodone is a newer antidepressant (Udabe,1973) which blocks the reuptake of 5-HT while having little effect on NA (Riblet et al.,1979). Thus any action which involves NA may be mediated by α_2 receptor blockade.

The three tricyclic antidepressants studied were not found to have any significant degree of α_2 blocking activity although of these, amitriptyline was the most potent. These results agree with those of Baumann and Maitre (1976), who measured stimulation-induced release of ^{3}H-NA from rat cortical slices. These workers found that amitriptyline had some degree of α_2 blocking activity, but desipramine and nortriptyline were without effect. Hughes (1978) also found amitriptyline to block α_2 receptors in the mouse vas deferens. Viloxazine did not block α_2 receptors either in the model used here or in that of Bauman and Maitre. Although it is apparent that α_2 receptor blockade is not a general property of antidepressants, it is probable that newer drugs may be introduced which do have this property in common.

The ability of the antidepressants to block postsynaptic α_1 receptors was also determined using the ano-coccygeus muscle. Amitriptyline was the most potent in this respect, being only 15 times less potent than phentolamine. The other drugs were considerably less potent, however, desipramine being non-competetive and viloxazine having no effect. Again, therefore, it is unlikely that this property is involved directly in the therapeutic action of antidepressants, since it is not a general property of such drugs. However, it may be important in mediating side-effects, such as sedation. U'Pritchard et al.,(1978) have found a correlation between the binding affinity of tricyclic antidepressants to α_1 receptors in rat brain and their capacity to relieve psychomotor agitation and induce sedation. A similar correlation can be seen between sedative action, which decreases in the order: amitriptyline > nortriptyline > desipramine; and postsynaptic α_1 receptor blockade in the present model. In addition, prazosin which is the most potent postsynaptic antagonist, induces a sedative behavioural syndrome in mice (see Chapter 3).

In view of the results obtained with antidepressants, which suggested that neither α_1 nor α_2 receptor blockade correlated with therapeutic activity, no further investigations using these drugs were undertaken. The α agonists and antagonists were further investigated for their effects on the SI release of NA from rat brain slices, prior to use in behavioural studies.

DRUG	ED_{50} at α_2 * receptor (mean ± SEM)	ED_{50} at α_1 + receptor (mean ± SEM)	RATIO $\alpha_2:\alpha_1$
Clonidine	0.42 ± 0.11 (7)	7.9 ± 3.3 (8)	18.8
Guanfacin	0.99 ± 0.16 (6)	113 ± 38 (6)	114.0
Diethyl-clonidine	1.22 ± 0.24 (6)	2.68 ± 1.4 (6)	2.19
Noradrenaline	815 ± 176 (6)	617 ± 214 (6)	0.76
α-methyl-noradrenaline	616 ± 141 (6)	558 ± 78 (6)	0.90
Methoxamine	972 ± 230 (4)	145 ± 42 (6)	0.15
Phenylephrine	> 3,000 (6)	60.9 ± 20 (6)	< 0.02

Table 1.1. Relative potencies of α agonists for pre- (α_2) and post-synaptic (α_1) receptors in rat peripheral tissues in vitro.

* ED_{50} for inhibition of the twitch response of the isolated rat vas deferens in ng/ml.

+ ED_{50} for contraction of the isolated rat anococcygeus muscle in ng/ml.

Numbers in parentheses refer to the number of experiments.

DRUG	ED_{20} for α_2 antagonism* (ng/ml)	Conc. for α_1 antagonism+ (ng/ml)	pA_2 at α_1 receptor mean ± SEM	Ratio α_2/α_1
Piperoxane	1.0 (6)	94.0 (7)	6.46 ± 0.14	94.0
Phentolamine	1.85 (6)	1.2 (5)	8.5 ± 0.16	0.65
Yohimbine	3.1 (6)	348.0 (8)	6.05 ± 0.16	112.0
Prazosin	1200 (7)	0.6 (6)	8.85 ± 0.20	0.0005
Mianserin	195.0(7)	55.0 (9)	6.74 ± 0.21	0.28
Trazodone	480.0(6)	67.0 (6)	6.75 ± 0.09	0.14
Amitriptyline	2700 (6)	18.0 (6)	7.24 ± 0.12	0.006
Nortriptyline	2800 (6)	255.0 (5)	6.07 ± 0.14	0.09
Desipramine	3000 (2)	- (4)	5.21 ± 0.14**	-
Viloxazine	3000 (2)	1000 (6)	4.44	-

* ED_{20} values for the reversal of cocaine-induced inhibition of the twitch response of the isolated rat vas deferens were used as some drugs did not produce 50% reversal

+ the concentration of antagonist which doubled the dose of NA required to give a 50% of maximal contraction on the isolated rat anococcygeus muscle

** pD'_2 value for non-competetive inhibition

Table 1.2. Relative potencies of α antagonists for pre- and postsynaptic α receptors peripherally in vitro.

Numbers in parentheses refer to the number of experiments.

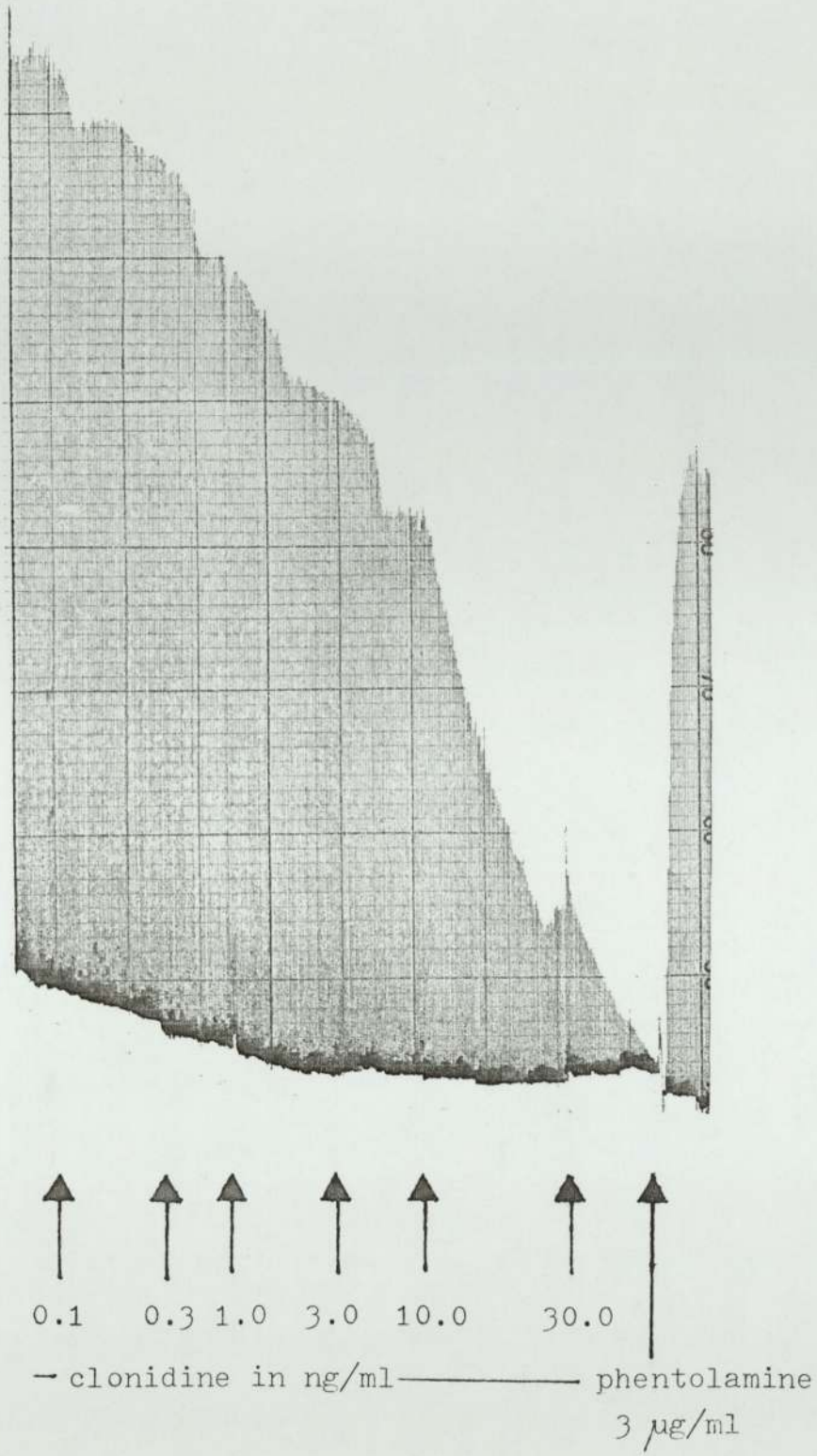


Fig 1.1. The effect of increasing concentrations of clonidine on the twitch response of the isolated rat vas deferens.

Phentolamine was added at the end of the experiment to ensure that the inhibition was due to α receptor stimulation.

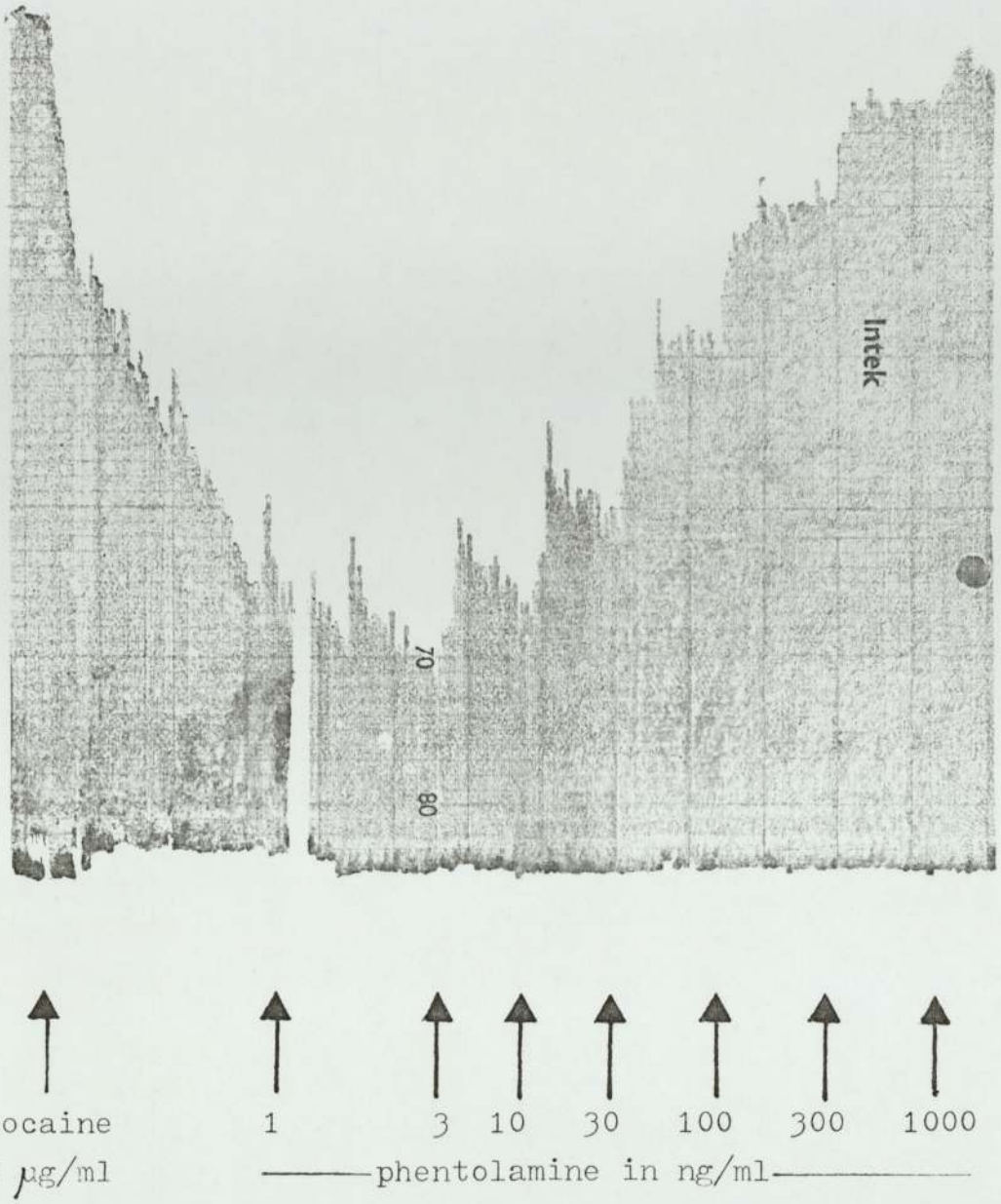


Fig 1.2. The effect of increasing concentrations of phentolamine on the cocaine-inhibited twitch response of the rat vas deferens.

After addition, the cocaine was present throughout the experiment.

Fig 1.3. Effect of α antagonists on the cocaine-inhibited twitch response of the isolated rat vas deferens.

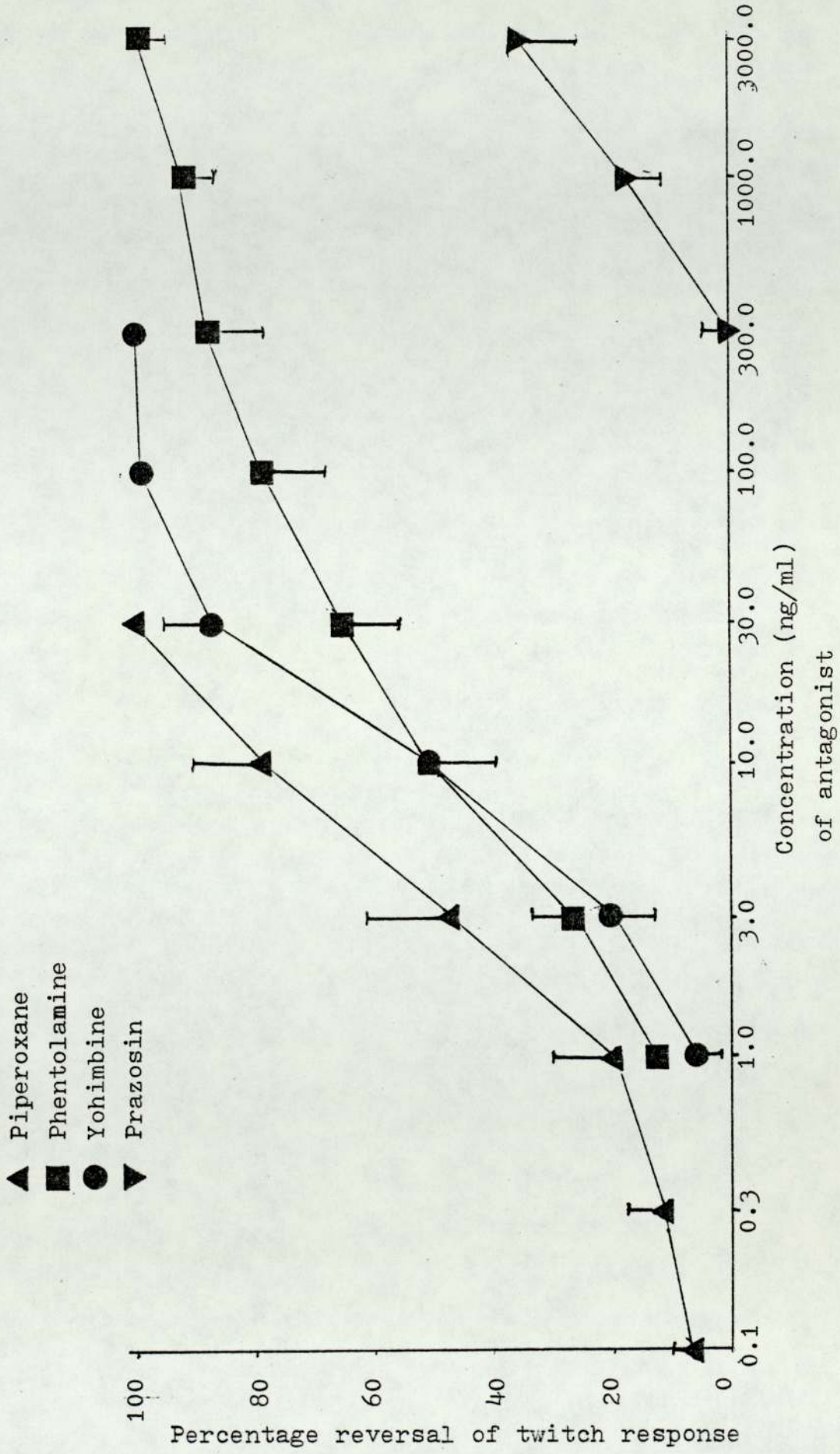
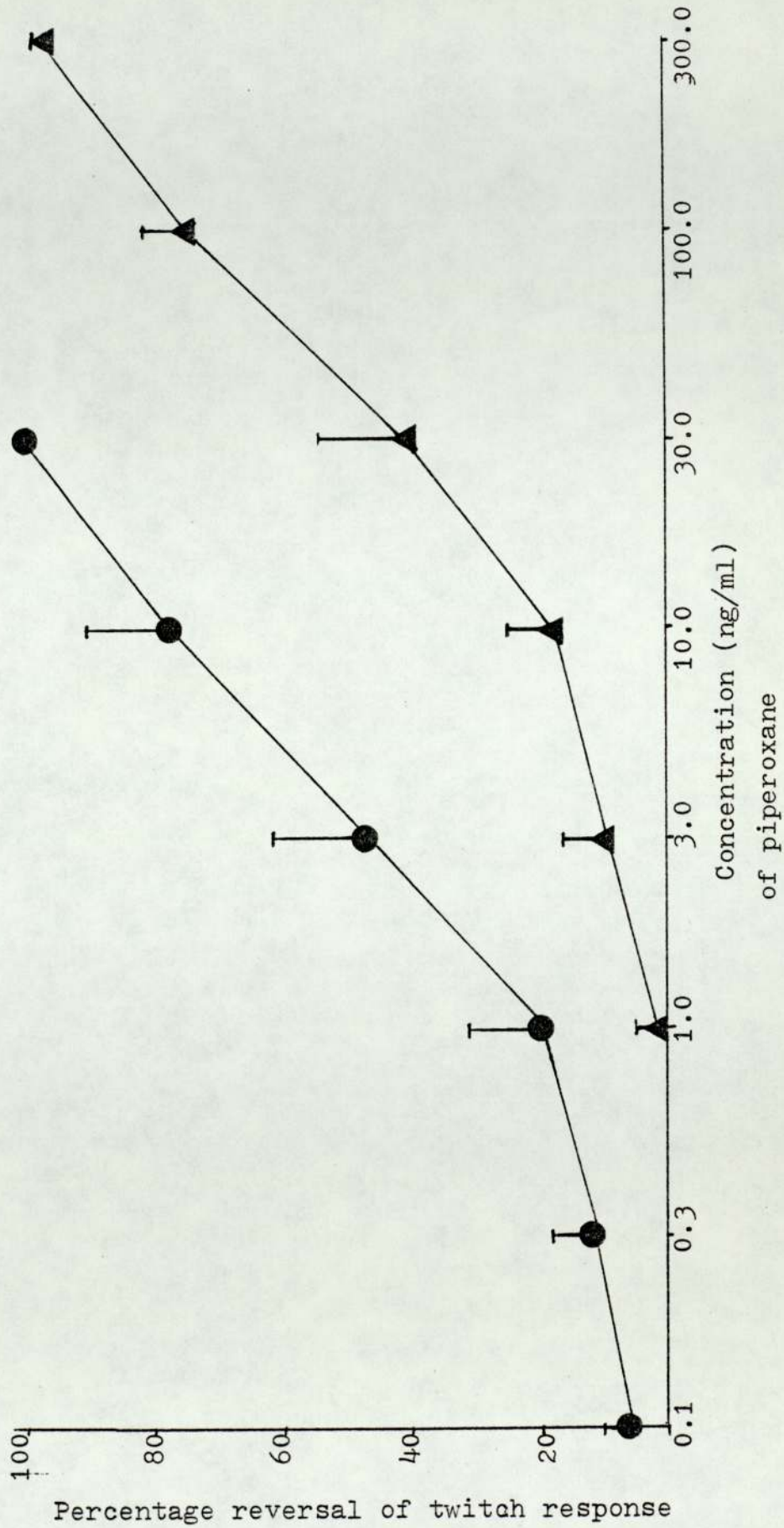


Fig 1.4. Effect of piperoxane on the cocaine (●) and clonidine (▲) inhibited twitch response of the isolated rat vas deferens.



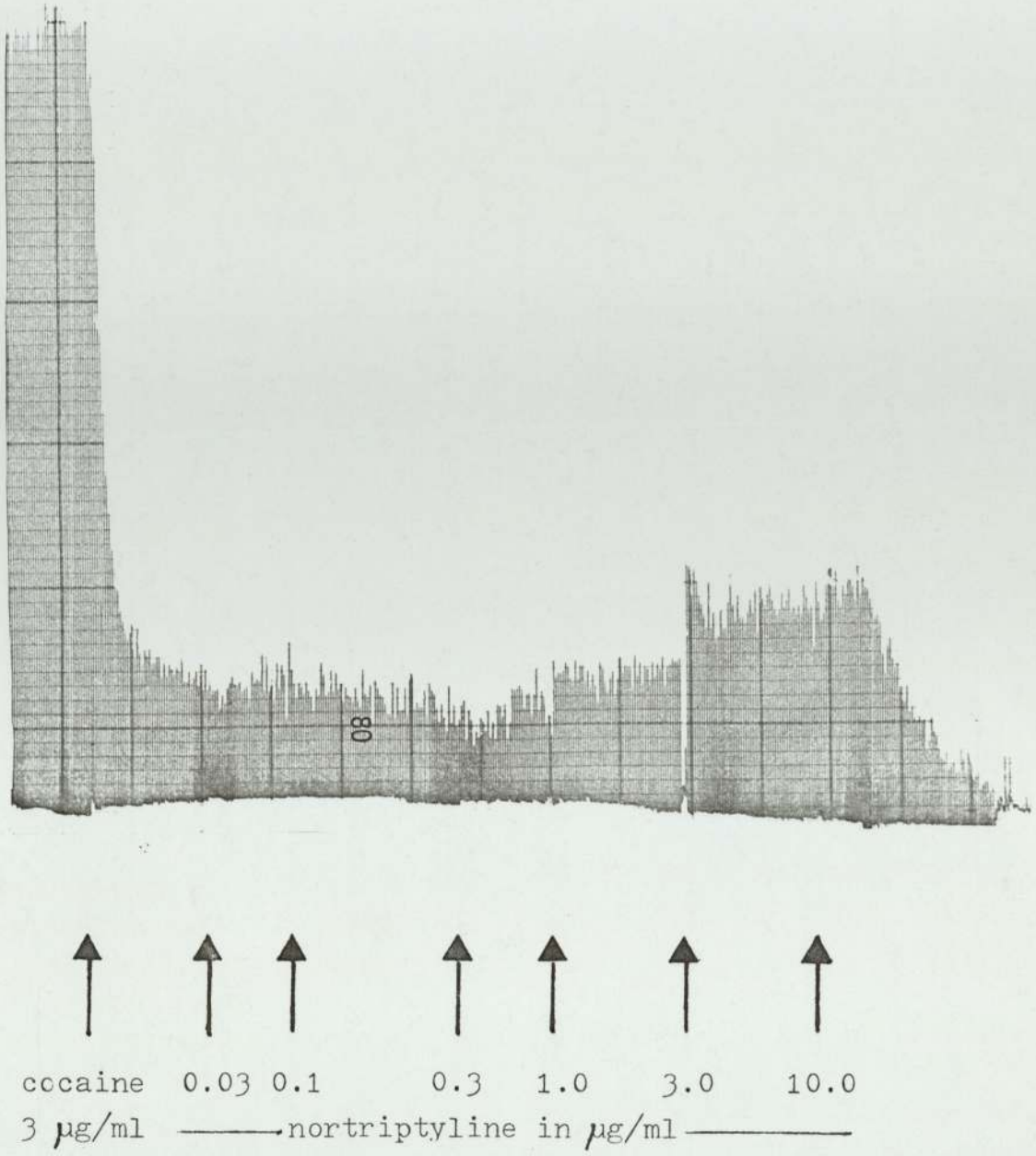


Fig 1.5. The effect of increasing concentrations of nortriptyline on the cocaine-inhibited twitch response of the isolated rat vas deferens.

After its addition, cocaine was present throughout the experiment.

Fig 1.6. Effect of antidepressants on the cocaine-inhibited twitch response of the isolated rat vas deferens.

(Phentolamine is also included for comparison)

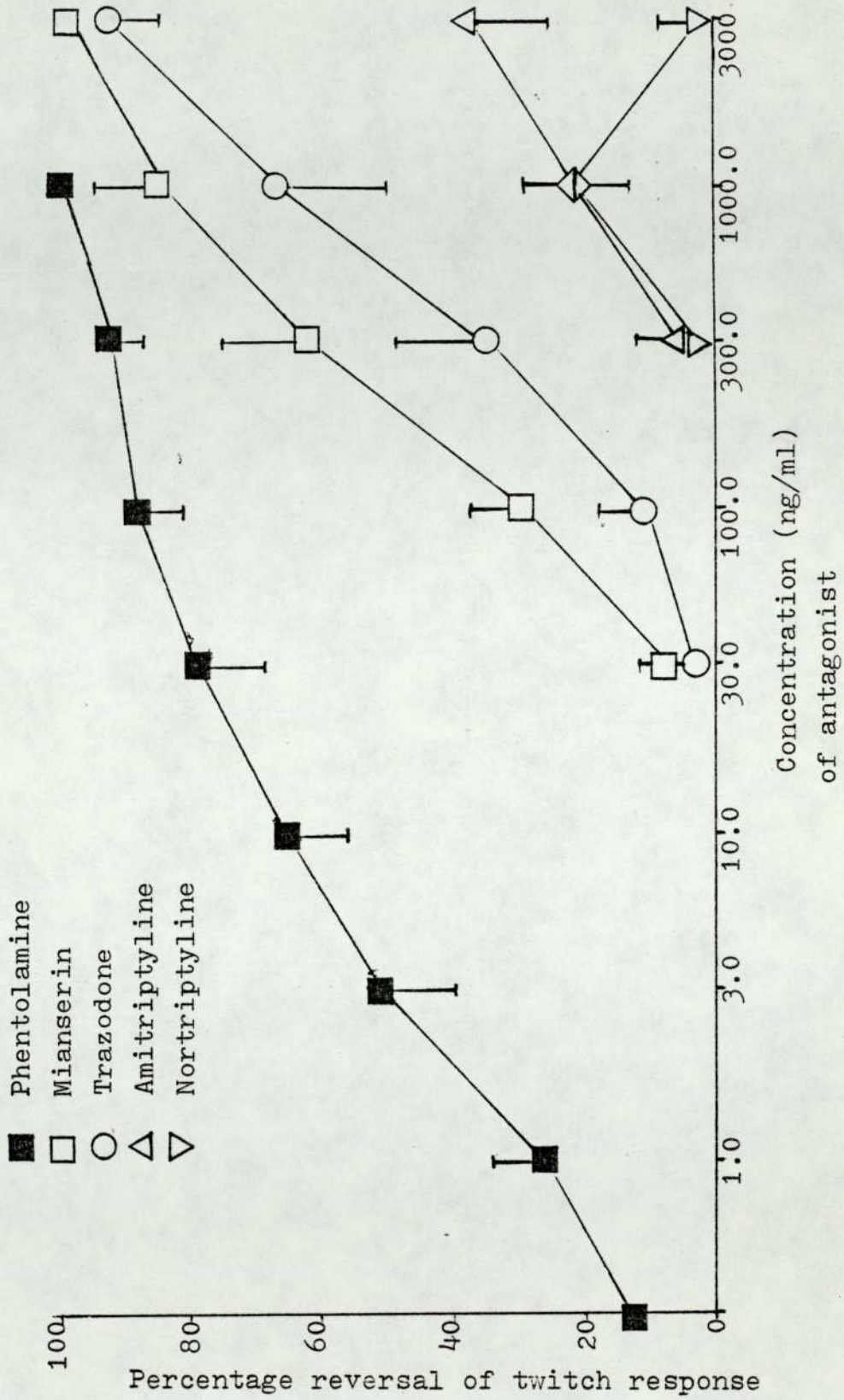
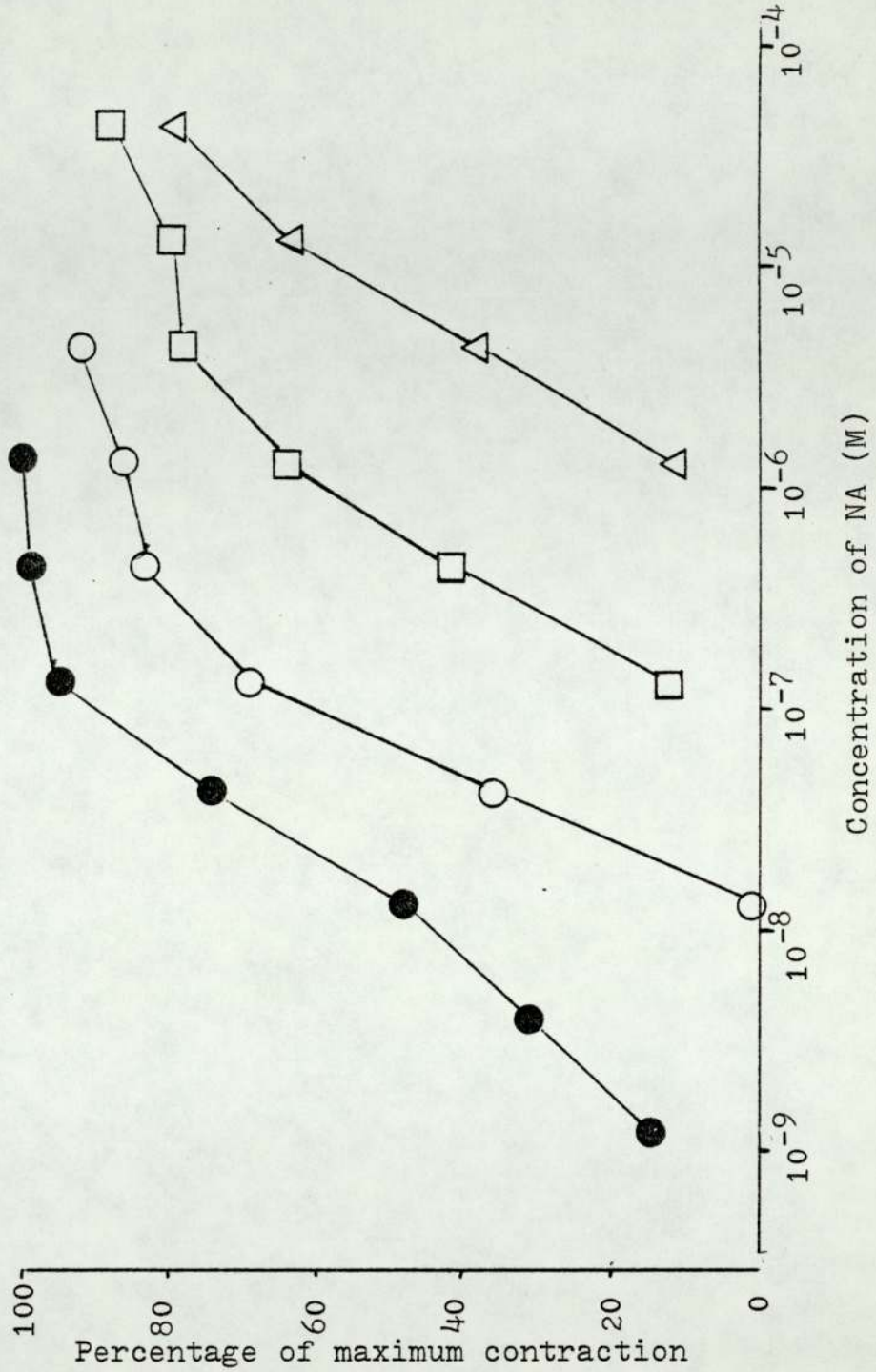
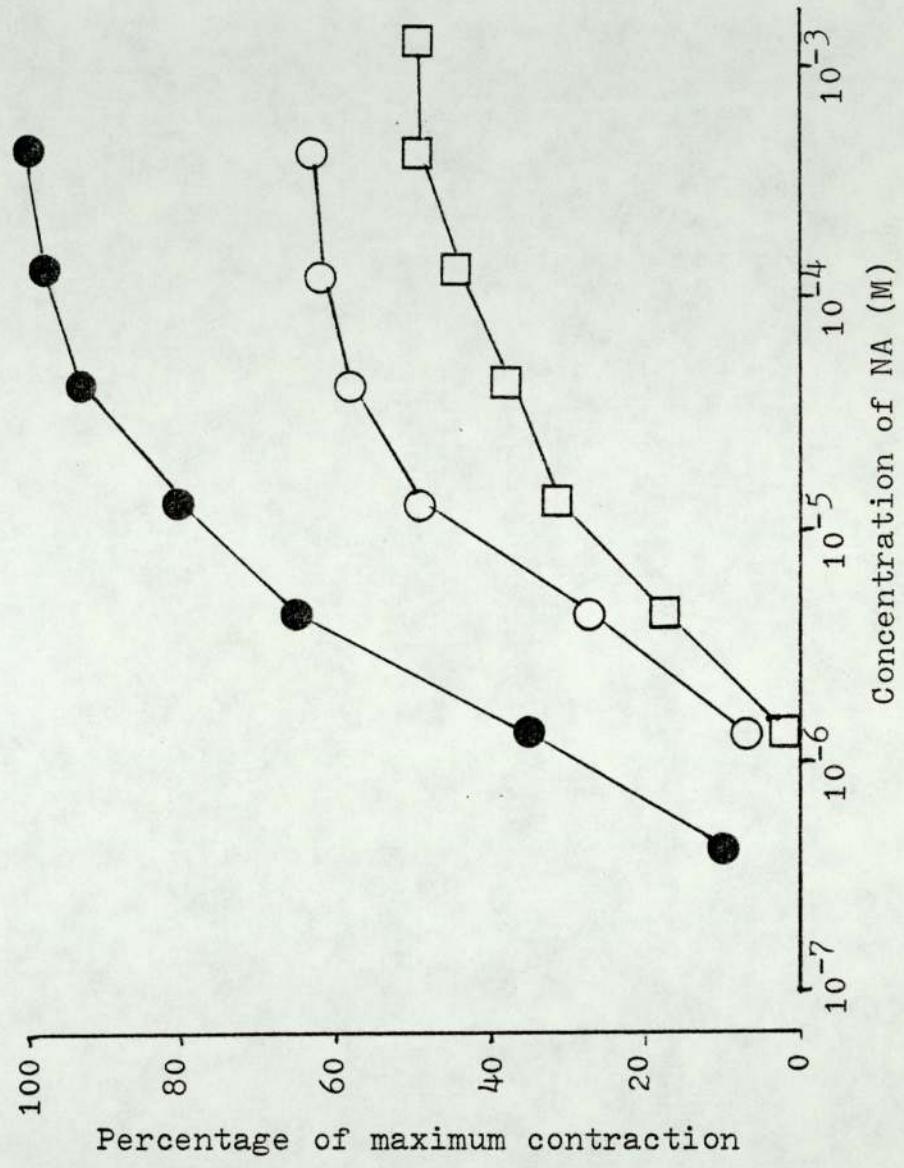


Fig 1.7. Effect of prazosin on the dose-response curve to NA on the isolated rat anococcygeus muscle.



(●) - NA alone: (○) - in the presence of prazosin 1ng/ml: (□) - in the presence of 10ng/ml: (△) - in the presence of 100ng/ml.

Fig 1.8. Effect of nortriptyline on the dose-response curve to NA on the isolated rat anococcygeus muscle.



(●) - NA alone: (○) - in the presence of nortriptyline 100ng/ml: (□) - in the presence of nortriptyline 1000ng/ml.

CHAPTER 2

EFFECTS OF α AGONISTS AND ANTAGONISTS ON THE STIMULATION-INDUCED RELEASE OF ^{3}H-NA FROM BRAIN SLICES

CHAPTER 2.

INTRODUCTION

It has been shown that different organs in the same species differ in their sensitivity to the effects of drugs acting on regulatory α_2 receptors (Pacha et al., 1975). Hence the extrapolation of drug actions from one tissue to another, even in the same species becomes speculative. Thus studies were undertaken to confirm that the drugs chosen for study were capable of exerting a similar regulatory effect on CNS neurones. Such studies were performed using rat cortical slices. Unfortunately, similar experiments on slices of mouse brain were not undertaken due to a lack of time. In addition, the lack of studies in the literature on mouse brain renders extrapolation from the results obtained here to the mouse difficult. However, it was felt that the experiments would still be of great value, since many of the drugs used had not been studied for their effects on NA overflow. In addition, *in vitro* studies on brain tissue can not be directly compared to *in vivo* results, since the absorption, metabolism and ability of the drug to penetrate the CNS may result in differences in the potency of their effects *in vivo*. Thus the studies performed here merely form a tentative basis for hypotheses which may be advanced in later chapters. However, since clonidine, for instance, has been found to reduce ^{3}H- NA release from many tissues in several species, the probability that a similar effect will occur in mouse brain is fairly high.

The method of incubating brain slices with tritiated neurotransmitters and studying their release has been in use for a considerable time. It is known that electrical stimulation or increasing the extracellular potassium ion (K^+) concentration causes an increase in the amount of transmitter released from the slices (Baldessarini & Kopin,1967). It is thought that this increase is similar to that occuring on the

arrival of a nerve impulse i.e. that the system is similar to the physiological situation, since it is calcium-dependent and is blocked by tetrodotoxin (Howd & Horita,1975).

Alpha-adrenergic stimulants have been found to decrease the stimulation-induced (SI) release of NA using this system, while α blocking drugs increase release (e.g.Farnebo & Hamberger,1971; Taube et al.,1977). However, data is not available for some drugs found to show more selectivity for α_1 or α_2 adrenoceptors than those already studied. Experiments were therefore carried out to determine the effects of the drugs to be used in studying behaviour upon SI release of NA from rat brain slices.

1. The effect of α agonists on ^{3}H-NA release.

Three α-adrenoceptor agonists found to show selectivity for α_1 (methoxamine) or α_2 (clonidine and guanfacin) receptors peripherally were investigated for their effects on the spontaneous efflux of ^{3}H-NA from unstimulated rat cortex slices and on the increased release produced by electrical stimulation, as shown in Fig.2.1.

Clonidine 10^{-8} - 10^{-7}M (2.6 - 26 ng/ml) had no effect on the spontaneous efflux of tritium. Methoxamine, however, did consistently increase spontaneous efflux at a concentration of 5.3×10^{-6}M (1 μg/ml), as did guanfacin at the highest concentration used, 4×10^{-7}M (100 ng/ml). Lower concentrations of the latter drug did not affect the spontaneous efflux of tritium.

The effects of the agonists on the SI efflux of tritium are shown in Table 2.1. Increasing concentrations of clonidine (10^{-8} - 10^{-7}M) produced a gradual overall decrease in the amount of tritium released from the brain slices on the second stimulation. As was found to occur with most of the drugs studied, however, some preparations produced the opposite effect to that seen in the majority of experiments. Guanfacin in concentrations of 4×10^{-8}M and 1.2×10^{-7}M also caused inhibition of the SI release of tritium of similar magnitude to that produced by clonidine 10^{-7}M. Guanfacin thus appeared to be slightly more potent than clonidine in this respect. A higher concentration of guanfacin, 4×10^{-7}M, had no overall effect on the SI release of tritium; a slight decrease being seen in 3 out of 6 experiments, while the remainder showed a slight increase. This lack of effect may be attributed to the increase in spontaneous efflux mentioned above. Methoxamine at a concentration of 5.3×10^{-6}M decreased SI efflux in 4 out of 8 experiments, the overall effect being one of a slight increase.

2. The effect of α antagonists on ^{3}H-NA release.

Of the four antagonists used, only phentolamine, 10^{-7}M, (37ng/ml) produced a slight transient increase in spontaneous efflux of tritium. This drug also markedly increased the SI release in 6 out of 10 experiments, although the overall effect was not significantly greater than in control experiments. The effect of phentolamine and the other antagonists on SI efflux are shown in Table 2.2. Piperoxane produced only a slight increase in SI efflux at 3.7×10^{-8} and 1.15×10^{-7}M (10 and 30 ng/ml); a more marked effect, however, was obtained with 3.7×10^{-7}M (100 ng/ml). Yohimbine 10^{-7} - 10^{-8}M (39 - 390 ng/ml) also increased the SI release of tritium, although this did not appear to be concentration-dependent. This may be due to the concentrations used all producing the maximal effect. Prazosin 10^{-7}M (42 ng/ml) had no overall effect on SI efflux, a slight increase being produced in only 3 out of 7 preparations.

DISCUSSION

A range of α agonists and antagonists which were found to differ in their selectivity for α_1 and α_2 receptors peripherally, have been studied for their effects on the release of tritium from perfused rat cortical slices, pre-incubated with ^{3}H-NA. This method has been used by many workers to study the effects of drugs on the release of transmitters from central neurones. Tritiated NA is taken up selectively into noradrenergic neurones (Farnebo & Hamberger,1971), whilst metabolites formed during incubation and perfusion are not taken up and retained by nerve terminals. The spontaneous efflux of tritium which occurs during perfusion consists mainly of tritiated metabolites; and the pattern of efflux with marked retention of tritium by the tissue is consistent with the mixing of exogenous NA with a relatively firmly bound form of endogenous NA (Baldessarini & Kopin,1967).

In addition, the distribution of tritium in brain slices has been shown to be mainly at synapses containing dense core vesicles (Baldessarini & Kopin,1967).

Electrical stimulation of the tissue leads to an increase in the amount of tritium in the perfusate. This increase is Ca^{2+} dependent and is blocked by tetrodotoxin, a drug which blocks axonal conduction (Farnebo & Hamberger,1971), thus has similarities to the physiological release of transmitter from nerve endings. High concentrations of K^+ are also able to increase tritium overflow, thus suggesting a further similarity to the physiological situation.

The tritium present in the perfusate immediately after stimulation is 70 - 75% unchanged NA (Baldessarini & Kopin,1967; Dismukes & Mulder,1976), thus may be taken as a measure of the amount of NA released from the nerve terminals. Raiteri et al.,(1975) using superfused synaptosomes, found that

stimulation by K^+ also released mainly ^{3}H-NA and did not increase the concentration of metabolites in the superfusate. Thus these workers suggested that stimulation releases ^{3}H-NA directly from synaptic vesicles, not from other intraneuronal pools. The method thus seems to be suitable for the study of drug effects on the release of NA from central noradrenergic neurones.

Of all the drugs studied, only guanfacin and methoxamine had any marked effect on the spontaneous overflow of tritium. A slight increase was produced by both drugs which, in the case of guanfacin, may have masked its inhibitory effect on SI release of NA. The increase may be due to displacement of NA from synaptic vesicles, release of tritiated metabolites from nerve terminals or release from extraneuronal sites. The use of an uptake blocker may be helpful in confirming the mechanism of this effect.

Of the three α agonists studied, only clonidine and guanfacin were found to affect the SI release of tritium from brain slices. Clonidine decreased release in a concentration-dependent manner, but only in concentrations greater than those which had previously been found to reduce the twitch response of the vas deferens by 100%. Lower concentrations of guanfacin were able to produce a similar degree of inhibition of tritium release, thus indicating that this drug was more potent than clonidine as an agonist at the α receptors involved.

The effect of clonidine on the SI release of ^{3}H-NA has been found previously (Farnebo & Hamberger,1971), while other agonists, including NA, have also been found to reduce release. (Dismukes & Mulder,1976; Taube et al.,1977). The decrease in release produced by oxymetazoline (Starke & Montel, 1973) or by NA (Taube et al.,1977) was prevented by phentolamine, thus indicating that α receptors are involved in these effects.

The lack of effect of methoxamine found in the present experiments suggests that the receptor may be of the α_2 type, since methoxamine was ineffective in stimulating these receptors peripherally. Dubocovich (1978) also found methoxamine to have no effect on SI release of NA from rat brain slices. Three of the antagonists tested, yohimbine, phentolamine and piperoxane, were found to increase the SI release of tritium from cortical slices. Prazosin was, however, ineffective at concentrations found to produce blockade of α_1 receptors peripherally. Such an increase has previously been shown for phentolamine, yohimbine and PBZ (Farnebo & Hamberger,1971; Taube et al.,1977). Phentolamine and PBZ may also increase overflow by interfering with the reuptake of transmitter or extraneuronal binding (Starke & Montel,1973); however, the antagonism of the effects of these drugs by oxymetazoline suggests that the mechanism involved in this action is one of α receptor blockade.

The lack of effect of prazosin, also found by Dubocovich (1978), may be interpreted as supporting the suggestion that this receptor is of the α_2 type. However, PBZ, which has been shown to be ineffective in blocking α_2 receptors (Doxey et al.,1977), does increase release, thus effects on extraneuronally bound NA and reuptake mechanisms may still contribute to the effect of this drug on release.

The increase in SI release by α antagonists has been postulated, therefore, to be due to blockade of the negative feedback system which would be stimulated by endogenous NA (Starke & Montel,1973). Wemer et al.,(1979) have calculated that endogenous NA inhibits release by 60% in brain slices, since the NA released on stimulation has to diffuse through the extracellular spaces before removal. This allows α blockers to inhibit the effect of NA on regulatory receptors and produce an

increase in outflow. If synaptosome preparations are used, however, diffusion of released transmitter is not delayed, the superfusate being able to effect removal more efficiently. Thus phentolamine and yohimbine do not increase SI release of tritium from such preparations (Mulder et al.,1978).

The results suggest that α receptors are present in the preparation, which have an inhibitory effect on the release of accumulated NA. The pre- or postsynaptic location of the receptors cannot be deduced from the results obtained here, since it is possible that neuronal feedback loops may be present in the slices, which may mediate the effects of drugs. However, the similarity of effect of clonidine and other agonists in reducing SI release from synaptosomal preparations (De Langen et al.,1979) suggests that such neuronal loops are not the means by which this effect is mediated. When synaptic levels of transmitter are high, such as in the presence of cocaine or after high frequency stimulation, α agonists are less potent in inhibiting release of NA (Starke & Montel,1973). This suggests that endogenous NA activates most of the receptors in such situations and together with the suggestion that α blockers increase NA efflux by inhibiting the effects of endogenous NA, provide evidence that the negative feedback system has a physiological basis.

The type of α receptor involved is probably α_2, since it is these receptors which have been found to modify transmitter release peripherally. The lack of effect of drugs which act mainly at α_1 receptors i.e. methoxamine and prazosin, supports this suggestion; although the effectiveness of PBZ in increasing release suggests that these receptors may differ slightly pharmacologically from those found peripherally. This is supported by the work of Wemer et al.,(1979), who found phentolamine to be more potent than yohimbine in increasing

release, while NA was more potent than oxymetazoline in reducing release. These workers suggest that oxymetazoline may preferentially stimulate postsynaptic receptors centrally.

Thus a similar mechanism controlling the amount of transmitter released from noradrenergic neurones appears to be present centrally to that which has been found peripherally. Similar receptor systems also seem to be present on dopaminergic and serotonergic neurones, whereby the transmitter involved can modify its own release (Farnebo & Hamberger,1971; Westfall et al.,1976). These systems appear to be activited only by the appropriate transmitter, thus DA or 5-HT do not affect release from noradrenergic neurones (Taube et al.,1977), while NA does not affect DA or 5-HT release (Farnebo & Hamberger,1971;1974a). However, differing results have been obtained by using slices from various brain regions (see Introduction), thus it seems possible that one transmitter may modify the release of another.

In addition, there does not appear to be a facilitatory feedback mediated by β receptors on noradrenergic neurones (Farnebo & Hamberger,1974b; Taube et al.,1977), such as has been demonstrated peripherally (Langer et al.,1975). However, Westfall et al.,(1976) did find such a regulatory receptor using slices from a different part of the cortex.

The differences which have been found in the characteristics of regulation of transmitter release both between brain regions (De Langen et al.,1979) and also in brain slices from a different species (Bryant et al,,1975) mean that the extrapolation of the effects found here to behavioural effects of these drugs in the mouse must be undertaken cautiously. Despite slight differences in drug potency, however, the overall effects of α agonists and antagonists in modifying release of NA from central nerve terminals are consistent. It is therefore acceptable to assume that clonidine may reduce and

yohimbine increase NA from noradrenergic neurones in mouse brain despite the lack of studies in this species. The studies undertaken here suggest that only drugs acting at α_2 receptors are capable of modifying this release. The results do not, however, give any indication of whether these drugs are capable of reaching the sites in the brain necessary to produce these effects, nor do they preclude the possibility that drugs acting at α_1 receptors may also modify release via neuronal feedback loops which are not intact in cortical slices.

The method used here was found to produce inconsistent increases in the amount of tritium released on stimulation, although this problem was overcome by the use of two stimulation periods for each set of slices. Thus drugs could be added after the first stimulatiom and their effect on the amount of tritium released studied by comparison between the two stimulations. This method also reduced errors due to the method used to estimate spontaneous overflow of tritium, since the eventual parameter used was a ratio of the amount released over the two stimulation periods. Improvements in the method may be achieved by the use of slices of the same size taken from the same brain regions, and the maintainence of a constant current for each stimulation. However, the method is still difficult to perform and an easier means of studying central α receptors may be to use the binding of α agonists and antagonists to brain membranes.

Using such a technique, U'Pritchard et al.,(1978) have shown that radioactive ligands can be obtained which bind to different types of receptors. Thus ^{3}H-clonidine and ^{3}H-catecholamines bind to receptors for which α agonists have a high affinity, while ^{3}H-WB4101 and ^{3}H-prazosin bind to receptors which have a higher affinity for most α antagonists. It has

been suggested that the two binding sites may correspond to agonist and antagonist states of the α receptor, however, highly selective α_2 antagonists have been shown to bind to ^{3}H-clonidine sites (Tanaka & Starke,1980), thus this does not seem to be the case.

The pharmacological potencies of α antagonists as estimated at postsynaptic α receptors in the rat vas deferens correlates with their affinity for the ^{3}H- prazosin binding site in the brain (Kapur et al.,1979), hence this site may represent a central α_1 receptor. The receptor which binds ^{3}H-clonidine is also present in rabbit duodenum, where its affinity for α agonists and antagonists correlates with their effects on presynaptic receptors in the rat vas deferens. However, no similar site has been found in the vas deferens, which is capable of binding ^{3}H-clonidine. This is unusual in view of the well-documented effects of α_2 agonists in this tissue.

The ^{3}H-clonidine binding site in the rat cortex is not reduced by 6-OHDA treatment, thus appears to be postsynaptic. The ^{3}H-WB4101 binding site is also located postsynaptically, thus none of the receptors studied by this method so far appear to correspond to the presynaptic α_2 receptor found to exist by pharmacological studies. However, the ^{3}H-clonidine binding site dose have similar pharmacological characteristics to peripheral α_2 receptors, while α antagonists display similar selectivity between the two receptors as has been found for α_1 and α_2 receptors peripherally (U'Pritchard et al.,1978; Greengrass & Bremner,1979).

Thus the presence of presynaptic α_2 receptors on central neurones has not yet been established by binding studies. However despite this, the method has produced much more accurate information on the effects of drugs on central α_1 and α_2 receptors than has been obtained by the study of SI release

of NA. The lack of identification of α receptors located presynaptically suggests that the method studied here may not, in fact, involve such receptors and that they may not be present on central neurones. However, receptors are present which are able to modify the release of NA. These receptors appear to be of the α_2 type, whether located pre- or postsynaptically; and hence behavioural effects of drugs which act at these receptors may be attributed to effects on NA release.

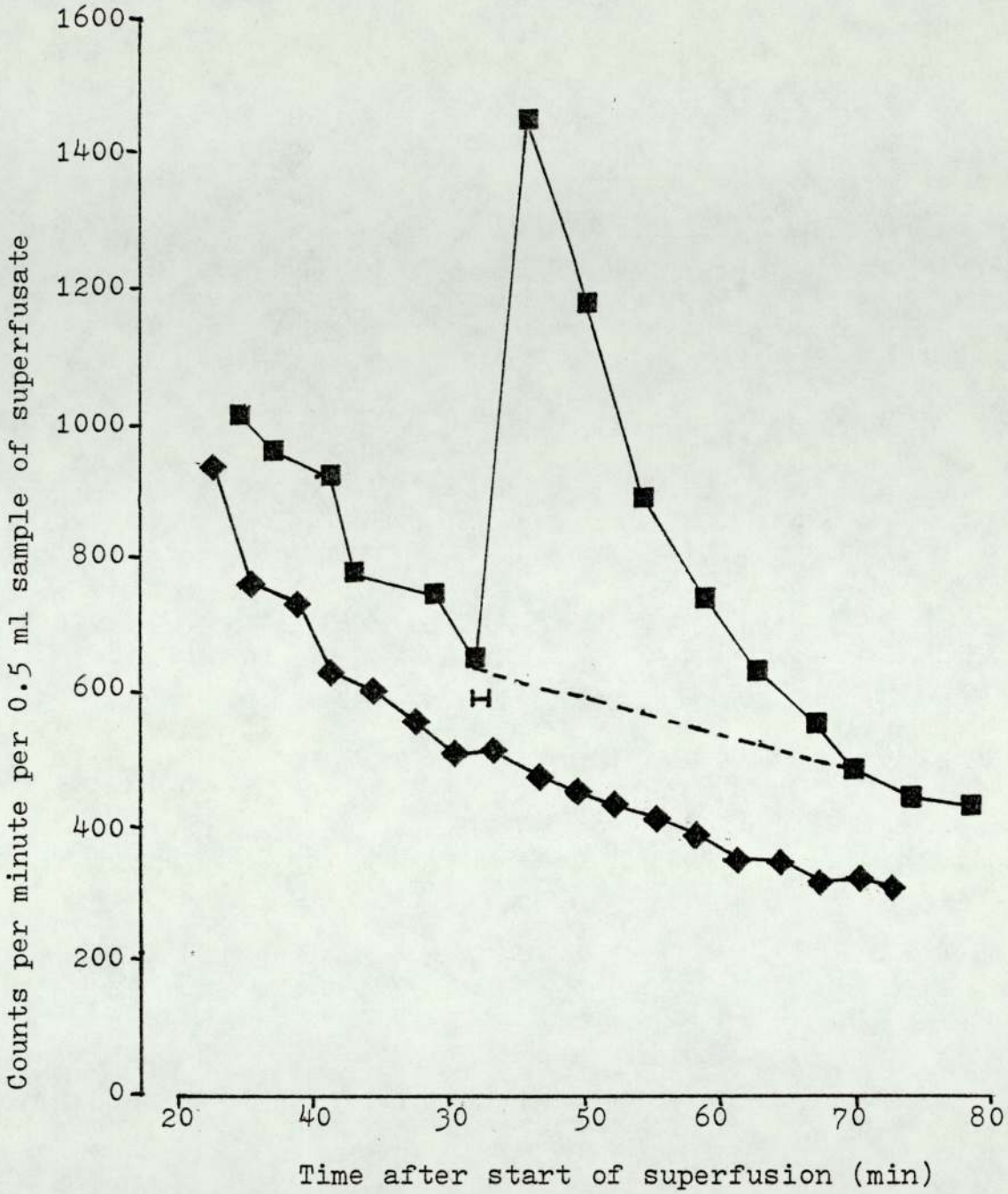


Fig 2.1. Tritium overflow from superfused rat cortical slices pre-incubated with ^{3}H-NA 10^{-7}M.

(◆) - spontaneous overflow; (■) - total overflow from slices stimulated for a period of 2 minutes (⊢——⊣); (- - - -) - estimated tritium overflow from stimulated slices.

DRUG	CONCENTRATION (M)	S_1/S_2 * Mean ± SEM	n	SIGNIFICANCE + LEVEL
None (control)	-	0.946 ± 0.043	19	N.S.
Clonidine	10^{-8}	0.92 ± 0.10	10	N.S.
	3×10^{-8}	0.90 ± 0.17	14	0.05
	10^{-7}	0.78 ± 0.14	10	0.05
Guanfacin	4.065×10^{-8}	0.81 ± 0.07	10	0.01
	1.22×10^{-7}	0.82 ± 0.09	8	0.025
	4.065×10^{-7}	1.02 ± 0.10	6	N.S.
Methoxamine	5.3×10^{-6}	1.13 ± 0.125	8	N.S.

Table 2.1. The effect of α agonists on the stimulation-induced overflow of ^{3}H-NA from superfused rat brain slices.

* ratio of the increase in tritium overflow after the first and second stimulations; the drug was added to the superfusing medium 20 minutes before the second stimulation.

+ significant differences from preparations in which no drug was added were calculated using a 't' test for paired samples.

DRUG	CONCENTRATION (M)	S_1/S_2 * Mean ± SEM	n	SIGNIFICANCE + LEVEL
None (control)	-	0.946 ± 0.043	19	N.S.
Yohimbine	10^{-7}	1.57 ± 0.19	11	0.01
	3×10^{-7}	1.42 ± 0.20	11	N.S.
	10^{-6}	1.625 ± 0.22	12	0.0025
Piperoxane	3.718×10^{-8}	1.15 ± 0.11	12	N.S.
	1.115×10^{-7}	1.23 ± 0.10	10	N.S.
	3.718×10^{-7}	1.43 ± 0.18	10	0.0125
Prazosin	10^{-7}	1.094 ± 0.10	7	N.S.
Phentolamine	10^{-7}	1.465 ± 0.26	10	N.S.

Table 2.2. The effect of α antagonists on the stimulation-induced overflow of ^{3}H-NA from superfused rat brain slices.

* ratio of the increase in tritium overflow after the first and second stimulations; the drug was added to the superfusing medium 20 minutes before the second stimulation.

+ significant differences from preparations in which no drug was added were calculated using a 't' test for paired samples.

CHAPTER 3

EFFECTS OF α AGONISTS AND ANTAGONISTS ON THE GENERAL BEHAVIOUR OF MICE

CHAPTER 3.

INTRODUCTION

The drugs which have been investigated for their effects on α_1 and α_2 receptors both peripherally and centrally *in vitro* were used in mice *in vivo* to attempt to distinguish behavioural effects which may be due to stimulation of these receptors. The general behavioural and autonomic effects of the drugs were observed using a modified form of the technique described by Irwin (1968) in an open field situation after subcutaneous (s.c.) or intracerebroventricular (i.c.v.) injection in mice of the T.O. strain.

Clonidine had been found to have actions on both α_1 and α_2 receptors (Chapter 1), hence was chosen for further study, along with methoxamine, which showed only α_1 activity. It was hoped that by this means, a tentative division could be made as to which behavioural effects of clonidine could be ascribed to actions at α_2 and which at α_1 receptors.

1. The effect of clonidine.

The behavioural effects of clonidine over the dose range 0.01 to 5.0 mg/kg s.c. were studied. It was found possible to divide all the measures affected by clonidine into three groups, as follows:

(i) effects on arousal, including reflexes and nociceptive responses

(ii) effects on activity

(iii) peripheral autonomic effects.

The effect of three doses of clonidine on these three groups of parameters is shown in Fig 2.1.

Low doses of clonidine (0.01 to 0.1 mg/kg) caused an increase in both arousal and activity with dose-dependent peripheral autonomic effects. The animals appeared fearful several minutes after injection, with increased startle and touch responses, increased reaction to tail pinch and flexor reflex testing and hyperalertness. Increased spontaneous locomotor activity was also present after 0.01 mg/kg, the animals being highly reactive and resisting handling (see Fig 3.1.a).

A very high dose of clonidine (5.0 mg/kg) also produced hyperreactivity, characterised by increased startle and touch responses, fearfulness and an increased body position. This however was accompanied by decreased activity, since the animals tended to 'freeze', both spontaneously and also on approach of a novel object or on handling. The pinna reflex was completely abolished at this dose and the corneal reflex depressed. Locomotion appeared abnormal due to hind limb splay; and peripheral sympathetic stimulation was marked with piloerection, pupil dilation and lacrimation (see Fig 2.1.c). The animals also had hypothermia and exophthalmos. Occasionally tremor and straubtail were seen, as was spontaneous maintainence

of abnormal postures resembling those seen in animals treated with haloperidol. The hyperreactivity was present for two hours, when the piloerection and exophthalmos also disappeared. The pinna reflex returned to normal after 220 minutes, as did the body temperature, corneal reflex and pupil dilation.

Intermediate doses of clonidine (0.5 to 1.0 mg/kg) produced a different syndrome, characterised by decreased alertness and reduced responses to startle, touch and tail-pinch and decreased spontaneous activity (Fig 2.1.b). Pinna, corneal and flexor reflexes were also inhibited by these doses and both hind and fore limb splay were present, resulting in abnormal rolling gait and decreased position of the ventral abdomen on locomotion. The mice tended to sit together in a characteristic hunched posture with marked piloerection and exophthalmos and slow, deep respiration. Occasional mice also had pupil dialtion, vasodilatation and/or hypothermia. Spontaneous abnormal posturing also occured in several mice and an increased catalepsy intensity compared to controls. This syndrome lasted for 2½ to 3 hours.

The two syndromes produced by different doses of clonidine could be differentiated mainly by the effects on arousal and nociceptive responses. Increased arousal with either an increase or decrease in activity was termed 'hyper-reactivity', whilst decreased arousal and activity may be termed 'sedation'. Most of the drugs subsequently investigated were found to produce one or the other of these syndromes.

2. The effect of other α-adrenoceptor agonists.

Noradrenaline

After s.c. injection NA 0.5 to 5.0 mg/kg produced a dose-dependent syndrome similar to the 'hyperreactivity' seen with clonidine 5.0 mg/kg. The behavioural effects of a dose of

2.5 mg/kg NA are shown in Fig 2.2.a. The animals were easily startled and had increased reflexes and nociceptive responses. Increased spontaneous activity, especially grooming was seen after 0.5 mg/kg, which appeared stereotyped after higher doses, consisting of short bursts of activity followed by 'freezing'. The animals resisted handling, but once held in a scruff grip tended to 'freeze' and not struggle. Peripheral sympathomimetic signs such as salivation and piloerection were marked at the higher doses, respiration was increased and hind limb splay was present. Again spontaneous maintainence of abnormal postures was seen, especially at 5.0 mg/kg.

Injection of NA directly into the cerebral ventricles at a dose of 5µg did not cause such marked hyperreactivity. Although injection of saline by this route produced a mild hyperreactivity, further slight increases in touch and tail-pinch responses were seen after NA. The pinna reflex was markedly inhibited, as was the corneal reflex in one or two animals. Respiration was markedly increased and hind limb splay and hypothermia were seen occasionally. Spontaneous posturing occured in two out of nine animals.

Methoxamine

Subcutaneous injection of methoxamine 2.5 to 10.0 mg/kg produced a marked dose-dependent 'hyperreactivity' syndrome with decreased spontaneous activity and prominent signs of peripheral sympathetic stimulation (Fig 2.2.b). Occasionally head twitches and straubtail were also observed, as was spontaneous abnormal posturing.

After i.c.v. injection of methoxamine over the dose range 0.5 to 20.0µg, marked 'hyperreactivity' was again seen, for 3 to 15 minutes after injection. Flexor and pinna reflexes were more brisk than controls; startle, touch and

tail-pinch responses were all increased; the animals appeared fearful and tended to 'freeze' both on startling and handling and there was marked exophthalmos and occasionally Straubtail. After 15 minutes, the syndrome had diminished slightly, but increased nociceptive responses were still present even up to 160 minutes after injection. Head twitches and writhing were quite marked after 20.0µg, as was spontaneous abnormal posturing. Piloerection was present from 5 minutes after injection, although not as marked as after peripheral administration of the drug.

Oxymetazoline

Doses of 1.0 and 2.5 mg/kg given s.c. produced a hyperreactivity syndrome similar to that of methoxamine, with increased nociceptive responses, but decreased spontaneous activity and marked peripheral sympathetic stimulation (Fig 2.2.c). Abnormal posturing was present after both doses, but catalepsy was only present after the higher dose. This dose also produced hind limb splay and marked lacrimation. Head twitches and stereotyped activity consisting of head-searching and grooming were also seen.

This syndrome was not produced after i.c.v. injection of oxymetazoline at a dose of 5µg. Initially after injection, all mice, including saline-treated, appeared fearful, although the drug-treated animals were more alert and active. Piloerection was present in all mice, by 5 minutes after injection; and exophthalmos appeared by 20 minutes. The drug-treated animals were active but not hyper-reactive and had raised body position on locomotion, with a hunched posture typical of clonidine-treated animals when stationary. By 40 minutes after injection, a marked stereotyped syndrome had developed with constant forward, sideways or backwards locomotion, downward-directed head-searching and some gnawing.

No grooming was seen at any time. After 50 minutes, the animals were placed in fresh cages to test their reactivity to a novel environment. Saline-injected animals explored the cage thoroughly for approximately 5 minutes, rearing, sniffing and digging in the sawdust. Oxymetazoline-treated animals showed constant locomotion with side-to-side head movements which became more rapid. Hind limb splay was present, rendering the locomotion somewhat jerky and difficult and the body position was lowered. The activity did not appear to be exploratory in nature and was maintained for 15 minutes, after which time the animals were returned to the observation box. Peripheral sympathetic stimulation had decreased by 150 minutes after injection and the animals remained still in the box until disturbed, when the stereotyped locomotion began again, persisting for at least 10 minutes without ceasing. This syndrome was still present 4½ hours after drug administration, by which time it appeared to have become more severe. (The eyes were completely closed and the mice appeared to be unable to open them, due to excess lacrimation, hence they may have had little sense of direction by this time.)

Guanfacin

Low doses of guanfacin (0.1 to 0.2 mg/kg) produced a slight hyperreactivity syndrome, similar to that produced by low doses of clonidine, which gradually diminished after 60 minutes, progressing to a syndrome more typical of sedative doses of clonidine. Higher doses (0.5 to 5.0 mg/kg) produced this sedative syndrome after only a transient initial hyperreactivity, which was not always present. The animals had decreased nociceptive responses and marked inhibition of pinna reflex, decreased alertness and activity. Piloerection, exophthalmos and hind limb splay were also present, as was the

typical clonidine-like hunched posture. Doses of 1.0 and 5.0 mg/kg also produced catalepsy. Peripheral sympathomimetic signs had disappeared by 3½ to 4 hours after injection, however the decreased activity and alertness was still present for up to 6 hours after 5.0 mg/kg.

Guanabenz

A similar picture to clonidine was again seen with this drug, a low dose producing mild hyperreactivity, but with a slight decrease in spontaneous activity; and higher doses (0.5 to 5.0 mg/kg) resulting in a dose-dependent sedative syndrome. The onset of the decreased nociception and activity was short as with clonidine, but the syndrome was of slightly longer duration, 3 to 4 hours.

3. The effect of α-adrenoceptor antagonists.

Yohimbine

After s.c. injection, yohimbine 0.2 to 5.0 mg/kg produced a marked 'hyperreactivity' (see Fig 3.3.a), characterised by increased startle, touch and tail-pinch responses, very brisk pinna and flexor reflexes, an increased body position and marked fearfulness. The animals resisted handling and tended to squeak and struggle when held in a scruff grip. At the lowest dose, there was an increase in all spontaneous activity, especially grooming, which became more stereotyped at higher doses. Head twitches, writhing and straubtail occured in several animals; and abnormal spontaneous posturing was quite marked. Exophthalmos and vasodilatation were present after all doses and slight piloerection also after 2.5 and 5.0 mg/kg. The animals were highly reactive to all types of noise or movement and tended to startle each other, setting off a type of 'chain reaction'. They also appeared to startle

when no external stimulus was present.

Piperoxane

Doses of 1.0 to 20.0 mg/kg produced a hyperreactivity syndrome very similar to that seen with yohimbine (Fig 3.3.b). Again activity was stereotyped, consisting of grooming and sniffing; posturing was not as marked however. The animals tended to close their eyes whilst in the observation box, but this was not true ptosis, since on transfer to a novel environment, animals appeared alert with eyes open.

Prazosin

In doses of 1.0 to 5.0 mg/kg, prazosin caused a marked depression of activity and alertness (Fig 3.3.c), although nociceptive responses were not decreased and reflexes were not inhibited at any dose. The animals had ptosis and vasodilatation; and remained still unless forced to move, when the gait was very slow, with the ventral abdomen touching the cage bottom.

Phentolamine

Peripheral administration of phentolamine at a dose of 5.0 mg/kg had very little effect on behaviour, although slight increases in startle and touch responses were seen. When injected centrally at a dose of 5ug, decreases in alertness and activity occured, along with decreased nociceptive responses The animals also had ptosis, piloerection and hypothermia. Catalepsy was quite marked and some writhing was present.

4. The effect of antagonists on the behavioural syndromes produced by clonidine 0.5 mg/kg and methoxamine 5.0 mg/kg.

The effect of yohimbine 5.0 mg/kg, piperoxane 10.0 mg/kg and prazosin 2.5 mg/kg on the sedative syndrome produced by clonidine 0.5 mg/kg and the hyperreactivity produced by methoxamine 5.0 mg/kg was investigated by pre-treating both groups with the agonist 15 minutes before administration of the antagonist or saline.

Clonidine consistently produced a decrease in arousal and activity, with marked signs of peripheral sympathetic activity. Yohimbine and piperoxane both caused the restoration of all nociceptive responses and reflexes, decreased the hind limb splay and increased alertness and activity. Prazosin led to a further decrease in alertness, touch response and body position; and potentiated clonidine-induced hypothermia. Passivity was also increased. Piloerection was however, reduced; and the inhibition of nociceptive reflexes slightly antagonised. Ptosis and vasodilatation were produced by the drug combination.

The hyperreactivity produced by methoxamine was reversed by prazosin, as was exophthalmos and to a slight extent hypothermia. Yohimbine and piperoxane did not antagonise the effects of methoxamine; in fact, yohimbine slightly potentiated startle and touch responses.

DISCUSSION

The behavioural effects of the drugs used were divided into three groups and a particular dose of a drug was said to produce either a 'hyperreactive' or a 'sedative' syndrome, on the basis of its effects on arousal and activity. Clonidine, guanfacin and guanabenz all produced qualitatively similar behavioural effects apart from the hyperreactivity due to a high dose of clonidine which was not seen with the other two drugs. These three drugs have all been found to have a selective action on α_2 receptors peripherally (Chapter 1 and Doxey, personal communication), although clonidine is more potent as an agonist of α_1 receptors than the other two.

It would seem possible therefore, that both the slight hyperreactivity and subsequent sedation seen with all three drugs may be due to stimulation of central α_2 receptors. The hyperreactivity seen with low doses may also,however, be related to a stimulatory action of the drugs on α_1 receptors, since α_1 agonists also produce hyperreactivity (see below). Possibly access to the α_1 receptors which mediate this response may be easier than to the α_2 receptors involved in the sedative effect, thus sedation would mask the hyperreactivity as drug concentrations increase. The time course of the behavioural effects of guanfacin lend support to this suggestion.

Both yohimbine and piperoxane, which are selective α_2 receptor antagonists (Chapter 1), were able to reverse the main features of the sedative syndrome due to clonidine 0.5 mg/kg, whilst prazosin, which peripherally was found to have no action on α_2 receptors, did not. Hence this finding supports the suggestion that the sedative actions of clonidine, guanfacin and guanabenz may be due to α_2 receptor stimulation. Delbarre and Schmitt(1973) found clonidine to

increase chloral hydrate sleeping time in mice, an effect which was prevented by yohimbine, but not by PBZ. Since the latter drug does not penetrate the CNS well, clonidine's action would appear to be centrally mediated. Further investigation of the sedative effect of clonidine was undertaken in an attempt to confirm the involvement of central α_2 receptors (see Chapter 4).

The behavioural syndrome which was produced by 5.0 mg/kg of clonidine may have been due to activation of central α_1 receptors, since similar effects were seen after methoxamine, which has little activity on α_2 receptors peripherally. Clonidine has been shown to worsen psychotic behaviour in schizophrenics at a dose of 900 to 2100 µg/day (Simpson et al.,1967) and produce irritability (Laverty & Taylor,1969). Extremely high doses (up to 50mg/kg) produce aggressive behaviour in mice (Morpugo,1968), as does chronic treatment in rats (Laverty & Taylor,1969). This was found to be inhibited by neuroleptics, but not by phentolamine at a dose of 20.0 mg/kg, hence the involvement of α receptors in aggression was excluded by this author. However, in view of the dose of clonidine used and the poor ability of phentolamine to penetrate the CNS, there is a possibility that α_1 receptor stimulation may be the cause of the aggression.

The receptors involved in the peripheral effects of clonidine which produce piloerection, lachrimation and pupil dilation are likely to be of the postsynaptic α_1 type, since peripheral injection of both methoxamine and NA also produced similar effects. In addition, these effects were reversed by prazosin, but not by piperoxane or yohimbine. Guanfacin and guanabenz also produced peripheral sympathomimetic activation, thus these drugs appear more able to activate

peripheral than central α_1 receptors.

All of the agonists which have marked actions on α_1 receptors peripherally produced a similar hyperreactive syndrome after s.c. injection. Alpha$_1$ receptors appear to be involved in this syndrome, since prazosin, but not yohimbine or piperoxane, reversed the behavioural effects of methoxamine. The syndrome was more marked after central injection of methoxamine, but this was not the case with NA. It is possible that the increased arousal seen after these drugs is mediated peripherally, since NA does not pass into the brain and oxymetazoline produced a different syndrome on central injection. Peripheral sympathetic activation may be producing a 'fearlike' sensation because of tachycardia and piloerection. Thus the sensation of symptoms which normally occur in fear-provoking situations may lead to behaviour such as would result from fear. Some effects, such as head-twitching after i.c.v. methoxamine and inhibition of the pinna reflex after i.c.v. NA, are likely to be mediated centrally, however. The stereotyped syndrome produced by i.c.v. oxymetazoline may not involve central α receptors, since none of the other agonists produced similar effects. Its delayed onset would tend to suggest that it may be due to a metabolite which may activate DA receptors, since apomorphine, a DA stimulant, also produces stereotypy (Randrup & Munkvad,1970).

Yohimbine and piperoxane both produced a marked hyperreactive syndrome with some stereotypy, spontaneous posturing and head twitches, hence may be acting on dopaminergic or serotonergic in addition to noradrenergic neurones. Alternatively, NA may modulate the activity of other types of neurones; and these drugs may therefore affect the

activity of other transmitters. Yohimbine is known to increase both brain and intestinal 5-HT concentrations in rats (Papeschi et al.,1971), hence the head-twitching and writhing could be due to these effects. Clonidine reduces 5-HT turnover (Anden et al.,1970) and depresses raphe neuronal firing (Svensson et al.,1975), hence it is possible that both of these drugs may affect 5-HT activity indirectly via α receptors. Further investigations of a central noradrenergic-serotonergic interaction have thus been carried out (Chapter 7).

The stereotyped behaviour produced by yohimbine and piperoxane may be due to a direct activation of central DA receptors, although this seems unlikely, since abnormal posturing and catalepsy were also produced, which normally result from blockade of these receptors. Papeschi (1974), however, found that yohimbine did not block stereotypy produced by l-DOPA plus a peripheral decarboxylase inhibtor, suggesting that it does not block DA receptors. Again these effects may be the result of an interaction between central noradrenergic and dopaminergic neurones; drugs acting at noradrenergic receptors modulating the activity of dopaminergic neurones. An investigation of this possibility using haloperidol-induced catalepsy was undertaken (Chapter 8).

The hyperreactivity produced by yohimbine and piperoxane may be analogous to anxiety-inducing effects of these drugs in man (Holmberg & Gershon,1961; Redmond, 1977). Since prazosin produces no such syndrome, α_2 receptors may mediate this effect. Phentolamine produced only a slight effect when given peripherally. This may be suggestive of a central effect of yohimbine and piperoxane, since phentolamine only penetrates the CNS poorly. It may, on the other hand, reflect the lack of selectivity of

for either α_1 or α_2 receptors. Since α_1 stimulants may be able to produce similar effects by peripheral actions (see above), it is possible that these drugs also act peripherally. Blockade of presynaptic α_2 receptors in the heart, although shown to be ineffective in the pithed rat (Drew,1976), may possibly lead to tachycardia in the mouse, leading to the possible sensation of anxiety. Piloerection was also present after high doses of yohimbine, probably due to increased peripheral NA release. However, central effects do appear to be present after yohimbine and piperoxane; and an attempt to quantify the hyperreactivity by the measurement of a fear-motivated behaviour was carried out. The possible peripheral influence on such behaviour was also studied (Chapter 9).

Prazosin and i.c.v. phentolamine, which were both shown to be potent α_1 receptor antagonists using peripheral tissues (Chapter 1), produced a sedative syndrome in mice. This was most marked with prazosin, although there was no decrease in reflexes, suggesting no 'neurological' impairment. The lack of peripheral activity of phentolamine suggests the action is central, although this is uncertain (see above). It was not possible to administer prazosin centrally, since it is insoluble in water, thus a peripheral action can not be ruled out. However, the sedative side effects of some anti-depressants has been postulated to be due to blockade of α_1 receptors centrally (Chapter1), hence prazosin may produce similar effects by the same mechanism.

The drugs investigated were found to have a wide range of effects on behaviour, which appeared to be tentatively divisible into those due to actions on α_1 or α_2 receptors. Several of the most marked effects were then chosen for closer study in an attempt to confirm these suggestions.

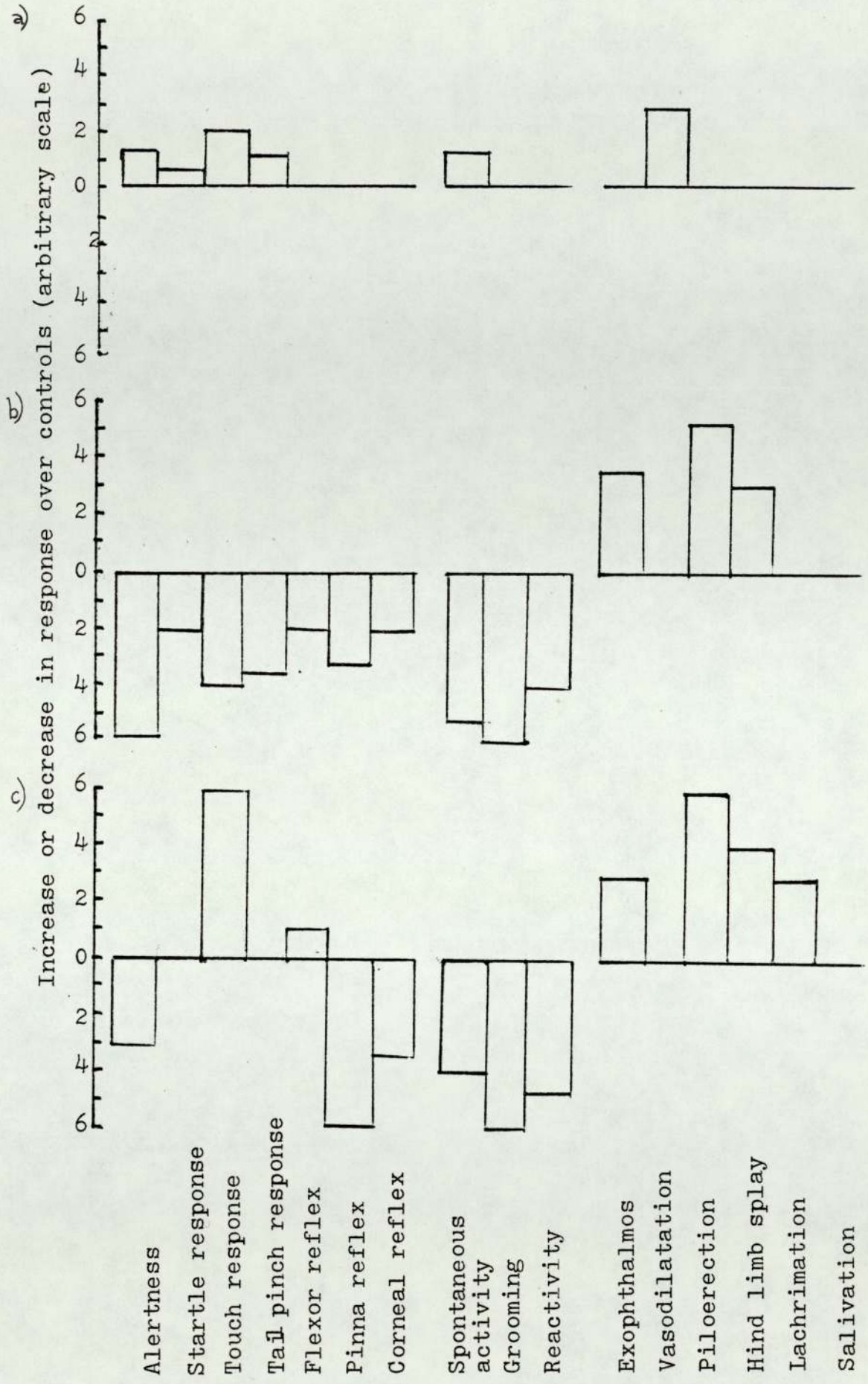


Fig 3.1. Behavioural effects of clonidine a) 0.01 mg/kg, b) 0.5 mg/kg c) 5.0 mg/kg.

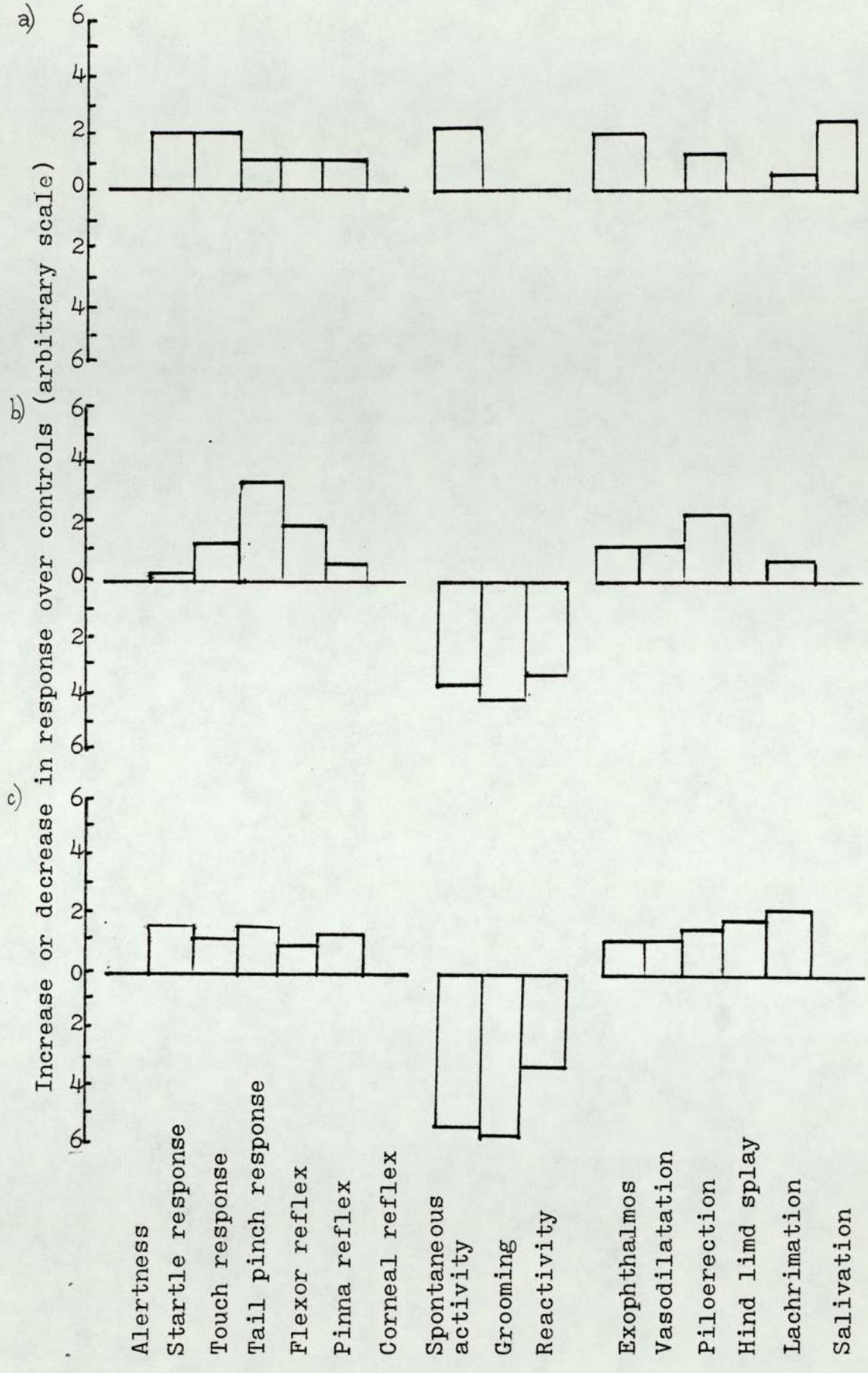


Fig 3.2. Behavioural effects of a) NA 2.5 mg/kg, b) methoxamine 5.0 mg/kg, c) oxymetazoline 2.5 mg/kg.

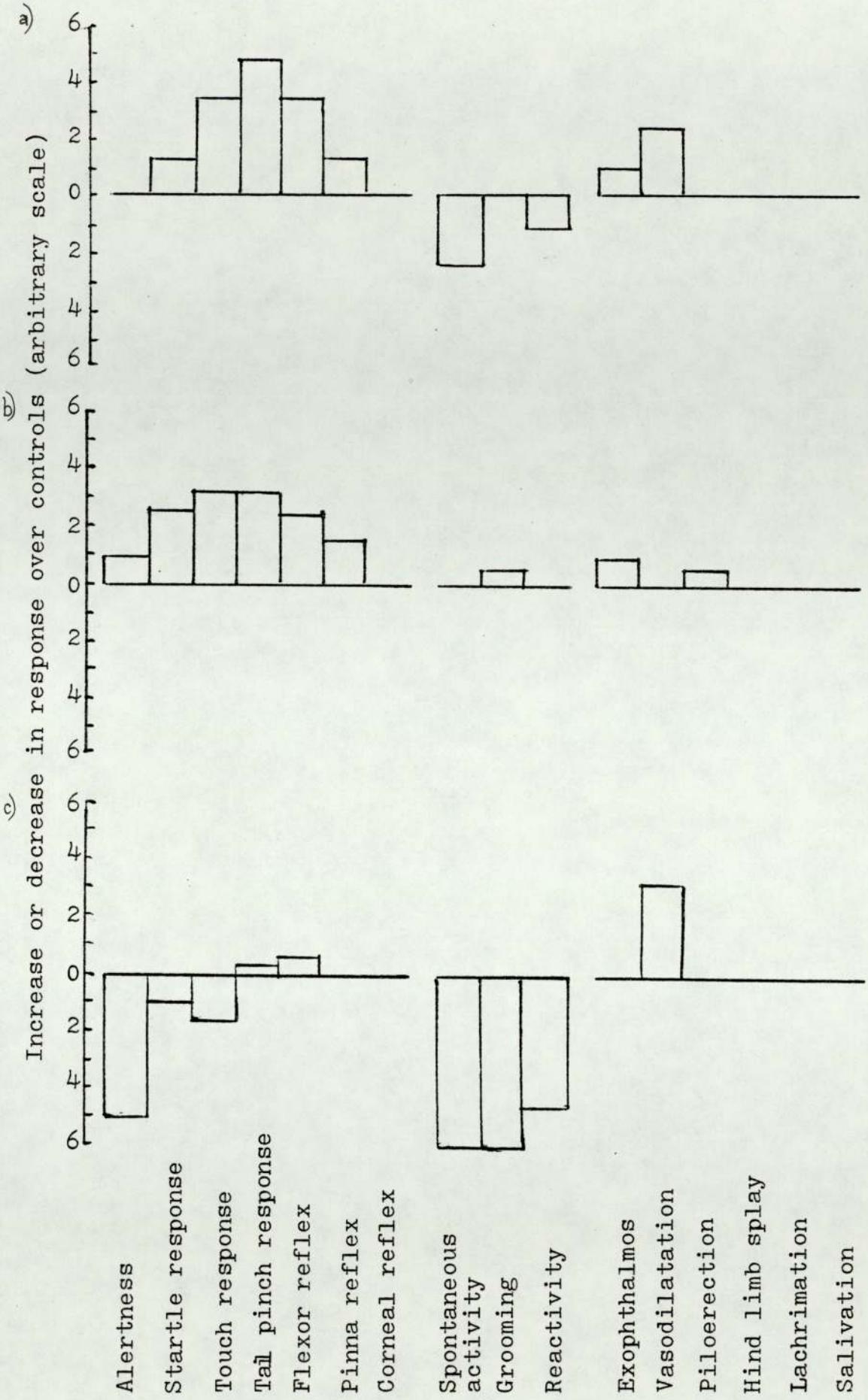


Fig 3.3. Behavioural effects of a) yohimbine 1.0 mg/kg, b) piperoxane 1.0 mg/kg, c) prazosin 2.5 mg/kg.

CHAPTER 4

ASSESSMENT OF THE SEDATIVE EFFECTS OF α AGONISTS AND ANTAGONISTS

CHAPTER 4.

INTRODUCTION

Central noradrenergic systems originating in the pontine reticular formation are known to play an important role in the state of arousal (Moruzzi,1954). Noradrenaline is involved in the behavioural excitation produced by amphetamine (Carlsson,1970) and the central injection of noradrenergic stimulants alone may also lead to excitation (see Chapter 3). Clonidine, however, has been found to produce sedation in animals and man (Delbarre & Schmitt,1971;1973; Autret et al.,1977) and sleep in chicks (Delbarre & Schmitt,1971; Fugner,1971). Both noradrenergic and serotonergic neurones may be involved in sleep (for review, see Morgane & Sterne, 1978) and since locus coeruleus (L.C.) lesions lead to a marked increase in sleep time (Jones et al.,1973), clonidine's sedative effect may be the result of inhibition of L.C. noradrenergic neurones (Cedarbaum & Aghajanian,1976).

Several workers have studied the sedative effect of clonidine by measuring sleeping time produced by a hypnotic in mice and/or sleep produced by clonidine alone in chicks. Alpha receptors are involved in this sedation, since α adrenoceptor antagonists such as yohimbine, phentolamine and piperoxane have been shown to prevent the action of clonidine (Delbarre & Schmitt,1973; Holman et al.,1971; Cavero & Roach,1978). Antagonists which act at α_1 receptors, however, have been shown to prolong sleeping time (Delbarre & Schmitt,1971; Cavero & Roach,1978), thus suggesting that clonidine sedation may be mediated via α_2 receptors.

The present study was undertaken to investigate the sedative effects of a wider range of agonists varying in their selectivity for α_1 or α_2 receptors, both in order to confirm the type of receptor involved and to assess the poss-

ibility of measuring sedation during the screening of new -adrenoceptor agonists. Various objective methods have been used to assess sedation, several of which are studied here. Measurement of locomotor activity using automatic activity meters has been used as an assessment of sedation, although it does not differentiate between effects on alertness and motor effects. The use of an accelerating Rotarod suffers from similar problems; drug effects on muscle tone and co-ordination being indistinguishable from effects on alertness and arousal. The use of young chicks, which lack a blood brain barrier, to assess sedation by loss of righting reflex would appear to be a good method of quantifying sedative effects. However, extrapolation to man could be difficult; and also, drugs which increase arousal can not be tested by this method. Similar methods using mice already sedated with another drug such as chloral hydrate may be more useful, although loss of righting reflex may not always be associated with a state of sleep, leading to false results. The most accurate method of measuring sleep is to study EEG patterns. However, as a routine method of screening for sedative side effects it has many obvious disadvantages. An observational method of studying sedative effects has also been investigated. This was included because of the marked behavioural difference which was found to exist between clonidine- and methoxamine-treated animals.

1. Assessment of the sedative properties of α-adrenoceptor agonists and antagonists by changes in the duration of sleep produced by chloral hydrate.

Chloral hydrate 300 mg/kg i.p. caused mice to become ataxic and appear sedated within 1 to 2 minutes of injection. When turned onto their backs, the majority of the animals had lost their righting reflex within 5 minutes. The duration of the loss was in general 20 to 40 minutes, although there was sometimes considerable variation both within the group and from day to day. In an attempt to reduce this variation, experiments were performed at the same time each day, by the same method, and animals all had a similar history prior to experimentation. Animals were all naive and were kept in the experimental room for at least three days before use. The variation in sleeping time was not found to be dependent on the day of the week.

1.1. Effects of α-adrenoceptor agonists.

Clonidine, guanfacin, guanabenz and oxymetazoline all produced a dose-dependent increase in the duration of loss of righting reflex induced by chloral hydrate. In order to compare the potencies of these drugs as sedatives, dose-response curves have been produced by expressing the increased sleeping time as a percentage increase over control sleeping times (Fig 4.1.). The dose response curves did not differ from parallel, indicating a possible similarity in the mechanism of the effect. The order of potency for the potentiation of sleeping time was found to be:

clonidine > guanabenz = guanfacin > oxymetazoline ≫ methoxamine.

Guanabanz and guanfacin were both 4.5 times less potent than clonidine in doubling sleeping time (100% increase, Fig 4.1.),

while oxymetazoline was 42.5 and methoxamine over 500 times less potent. The latter drug produced only 38% potentiation at a dose of 10.0 mg/kg, lower doses being ineffective.

Injection of saline into the cerebral ventricles caused a marked increase in sleeping time over that produced after i.p. injection of saline (Fig.4.2.). Oxymetazoline potentiated this by only 25% at a dose of 5µg i.c.v., 10µg having no effect. Methoxamine in doses of 5,10 and 20µg i.c.v. had no effect on chloral hydrate sleeping time.

1.2. Effect of α-adrenoceptor antagonists.

Drugs were also studied for their effects on a lower dose of chloral hydrate during subsequent investigations into the reversal of potentiated sleeping time. Yohimbine potentiated the effect of chloral hydrate 300mg/kg at 1.0 and 2.5 mg/kg (Fig 4.3.), lower doses having no effect. However, when tested with 250 mg/kg of chloral hydrate, doses of 0.25, 0.5 and 2.5 mg/kg increased the duration of sleep (fig 4.5.). Similar inconsistencies arose between the effects of prazosin and piperoxane on sleeping time produced by different doses of chloral. Piperoxane had no effect at doses of 2.5 to 10.0 mg/kg on the higher dose (Fig 4.3.), but produced slight potentiation at much lower doses (0.1 to 1.0 mg/kg) in combination with chloral hydrate at 250 mg/kg (Figs 4.5. and 4.6.). Prazosin had a marked effect on sleeping time produced by chloral hydrate 300 mg/kg at doses of 0.5 to 2.5 mg/kg(Fig 4.3.), but this effect was much reduced at the lower dose of chloral hydrate.(Figs 4.5. and 4.6.).

It was noted that there was still considerable variation in the duration of sleep produced by chloral hydrate 250 mg/kg, some animals failing to lose their righting reflex at all. The effect of agonists on this dose of chloral hydrate

was also markedly inconsistent, hence the results may not be of any great value.

The effect of antagonists on the sleeping time due to chloral hydrate 300 mg/kg was one of potentiation, the order of potency being:

prazosin > yohimbine > piperoxane.

However, in view of the above-mentioned results obtained using a lower dose of the hypnotic, this potency order can not be taken as a generalised effect for other doses of chloral hydrate or other hypnotic drugs.

1.3. Effect of α-adrenoceptor antagonists on the potentiation of sleeping time produced by clonidine and guanabenz.

Initial experiments suggested that piperoxane may lead to further potentiation of the increased sleeping time produced by the two agonists (for example, see Fig 4.4.). The doses of both chloral hydrate and the α agonists were therefore reduced to decrease the duration of the experiment. Lower doses of piperoxane were also used in further experiments as it was thought to be less likely that such doses may potentiate sleeping time themselves. Doses of 0.1 to 1.0 mg/kg had no effect on the potentiation of sleeping time produced by clonidine 0.1 mg/kg (Fib 4.5.b), although 0.5 mg/kg did reduce the effect of guanabenz 0.25 mg/kg (Fig 5.6.b). Yohimbine had a slight, but not statistically significant, inhibitory effect on clonidine potentiation of sleeping time, which was most marked at 0.25 mg/kg (Fig 4.5,a), This dose had no effect on potentiation by guanabenz, (Fig 4.6.a), but a lower dose 0.1 mg/kg significantly reduced this potentiation.Prazosin had no effect on the increase in sleeping time by either clonidine or guanabenz at a dose of 0.5mg/kg, whereas 1.0 mg/kg caused a marked further potentiation of both drugs (Figs4.5.c and 4.6.c).

2. The effect of α-adrenoceptor agonists and antagonists on the ability of mice to remain on an accelerating Rotarod.

The accelerating Rotarod has been used by Drew et al.,(1979) to provide an objective measure of sedation, hence the method was investigated here. Mice have previously been found to show some adaptation to the Rotarod, hence were tested three times before treatment in order to minimise this effect. All figures show the third pretreatment test for comparison.

2.1. Effect of α-adrenoceptor agonists.

Clonidine (0.25 to 1.0 mg/kg) given s.c. 20 minutes before the following test, produced a dose-dependent decrease in the time mice remained on the Rotarod (Fig 4.7.). The effect of a dose of 0.25 mg/kg lasted for only 60 minutes, but the higher doses were still effective at 120 minutes. Guanabenz had no effect on time on the Rotarod at a dose of 0.5 mg/kg (Fig 4.8.). The group of mice injected with 1.0 mg/kg of guanabenz had an abnormally low ability to remain on the Rotarod before drug treatment. However, the drug did inhibit the continuing increase which normally occured after saline injection; and, in fact, further depressed the time at 40 to 120 minutes after injection.

Neither methoxamine (5.0 and 10.0 mg/kg) nor oxymetazoline (2.5 and 5.0 mg/kg) had any effect on time on the Rotarod when injected s.c. Intracerebroventricular injection of saline decreased the time on the Rotarod for at least 90 minutes after injection (Fig 4.9.). Methoxamine, when administered i.c.v. at a dose of 5μg, had no significant effect on this decreased time. A higher dose, 10μg, however, further depressed the time at 20, 40 and 60 minutes after injection (Fig 4.10.).

2.2. Effect of α-adrenoceptor antagonists.

Yohimbine 0.5 mg/kg decreased the time mice remained on the Rotarod (Fig 4.11.). The effect was not very marked, but was significant at 60 and 90 minutes after injection. Piperoxane had no significant effect at 0.5 mg/kg (Fig 4. 11), but a dose of 2.5 mg/kg had a similar effect to yohimbine, decreasing the time at 60 and 90 minutes. Prazosin (0.5 mg/kg) produced a marked decrease only at 40 minutes after injection, animals having returned to control times by 60 minutes (Fig 4.11). A higher dose (1.0 mg/kg) however, depressed the time mice remained on the Rotarod for at least 120 minutes.

2.3. Effect of antagonists on the decrease in time on the Rotarod produced by clonidine.

Pretreatment of animals with yohimbine 0.5 mg/kg restored their ability to remain on the Rotarod after clonidine 0.25 mg/kg (Fig 4.12). Piperoxane 0.5 and 1.0 mg/kg was ineffective in this respect, as was prazosin 0.5 mg/kg. The latter drug further decreased time at 90 and 120 minutes after injection of clonidine, when animals treated with clonidine alone had returned to control times (Fig 4.13.).

3. Assessment of the sedative effects of α -adrenergic agonists and antagonists by observation.

This method was modified from that of Drew et al.,(1979) to include observation of sedation and arousal. Saline, when administered either s.c. or i.c.v., gave a score midway between the minimum (0 - extreme sedation) amd maximum (36 - extreme arousal).

3.1. Effect of α-adrenoceptor agonists.

Clonidine 0.1 to 0.5 mg/kg produced a gradual decrease in the total activation score (Table 4.1.), though this was not very marked (for comparison, see Table 9.1. for diazepam). The decrease in the total score due to clonidine was mainly due to a decrease in activity and transfer arousal, although body position was also decreased. This was somewhat compensated for by a slight increase in muscle tone at the two lower doses and a decrease in ptosis at 0.25 and 0.5 mg/kg. Guanfacin 1.0 mg/kg produced a total score similar to that of clonidine 0.25 mg/kg, suggesting that it is approximately 4 times less sedative than clonidine. These animals did exhibit ptosis and were also less active, although transfer arousal was not markedly reduced. Muscle tone was again increased, even more so than by clonidine. Guanabenz 1.0 mg/kg produced a total score between that of clonidine 0.25 and 0.5 mg/kg, suggesting that it is approximately 3 times less potent as a sedative. Animals again had ptosis and also showed a decrease startle response and decreased activity.

Methoxamine, even at the very high dose tested, 25 mg/kg, had no sedative activity as shown by the total score. Body position and spontaneous activity were, however, reduced, as was transfer arousal, although visual placing and muscle tone were increased, suggesting an increase in arousal.

Injection of saline into the ventricles resulted in a total activation score similar to that produced by s.c. saline (Table 4.2.). Activity was increased in amount, however, while muscle tone, transfer arousal and body position were very slightly decreased. When administered by this route, methoxamine, again in a high dose, 15μg, had a slight sedative effect, but this was less than that produced by clonidine 0.1 mg/kg s.c. Examination of the data shows that the decrease in the total score was almost entirely due to an absence of activity, while body position and muscle tone were increased. NA (2.9μg) and oxymetazoline (3μg) both caused an increase in arousal as shown by the total score. Both drugs increased visual placing, body position and muscle tone, while NA also increased the startle response and transfer arousal.

3.2. Effect of α-adrenoceptor antagonists.

Yohimbine 1.0 and 2.5 mg/kg increased the total activation score, suggesting an increase in arousal, while 5.0 mg/kg slightly decreased the overall score (Table 4.3.). All doses increased the startle response and muscle tone, but decreased spontaneous activity and body position. Only 2.5 and 5.0 mg/kg caused a slight decrease in transfer arousal. Prazosin, on the other hand, produced a marked decrease in the total activation score at both 1.0 and 2.5 mg/kg. Every parameter tested was decreased by this drug, suggesting a strongly sedative effect.

DISCUSSION

Three methods have been used to assess the sedative properties of α-agonists and antagonists in mice; namely, potentiation of the duration of sleep induced by chloral hydrate, decreased ability to stay on an accelerating Rotarod and visual assessment of behavioural depression. The results obtained for the sedative effect of α-agonists were qualitatively similar over all three methods, the order of potency in each case being:

clonidine > guanabenz = guanfacin >> oxymetazoline >> methoxamine;

with potency ratios:

1 : 4.5 : 4.5 : 42.5 : > 1000.

Other workers have found guanabenz to be 10 times less sedative than clonidine (Baum et al.,1970), while guanfacin has been reported to be 100 times less sedative than clonidine (Scholtysik et al.,1975).

The order of potency of the agonists suggests that their sedative action may involve stimulation of α_2 receptors; since clonidine, guanabenz and guanfacin have all been found to act selectively on these receptors, while oxymetazoline, although having potent α_2 stimulant properties *in vitro*, displays predominantly α_1 effects *in vivo*; and methoxamine has little α_2 activity (see Chapter 1). These results are in general agreement with those of other workers, who found clonidine to be the most potent sedative of a range of imidazolines in chicks (Delbarre & Schmitt,1971), while oxymetazoline was 23 times less potent and NA and phenylephrine, which acts mainly on α_1 receptors, did not abolish the righting reflex.

Delbarre and Schmitt (1971) also found yohimbine

to antagonise the effect of clonidine in both chicks and mice, as did piperoxane and phentolamine. The effect of phentolamine has been shown by several other workers (Fugner, 1971; Holman et al.,1971; Cavero & Roach,1978). The latter workers also studied prazosin, which was found to further potentiate the effect of clonidine. The results obtained here are in agreement with this. Phenoxybenzamine which, like prazosin, has little effect on α_2 receptors, has also been shown to potentiate clonidine, and to increase sleeping time when given alone. (Delbarre & Schmitt,1971). Such an action was found for prazosin in these experiments and to a lesser extent for yohimbine. In the cases of prazosin and PBZ, this may be due to α_1 receptor blockade, which would have a similar overall effect to α_2 receptor stimulation i.e. a decrease in neuronal activity. Yohimbine may also be acting in this manner, although any such effect would probably be masked by α_2 receptor blockade, since this drug acts selectively on these receptors. However, yohimbine has also been found to affect 5-HT neurones (Papeschi et al., 1971), stimulation of which would tend to prolong sleeping time (see below).

The prolongation of chloral hydrate-induced sleep was found to be extremely variable. Similar variability was also apparent in the work of Delbarre and Schmitt. Possibly the use of young chicks which lack a blood brain barrier, thus avoiding the need for an additional drug to induce sleep may produce more consistent results, although decreases in the effect of chloral hydrate which may be seen using the method studied here, would not be seen in chicks.

Drew et al.,(1979) used the Rotarod to determine sedative effects and obtained a similar order of potency of

the α agonists to that found here, with clonidine and xylazine being much more potent than methoxamine and phenylephrine, which are both selective agonists of α_1 receptors. These workers did not find an inhibitory effect of yohimbine, piperoxane or prazosin on Rotarod performance, although thymoxamine and tolazoline which are, like prazosin, selective antagonists at α_1 receptors, did reduce performance. Yohimbine, piperoxane and phentolamine were able to prevent the effect of clonidine, while thymoxamine and prazosin were ineffective, thus further supporting the involvement of α_2 receptors in clonidine sedation. However, the method used may not be a suitable measure of sedation despite the agreement in results. Noradrenergic stimulation appears to increase muscle tone and several of the agonists have been found to produce hind limb splay (Chapter 3), both of which effects may affect the ability of an animal to remain on an accelerating Rotarod. Other central effects of these drugs, for instance on the cerebellum or on dopaminergic neuronal systems, may contribute to their effects on Rotarod performance. The similarity in the results obtained by this method and by potentiation of chloral hydrate sleeping time may thus not be due to effects on the same neuronal system, although the same class of receptors appears to be involved. This method is therefore theoretically less suitable for routine detection of sedative effects of α-adrenoceptor agonists, as it is not specific for effects on alertness, being more sensitive to muscle relaxants and drugs which produce ataxia.

The use of visual assessment of behavioural depression may be more useful, since parameters known to be associated with α receptor stimulation, such as exophthalmos, may be included and hence there is more likelihood of detecting this type of drug action among a series of

unknowns. The parameters measured in these studies were chosen from the wide range used by Irwin (1968) in his observational analysis of behaviour. The startle response and visual placing/tracking both reflect the level of arousal of the animal, but probably do not involve any emotional response of the animal towards the investigator. Drew et al., (1979) included passivity and depressed touch response, both of which may involve emotional responses such as fear and, in the case of touch response, the degree of motor co-ordination. This latter measurement is also implicit in the assessment of slow gait which Drew used. Here, both the speed and amount of spontaneous locomotor activity were measured, which makes some allowance for both lack of locomotion and the possibility of decreases in motor co-ordination and muscle tone, rather than sedation producing a slow gait.

In the present experiments, the muscle tone was measured separately, since it would be expected to decrease with sedation or relaxation and increase with apprehension of excitement. The results show, in fact, that α-receptor stimulation produced an increase in muscle tone, which may be due to such emotional effects. Ptosis and body position were measured both in the home cage and after transfer to a novel environment. This was because it had been previously observed that after treatment with certain drugs, such as yohimbine, animals tended to lie with eyes closed in the home cage, but on transfer to a new cage, had an increased body position and their eyes fully open. Prazosin-treated animals, however, still had a lowered body position and ptosis on transfer. Thus the dual measurement was able to detect a difference in response to an arousing stimulus between sedated and non-sedated animals. The appearance

of the animal on transfer was also measured, to give an indication of the state of arousal , whether alert and interested or indifferent. This method of observation thus is particularly suitable for distinguishing between generalised CNS depression, such as occured with prazosin, and effects which are similar in direction but may be part of a differing behavioural syndrome, such as the decrease in locomotor activity produced by yohimbine.

The total of all the scores gives a useful guide to the direction and magnitude of reaction produced by a drug i.e. sedation or increased arousal. It is important, however, to examine all the parameters individually, since, for example, α-receptor stimulation by clonidine may cause sedative effects, but was also found to cause exophthalmos and an increase in muscle tone, both of which are not typical of a 'sedative' drug and tended to increase the total score. If total activation score is the only measure used, therefore, clonidine would not appear ss sedative as it actually is.

The results obtained using the observational analysis were in agreement with the hypothesis that clonidine sedation is mediated by α_2 receptors, since guanabenz and guanfacin were also sedative, whilst methoxamine was not. Administration of methoxamine centrally produced a slight decrease in the total score, which was almost entirely due to a lack of spontaneous activity. This may have been due to apprehension in view of an increased body position and markedly increased muscle tone. Oxymetazoline and NA had no sedative action, in fact, increased arousal. Prazosin, an α_1 receptor antagonist, had a marked sedative effect, while yohimbine, which acts mainly on α_2 receptors, produced slight excitation at 1.0 and 2.5 mg/kg. This method was the only one

of the three which was able to detect this effect of yohimbine and of α_1 agonists, hence is most suitable for investigating sedative effects of drugs acting at α receptors, since it appears to be more specific. Problems in objectivity could be avoided by operator training and the use of 'blind'studies and further analysis of the results may provide a more accurately combined group of parameters which should be measured to indicate sedation.

The results obtained here agree with those of other workers who suggest that α_2-adrenoceptors are involved in the sedative effect of clonidine. Serotonergic systems may also be involved, however, although indirectly. Destruction of the anterior raphe nuclei or the administration of parachlorophenylalanine (pCPA) leads to behavioural and EEG arousal which is negatively correlated with brain 5-HT levels. (Fuxe & Lidbrink,1972). Thus 5-HT systems appear to be of prime importance for the induction of sleep. However, the dorsal noradrenergic pathway has been implicated in the control of the 5-HT pathways involved in sleep. Destruction of this pathway leads to hypersomnia with an increase in slow wave sleep (SWS) (Jones et al.,1973). Biochemically, such an action produces a decrease in brain NA levels, but an increase in 5-HT turnover, suggesting that NA may normally inhibit the activity of 5-HT neurones. An inhibitory pathway from the L.C. to the raphe nucleus has been demonstrated (Morgane & Sterne,1978), which may thus be involved in the maintainence of tonic arousal. Clonidine may thus act by inhibiting neuronal activity in this pathway, leading to a subsequent increase in 5-HT activity.

Noradrenaline may also be involved in rapid eye movement (REM) sleep, since L.C. lesions lead to a decrease in

REM sleep (Jouvet,1969), while the firing rate of L.C. neurones has been shown to increase during REM sleep (Chu & Bloom, 1973). Clonidine has been found to decrease REM sleep in rats (Kleinlogel et al.,1975), cats (Putkonen et al.,1977) and humans (Autret et al.,1977), whilst increasing the total duration of sleep.This effect of clonidine may also involve α_2 receptors, since yohimbine is able to reverse clonidine depression of REM sleep (Autret et al.,1977); and guanfacin also inhibits REM sleep (Kleinlogel et al.,1975). However, thymoxamine, a selective α_1 receptor antagonist (Drew,1976), has been found to increase REM sleep (Adam et al.,1971 quoted in Kleinlogel et al.,1973), as does inhibition of catecholamine synthesis by α-methyl-para-tyrosine (α-mpt) (Wyatt et al.,1971). Hence a further noradrenergic system which has an inhibitory effect on REM sleep may also exist.

Studies using EEG measurements would appear to be the most accurate in determining drug effects on sleep, since waking, dozing and all stages of sleep may be distinguished (Kleinlogel et al.,1973). Both these workers and Putkonen et al.,(1977) found clonidine to increase drowsiness or dozing, but to produce a decrease in SWS. This effect is not easily distinguishable by measuring the duration of the loss of righting reflex. Both this and effects on REM sleep may well be of importance clinically. However, the disadvantage of EEG measurements is the availability of equipment and personnel trained in its use. Such methods as have been used here are able to provide useful preliminary data on the sedative effects of most drugs. Unfortunately, none of these methods can distinguish satisfactorily between drugs acting at α receptors and any other central depressants. The measurement of sedation is therefore of little use as a primary screen for

central α_2 receptor activation alone, but reversal of clonidine-induced sedation may be specific enough to use as a method of assessment of α_2 receptor blockade. These methods may however be useful for the assessment of relative potency of new α_2 agonists in relation to their antihypertensive effects.

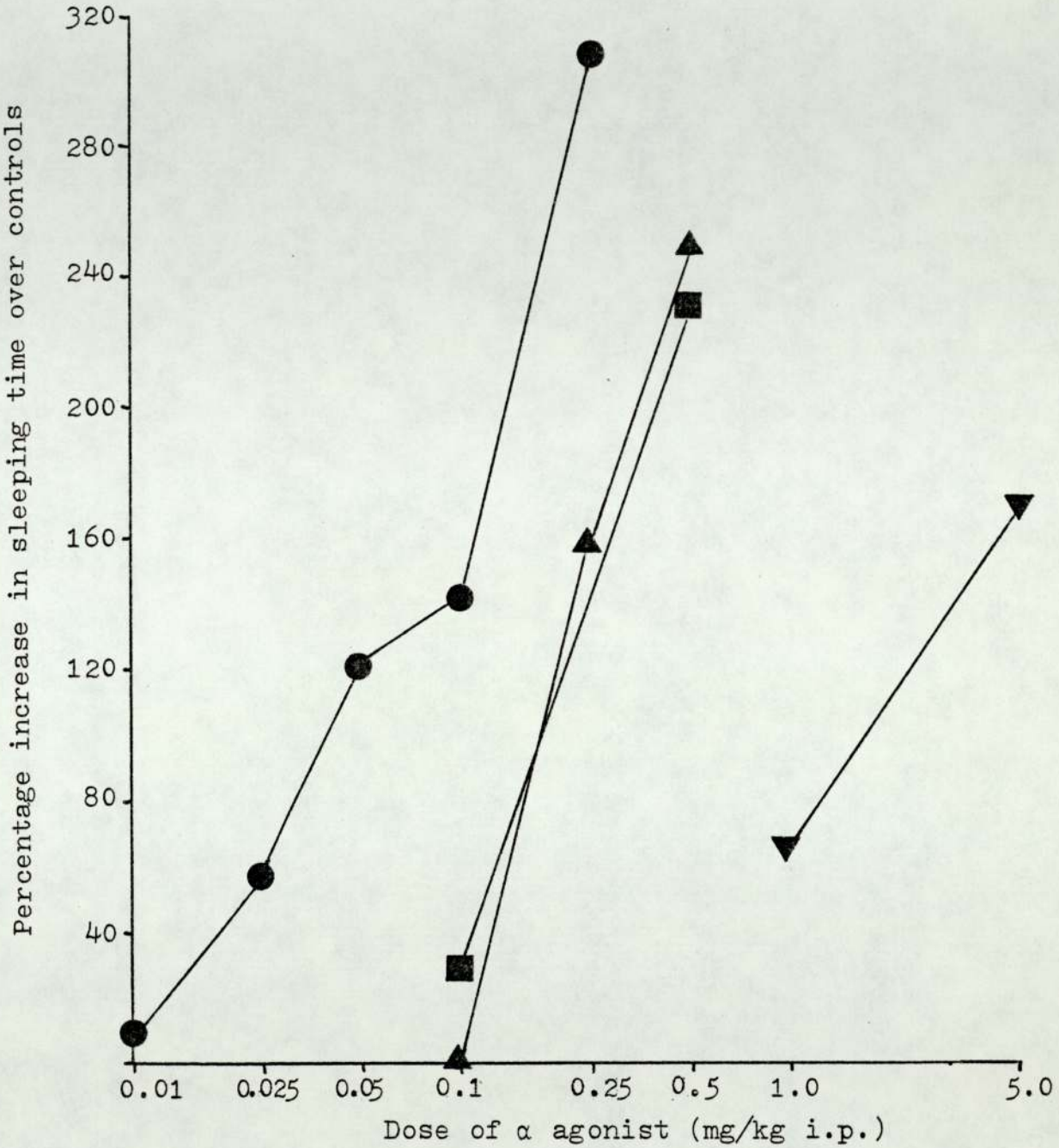


Fig 4.1. The effect of α-adrenoceptor agonists on sleeping time after chloral hydrate 300 mg/kg i.p.

(●) - clonidine; (■) - guanabenz; (▲) - guanfacin; (▼) - oxymetazoline.

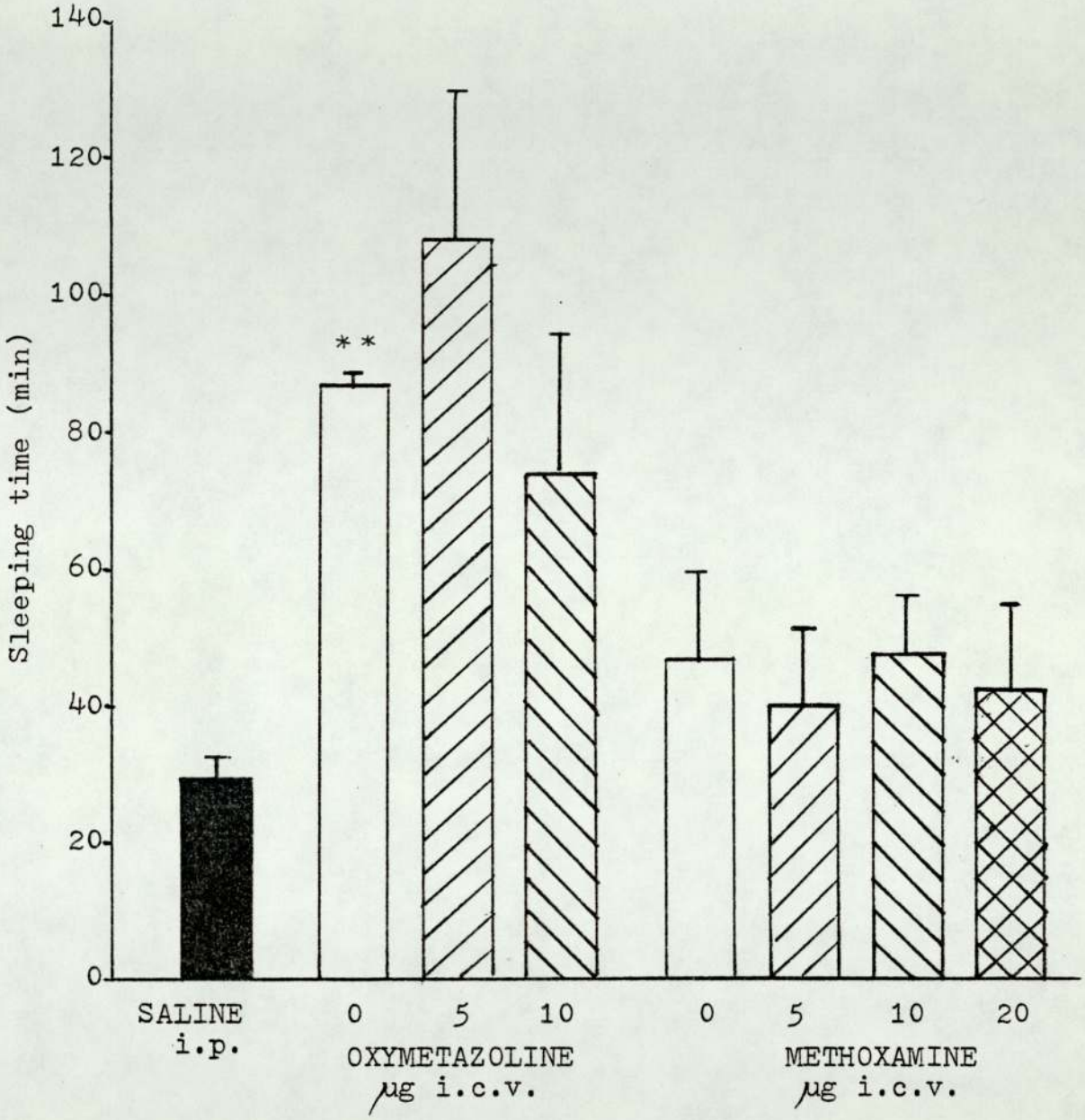


Fig 4.2. The effect of saline, oxymetazoline and methoxamine injected i.c.v. on sleeping time after chloral hydrate 300 mg/kg.

* * $p < 0.01$ difference from saline

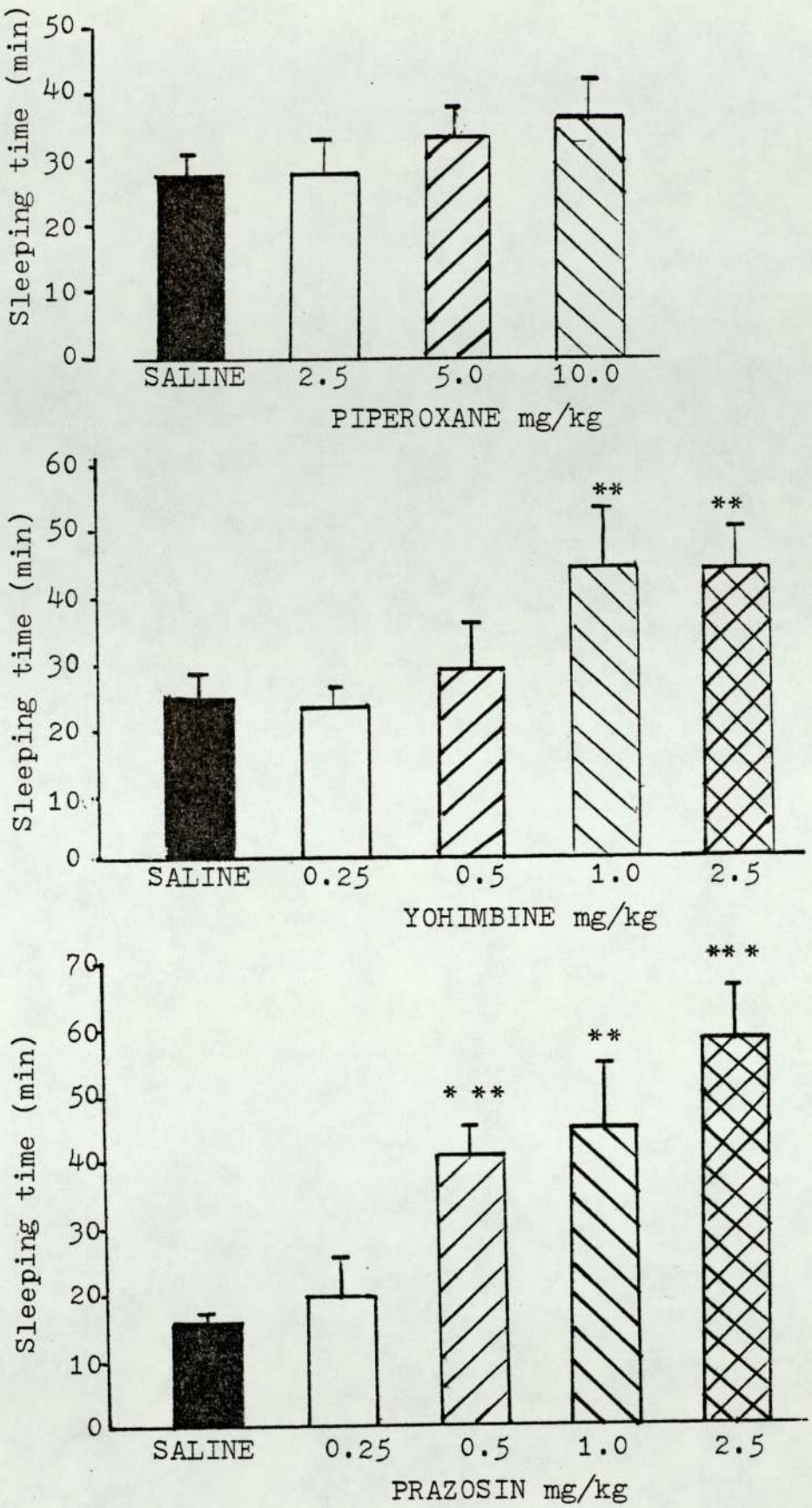


Fig 4.3. The effect of α-adrenoceptor antagonists on the duration of sleep after chloral hydrate 300 mg/kg i.p.

** $p < 0.05$, *** $p < 0.001$ difference from saline-treated controls (Student's 't' test).

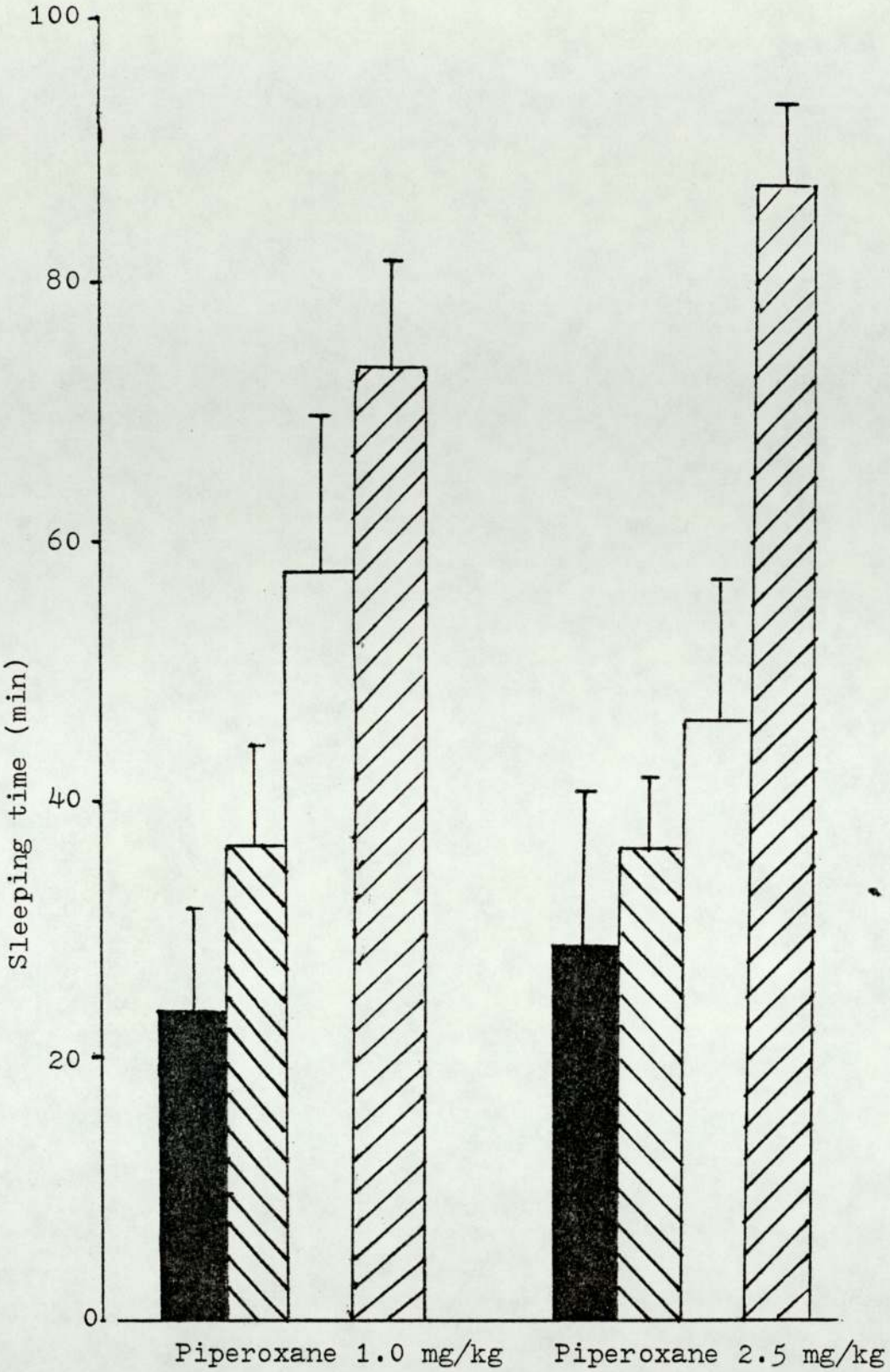


Fig 4.4. The effect of piperoxane 1.0 and 2.5 mg/kg on sleep produced by chloral hydrate 300 mg/kg and its potentiation by guanabenz 0.5 mg/kg.

chloral hydrate 300 mg/kg

piperoxane + chloral hydrate

guanabenz + chloral hydrate

piperoxane + guanabenz + chloral hydrate

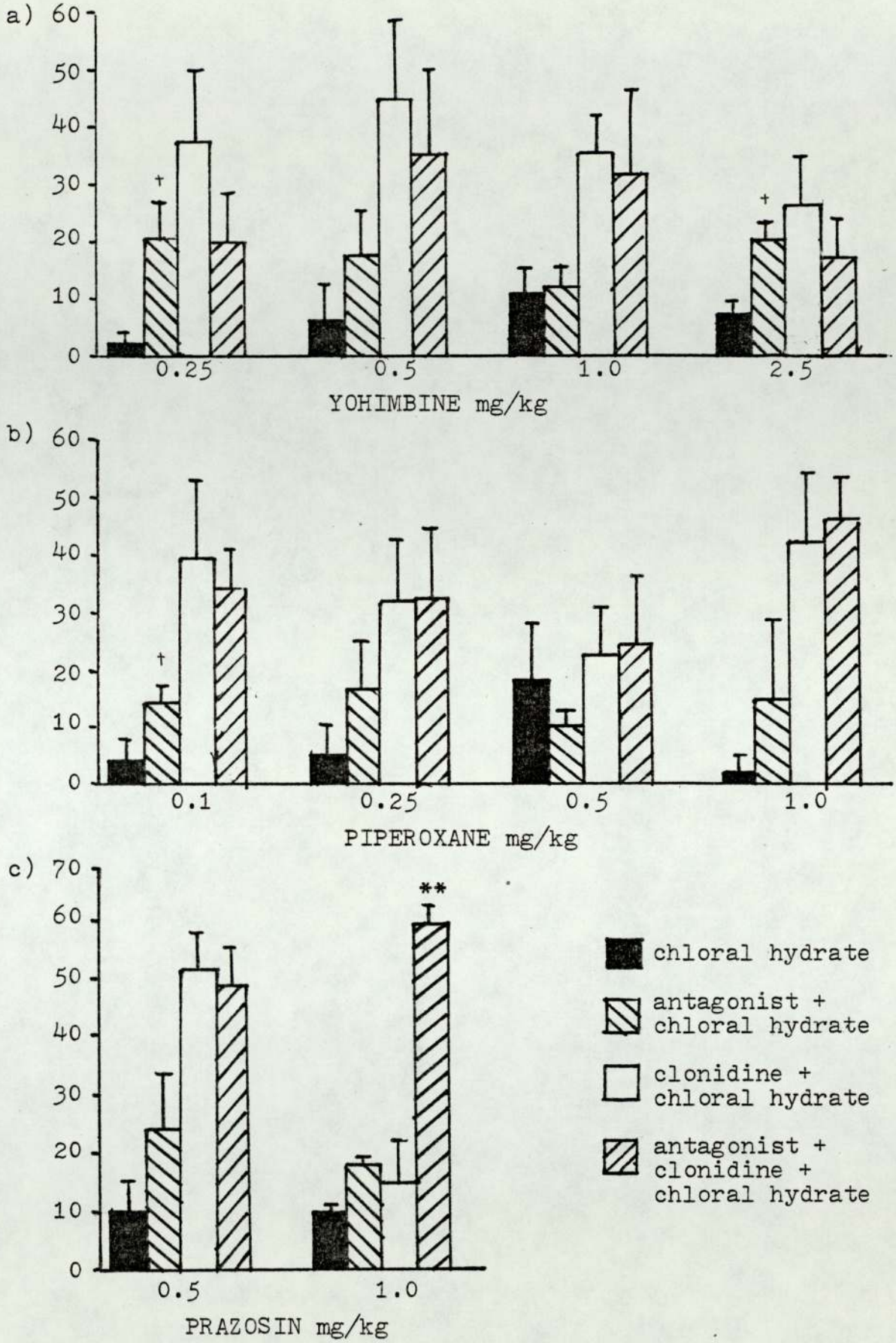


Fig 4.5. The effect of α antagonists on the duration of sleep produced by chloral hydrate 250 mg/kg and its potentiation by clonidine 0.1 mg/kg. † $p < 0.05$ difference of antagonist from saline.

**$p < 0.005$ difference from clonidine + chloral hydrate (student's 't' test)

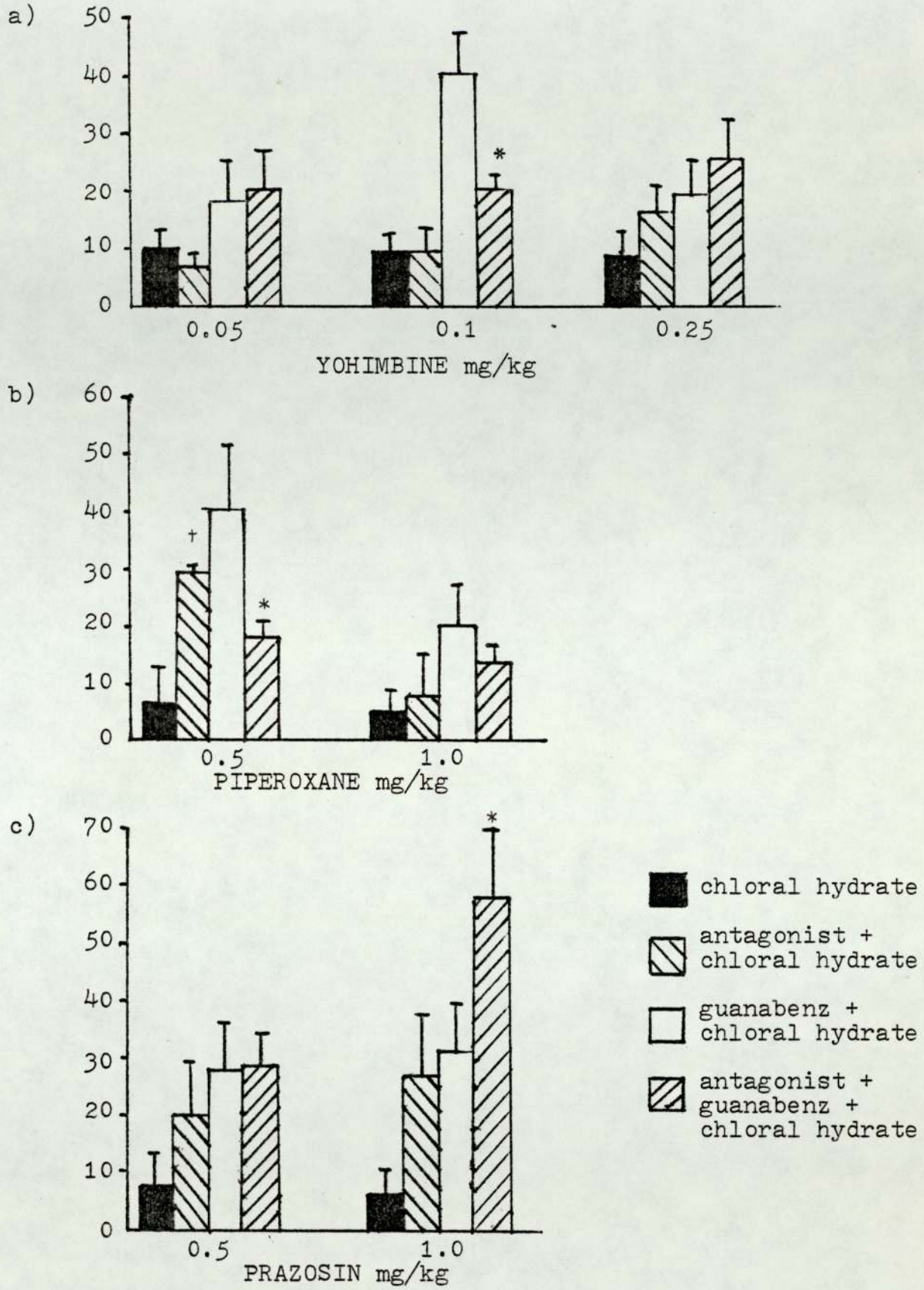


Fig 4.6. The effect of α antagonists on the duration of sleep produced by chloral hydrate 250 mg/kg and its potentiation by guanabenz 0.25 mg/kg. † $p<0.05$ difference of antagonist from saline.

* $p < 0.05$ difference from guanabenz + chloral hydrate (Student's 't' test).

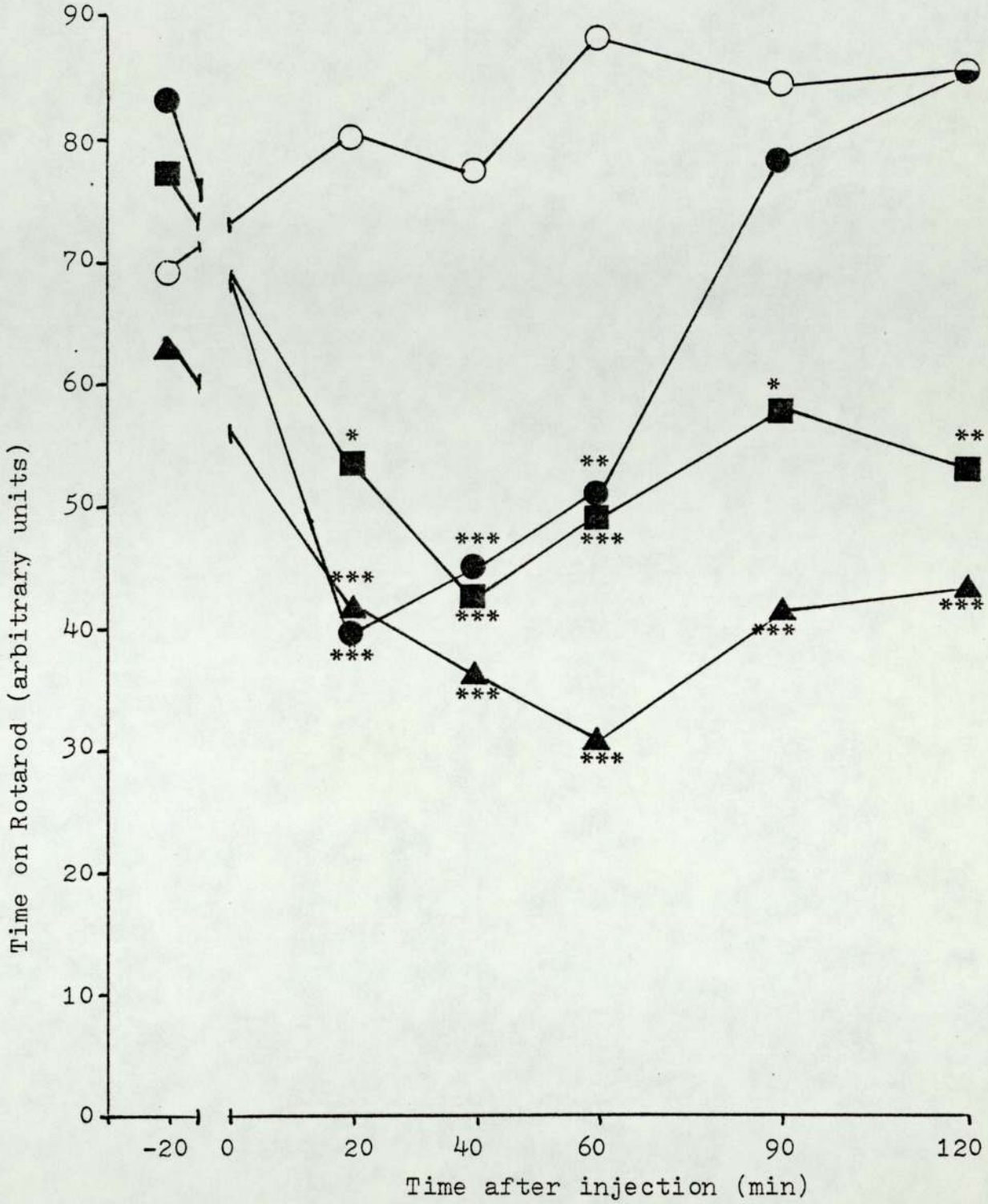


Fig 4.7. The effect of clonidine on time on an accelerating Rotarod. In each group of animals, the first score represents the time on the Rotarod at 20 minutes before injection.
(○) - saline; (●) - clonidine 0.25 mg/kg; (■) - 0.5 mg/kg; (▲) - 1.0 mg/kg.
* $p < 0.01$, ** $p < 0.005$, *** $p < 0.0005$ difference from saline (Student's 't' test).

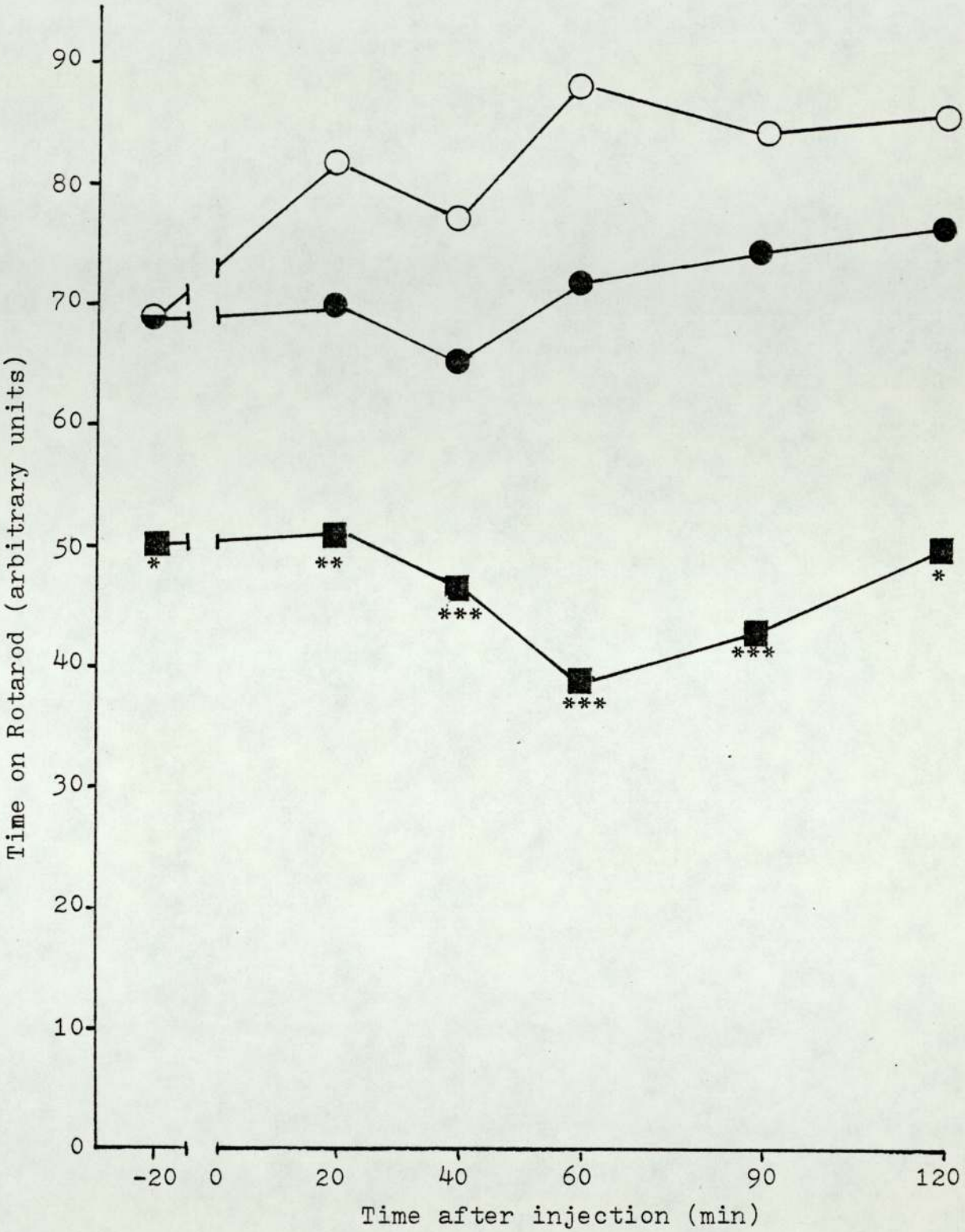


Fig 4.8. The effect of guanabenz on time on an accelerating Rotarod.

(○) - saline; (●) - guanabenz 0.5 mg/kg; (■) - 1.0 mg/kg.

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$ difference from saline (Student's 't' test).

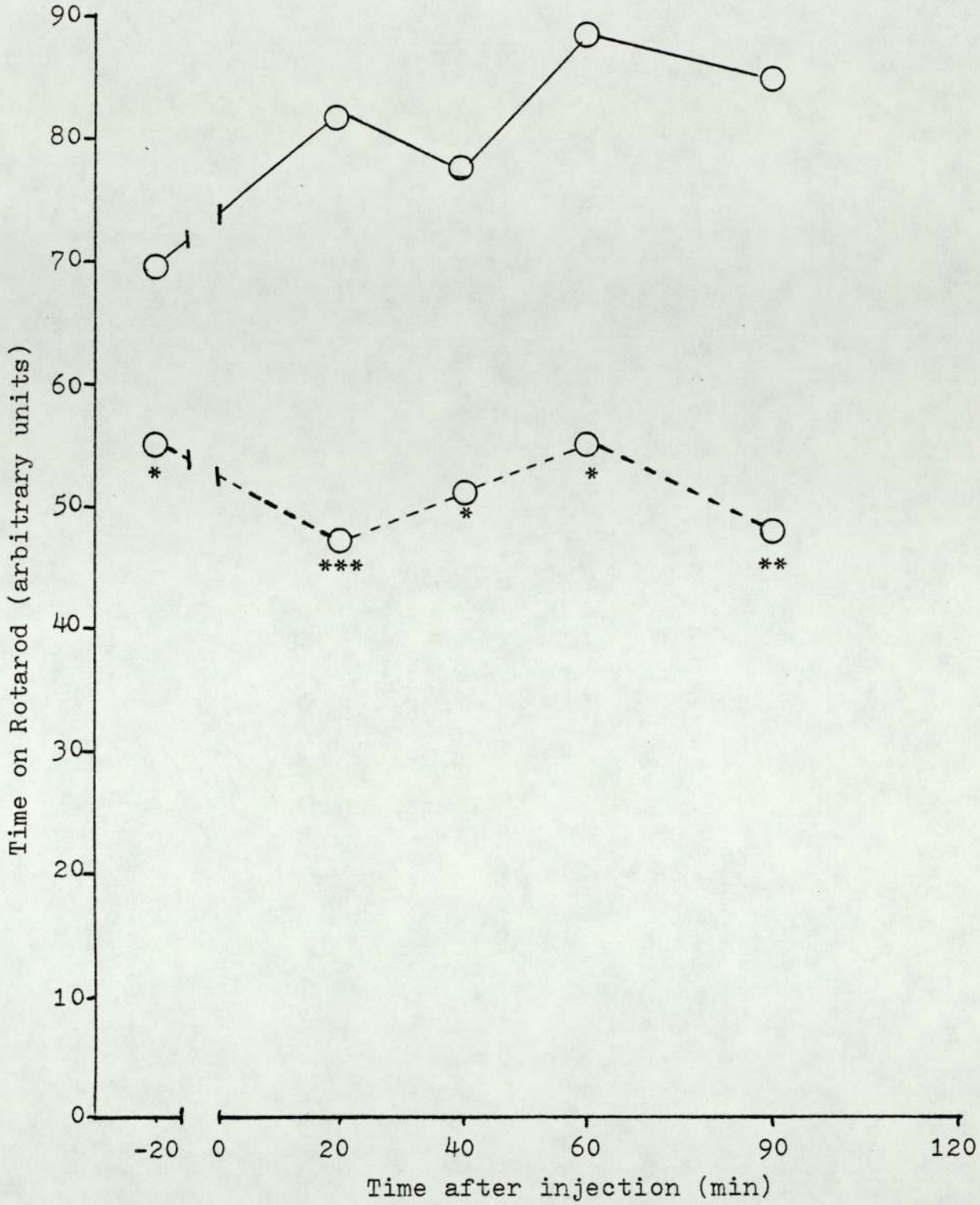


Fig 4.9. The effect of administration of saline i.c.v. on time on an accelerating Rotarod.

solid line - saline s.c.; dashed line - saline i.c.v.

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$ difference from saline s.c. (Student's 't' test).

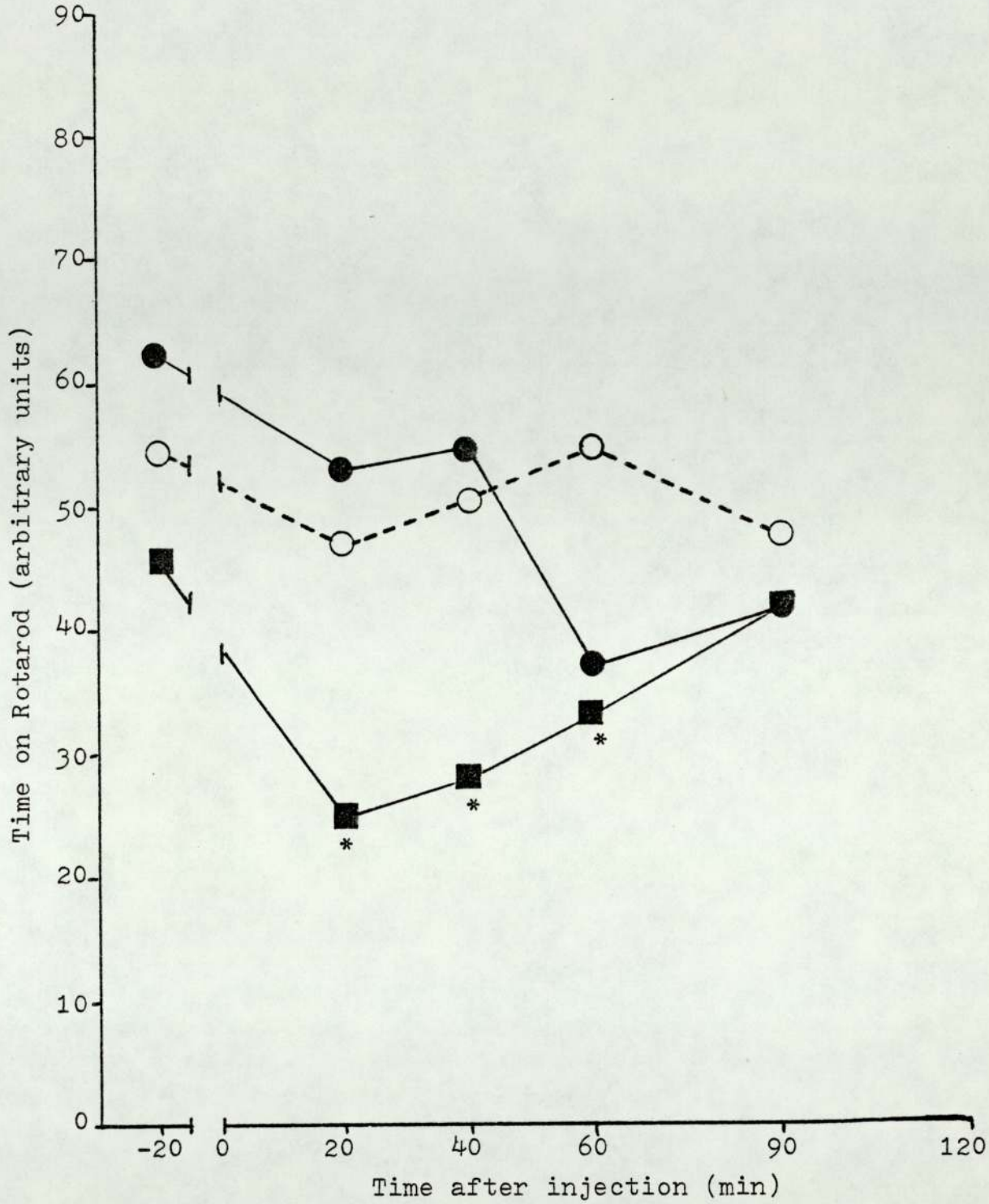


Fig 4.10. The effect of i.c.v. methoxamine on time on an accelerating Rotarod.

(○) - saline i.c.v.; (●) - methoxamine 5 μg; (■) - 10 μg i.c.v.

* $p < 0.05$ difference from saline i.c.v. (Student's 't' test).

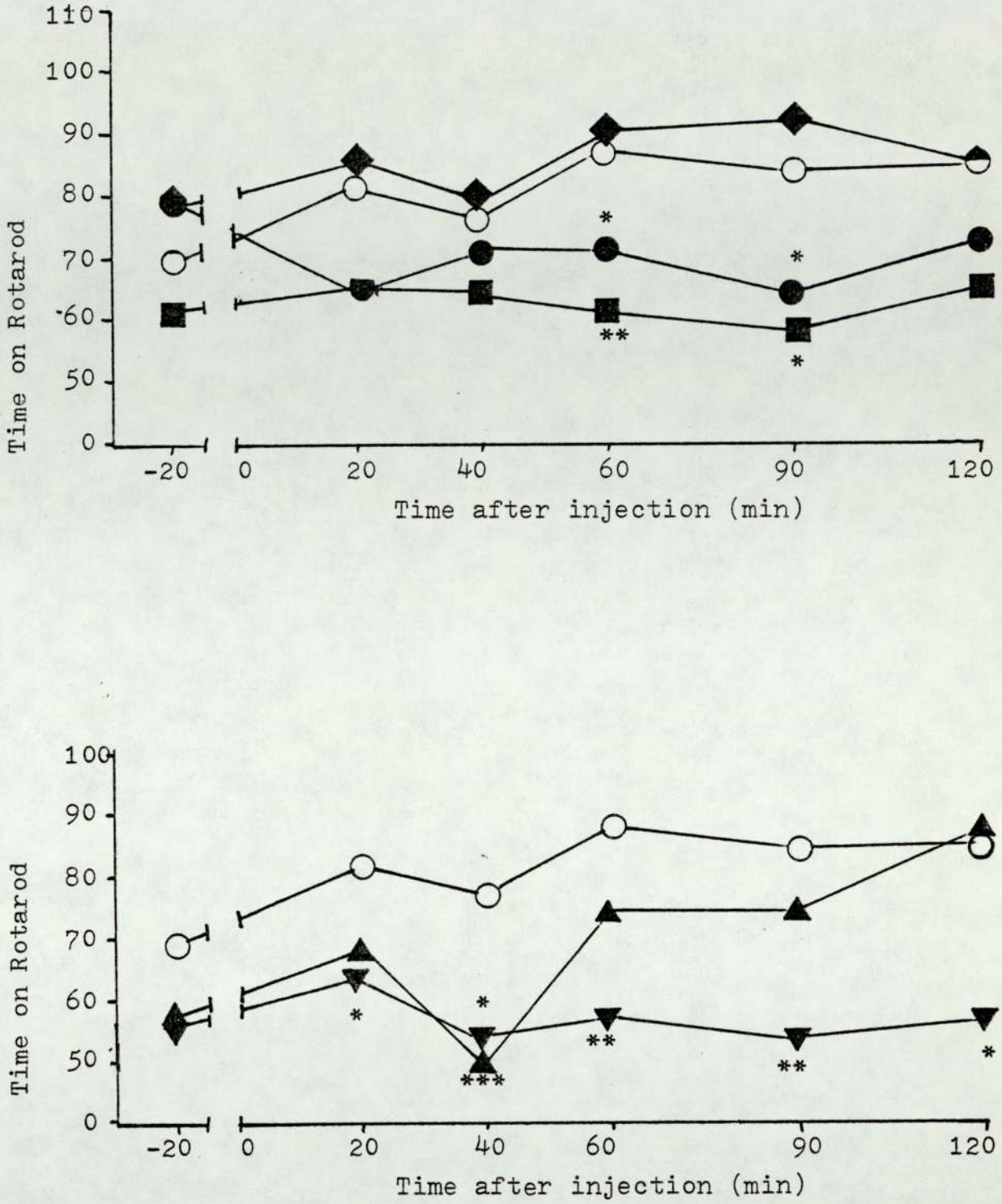


Fig 4.11. The effect of α antagonists on time on an accelerating Rotarod.

(○) - saline; (●) - yohimbine 0.5 mg/kg; (◆) - piperoxane 0.5 mg/kg; (■) - piperoxane 2.5 mg/kg; (▲) - prazosin 1.0 mg/kg; (▼) - prazosin 2.5 mg/kg.

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$ difference from saline (Student's 't' test).

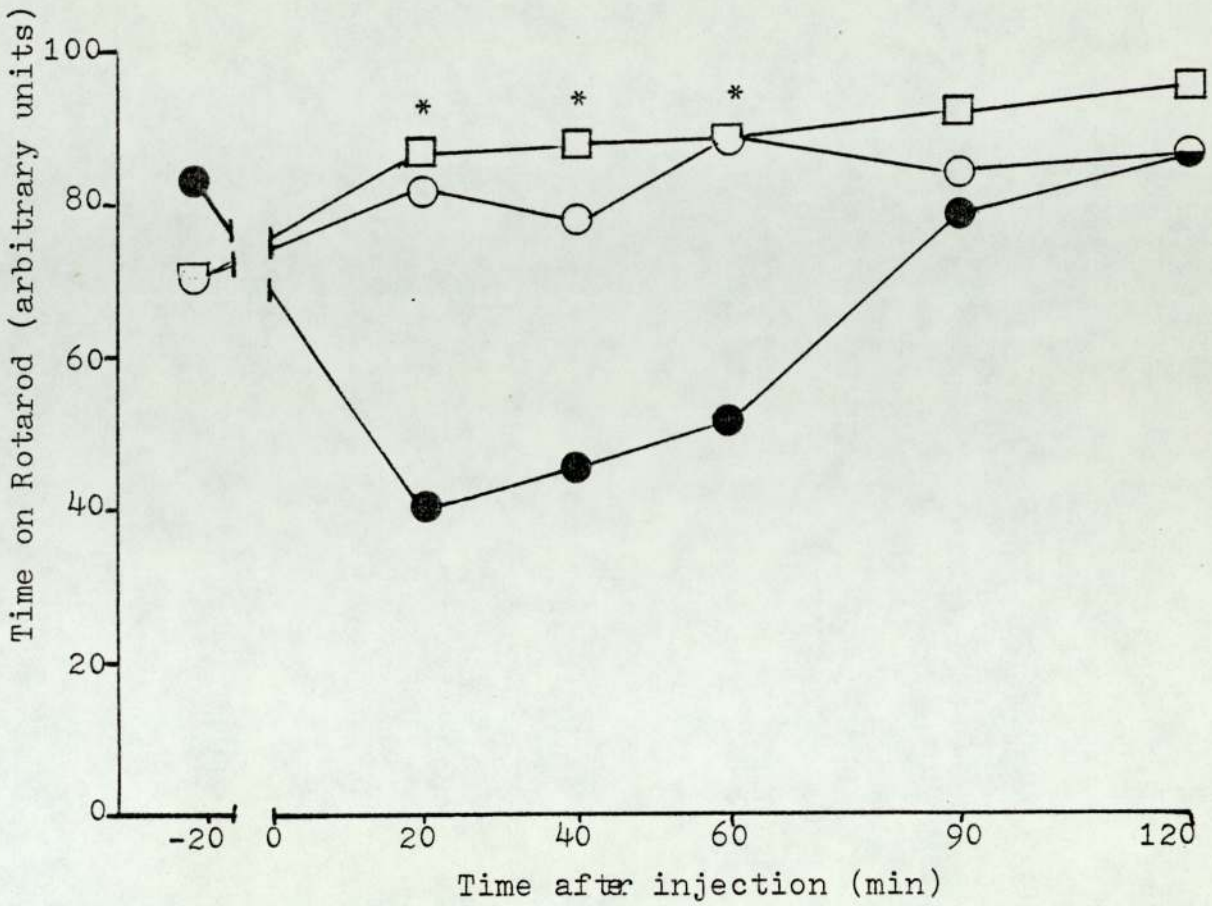


Fig 4.12. The effect of pretreatment with yohimbine 0.5 mg/kg on the decrease in time spent on an accelerating Rotarod produced by clonidine 0.25 mg/kg.

(○) - saline; (●) - saline + clonidine 0.25 mg/kg; (□) - yohimbine 0.5 mg/kg + clonidine 0.25 mg/kg.

* $p < 0.05$ difference from saline + clonidine (Sudent's 't' test).

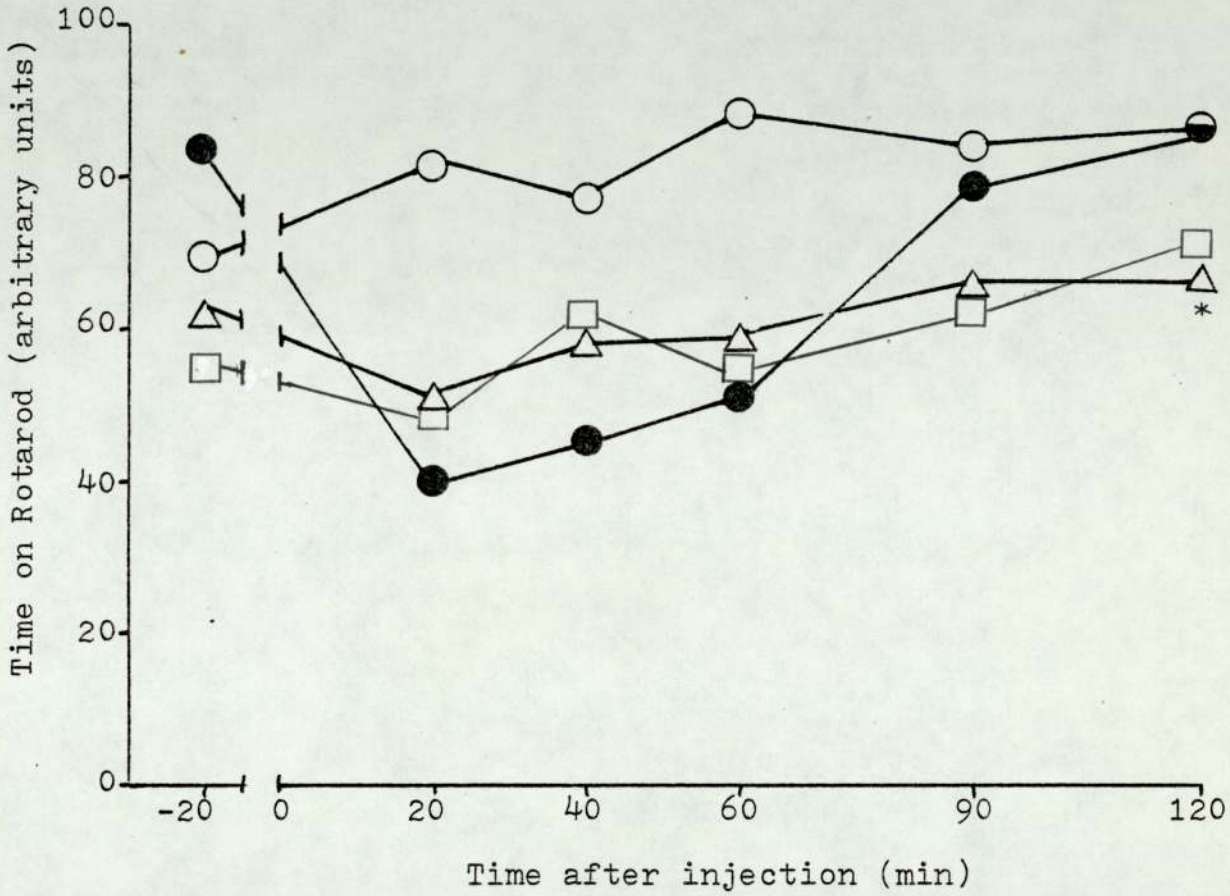


Fig 4.13. The effect of pretreatment with prazosin 0.5 mg/kg on the decrease in time spent on an accelerating Rotarod produced by clonidine 0.25 mg/kg.

(○) - saline; (●) - saline + clonidine 0.25 mg/kg; (△) - prazosin 0.5 mg/kg + clonidine 0.25 mg/kg.

(□) - piperoxane 1.0 mg/kg + clonidine 0.25 mg/kg.

* $p < 0.05$ difference from saline + clonidine (Student's 't' test).

ACTIVATION SCORES (each score is the mean of 3 animals)

DRUG AND DOSE	Ptosis 0 - 4	Body position 0 - 4	Activity 0 - 8	Startle response 0 - 4	Transfer arousal 0 - 8	Visual placing 0 - 4	Muscle tone 0 - 4	TOTAL Max. 36
Saline	3.83	2.25	1.33	2.0	5.0	2.0	2.0	18.41
Clonidine 0.1 mg/kg	3.0	2.08	0.33 *	2.0	4.33	2.0	2.66	16.40 *
Clonidine 0.25 mg/kg	4.0	1.92	0.0 *	1.66	3.66 *	1.66	2.66	15.56 *
Clonidine 0.5 mg/kg	4.0	2.0	0.34 *	1.66	2.33 *	1.66	1.33	13.32 *
Guanabenz 1.0 mg/kg	2.0	2.17	0.83	1.0 *	4.66	2.0	2.0	14.66 *
Guanfacin 1.0 mg/kg	1.67 *	2.08	0.17 *	2.0	4.0	2.0	3.33 *	15.25 *
Methoxamine 25.0 mg/kg	3.66	1.75	0.5 *	2.0	4.0	2.66	3.66 *	18.23

Table 4.1. Activation scores for α agonists administered s.c.

Animals were observed 30 minutes after injection, except in the case of guanfacin, when animals were observed 90 minutes after injection.

* $p < 0.05$ difference from saline (Mann Whitney 'U' test).

ACTIVATION SCORES (each score is the mean of 3 animals)

DRUG AND DOSE	Ptosis 0 - 4	Body position 0 - 4	Activity 0 - 8	Startle response 0 - 4	Transfer arousal 0 - 8	Visual placing 0 - 4	Muscle tone 0 - 4	TOTAL Max. 36
Saline i.c.v.	3.83	2.08	2.33	2.0	4.33	2.0	1.66	18.23
Methoxamine 15µg i.c.v.	4.0	2.17	0.0 *	1.0 *	4.33	2.0	3.33	16.83
Noradrenaline 2.5µg i.c.v.	3.83	2.33	2.0	3.0 *	4.66	4.0 *	3.66 *	23.48 *
Oxymetazoline 3.0 µg i.c.v.	4.0	2.17	2.83	1.66	4.33	2.66	2.66	20.31

Table 4.2. Activation scores for α agonists after i.c.v. administration.

Animals were observed 20 minutes after injection

* $p < 0.05$ difference from saline (Mann Whitney 'U' test).

ACTIVATION SCORES (each score is the mean of 3 animals)

DRUG AND DOSE	Ptosis 0 - 4	Body position 0 - 4	Activity 0 - 8	Startle response 0 - 4	Transfer arousal 0 - 8	Visual placing 0 - 4	Muscle tone 0 - 4	TOTAL Max. 36
Saline	3.83	2.25	1.33	2.0	5.0	2.0	2.0	18.41
Yohimbine 1.0 mg/kg	3.83	2.08	0.58	3.66 *	5.0	2.33	3.0	20.48
Yohimbine 2.5 mg/kg	3.66	2.08	0.5	3.0 *	4.66	2.0	3.66 *	19.56
Yohimbine 5.0 mg/kg	3.5	1.75	0.5	3.0 *	4.0	2.0	2.66	17.41
Prazosin 1.0 mg/kg	1.33 *	2.0	0.17 *	0.66 *	4.0	1.33	1.33	12.82 *
Prazosin 2.5 mg/kg	1.33 *	1.92	0.17 *	1.0 *	3.33 *	1.33	1.66	10.74 *

Table 4.3. Activation scores for α antagonists administered s.c.

Animals were observed 30 minutes after injection.

* $p < 0.05$ difference from saline (Mann Whitney 'U' test).

CHAPTER 5

EFFECTS OF α AGONISTS AND ANTAGONISTS ON MOTOR ACTIVITY

CHAPTER 5.

INTRODUCTION

While activation of dopaminergic systems has been found to be fundamental to the initiation of motor activity (Anden et al.,1973), NA receptor activation may modify this activity, both qualitatively and quantitatively. Apomorphine, a DA receptor agonist, has been found to have a biphasic effect on locomotor activity (Strombom,1976). Low doses (0.025 to 0.2 mg/kg) decreased the initial high motor activity caused by placing animals in a new environment; whilst higher doses either were ineffective (Strombom,1976) or produced a slight enhancement of activity (Handley & Thomas,1978; Strombom,1975).

In animals treated with reserpine, however, apomorphine caused a marked stimulation of locomotor activity (Anden et al.,1970) accompanied by stereotypy (Anden et al., 1973). Clonidine has been found to potentiate the stimulant action of apomorphine both in untreated (Handley & Thomas,1978) and reserpinised mice (Anden et al.,1970), whilst simultaneously improving motor co-ordination and reducing stereotypy. The increase in locomotor activity produced by low doses of d-amphetamine was also potentiated by clonidine and i.c.v. NA or α-methyl-NA (Handley & Thomas,1978).

When given alone, however, clonidine has been found to inhibit activity (Strombom,1976; Delini-Stula et al. ,1979; Clineschmidt et al.,1979). Both this effect and the depression of activity by low doses of apomorphine may be attributable to activation of regulatory autoreceptors of noradrenergic and dopaminergic systems respectively., thus leading to a decrease in neuronal activity. Delini-Stula et al.,(1979) found the inhibition of locomotor activity due to clonidine in the rat to be antagonised by tolazoline, yohimbine and piperoxane, but not by phentolamine or PBZ, hence it seems likely that the receptor involved is of the α_2 type. Clineschmidt et al.,(1979) also

found the effect of clonidine to be due to α_2 receptor stimulation, and further showed that the α_1 receptor agonists, phenylephrine and methoxamine, caused an increase in initial high activity in a new cage. This effect was antagonised by prazosin and azepetine, but not by yohimbine or piperoxane.

Noradrenaline may also cause an increase in locomotor activity (Herman, 1970), presumably by α_1 receptor activation. Clonidine only produces such an increase in animals whose neuronal systems are depleted of transmitters (Zebrowska-Lupina et al.,1977) or may not be fully developed (Nomura & Segawa,1979). This may be due to the selective action of the drug on regulatory α_2 receptors, which may override any direct stimulation via postsynaptic α_1 receptors except when regulation of transmitter release is ineffectual.

The present experiments were performed in an attempt to confirm the type of receptors involved in both the depressant and the stimulant actions of clonidine, and to study the effects of α receptor antagonists on locomotor activity. In addition, the measurement of locomotor activity as a means of assessing sedation was studied.

1. Effect of α agonists and antagonists on exploratory activity.

Animals injected with saline and subsequently placed in a new cage in a novel environment with unusual objects present in the cage were found to have a high locomotor activity score forapproximately 60 minutes (Fig 5.1). The activity then decreased gradually, until by 120 minutes, there was very little locomotion occurring.

Clonidine (0.05 to 1.0 mg/kg) was found to produce a dose-dependent decrease in the initial high activity (Fig 5.1), but the lowest dose produced a marked increase in activity 100 to 220 minutes after the start of the experiment, at a time when controls and animals treated with higher doses were relatively inactive (Fig 5.1.a).

Yohimbine (1.0 to 5.0 mg/kg) also inhibited the initial high activity in a dose-dependent manner (Fig 5.2a), although the higher doses increased activity 80 to 140 minutes after placement in the cage. This increase in activity also appeared to be dose-dependent. Piperoxane 5.0 mg/kg had a similar effect to that of yohimbine, causing an initial decrease followed by a slight increase in activity. All the doses of prazosin studied (0.25 & 2.5 mg/kg) decreased activity over the first 60 minutes (Fig 5.2b). There was however, no subsequent large increase in activity after this time. The highest dose of prazosin totally abolished activity throughout the whole duration of the experiment (4 hours).

A closer study of the initial high motor activity occurring after placement in a new cage was then undertaken, since this seemed to be sensitive to all the drugs so far tested and also since this type of activity has been studied by other workers (see Introduction). These experiments were carried out by placing animals in a new cage in a room where they had been

kept for at least 5 days prior to use. Thus only the cage, which was devoid of objects, was novel, not the laboratory environment. The experiments covered a period of 2 hours, over which time control animals showed a gradual decline in locomotor activity.

Clonidine in doses as low as 0.025 mg/kg inhibited activity for the first 60 minutes, after which time, animals treated with doses of 0.025 and 0.05 mg/kg all showed an increase in activity over controls (Fig 5.3a). A higher dose (0.5 mg/kg) produced a more marked inhibition, which was maintained throughout the experiment (Fig 5.4). A very high dose of clonidine (5.0 mg/kg), which had been found to produce signs of behavioural excitation (Chapter 3), had a similar effect to 0.5 mg/kg i.e. it decreased activity for the whole 2 hours (Fig 5.3a). Methoxamine 10.0 mg/kg also markedly inhibited acvity for 2 hours (Fig 5.3b), as did oxymetazoline 2.5 mg/kg.

2. The effect of α antagonists on the decreased activity due to clonidine 0.5 mg/kg.

Yohimbine in doses of 1.0 and 2.5 mg/kg produced some reversal of the effect of clonidine 0.5 mg/kg on motor activity over a 2 hour period (Fig 5.4a). Animals still had marked piloerection and exophthalmos after the drug combination, but appeared rather 'jumpy' and less sedated than animals treated with clonidine alone. A higher dose of yohimbine (5.0 mg/kg) was found to be ineffective in reducing the effect of clonidine (Fig 5.4b), although the animals were not any more sedated in appearance.

Piperoxane 5.0 mg/kg produced only a slight increase in activity in clonidine-treated animals (Fig 5.5a), while prazosin 1.0 and 2.5 mg/kg further reduced activity (Fig 5.5b).

3. The effect of clonidine and methoxamine on home cage activity.

Since none of the α agonists used were found to produce any increase in the initial high motor activity as had been found by other workers (see Introduction), a further type of activity was measured in order to see whether an increase in activity could be detected by this method. Some agonists had been found to produce a 'hyperreactivity' syndrome (Chapter 3), which it was thought may lead to the decrease in initial locomotor activity, thus animals were tested in a situation designed to reduce 'fear' of the novel situation as much as possible. Animals were kept in cages in groups of 5 for at least 5 days prior to use. Experiments were carried out using these same cages in the same room during the dark phase of the light cycle.

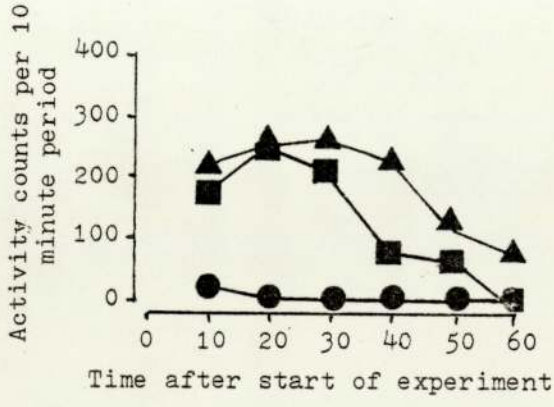
In animals treated with saline, three distinct peaks of activity were distinguishable (Fig 5.6a), the first on initial removal of light, the second at 40 to 80 minutes after the start of the experiment, and the third at 140 to 180 minutes. Clonidine markedly reduced all activity especially the first and second peaks, but the effect did not appear to be dose-related (Fig 5.6b). Methoxamine at a dose of 10.0 mg/kg totally eliminated the first peak (Fig 5.6c), although the inhibitory effect was less on the second peak; and by 140 minutes, activity was close to control levels. A higher dose, 25.0 mg/kg, however, markedly decreased activity at all times and eliminated the cycle seen in control mice (Fig 5.6c).

4. Activity in reserpinised animals.

Reserpine 5.0 mg/kg totally abolished activity of mice placed in a new cage 24 hours later (see p 145). Apomorphine 0.5 and 1.0 mg/kg produced a marked increase in activity, which varied in duration, not intensity, with the dose (see p 145). This activity consisted of compulsive locomotion, stereotyped head-searching and some gnawing. Clonidine 1.0 mg/kg caused a marked

potentiation of activity, including short bursts of running and jumping (Fig 5.7a); and produced piloerection and exophthalmos. Guanabenz 2.5 mg/kg was ineffective and 5.0 mg/kg only produced a slight potentiation of activity 40 to 60 minutes after the start of the experiment, when the effect of the apomorphine was diminished (Fig 5.7c and d). Oxymetazoline 2.5 mg/kg caused a marked potentiation of apomorphine, especially during the first 15 to 20 minutes after apomorphine (Fig 5.7b). Methoxamine was only slightly effective after peripheral injection of 10.0 mg/kg (Fig 5.7e), but did potentiate the effect of apomorphine after central administration (Fig 5.7f).

All of the α antagonists tested prevented the increase in activity due to clonidine . Piperoxane 5.0 mg/kg (Fig 5.10a) and prazosin 2.5 mg/kg (Fig 5.8a) both reduced the activity to levels seen in animals treated with only reserpine and apomorphine i.e. only abolished the effect of clonidine. Neither drug had an effect on activity produced by apomorphine alone in reserpinised animals (Figs 5.8b and 5.10b). Yohimbine 5.0 mg/kg, however, markedly attenuated the increased activity produced by both apomorphine alone (Fig 5.9a) and its potentiation by clonidine (Fig 5.9b).



The effect of reserpine and apomorphine on locomotor activity in mice.

(●) - reserpine 5 mg/kg 24 hours before + saline 5 minutes before start of experiment; (■) reserpine + apomorphine 0.5 mg/kg; (▲) - reserpine + apomorphine .1.0 mg/kg 5 minutes before start of experiment.

DISCUSSION

Clonidine has been found to inhibit exploratory locomotor activity in doses of 0.025 to 5.0 mg/kg. This effect has been seen by other workers and may be indicative of the sedative effect of the drug. Yohimbine 1.0 to 5.0 mg/kg was able to reduce the decrease in activity due to clonidine 0.5 mg/kg, although this effect was not very marked. This may be due in part to the inhibition of initial exploratory activity which occurred with yohimbine alone. However, other workers have found yohimbine to markedly increase activity in clonidine-treated rats (Delini-Stula et al.,1979; Clineschmidt et al.,1979), although these workers were using only short (15 to 20 minutes) periods of measurement. In the present experiments, yohimbine was found to potentiate activity by over 100% over the first 20 minutes.

Piperoxane was also only effective in producing an increase in locomotor activity over the first 20 minutes at a dose of 5.0 mg/kg. Delini-Stula et al.,(1979) found this drug to be ineffective in rats in doses below 10.0 mg/kg, while Clineschmidt et al.,(1979) also found it to be less potent than yohimbine.

Drugs which are known to block α_1 receptors were ineffective in reducing the depressant effect of clonidine both in mice and rats Clineschmidt et al.,1979; Delini-Stula et al., 1979). Further decreases in activity were, in fact, produced by all three drugs studied: prazosin, phentolamine and PBZ (Delini-Stula et al.,1979). These workers also found that both tolazoline and yohimbine further depressed activity in clonidine-treated rats when given in high doses.

The results found here suggest that the decrease in activity by clonidine may be due to stimulation of α_2 receptors, since it is reversed by yohimbine and, to a lesser extent,

piperoxane, but not by drugs which are able to block α_1 receptors i.e. prazosin, phentolamine and PBZ. These latter drugs, in fact, potentiate the effect of clonidine; and also reduce activity themselves (Delini-Stula et al.,1979). It would thus appear that a complete noradrenergic α synapse may be involved in activity. A decrease in the functioning of this synapse by either a reduction in the release of NA by clonidine, or blockade of the postsynaptic receptor by prazosin, leads to a decrease in locomotor activity.

It is possible that the reduction in activity may be a consequence of the sedative effects of both clonidine and prazosin, since a similar synaptic arrangement could be responsible for sedation. However, NA has been found to be of importance for the mediation of activity (Corrodi et al.,1970), hence these drugs may, in addition, be acting on a different neuronal system not involved in sedation, since increased arousal only would not necessarily lead to an increase in activity.

The decrease in initial high activity found with yohimbine may reflect an action of the drug on α_1 receptors, since prazosin also reduced activity. This seems unlikely, however, in view of the selectivity for α_2 receptors which yohimbine possesses (Starke et al.,1975a). It is possible that the 'hyperreactivity' syndrome induced by the drug may lead to 'fear' and thus decrease exploration (Chapter 9). The behavioural effect of yohimbine is one of arousal, thus a sedative effect is unlikely to be responsible for the decreased activity. This is supported by the observations of Papeschi et al.,(1971), who found that despite a reduction in locomotor activity, yohimbine-treated rats did not appear sedated, in fact, were overreactive to external stimuli.

Low doses of clonidine were found to increase locomotor activity after the initial decrease, although studies

were not undertaken to determine whether this was due to α_1 or α_2 receptor stimulation. Yohimbine and piperoxane were found to have similar effects, thus all three drugs may produce this effect by increasing noradrenergic activity. Low doses of these drugs have been found to increase observed spontaneous activity (Chapter 3); however, the delay in the onset of the increased locomotor activity suggests that the two effects may not be the same. In addition, clonidine is unlikely to increase locomotor activity by α_1 receptor stimulation, since a very high dose of the drug, which does appear to affect α_1 receptors (Chapter 3), had no such effect . Methoxamine, when administered either peripherally or centrally, and oxymetazoline were also ineffective in stimulating locomotion throughout the experiment. It is possible, therefore, that the increase in activity may be due to a rebound effect occurring after the effects which result in depression of activity have subsided i.e. a delayed initiation of exploration.

The lack of effect of α_1 agonists in increasing locomotor activity was surprising in view of the marked depression of activity seen after α_1 antagonists. Other workers have found α_1 agonists to increase activity in rats (Clineschmidt et al.,1979; Herman,1970; Segal & Mandell, 1972); however, methoxamine, oxymetazoline and a high dose of clonidine all produced a decrease in exploratory activity in mice. This lack of stimulation was not due to an inability of the method used to detect increases in activity, since diazepam and amylobarbitone both markedly increased exploratory activity. All of the α_1 agonists used have been found to result in a 'hyperreactivity' syndrome (Chapter 3), which it was thought may lead to decreased exploration due to increased 'fear' of the novel environment. Measurement of locomotor activity in a familiar environment was therefore undertaken to minimise the possibility of this effect

occurring. Experiments were carried out during the dark phase, when activity had been found to be high. Neither clonidine nor methoxamine produced any increase in activity; in fact, marked decreases were again seen.

This lack of stimulatory effect of drugs which have been shown to activate α_1 receptors peripherally may indicate that α_1 receptor stimulation is a prerequisite for exploratory activity to occur and that no further stimulation is possible. Although α receptor stimulation is not a prerequisite for motor activity (Anden et al.,1970), α_1 receptor stimulation does potentiate apomorphine-induced activity and render the activity less stereotyped and more similar to normal exploratory activity (ibid). In support of this suggestion, inhibition of activity by α_2 receptor stimulation affects rearing more than ambulation (Delini-Stula et al.,1979), thus it appears that exploration is more affected by a reduction in noradrenergic function than is other activity. In addition, inhibition of NA synthesis by FLA 63 reduces exploratory behaviour, rendering activity stereotyped (Corrodi et al.,1970).

Methoxamine may thus decrease exploratory activity either by an effect on α_2 receptors, which may occur at high doses; or by competing with NA for α_1 receptors. If the drug had a lower intrinsic activity and greater receptor affinity than NA, this would result in a decrease in locomotor activity. Since both oxymetazoline and a high dose of clonidine have similar effects, it is, however, unlikely that all three drugs would possess the same characteristics; thus α_2 receptor stimulation is more likely to be responsible for the decrease in activity seen with these drugs. The increase in activity found by other workers may be due to differences in species or in the methods used to measure activity. Alternatively, methoxamine may activate α_2 receptors more readily in the mouse, thus

blockade of such receptors may reveal a stimulatory effect of methoxamine on activity.

Stimulation of α_1 receptors has been found to increase activity in animals in which α_2 receptor stimulation is ineffectual. Thus, clonidine, methoxamine and oxymetazoline were all found to potentiate the apomorphine-induced increase in activity in reserpinised mice, while guanabenz, which acts mainly at α_2 receptors, was only effective at a high dose 50 to 60 minutes after injection. Clonidine has also been shown to potentiate the effect of apomorphine in mice in which only NA synthesis was inhibited (Maj et al.,1972), hence NA receptor activation does appear to mediate this effect.

Anden and Strombom, (1974) found PBZ 20 mg/kg abolished the potentiation of activity by clonidine in reserpinised animals without affecting the action of apomorphine. Phentolamine, however, decreased both apomorphine-induced activity and its potentiation by clonidine. Prazosin and piperoxane had a similar effect to that found for PBZ by these workers i.e. both drugs reduced the potentiating effect of clonidine. Activity levels were similar to those in animals treated with reserpine and apomorphine alone after this drug combination. Yohimbine, however, reduced activity to levels below those produced by apomorphine and also reduced the effect of this drug alone.

The results obtained here suggest that blockade of α_1 receptors reduces the effect of clonidine, since prazosin acts only on these receptors, while piperoxane and yohimbine, although showing a selective effect at α_2 receptors, are also capable of blocking α_1 receptors. In reserpinised animals, where blockade of α_2 receptors would have no effect on transmitter release, α_1 receptor block would be the predominant effect. The work of Anden and Strombom (1974) supports this suggestion,

since both PBZ and phentolamine are able to block α_1 receptors but PBZ shows selectivity for these receptors (Doxey et al.,1977; Dubocovich & Langer,1974).

Stimulant effects of clonidine found by other workers both in reserpinised rats (Zebrowska-Lupina et al.,1977) and in 7 day old rats (Nomura & Segawa,1979) also appear to be mediated by α_1 receptor stimulation. It is possible that in young animals, α_2 receptors for the regulation of transmitter release are not yet fully developed, thus enabling clonidine to stimulate only α_1 receptors.

In addition to reducing the stimulant effect of clonidine, yohimbine and phentolamine antagonised the effect of apomorphine alone. Since piperoxane did not similarly affect apomorphine-induced activity, this is unlikely to be due to any effect on α_2 receptors. Yohimbine does not appear to block DA receptors in the rat (Papeschi & Theiss,1975), hence the antagonism of apomorphine may not be due to a direct effect on DA receptors. Anden and Strombom,(1974) suggested that phentolamine reduced activity by a 'non-specific behavioural suppression'. This seems unlikely in the case of yohimbine, as, when given alone, this drug produces slight behavioural excitation (Chapter 4). Thus, the inhibition of apomorphine-induced activity by yohimbine remains unexplained.

The results suggest therefore, that decreases in noradrenergic function decrease activity either via sedation or directly; and that, if presynaptic inhibitory effects are eliminated, noradrenergic stimulation can result in an increase in activity. Clonidine thus appears to reduce activity by stimulation of α_2 receptors, but in reserpinised animals, produces an increase in activity by stimulation of α_1 receptors. As a model for the study of either sedation or α_2 receptor stimulation, locomotor activity itself is unreliable, since

methoxamine also reduced activity. This drug does not stimulate α_2 receptors selectively, nor does it produce behavioural sedation (Chapter 4). Similar effects were seen with drugs which block α_2 receptors ,when given alone. However, reversal of the effect of α_2 agonists by α_2 antagonists could be used to confirm that α_2, not α_1, receptors were involved in the effect, although there is a possibility that such treatment may also reverse the effects of methoxamine. Thus locomotor activity appears to be an unsuitable method of studying sedation and also as a means of quantifying drug effects at α_2 receptors.

The potentiation of apomorphine-induced activity in reserpinised mice does appear to be a suitable model for the study of α_1 agonists. The main disadvantage of this model, however, is the large number of drugs administered simultaneously and the consequent lack of similarity to the physiological situation.

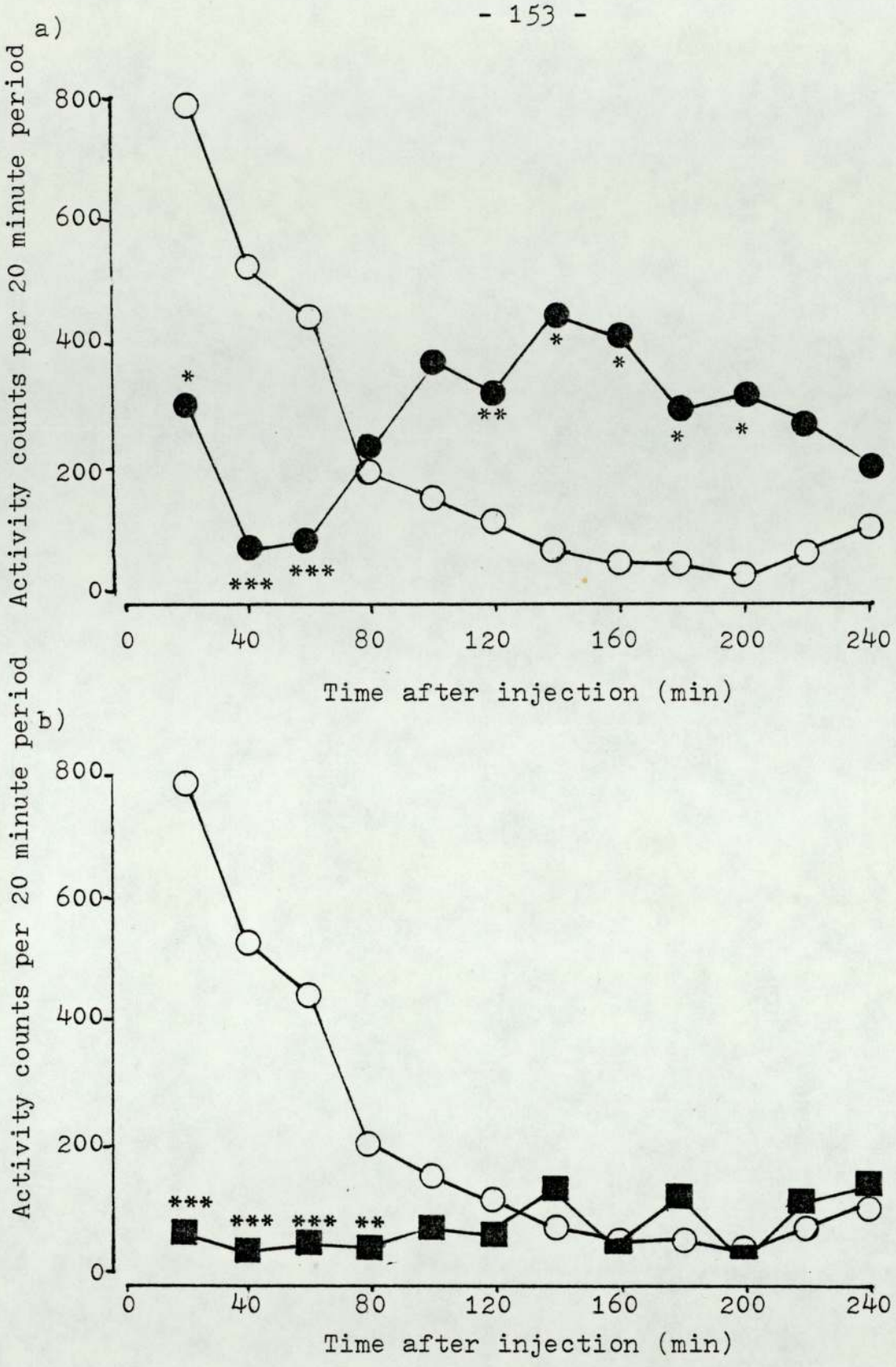


Fig 5.1. The effect of clonidine on locomotor activity in a novel environment, measured from 5 minutes after injection.

(○) - saline; (●) - clonidine 0.05 mg/kg; (■) - clonidine 0.5 mg/kg.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ difference from saline (Student's 't' test).

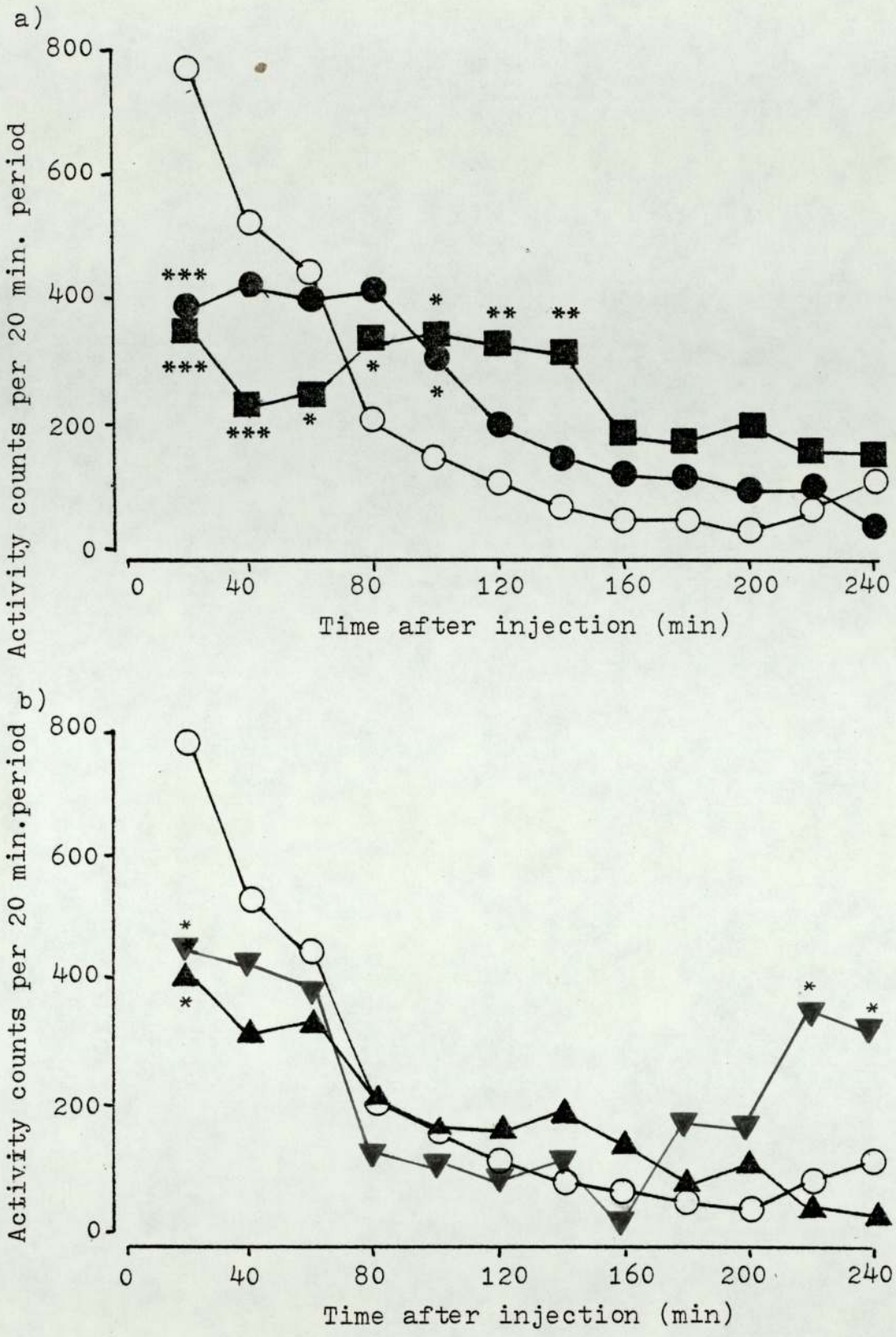


Fig 5.2. The effect of a) yohimbine and b) prazosin on locomotor activity in a novel environment.

(○) - saline; (●) - yohimbine 2.5 mg/kg; (■) - yohimbine 5.0 mg/kg; (▲) - prazosin 0.25 mg/kg; (▼) - piperoxane 5.0 mg/kg.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ difference from saline (Student's 't' test).

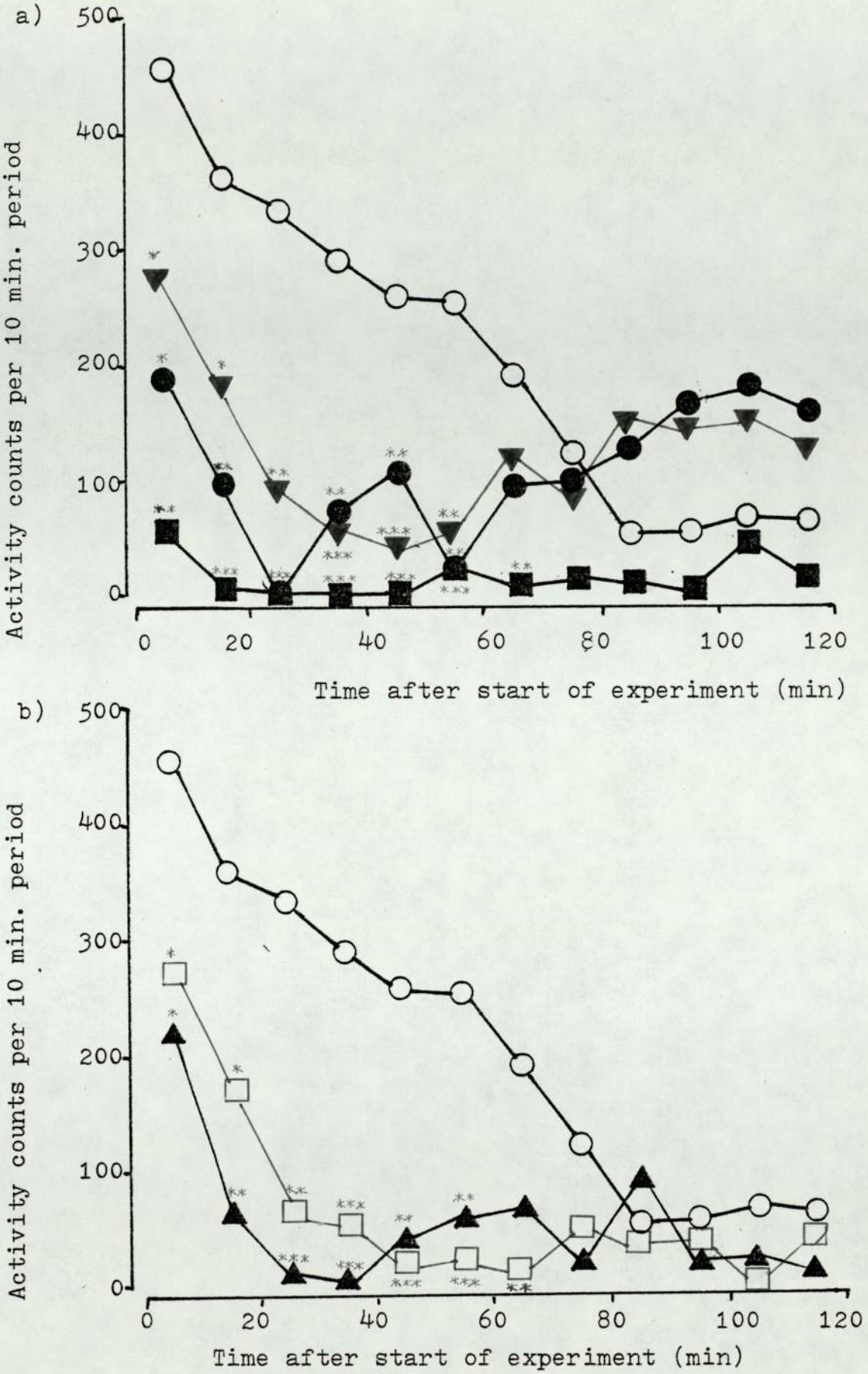


Fig 5.3. The effect of a) clonidine and b) methoxamine on the initial high motor activity in mice.(▼) - clonidine 0.025mg/kg (○) - saline; (●) - clonidine 0.05 mg/kg; (■) - clonidine 5.0 mg/kg; (▲) - methoxamine 10.0 mg/kg (□) -oxymetazoline 2.5mg/kg

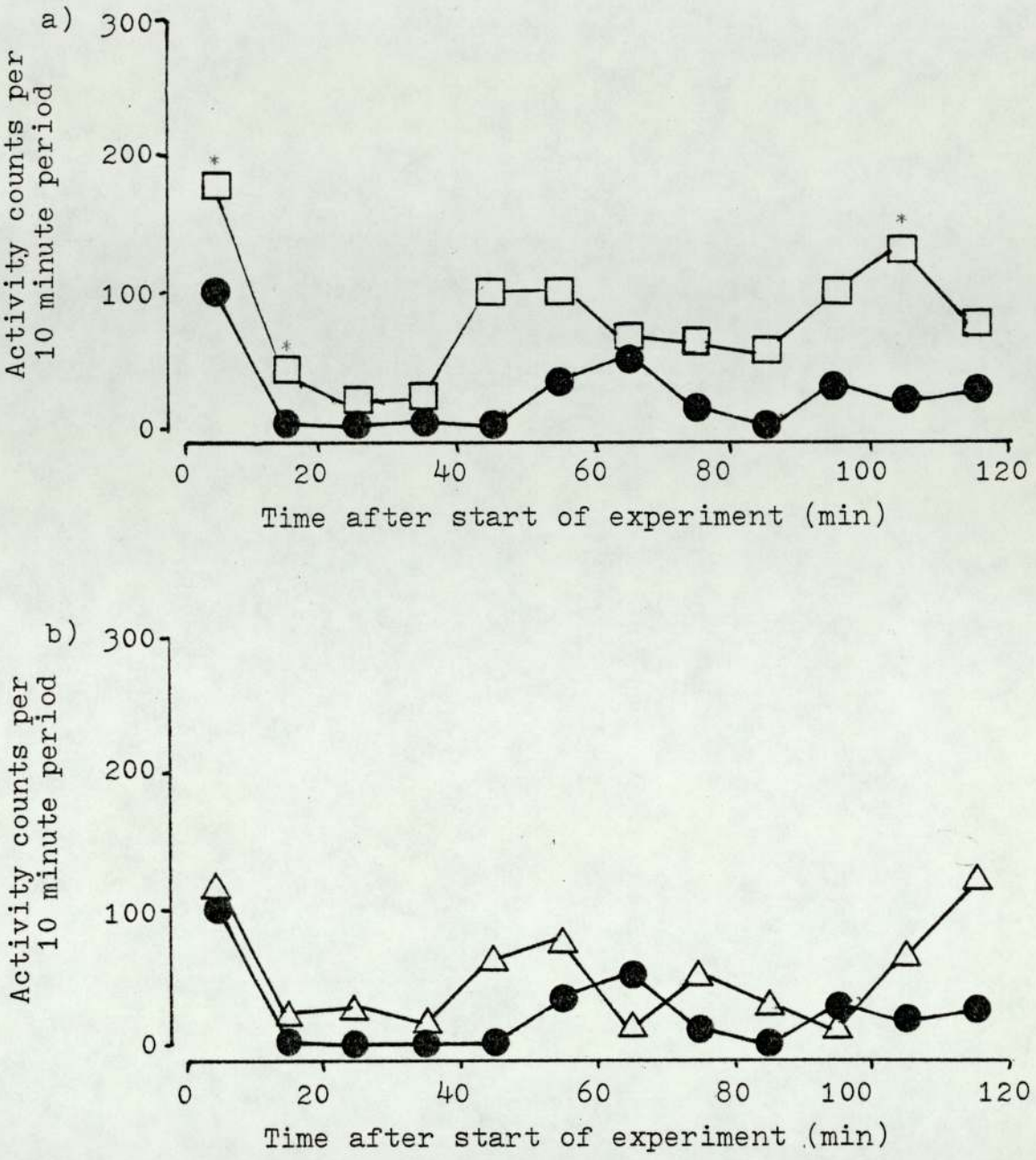


Fig 5.4. The effect of two doses of yohimbine on the decrease in initial high motor activity produced by clonidine 0.5 mg/kg.

(●) - clonidine 0.5 mg/kg; (□) - yohimbine 1.0 mg/kg + clonidine 0.5 mg/kg; (△) - yohimbine 5.0 mg/kg + clonidine 0.5 mg/kg. Yohimbine was administered 15 minutes before clonidine and animals were placed on the Animex 30 minutes later.

* $p < 0.05$ difference from clonidine 0.5 mg/kg (Student's 't' test).

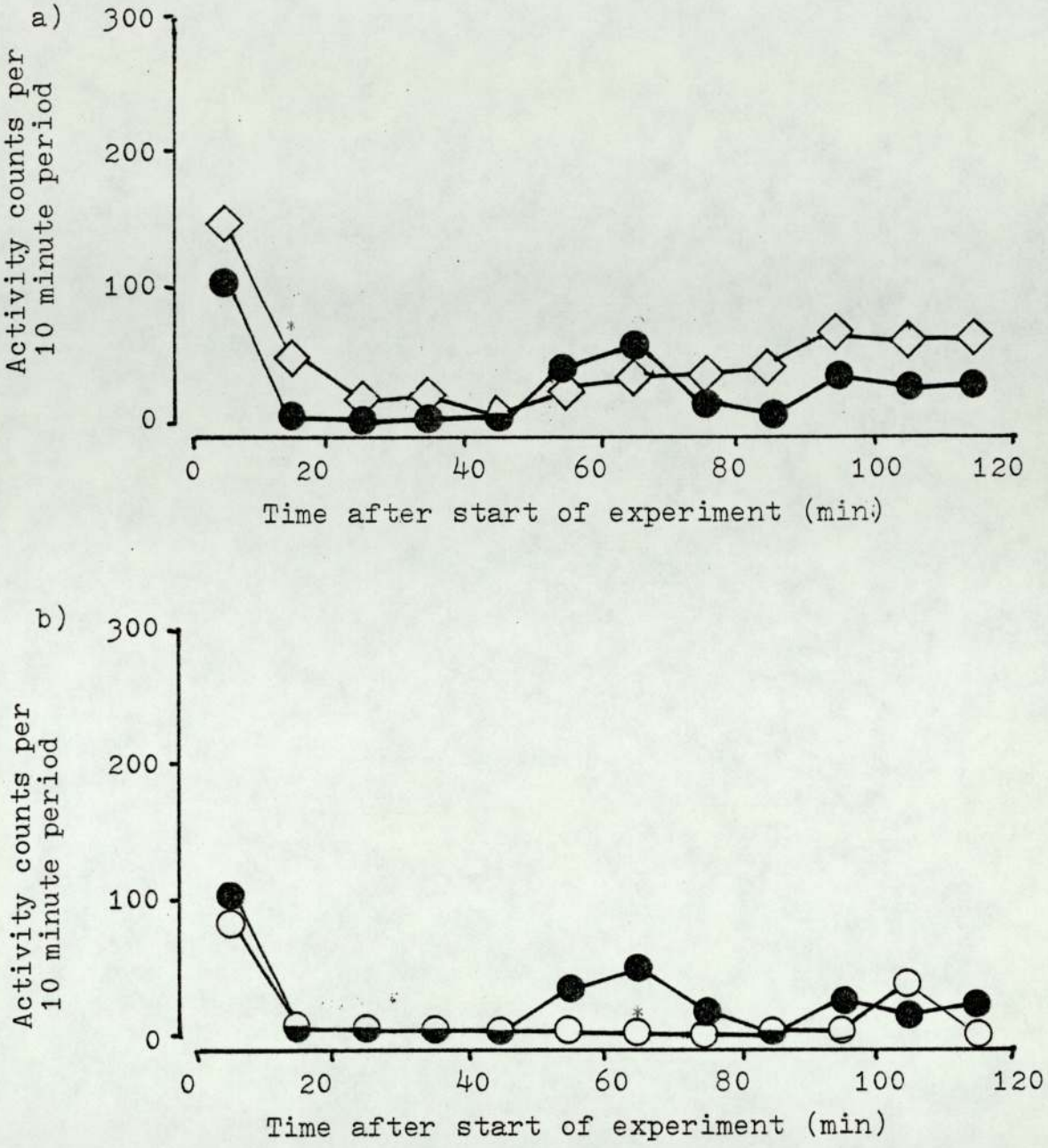


Fig 5.5. The effect of a) piperoxane and b) prazosin on the decrease in initial high motor activity produced by clonidine 0.5 mg/mg.

(●) - clonidine 0.5 mg/kg; (◇) - piperoxane 5.0 mg/kg + clonidine 0.5 mg/kg; (○) - prazosin 1.0 mg/kg + clonidine 0.5 mg/kg. The antagonist was administered 15 minutes before clonidine and the animals placed on the Animex 30 minutes later.

* $p < 0.05$ difference from clonidine 0.5 mg/kg (Student's 't' test).

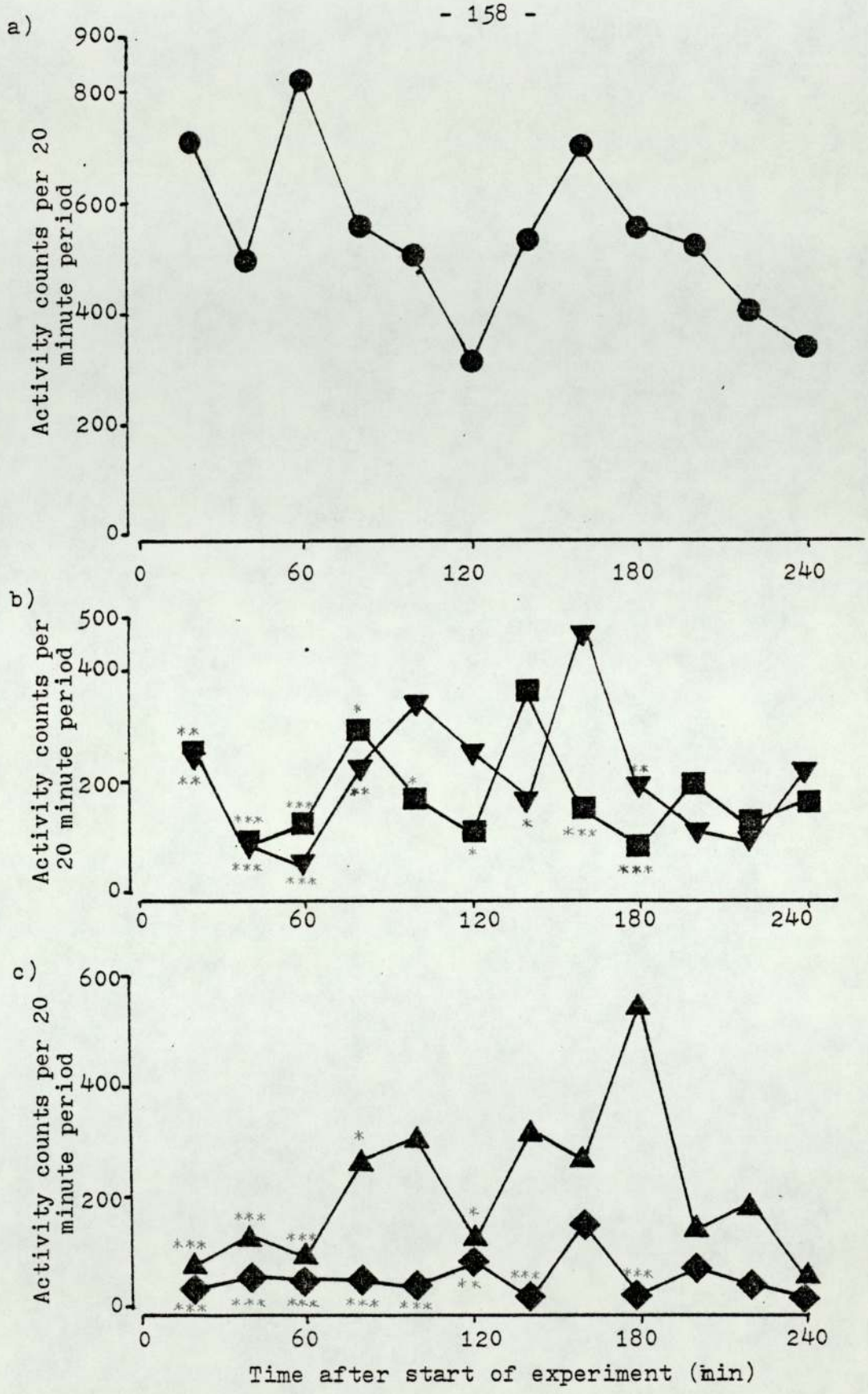


Fig 5.6. The effect of clonidine and methoxamine on home cage activity during the dark phase.

(●) - saline; (■) - clonidine 0.1 mg/kg; (▼) - 0.5 mg/kg; (▲) - methoxamine 10 mg/kg; (◆) - 25 mg/kg.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ difference from saline (Student's 't' test).

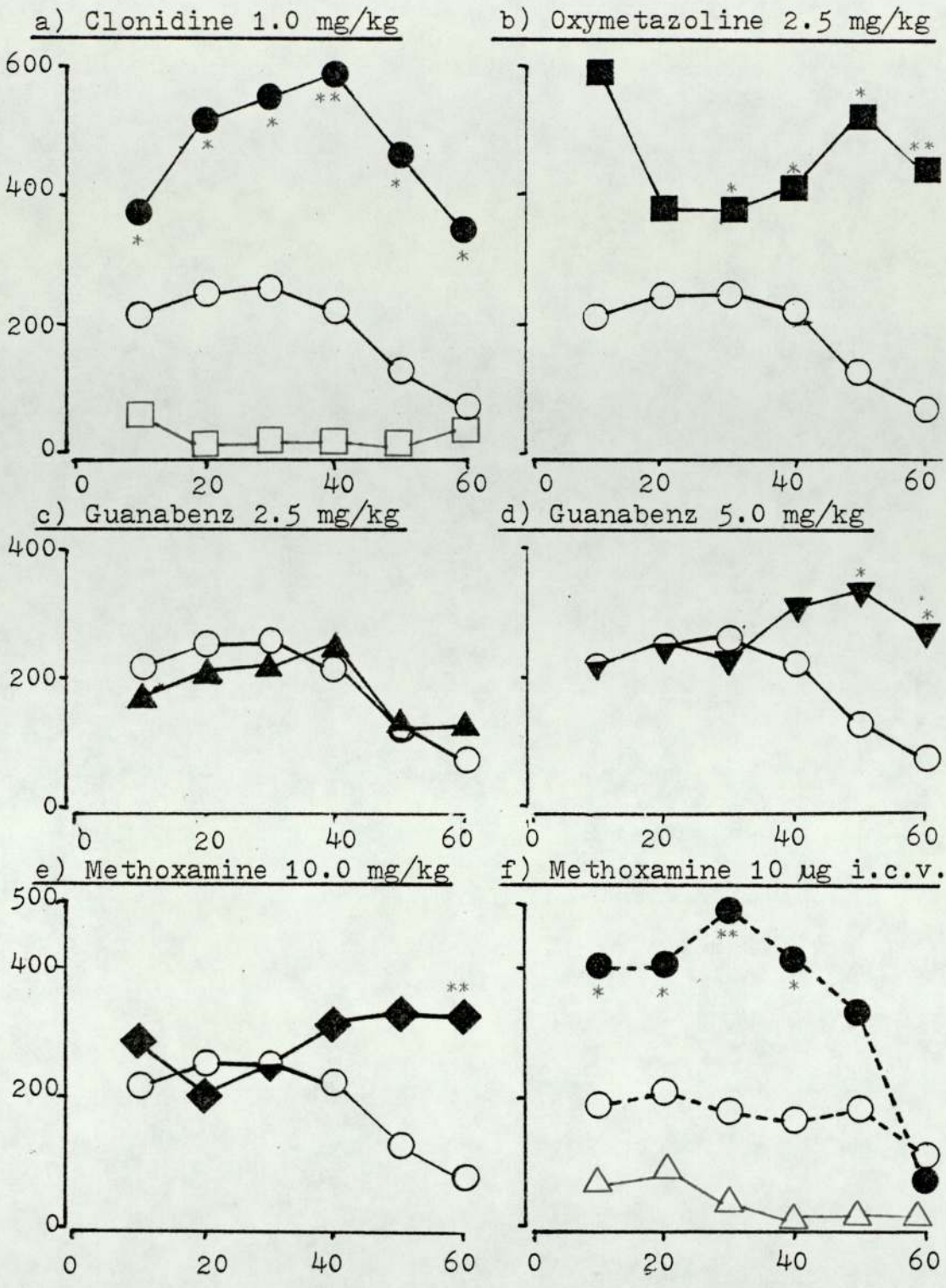
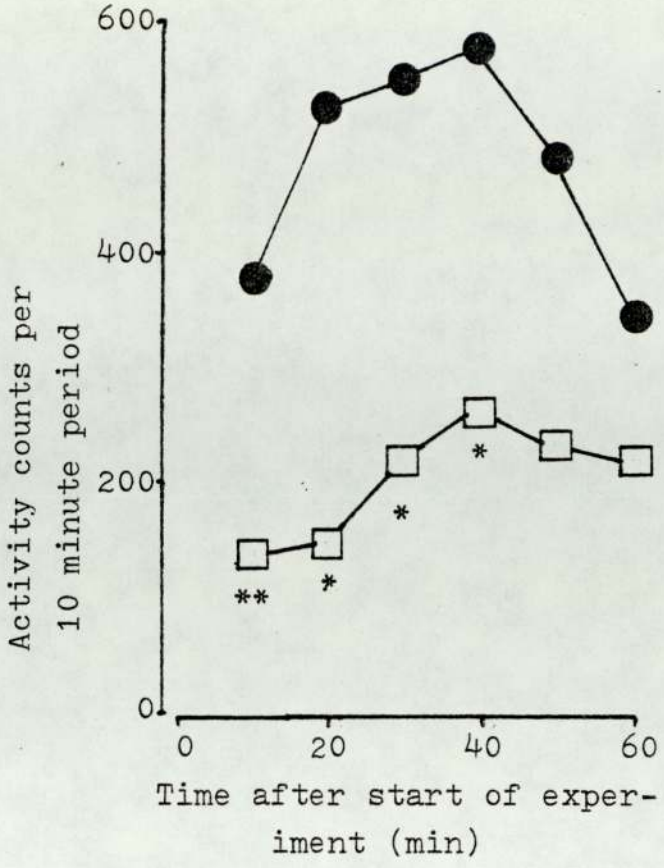


Fig 5.7. The effect of α agonists on apomorphine-induced activity in reserpinised animals.

Abscissa = time after start of experiment (min); **ordinate** = counts per 10 minute period. Animals were given reserpine 5 mg/kg 24 hours before, apomorphine 5 minutes before and the α agonist 15 minutes before the start of the experiment. (□) - clon + res (△) - meth + res (○) - reserpine + apomorphine 1 mg/kg + saline s.c.; dashed line - res + apo + saline i.c.v. *$p < 0.05$; **$p < 0.01$ difference from res + apo + saline (Student's 't' test).



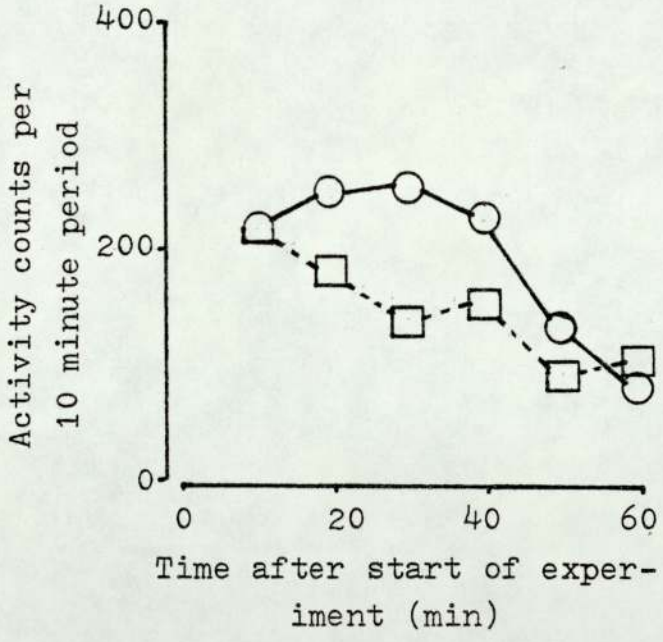


Fig 5.8. The effect of prazosin 2.5 mg/kg on apomorphine-induced activity in reserpinised animals and its potentiation by clonidine 1.0 mg/kg.

(○) - res + apo; (●) - res + apo + clon; (□) res + apo + prazosin (dashed line), res + apo + clon + prazosin (solid line). * $p < 0.05$, ** $p < 0.01$ difference from res + apo + clon (Student's 't' test).

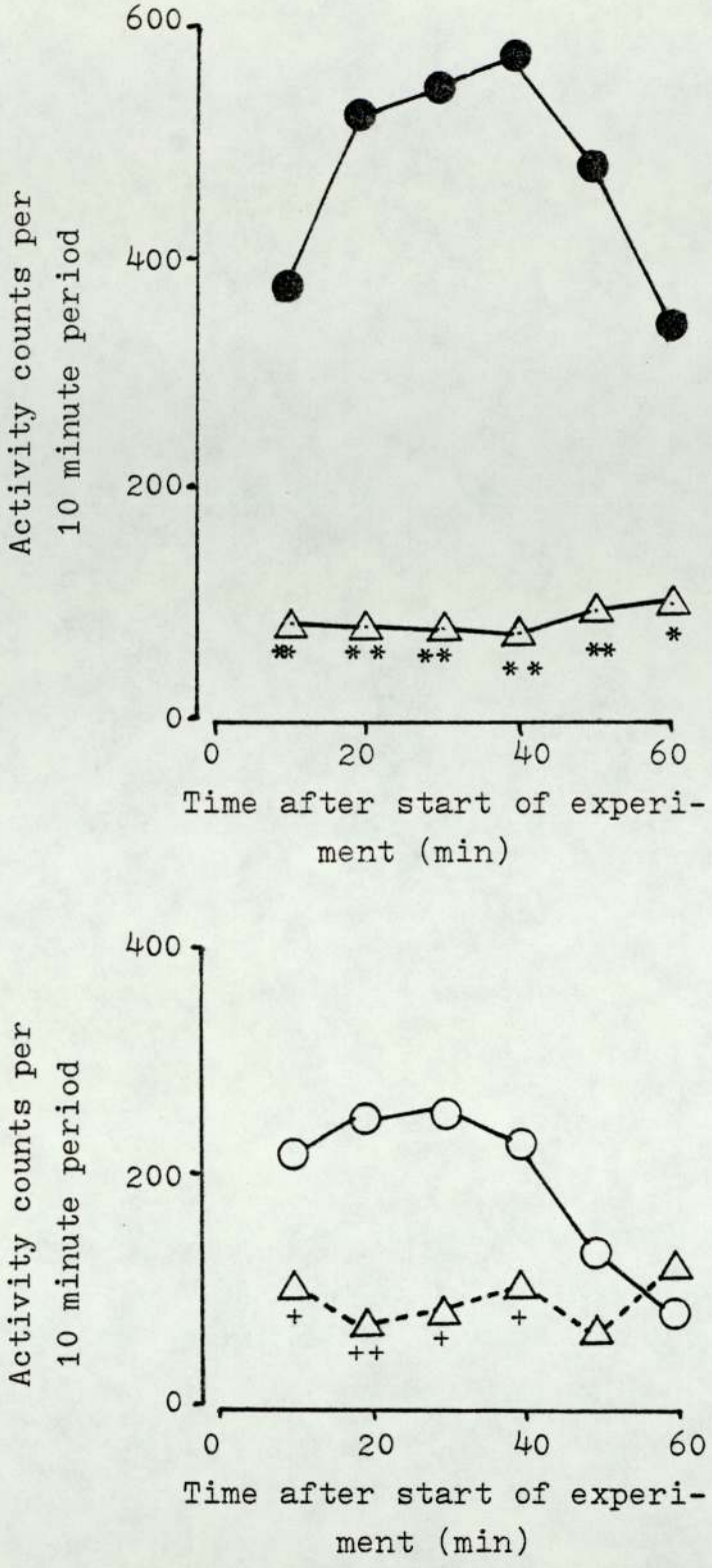


Fig 5.9. The effect of yohimbine 5.0 mg/kg on apomorphine-induced activity in reserpinised animals and its potentiation by clonidine 1.0 mg/kg.

(○) - res + apo; (●) - res + apo + clon; (△) - res + apo + yohimbine (dashed line), res + apo + clon + yohimbine (solid line). * $p < 0.05$, ** $p < 0.01$ difference from res + apo + clon; + $p < 0.05$, ++ $p < 0.01$ difference from res + apo (Student's 't' test).

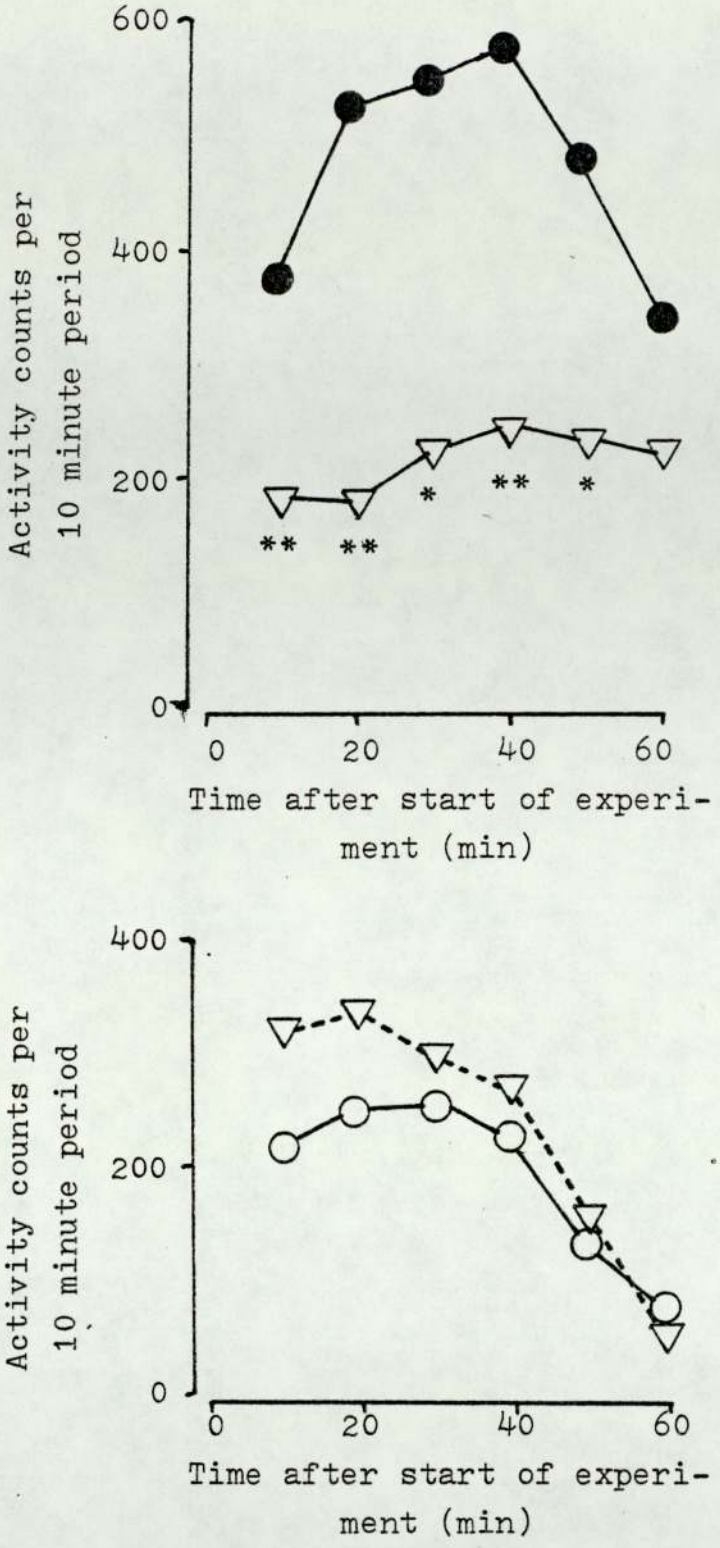


Fig 5.10. The effect of piperoxane 5.0 mg/kg on apomorphine-induced activity in reserpinised animals and its potentiation by clonidine 1.0 mg/kg.

(○) - res + apo; (●) - res + apo + clon; (▽) - res + apo + piperoxane (dashed line), res + apo + clon + piperoxane (solid line).

* $p < 0.05$, ** $p < 0.01$ difference from res + apo + clon, (Student's 't' test).

CHAPTER 6

INVESTIGATION OF THE ANALGESIC EFFECT OF α AGONISTS

CHAPTER 6.

INTRODUCTION

The analgesic activity of clonidine is well documented (e.g. Schmitt et al.,1974), and the involvement of central noradrenergic systems in opiate analgesia has been studied by many workers (for review see Blasig,1978). Opiates affect the turnover of NA and drugs which affect noradrenergic systems may alter opiate analgesia. Many conflicting reports are available on this topic, most of which suggest that an increase in noradrenergic function decreases morphine analgesia. Noradrenaline itself appears to attenuate analgesia due to morphine and other opiates after i.c.v. injection (Sewell & Spencer,1975), but may cause analgesia itself (Handley,1970; Gardella et al.,1970). The involvement of spinal noradrenergic neurones in opiate analgesia has been postulated by Zemlan et al.,(1980), but supraspinal centres also appear to be involved. Lesions of the L.C. in the pons inhibited morphine analgesia in the rat (Sasa et al.,1977), while application of a noxious stimulus caused an increase in firing of L.C. neurones, also in the rat, which was blocked by morphine (Segal,1979). Both clonidine and morphine inhibit spontaneous firing of L.C. neurones, hence this nucleus may be the site of a possible interaction. Clonidine is also able to mimic the action of morphine in relieving symptoms of opiate withdrawal without producing any tolerance itself (Fielding et al.,1977; Gold et al.,1978). It is therefore of interest both as a drug for use in withdrawal and as a potential analgesic without the disadvantages of opiates. It would be useful to determine whether the receptors involved in clonidine's analgesic activity were of the α_1 or α_2 type, since the drug does not act on opiate receptors (Farsang & Kunos,1979).

Selection of a test situation appears to be impor-

tant for experiments involving noradrenergic function and nociception, since the method of measuring analgesia may affect the results obtained (Ross & Ashford,1967). Handley, (1970) found NA to produce analgesia after peripheral injection in mice using the tail clip method, as did Colville & Chaplin (1964) in rabbits using the inflamed foot method. Hence it would appear that these methods are unsuitable for detection of centrally-mediated analgesia. The hot plate method only detected analgesia in mice after NA when injected centrally, not peripherally, hence may seem to be more suitable (Handley,1970). However, Ross and Ashford (1967) suggested that peripheral actions of drugs may be important using methods involving heat stimuli. In the present experiments, inconsistent results were obtained using the tail clip method, since certain of the drugs caused animals to 'freeze' on application of the tail clip, hence the hot plate method as described in Methods was used.

1. Effects of α-adrenoceptor agonists.

Of the agonists tested, only clonidine, guanfacin and guanabenz produced any consistent degree of analgesia. The dose response curves for these three drugs are shown in Fig 6.1. Reaction time on the hot plate was prolonged after s.c. injection of clonidine in doses of 0.1 to 5.0 mg/kg, the effect being dose-dependent. The dose-response curve was shallow up to 0.5 mg/kg, then rose very steeply up to 5.0 mg/kg Higher doses were not used as marked signs of behavioural stimulation were present at this dose. The peak effect of clonidine on reaction time occured 30 minutes after injection. At doses below 5.0 mg/kg, animals showed signs of overt sedation, but were not incapable of motor activity.

After i.c.v. injection, a dose of 1.0μg produced analgesia only after 30 minutes (Fig 6.2.), whilst doses of 0.05 to 0.5μg i.c.v. had no analgesic effect.

Guanfacin in doses of 0.25 to 5.0 mg/kg s.c. prolonged reaction time on the hot plate. A similar shaped dose-response curve to that produced by clonidine was obtained. The potency ratio as compared to clonidine for a 30% increase in reaction time i.e. the shallow part of the curve was 1:5.7; and for a 100% increase on the steep part of the curve was 1:4.

Guanabenz produced analgesia in doses of 0.25 to 5.0 mg/kg s.c. Again a very steep dose-response curve was obtained with potency ratios against clonidine of 1:5.24 for a 30% increase, but 1:0.9 for a 100% increase.

Methoxamine after peripheral injection at a dose of 2.5 mg/kg, produced slight, non-significant analgesia, higher doses having no effect. Central administration of 2μg was ineffective, but both 10 and 20μg produced marked analgesia 5 minutes after injection, 20μg being more effective than 10μg (Fig 6.3.). This effect of methoxamine had disappeared

by 10 minutes after injection of 10μg, but not of 20μg. Hyperalgesia was present 60 minutes after a dose of 10μg; the mice had markedly increased startle and touch responses at both 30 and 60 minutes.

Peripheral injection of NA 0.5 and 1.0 mg/kg produced slight, non-significant hyperalgesia when measured 10 minutes after injection. (Fig 6.4a.). These mice were retested at 30 minutes, when marked hyperalgesia was seen, although this was not dose-dependent (Fig 6.4b.). Animals had increased startle and touch responses at both times. Central injection of NA in doses of 0.25 to 2.0 μg had no significant effect on reaction time, but 10μg produced marked analgesia 15 minutes after injection.

2. Effects of α-adrenoceptor antagonists.

The effects of four α-adrenoceptor antagonists on hot plate reaction time are shown in Fig 6.5. Yohimbine 1.0 and 2.5 mg/kg produced a significant decrease in reaction time, while piperoxane 1.0 to 10.0 mg/kg caused adecrease of similar magnitude which did not appear to be dose-dependent. Increased startle and touch responses were seen after both drugs. Neither phentolamine not prazosin had any significant effect on reaction time after peripheral administration, although phentolamine produced marked analgesia when gived centrally in doses of 5 and 10μg.

3.Effect of α-adrenoceptor antagonists and naloxone on clonidine-induced analgesia.

Clonidine at a dose of 1.0 mg/kg s.c. caused marked analgesia, animals remaining on the hot plate for a mean duration of 15.18 seconds as compared to that of saline controls, which was 6.7 seconds. The effect of α-adrenoceptor antagonists on this analgesia are shown in Fig 6.6. Pretreatment with yohimbine in doses of 0.5 to 2.5 mg/kg had no significant

effect on this analgesia. Piperoxane only produced a significant diminution of the analgesia at 10.0 mg/kg. Both phentolamine and prazosin potentiated clonidine analgesia, although the effect of phentolamine was not significant. The potentiation due to prazosin 0.5 to 2.5 mg/kg was marked, however. Animals appeared sedated, but all showed a co-ordinated reaction to the heat stimulus, hence were not suffering from neurological impairment. Naloxone, which blocks opiate receptors and reverses the analgesic effect of morphine, had no effect on clonidine analgesia in doses of 1.0 to 5.0 mg/kg (fig 6.6).

DISCUSSION

Clonidine produced analgesia as determined by the hot plate test in mice in doses of 0.1 to 5.0 mg/kg, a 100% increase in reaction time being produced by 0.8 mg/kg. The lack of analgesic activity of low doses of the drug after central administration may be due to testing at the wrong time after injection or to an inability of clonidine to reach its site of action from the ventricular system. Higher doses have been found to cause analgesia after i.c.v. injection using the tail flick method in mice (Lipman & Spencer,1979) while Schmitt et al.,(1974) found clonidine in doses of 0.3 to 1.0 µg i.c.v. to be analgesic in the mouse hot plate test. Hence strain differences also may be important in the lack of effect found here. Such differences among rats have been found to be important in other behavioural effects of clonidine (Laverty & Taylor,1969). A further possibility is that the analgesic effect of clonidine does not involve the CNS. The drug can produce analgesia without the involvement of supraspinal centres, as demonstrated by Spaulding et al.,(1979) in the spinalised rat. Such analgesia may be blocked by PBZ (Zemlan et al.,1980), hence either spinal or peripheral α receptors are involved in this effect. Contrary to these reports is the finding that L.C. lesions attenuate clonidine analgesia in mice (Kostowski & Jerlicz,1978) and St 91, a clonidine analogue which does not penetrate the CNS, only produces analgesia after i.c.v. injection (Lipman & Spencer,1979). Hence it would appear that clonidine may produce analgesia by spinal, supraspinal or peripheral mechanisms or any combination of these.

The type of receptor activated by clonidine to produce analgesia in this model would appear to be α_2, since guanfacin and guanabenz were also analgesic, while methoxamine

and NA both caused hyperalgesia, although this was not statistically significant. Blockade of α_2 receptors by yohimbine and piperoxane also resulted in hyperalgesia and but did not attenuate the effect of clonidine. The lack of effect of low doses of these drugs on clonidine analgesia was surprising in view of their potency as antagonists of the pinna reflex inhibition produced by the same dose (Chapter 7). However, Schmitt et al.,(1974) found piperoxane, yohimbine, tolazoline and phentolamine to antagonise xylazine analgesia: and Paalzow and Paalzow (1976) found yohimbine to block the effect of clonidine on vocalisation in rats, whereas PBZ potentiated it. Again, species differences may be responsible for the lack of effect in this model, although this does seem unlikely in view of the above-mentioned pinna reflex results. The lack of effect on clonidine analgesia by peripherally injected phentolamine suggests that clonidine may be acting at a site which is relatively inaccessible to α_2 antgaonists, since phentolamine would be expected to reverse the effect of clonidine. This further suggests that a peripheral action is involved, since phentolamine does not penetrate the CNS. The potentiation of analgesia by prazosin supports the involvement of α_2 rather than α_1 receptors in clonidine's action.

Naloxone was ineffective in reversing the analgesia produced by clonidine in this model. Other workers have also shown a lack of antagonism by naloxone in mouse tail flick (Fielding et al.,1977), phenylquinone writhing (Bentley, 1978) or inhibition of vocalisation in rats (Paalzow & Paalzow 1977). Bentley suggested that morphine may produce local analgesia in writhing experiments by the involvement of α receptors, since piperoxane was able to reduce the effect of morphine. Stimulation of opiate receptors may thus lead to an increased stimulation of α_2 receptors to produce an antinocicep-

tive effect. Opiate analgesia would thus be reduced by α_2 receptor blockade, while clonidine analgesia would be unaffected by opiate antagonists. Experiments on the co-axially stimulated guinea pig ileum have however shown that naloxone is able to reverse the inhibitory effect of clonidine under certain conditions (Mithani,1980). The antihypertensive effect of clonidine is also naloxone-reversible (Farsang & Kunos, 1979), thus clonidine may also release endogenous opiates. However, the lack of effect of naloxone in reversing the analgesic effect of clonidine suggests that this effect is not mediated by such release.

Experiments on individual neurones of the L.C. have shown that, in addition, there is no competition for receptor sites. Thus, naloxone is able to reverse the inhibition of L.C. firing due to morphine, but not amphetamine; while clonidine-induced inhibition of L.C. firing is reversible by piperoxane but not naloxone (Aghajanian,1978). Receptor binding studies have also shown that there is no competition for receptors between opiates and α agonists (Farsang & Kunos,1979), thus the analgesic effect of clonidine does not appear to involve endogenous opiates.

Although this effect of clonidine appears to be mediated via α_2 type adrenoceptors, the peripheral or central location of these receptors can not be established from the results obtained here using the hot plate test in mice. The strain of mouse used may have contributed to the difficulties found in obtaining consistent and therefore significant results due to their apparent hyperreactivity. Hence other strains and other methods of testing may be more useful in elucidating the site of action. It seems likely that both peripheral and central mechanisms are involved in clonidine analgesia, hence it is unsuitable for screening for centrally-acting α_2 agonists.

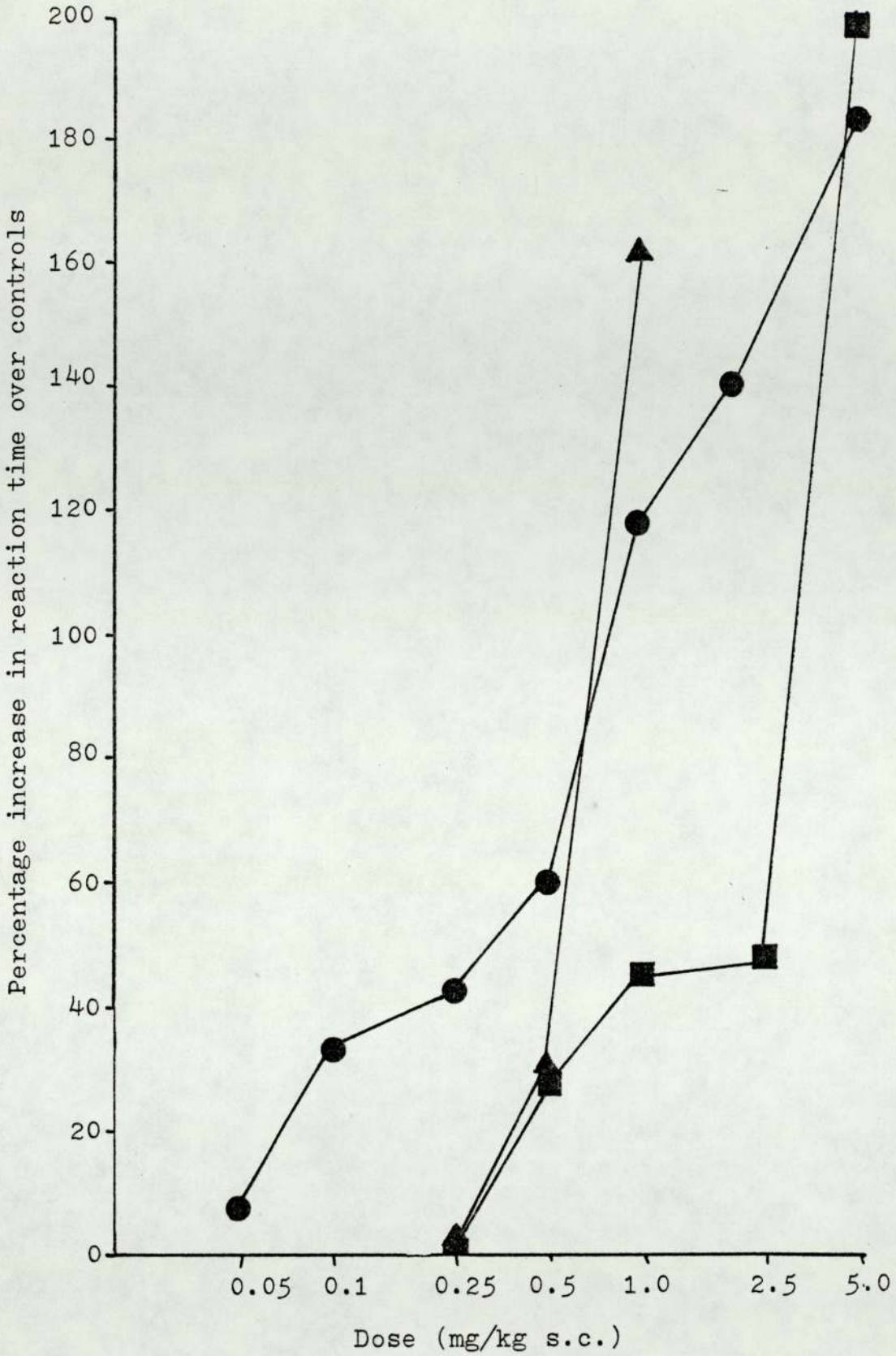


Fig 6.1. The effect of three α agonists on hot plate reaction time, expressed as a percentage of saline-treated animals.

(●) - clonidine; (▲) - guanabenz; (■) - guanfacin.

Animals treated with clonidine and guanabenz were tested 30 minutes after injection, those with guanfacin were tested 90 minutes after injection.

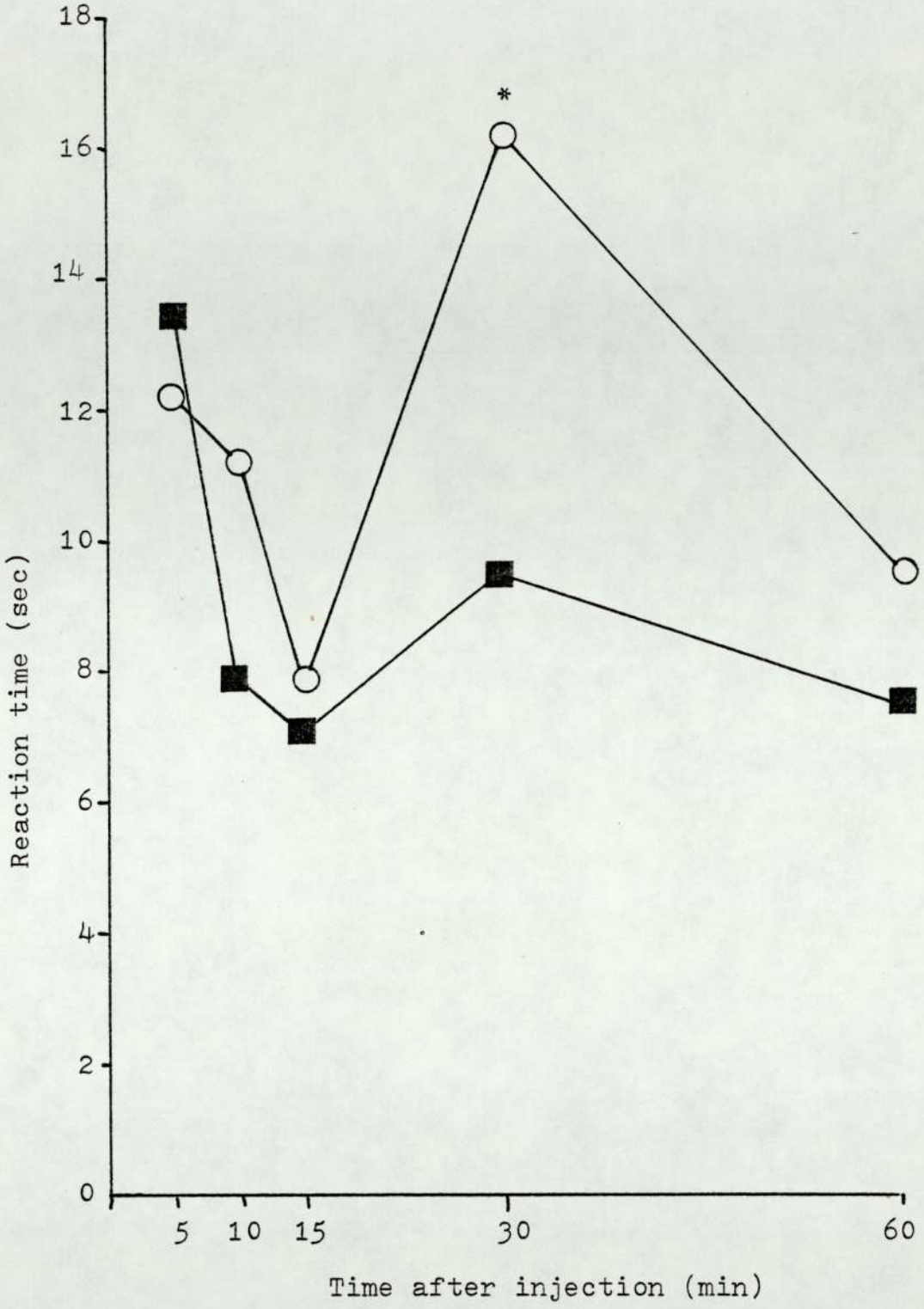


Fig 6.2. The effect of saline and clonidine injected i.c.v. on hot plate reaction time.

(■) - saline; (○) - clonidine 1.0 µg i.c.v.

* $p < 0.05$ difference from saline i.c.v. (Student's 't' test).

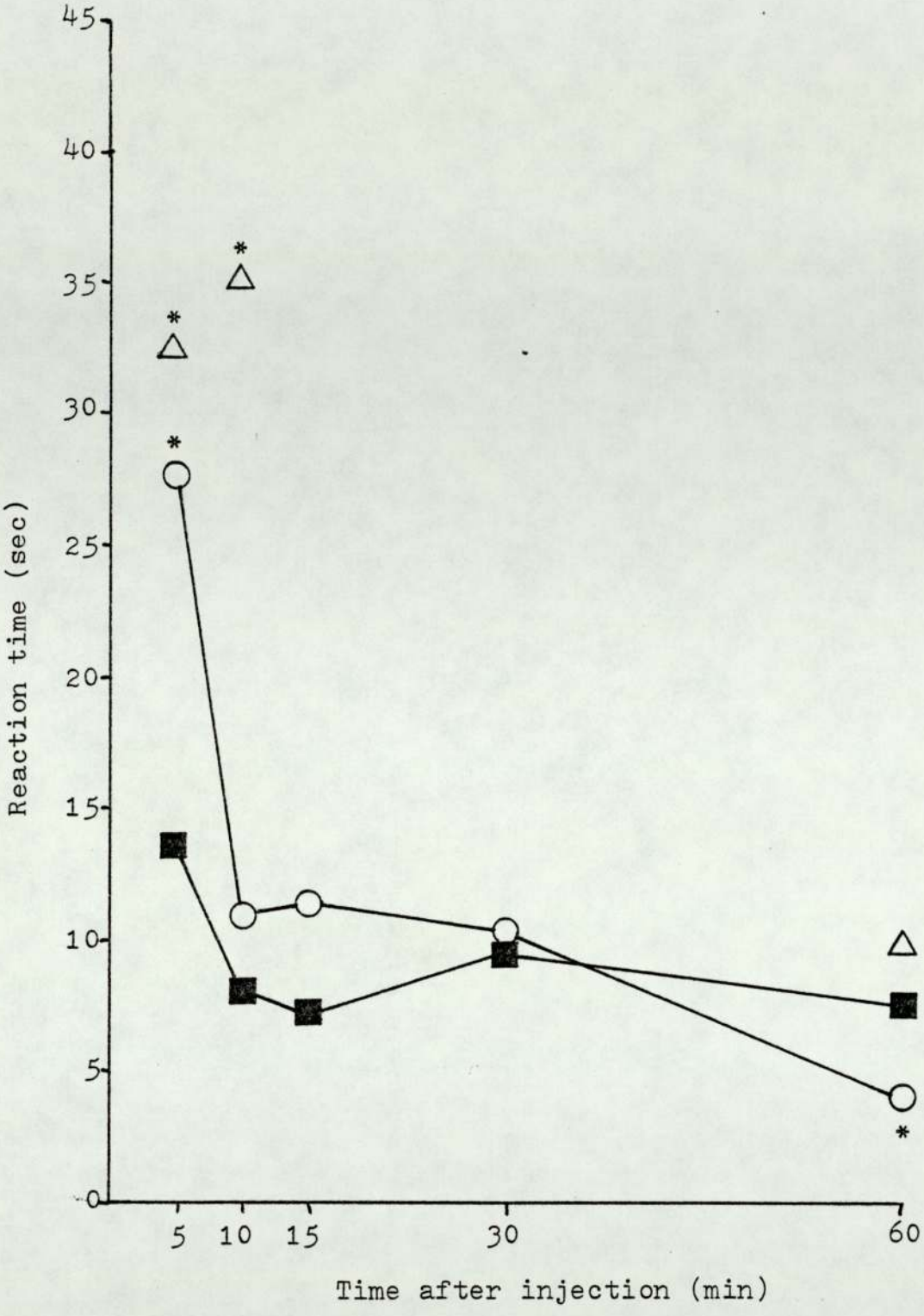


Fig 6.3. The effect of methoxamine injected i.c.v. on hot plate reaction time.

(■) - saline; (○) - methoxamine 10 μg; (△) - methoxamine 20 μg i.c.v.

* $p < 0.05$ difference from saline i.c.v. (Student's 't' test).

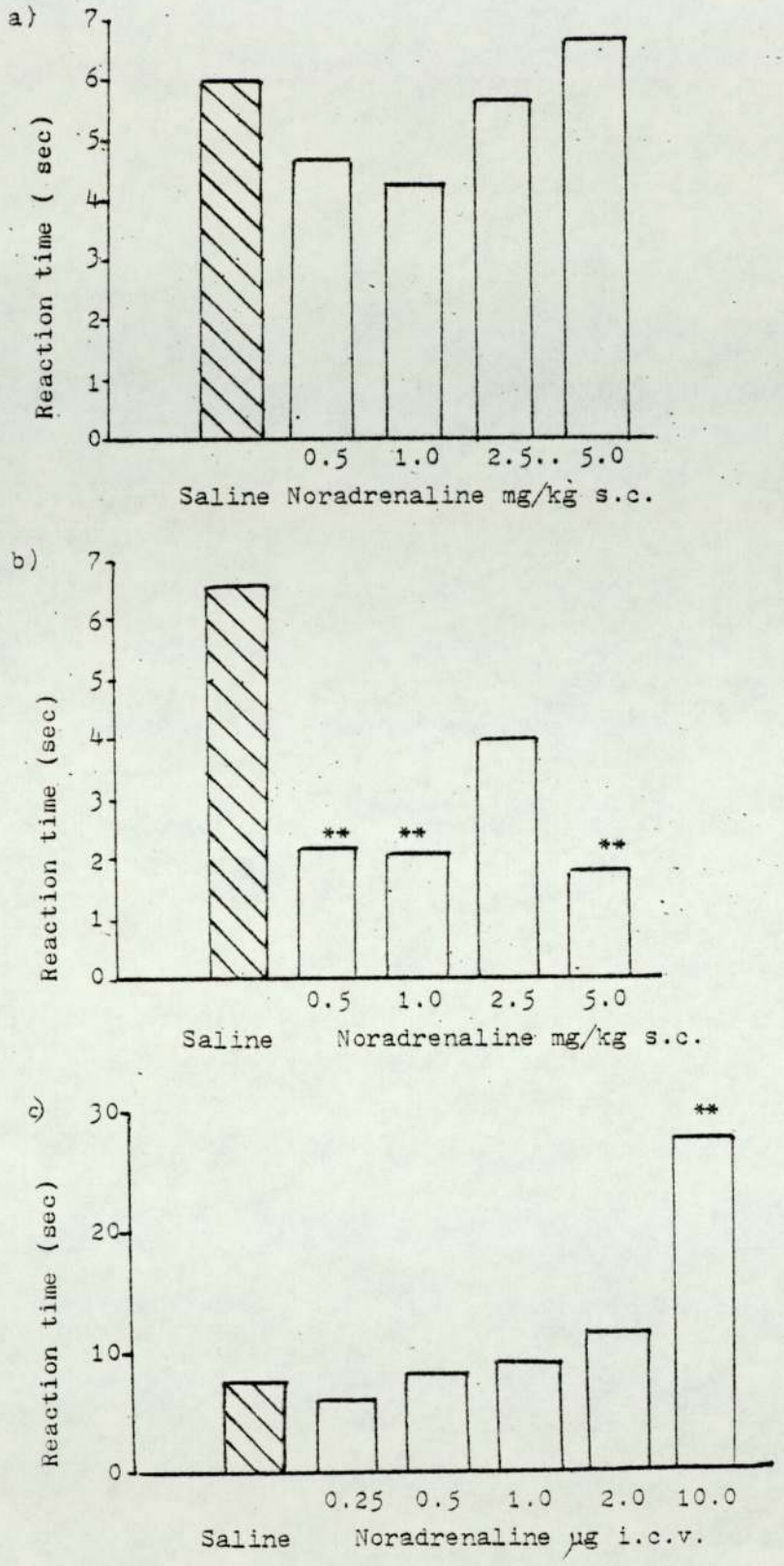


Fig 6.4. The effect of noradrenaline on hot plate reaction time measured a) 10 and b) 30 minutes after s.c. injection in the same animals. and c) after i.c.v. injection.

** $p < 0.005$ difference from saline-treated animals (Student's 't' test).

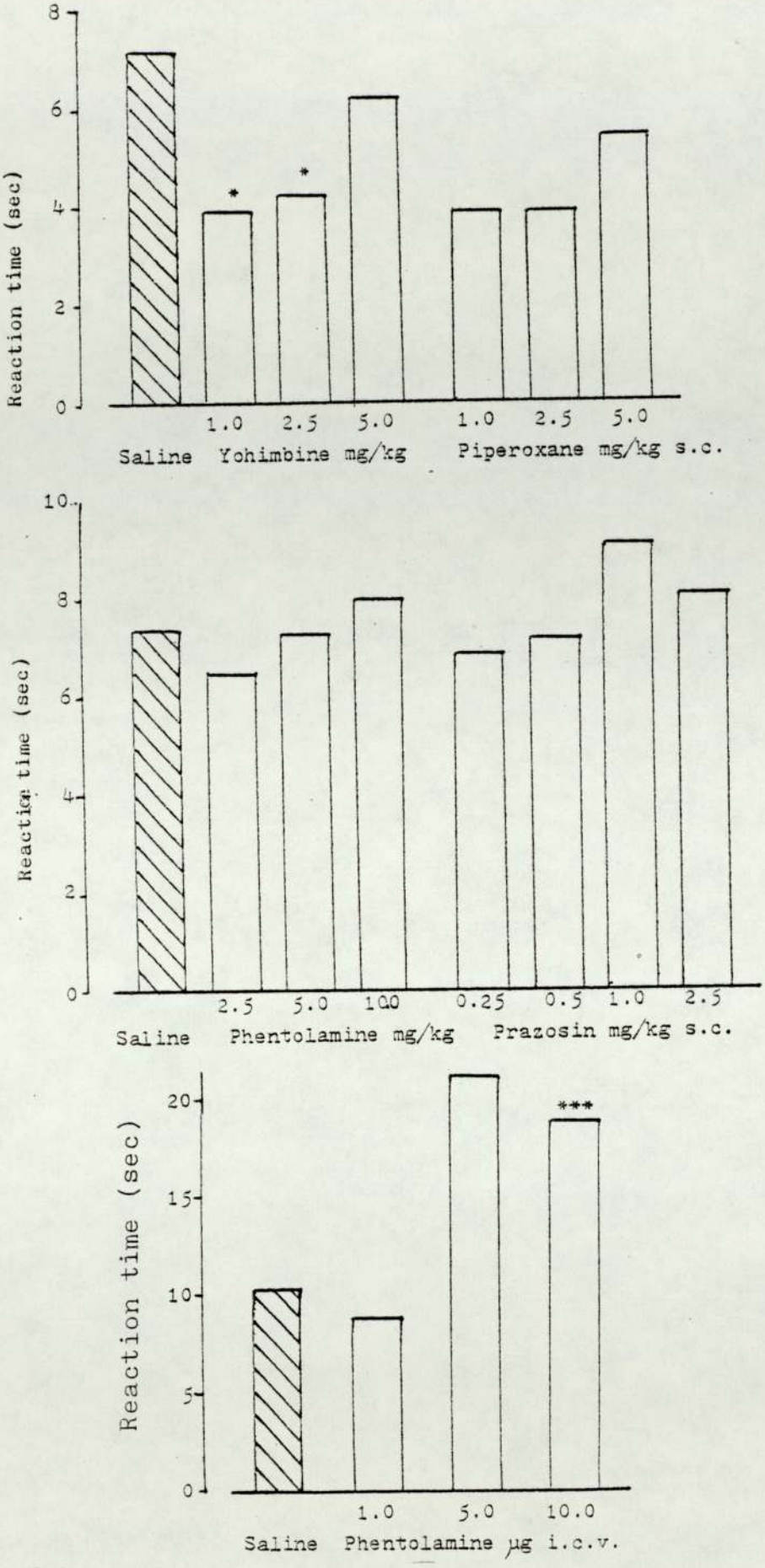


Fig 6.5. The effect of four α antagonists on hot plate reaction time, measured 30 minutes after injection.

* $p < 0.01$ difference from saline-treated animals (Student's 't' test).

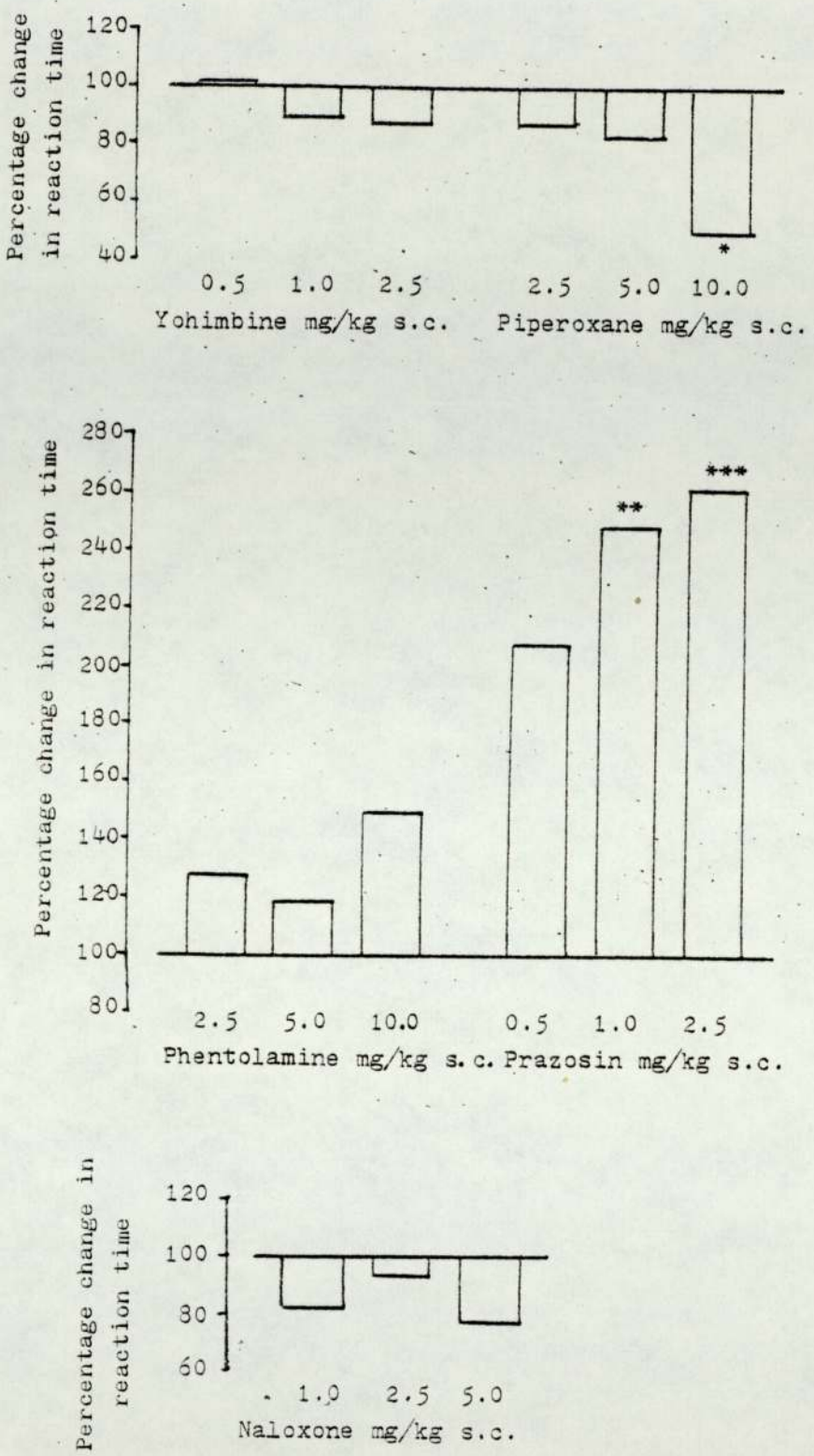


Fig 6.6. The effect of α antagonists and naloxone on analgesia produced by clonidine 1.0 mg/kg (100% level).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ difference from clonidine 1.0 mg/kg (Student's 't' test).

CHAPTER 7

EFFECTS OF α AGONISTS ON THE PINNA REFLEX AND THE 5-HT-INDUCED HEAD TWITCH - A POSSIBLE INTERACTION WITH 5-HT

CHAPTER 7.

INTRODUCTION

"The pinna reflex involves a twitch, tremor or laying back of the ear when the external auditory meatus is stimulated." (Goodsell et al.,1954). The test may be used as an indication of the state of consciousness of an animal, along with the corneal and other reflexes. It was included in the general behavioural observations where it was noted that α_2, but not α_1, agonists were capable of inhibiting the reflex at fairly low doses. Many classes of centrally-acting drugs, such as hypnotics, analgesics, muscle relaxants, some antidepressants and neuroleptics are able to block the pinna reflex (Corne et al.,1963; Witkin et al.,1959). However, very high doses which produce severe neurological impairment are required of all but neuroleptics and muscle relaxants. At the ED_{50} for pinna reflex inhibition of these drugs, however, animals were sedated. The potency of neuroleptics in inhibiting the pinna reflex suggests that DA receptors may be involved in its production. Clonidine has been shown to decrease DA turnover in the rat (Strombom,1975), hence is unlikely to be acting in a similar manner to neuroleptics.

The similarity of the pinna reflex to the head twitch induced by administration of 5-HTP to mice suggests a possible involvement of 5-HT neurones in the reflex. Clonidine also decreases the turnover of 5-HT (Anden et al.,1970), but this is thought to be an indirect effect mediated via α_2 receptors (Svensson et al.,1975). Hence it would seem that α_2 receptors are involved in the inhibition of pinna reflex by clonidine. Further studies were thus carried out in an attempt to confirm this. All experiments were performed on unrestrained mice , since even slight restraint has been shown to inhibit the reflex (Boulton & Handley,1973). The effects of drugs on 5-HT-induced head twitches were also studied.

1. Effect of α-adrenoceptor agonists on the pinna reflex.

Clonidine, guanfacin and guanabenz all depressed the pinna reflex in a dose-dependent manner after s.c. injection (Fig 7.1.). High doses were required to cause inhibition in 100% of animals and sedation was marked at these doses. However, only mild sedation as assessed by behavioural observation was produced by doses close to the ED_{50} value for these drugs (Table 7.3.). Clonidine at all doses produced inhibition of the reflex in some animals by 20 minutes after injection (Fig 7.2.). The peak effect occurred at 40 minutes and the inhibition was maintained for 150 minutes after the highest dose. Clonidine also depressed the pinna reflex after i.c.v. administration (Fig 7.5.).

Guanabenz was also active in some animals 20 minutes after injection (Fig 7.3.), but was approximately 4 times less potent than clonidine. The peak effect did not occur until 60 minutes after injection and the inhibition was maintained for at least 180 minutes after 2.5 mg/kg. Guanfacin wss found to be equipotent with guanabenz in inhibiting the reflex (ED_{50}'s measured at time of peak activity), but it had a delayed onset of action, significant inhibition only occurring 60 minutes after injection (Fig 7.4.). The reflex was still absent in some animals 4 hours after administration of guanfacin.

Methoxamine, oxymetazoline and NA were ineffective in inhibiting the reflex after peripheral administration, hence were injected centrally (Fig 7.5.). Noradrenaline then produced a dose-dependent inhibition of the reflex (Table 7.1) which lasted for 40 to 60 minutes. Methoxamine only partially inhibited the reflex, producing 50% inhibition at both 10 and 20 μg. At these doses, the mice showed behaviour characteristic of extreme fearfulness, remaining immobile with ears

pressed back. Oxymetazoline 2.5 and 5.0 μg i.c.v. had no effect on the pinna reflex until 60 minutes after injection, when inhibition appeared, progressing to 100% by 180 minutes. A marked behavioural syndrome developed over the same time course, consisting of stereotyped head-searching, sniffing, compulsive locomotion, either forwards or backwards, and severe hind limb splay. Both this syndrome and the pinna reflex inhibition were maintained for at least 5 hours. The behavioural syndrome was assessed before each test for pinna reflex as follows: the animals were observed for 2 minutes and were allocated a score of 0 if they remained still; 1, if they showed imtermittent locomotion with head-searching or sniffing , or 2 if such activity was continuous throughout the 2 minutes. The occurrence of this syndrome was shown not to be associated with the occurrence of pinna reflex inhibition using Fisher's exact test (Siegel, 1956).

2. Sedative effects of α agonists and chlorpromazine.

In order to determine whether the inhibition of pinna reflex by α agonists was a specific effect or due to a general depressant activity, the activation scores produced by the ED_{50} doses of all agonists were studied. Chlorpromazine, which has been found to have a fairly specific action on the pinna reflex, was also included in this study. The activation scores obtained are shown in Table 7.3. At dosages which inhibited the pinna reflex in 50% of animals, clonidine, guanfacin and guanabenz produced only mild sedation. The effects of NA, methoxamine and oxymetazoline were studied after i.c.v. administration. Methoxamine did produce mild sedation, but NA increased the activation score. The effect of oxymetazoline was measured 120 minutes after injection when sedation did appear to be present, although even this drug did not produce

such a marked effect as chlorpromazine. A dose of 5 mg/kg was required to inhibit the reflex in mice, at which dose, all parameters measured for the activation score were markedly reduced. The property of pinna reflex inhibition was not therefore associated with sedation by all drugs.

3. Effect of α-adrenoceptor antagonists on the pinna reflex.

When given alone, yohimbine (0.05 to 2.5 mg/kg), piperoxane (0.1 to10.0 mg/kg) and prazosin (0.5 to 5.0 mg/kg) s.c. did not have any effect on the incidence of the pinna reflex. However, it appeared to be more readily elicited and of greater amplitude after yohimbine and piperoxane than in saline-treated animals. Since this effect could not be quantitated, the effects of the antagonists on inhibition produced by submaximal doses of the agonists were studied (see Table 7.2.). Yohimbine and piperoxane produced a dose-dependent reversal of the inhibition due to clonidine, guanfacin, guanabenz and oxymetazoline and also prevented the partial inhibition caused by methoxamine 20μg i.c.v. The doses of the antagonists required were much higher after oxymetazoline than after the other agonists. Prazosin at a dose of 2.5 mg/kg partially (22%) reversed the clonidine-induced inhibition of pinna reflex, 5.0 mg/kg having no further effect. This drug had no effect on the inhibition due to guanfacin, guanabenz, methoxamine and oxymetazoline. It was, however, partially effective in reversing the behavioural syndrome produced by i.c.v. oxymetazoline at a dose of 5.0 mg/kg. Piperoxane was also able to attenuate the syndrome dose-dependently, while yohimbine was less effective. Haloperidol 0.1 mg/kg was also used in an attempt to determine the type of receptors involved, but was not as potent as prazosin in reversing the syndrome.

4. Effects of β antagonists on the pinna reflex.

Propranolol 1mg/kg, d-propranolol 1mg/kg and sotalol 10mg/kg had no effect on the incidence of the pinna reflex after s.c. injection. All mice treated with these drugs possessed a pinna reflex in one or both ears.

5. Effects of α agonists and antagonists on the 5-HT head twitch.

The effects of drugs were studied on head twitches induced by the central administration of 5-HT 50µg, since peripheral injection of 5-HTP as a method of inducing twitches may be less specific. This method may lead to the formation of 5-HT in noradrenergic and dopaminergic neurones, hence may interfere with the effects of drugs which act on these neurones. Clonidine produced a dose-dependent inhibition of the head twitch as measured by percentage reduction in the total number of twitches in a period of 24 minutes. The ED_{50} was found to be 0.049 (0.03 - 0.079) mg/kg, thus clonidine was 5 to 6 times more potent in inhibiting the head twitch induced by 5-HT 50µg than the pinna reflex. However, the relative potency is not absolute, since ED_{50} values may depend on the dose of 5-HT used. Methoxamine at a dose of 10µg also inhibited the 5-HT head twitch by 68% when the two drugs were administered together.

Yohimbine markedly potentiated the number of twitches produced by 5-HT in a dose-dependent manner over the dose range 0.0312 to 1.0 mg/kg. The dose which potentiated the head twitch by 100% was 0.047(0.027 - 0.083)mg/kg (95% confidence limits Documenta Geigy, p 176,1962), thus this drug also

showed increased potency over its effects on the pinna reflex. Prazosin produced a dose-dependent inhibition of the 5-HT head twitch, with an ED_{50} of 0.88(0.615 - 1.26) mg/kg.

DISCUSSION

Drugs which stimulate α-adrenoceptors have been shown to inhibit the pinna reflex in mice. The order of potency was found to be:

clonidine > guanfacin = guanabenz >> noradrenaline = oxymetazoline > methoxamine

after peripheral administration, with potency ratios of:

1 : 4 : 4 : > 36.3 : > 36.3 : > 90,

the latter three drugs being ineffective. After central administration, NA, oxymetazoline and methoxamine did inhibit the reflex, NA and oxymetazoline being approximately equipotent, but 6 times less potent than clonidine, while methoxamine was approximately 34 times less potent than clonidine i.c.v. The order of potency suggests that α_2 receptors may be involved in this effect, since clonidine, guanfacin and guanabenz all selectively stimulate α_2 receptors. Noradrenaline has also been found to have an effect on α_2 receptors, although it does not display any selectivity. Oxymetazoline, while being a relatively potent α_2 agonist *in vitro* (Doxey, 1979), displays selectivity for α_1 receptors (Drew,1976; Doxey,1979), thus α_2 effects are less detectable *in vivo*. Methoxamine has been found to possess very little α_2 stimulant activity; and high doses centrally administered were required to inhibit the pinna reflex. When inhibition did occur after methoxamine, it appeared to be associated with behaviour characterestic of extreme fearfulness, which may have a similar inhibitory effect on the reflex to that of actual physical restraint.

The inhibition of pinna reflex produced by oxymetazoline after i.c.v. injection had a delayed onset, which suggests that a metabolite may be responsible. A stereotyped

behavioural syndrome also occurred with a similar time course to pinna reflex inhibition, though the two were not associated. Prazosin 5 mg/kg was able to prevent this syndrome to some extent, as was piperoxane 10 mg/kg. Lower doses of piperoxane and yohimbine were less effective, suggesting the possible involvement of α_1 receptors in the syndrome. However, this seems unlikely in view of the lack of similar behavioural effects on administration of other α_1 stimulants. Since DA has been shown to be associated with stereotyped behaviour (Randrup & Munkvad, 1970), haloperidol 0.1 mg/kg was also included in the study of this syndrome, but was found to have only a slight inhibitory effect.

The effect of oxymetazoline on the pinna reflex was however shown to be due to α_2 receptors, since yohimbine and piperoxane, which selectively block these receptors, were able to reverse this inhibitory effect, while prazosin, which blocks α_1 receptors, was inactive. Similar effects were shown for the inhibition of pinna reflex by clonidine, guanfacin and guanabenz and also for the partial inhibition occurring after methoxamine i.c.v. Inhibition of pinna reflex in the mouse may thus be achieved by stimulation of central α_2 receptors. If a presynaptic α_2 adrenoceptor is involved, causing inhibition via reduced release of NA, blockade of the postsynaptic receptor would also be expected to inhibit the reflex. Prazosin, however, which blocks postsynaptic α_1 receptors *in vitro*, had no inhibitory effect on the reflex. The possibility that the postsynaptic receptor may be β-adrenergic was also investigated. Propranolol and sotalol were found to have no effect when given alone, although some potentiation of inhibition by α_2 agonists was seen. There was, however, some antagonism between these β blockers and clonidine. This action may not

involve β receptor blockade, since d-propranolol, which has no such activity, also potentiated the effect of clonidine. Weinstock et al.,(1977) found the ED_{50}'s for mouse pinna reflex inhibition by a series of β blockers to be in the range 40 to 100 mg/kg s.c. In view of this high dosage and the fact that d-propranolol also inhibited the reflex at these doses, it is unlikely that a postsynaptic β receptor could be involved in the pinna reflex. The effects of β blockers shown by Weinstock et al. may be due to a local anaesthetic action of the drugs.

Since neither α_1 nor β adrenoceptor blockade inhibited the pinna reflex, the α_2 receptor, stimulation of which does produce inhibition, may be located postsynaptically to a noradrenergic neurone. Although reserpine inhibits the reflex (Corne et al.,1963), this may not be due to a reduction in NA levels, but could involve either 5-HT or DA. A postsynaptic inhibitory α_2 receptor located on either a serotonergic or dopaminergic neurone may thus produce a similar effect to reserpine. Dopamine is the more likely transmitter to be involved, since blockade of DA receptors inhibits the reflex (Witkin et al.,1959), whereas cyproheptadine and methysergide, which block 5-HT receptors, are only active in very high doses (Corne et al.,1963: Weinstock et al.,1977).

The probable lack of involvement of 5-HT in the pinna reflex seems surprising in view of the close similarity in appearance of this reflex to head twitches induced by 5-HT stimulants, and their mutual inhibition by clonidine. The effect of clonidine on head twitches induced by i.c.v. 5-HT may also invlove α_2 receptors, since yohimbine, which blocks these receptors, was found to strongly potentiate twitching. Yohimbine and piperoxane both produced occasional head twitches

alone (Chapter 3), an effect also seen with yohimbine by other workers (Corne & Pickering,1967). This drug may directly stimulate 5-HT receptors (Sanghvi & Gershon,1974) since it increases brain 5-HT levels (Papeschi et al.,1971) and reduces turnover (Papeschi & Theiss,1975). However, clonidine is also able to affect 5-HT turnover (Anden et al.,1970), leading to a decrease, probably indirectly via stimulation of α_2 receptors. This effect of clonidine may be due to a decrease in the firing rate of raphe neurones (Svensson et al.,1975) caused by a reduction in noradrenergic activity. Yohimbine may therefore similarly affect 5-HT neurones indirectly via an increase in NA release.

Clonidine has also been shown to inhibit 'wet dog shakes' in rats produced by 5-HTP (Bednarczyk & Vetulani,1978). The withdrawal of morphine from dependent rats results in a similar syndrome (Martin et al.,1963), which may involve central 5-HT (Green & Grahame-Smith,1975), although 5-HT antagonists are unable to reduce this. (Vetulani & Bednarczyk, 1977). Clonidine is, however, able to antagonise this shaking behaviour, both when induced by nalorphine in morphine-dependent rats (Vetulani & Bednarczyk,1977) or on withdrawal of morphine (Bednarczyk & Vetulani,1978). Since morphine is able to inhibit head twitches induced by LSD (Vetulani et al., 1979), it would appear that both opiate and α_2 receptors are present in the neuronal pathway leading to the production of head twitches. Morphine dependence may thus lead to 5-HT receptor supersensitivity by suppression of 5-HT release. Withdrawal of morphine would then have a similar effect to that of administration of a 5-HT agonist. Since clonidine is able to block this effect, the morphine withdrawal syndrome may be antagonised without the stimulation of opiate receptors.

Such a method has been used successfully in the clinic to treat methadone addicts (Gold et al.,1978).

Prazosin was also found to inhibit the 5-HT head twitch, as do phentolamine and PBZ (Maj et al.,1978b), It thus appears that α_1 receptors do play a part in the mediation of this response, in addition to α_2 receptors, unlike the pinna reflex. Methoxamine, despite inducing twitches alone (Chapter 3), produced inhibition of the head twitch at a dose of 10µg i.c.v. Previous work has shown that methoxamine produced a slight potentiation of head twitches induced by 5-HTP at low doses, but this was masked by inhibition as the dose was increased (Handley & Powell, unpublished observations). This inhibitory effect of methoxamine may be explained by its β-blocking activity (Karin,1965), since blockade of β receptors has been shown to inhibit the 5-HTP head twitch (Weinstock et al.,1977). Propranolol was however found to be ineffective in reducing the 5-HT head twitch in these experiments. It is possible that the α_2 and α_1 receptors may be located at different sites in the neuronal pathway and that stimulation of α_1 receptors is a prerequisite for the head twitch to occur; thus α_1 receptors may be already fully occupied by NA. Blockade of these receptors would thus be expected to decrease the head twitch, whereas further stimulation may be ineffective. Methoxamine may thus either stimulate α_2 receptors at high doses or, if the drug had a higher affinity for α_1 receptors and a lower intrinsic activity than NA, displace NA to reduce receptor stimulation. Both of these effects would lead to a decrease in the head twitch response to 5-HT stimulation. Experiments have since been performed which show that if α_2 receptors are blocked by yohimbine, methoxamine does potentiate the head twitch. The inhibitory effect of the

drug thus appears to be due to α_2 receptor stimulation.

The very low doses of clonidine, yohimbine and prazosin required to inhibit the 5-HT head twitch suggest that this a specific action on 5-HT systems. The depressant effect of clonidine on the pinna reflex may, however, reflect a more general sedative effect of the drug. Although, in view of the high doses of many other drugs with sedative properties which are required to inhibit the reflex, this seems unlikely e.g. ED_{50}'s for promethazine - 140 mg/kg; chlorpheniramine - > 400 mg/kg; amitriptyline - 120 mg/kg; thioridazine - 140 mg/kg; chlordiazepoxide - > 400 mg/kg (Corne et al.,1963). In addition, the ED_{50} doses of clonidine, guanfacin and guanabenz produced only a slight reduction in activation score. Thus the inhibitory effects of clonidine on both the pinna reflex and 5-HT head twitch appear to be specific and due to stimulation of α_2 adrenoceptors. It is possible that the neuronal pathway involved in the pinna reflex may be further stimulated by 5-HT although the 5-HT neurones involved may normally be inactive. Thus blockade of 5-HT receptors would have no effect on the pinna reflex, while their stimulation may potentiate the activity of the pathway to such an extent that the characteristic twitch of the head occurs without physical stimulation.

Inhibition of the 5-HT head twitch, although a specific action of α_2 receptor agonists, may also be produced by 5-HT antagonists, neuroleptics and analgesics (Corne et al.,1963). This, combined with the difficulty in obtaining consistent results, the need for a constant, quiet environment and some training in the handling of animals renders this method unsuitable for the detection *in vivo* of central α_2 adrenoceptor stimulants. The inhibitory effect of prazosin also suggests that care should be taken in the interpretation of results obtained from drugs capable of blocking α_1 recept-

ors when using this method, for example, mianserin and amitriptyline (Maj et al.,1978a;1979), since inhibition of the head twitch may be due to either α_1 blockade or antagonism of 5-HT receptor activation.

The inhibition of the pinna reflex, on the other hand, appears to be a good test for the detection of central α_2 receptor agonists. Drugs which act solely on α_1 receptors have very little effect on the reflex, hence it may be used to demonstrate drug selectivity. The test may easily be incorporated into existing behavioural investigations, providing the animals are not restrained. The method used here is suitable for the determination of information concerning the ability of a drug to penetrate the CNS, any obvious observable side effects, such as sedation and the duration of action. It is also simple to perform, requires no prior training and may be used quantitatively.

DRUG	ED_{50} for inhibition of pinna reflex, with 95% confidence limits, measured at time of peak inhibitory effect	Time after injection (min)
Clonidine	0.275 (0.17 - 0.43) mg/kg s.c.	40
	0.44 (0.275 - 0.7) µg i.c.v.	20
Guanfacin	1.12 (0.67 - 1.88) mg/kg s.c.	180
Guanabenz	1.12 (0.65 - 1.94) mg/kg s.c.	60
Noradrenaline	10.0 mg/kg s.c.	
	2.7 (1.81 - 3.82) µg i.c.v.	20
Oxymetazoline	10.0 mg/kg s.c.	
	2.9 (1.84 - 4.58) µg i.c.v.	150
Methoxamine	25.0 mg/kg s.c.	
	approx. 15.0 µg i.c.v.	20

Table 7.1. The potencies of a range of α agonists in inhibiting the pinna reflex in mice.

AGONIST	ID_{50} for reversal of inhibition of pinna reflex with 95% confidence limits, measured at time of peak activity of agonist * (mg/kg s.c.)		
	Yohimbine	Piperoxane	Prazosin
Clonidine 1.0 mg/kg	0.85(0.50 - 1.45)	0.2(0.11 - 0.36)	14.2
Guanfacin 2.5 mg/kg	0.14(0.08 - 0.24)	1.3(0.62 - 2.73)	-
Guanabenz 2.5 mg/kg	0.58(0.34 - 2.13)	0.23(0.15 - 0.36)	-
Oxymetazoline 5.0 µg i.c.v.	1.45(0.99 - 2.13)	5.4(4.06 - 7.18)	-
Methoxamine 20.0 µg i.c.v.	0.9(0.62 - 1.31)	approx. 2.0	-

Table 7.2. The potencies of α antagonists in reversing the inhibition of pinna reflex produced by various α agonists.

* see Table 7.1. for the times of peak activity of the agonists.

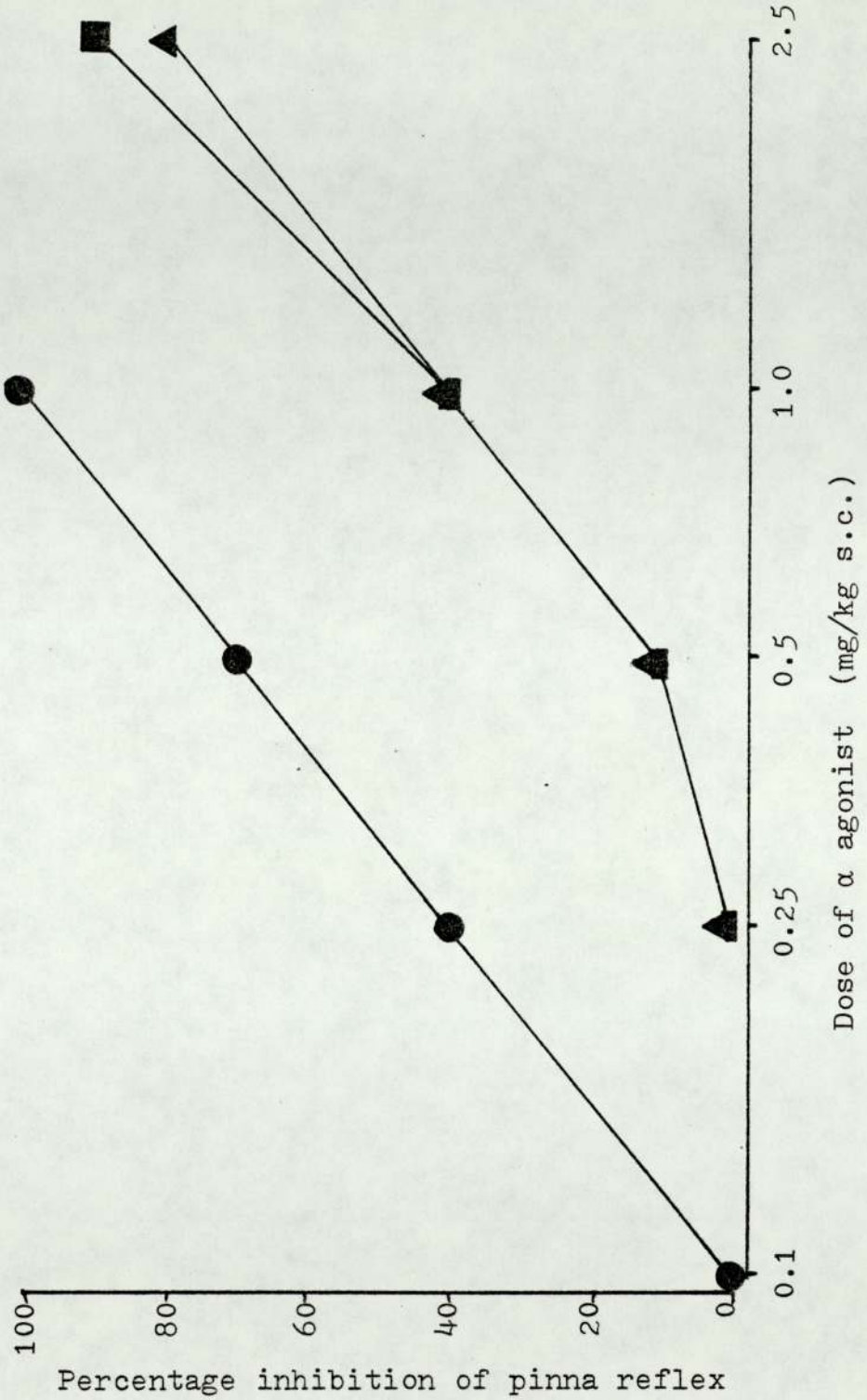


Fig 7.1. The effect of α agonists on pinna reflex after s.c. injection, measured at time of peak inhibitory effect.

(●) - clonidine; (■) - guanabenz; (▲) - guanfacin.

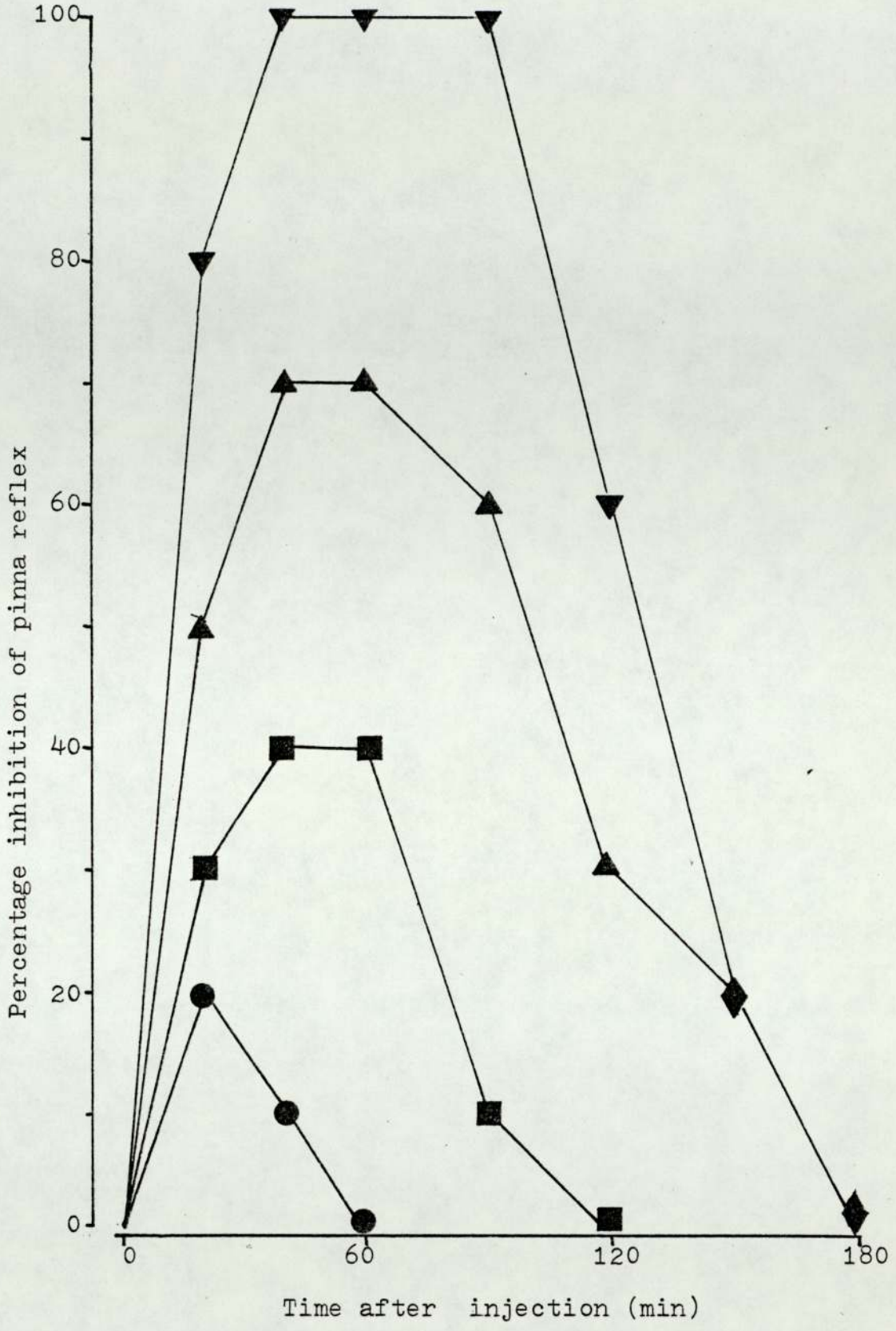


Fig 7.2. The effect of clonidine on pinna reflex after s.c. injection.

(●) - 0.1 mg/kg; (■) - 0.25 mg/kg; (▲) - 0.5 mg/kg; (▼) - 1.0 mg/kg.

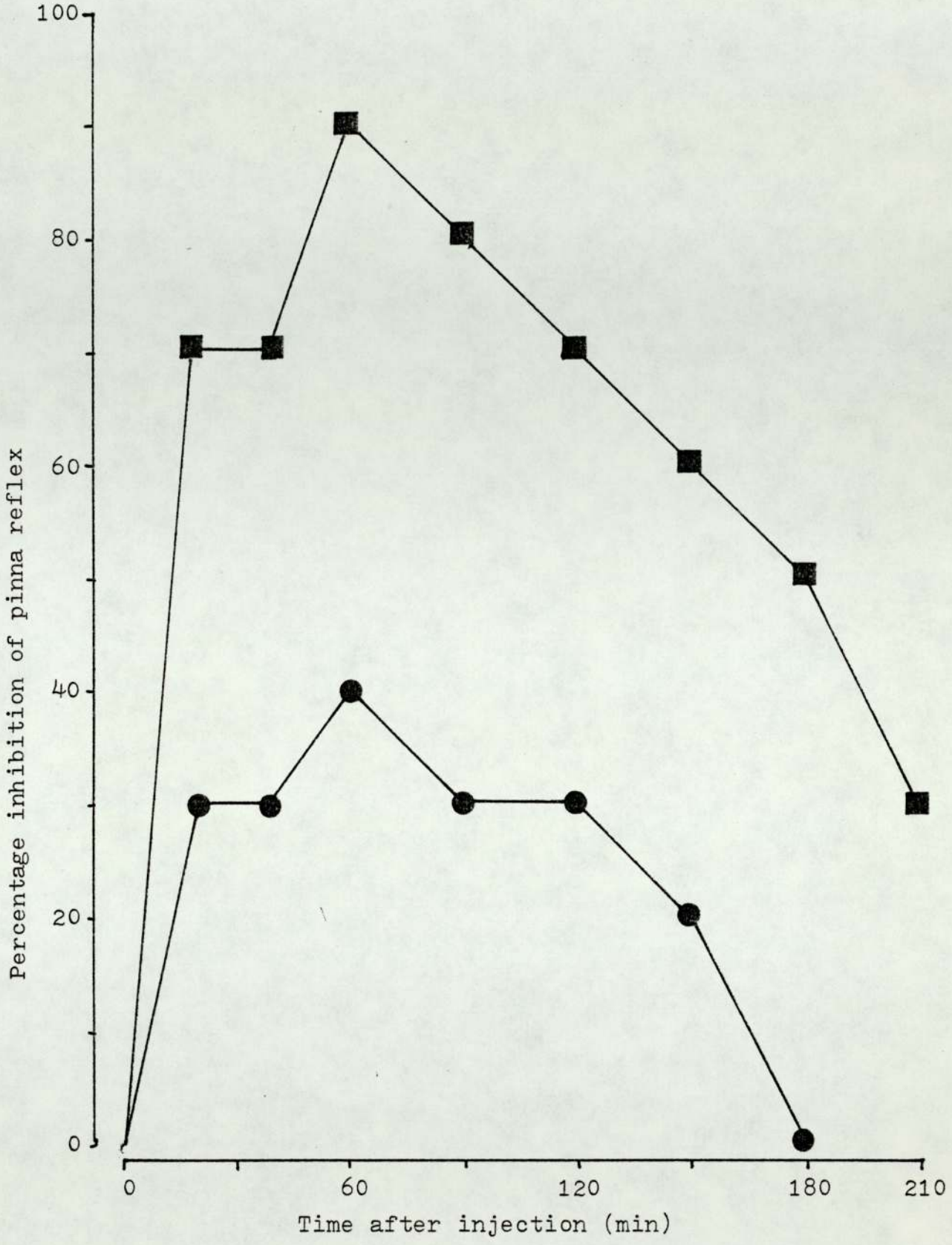


Fig 7.3. The effect of guanabenz on pinna reflex after s.c. injection.

(●) - 1.0 mg/kg; (■) - 2.5 mg/kg.

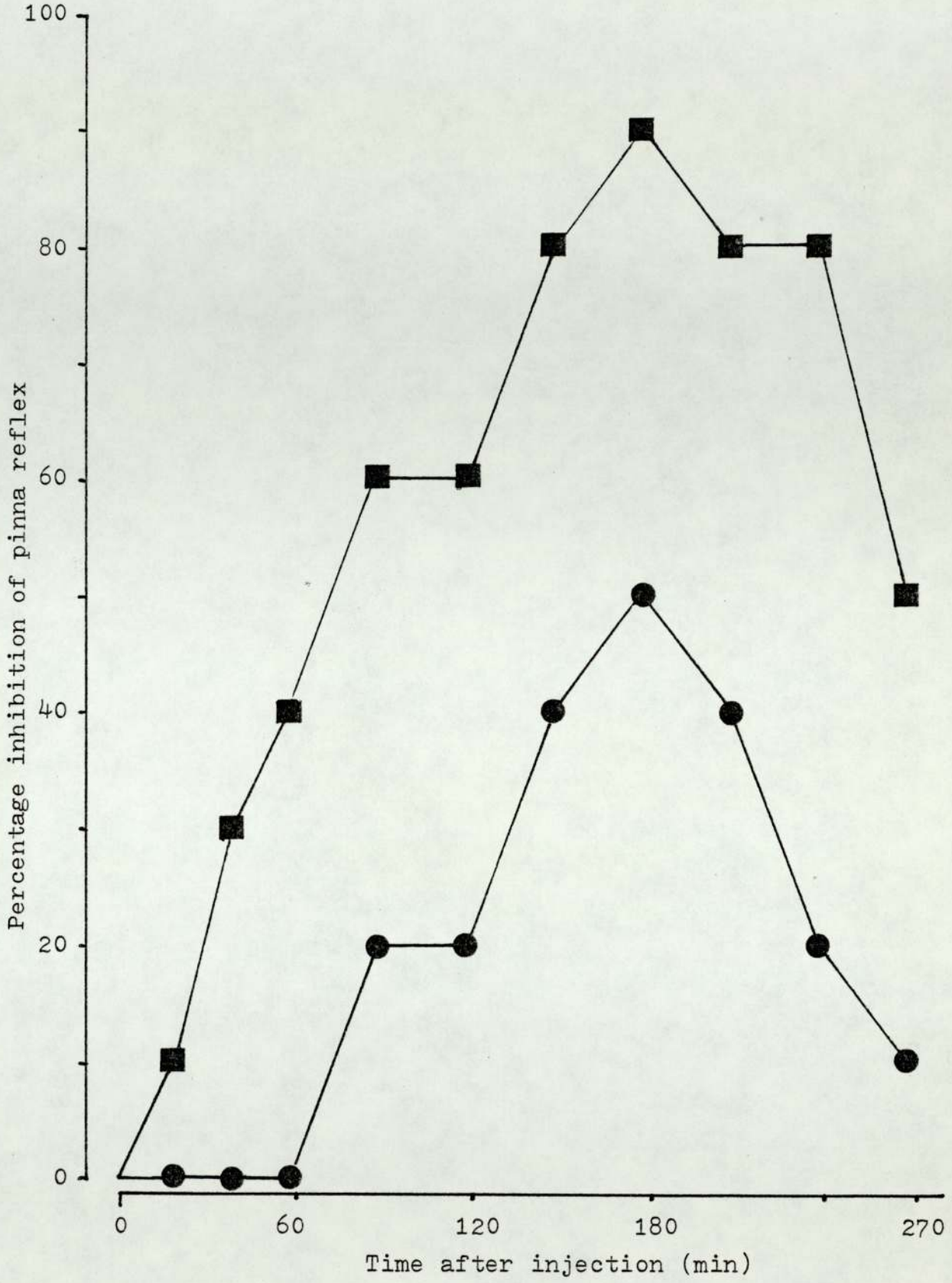


Fig 7.4. The effect of guanfacin on pinna reflex after s.c. injection.

(●) - 1.0 mg/kg; (■) - 2.5 mg/kg.

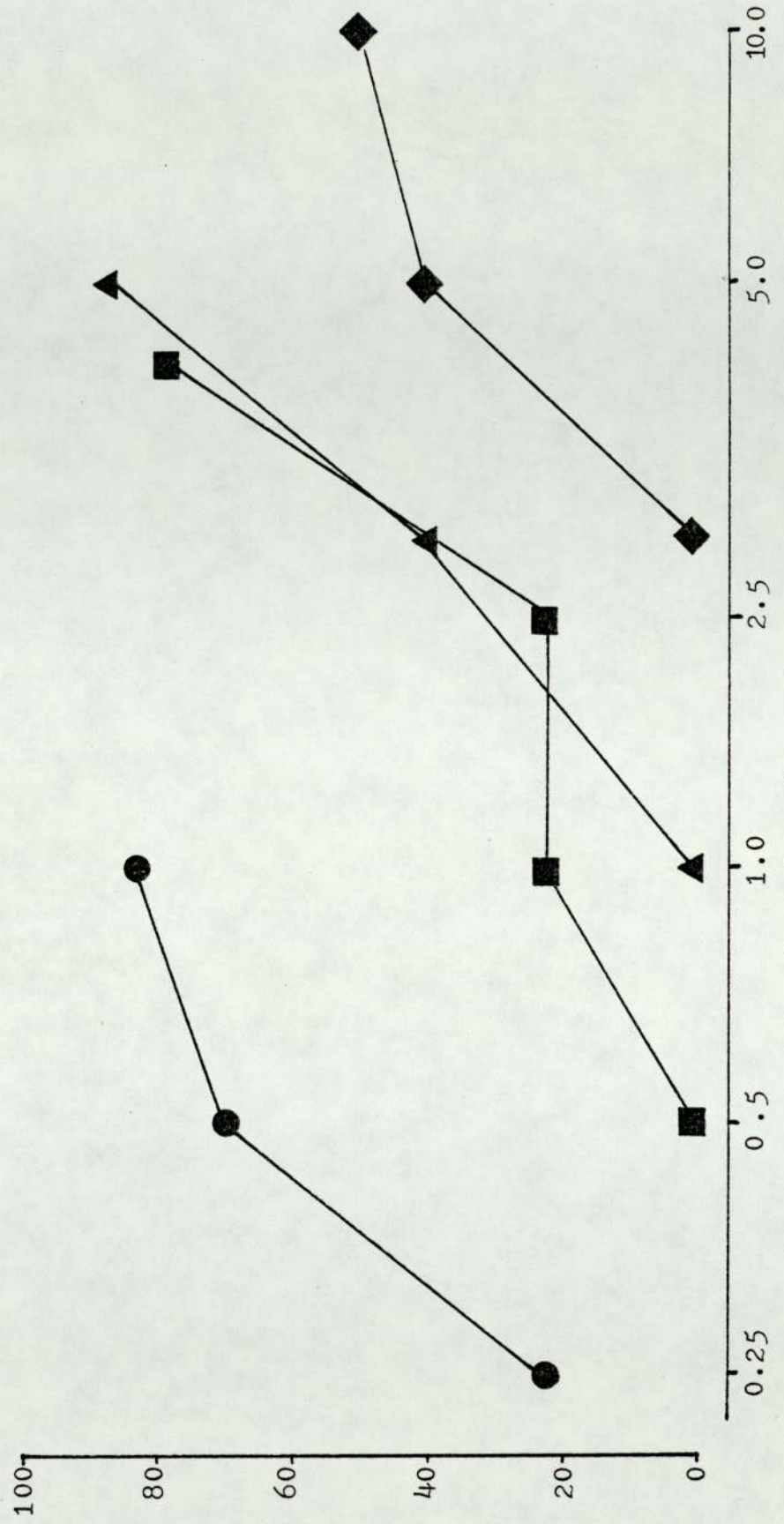


Fig 7.5. The effect of various α agonists on pinna reflex after i.c.v. injection, measured at time of peak inhibitory effect.

ACTIVATION SCORES (each score is the mean of 3 animals)

DRUG AND DOSE (ED_{50} for pinna reflex inhibition)	Ptosis 0 - 4	Body position 0 - 4	Activity 0 - 8	Startle response 0 - 4	Transfer arousal 0 - 8	Visual placing 0 - 4	Muscle tone 0 - 4	TOTAL Max. 36
Saline s.c.	3.83	2.25	1.3	2.0	5.0	2.0	2.0	18.41
Saline i.c.v.	3.83	2.08	2.33	2.0	4.33	2.0	1.66	18.23
Clonidine 0.25 mg/kg	4.0	1.92	0.0	1.66	2.66	2.66	1.66	15.56
Guanabenz 1.1 mg/kg	2.0	2.10	0.83	1.0	4.66	2.0	3.0	15.66
Guanfacin 1.1 mg/kg	3.33	2.08	0.16	2.0	4.0	2.0	3.33	16.90
Noradrenaline 2.5 ug i.c.v.	3.83	2.33	2.0	3.0	4.66	4.0	2.66	22.48
Oxymetazoline 3.0 ug i.c.v.	3.33	1.5	0.56	0.66	2.0	1.0	2.66	11.71
Methoxamine 15 ug i.c.v.	4.0	2.17	0.0	1.0	4.33	2.0	2.33	15.83
Chlorpromazine 5.0 mg/kg	1.5	1.25	0.08	1.33	0.5	0.83	1.0	6.49

Table 7.3. Activation scores of drugs which inhibit the pinna reflex, measured at time of maximal pinna reflex inhibition

CHAPTER 8

EFFECTS OF α AGONISTS AND ANTAGONISTS ON CATALEPSY PRODUCED BY HALOPERIDOL AND BY TRAINING - A POSSIBLE INTERACTION WITH DA

CHAPTER 8.

INTRODUCTION

During observation of the general behaviour of animals treated with drugs acting at α receptors, it was noted that certain drug treatments caused animals to assume and maintain abnormal postures in the observational area. When tested for catalepsy by placing the forelimbs on a 4 cm high bar, such animals frequently remained in this position, some for at least 60 seconds.

Catalepsy may be induced in animals by several types of drugs; namely, neuroleptics, cholinomimetics, opiates and rsserpine. Neuroleptic-induced catalepsy is thought to be due to blockade of DA receptors in the striatum (Fog,1972), thus reducing dopaminergic transmission in the nigro-striatal pathway. Reserpine may produce catalepsy by the depletion of DA in this pathway, while stimulation of cholinergic receptors to induce catalepsy may also involve striatal neurones. Nigro-striatal dopaminergic neurones may also be involved in opiate-induced catalepsy (Kuschinsky & Hornykiewicz,1972; Van Loon & Kim,1978). However, although decreased dopaminergic transmission appears to be involved in the induction of catalepsy by several classes of drugs, drugs which act upon other neuronal systems may modulate catalepsy, possibly via actions which affect dopaminergic neurones.

Thus 5-HT has been shown by many workers to facilitate neuroleptic-induced catalepsy (e.g. Fuenmayer & Vogt,1979), as does GABA (Worms & Lloyd,1978), while NA may have an inhibitory effect (Honma & Fukushima,1977). It is possible therefore that drugs acting on α receptors may affect dopaminergic transmission indirectly, thus inducing catalepsy. The effects of these drugs were therefore investigated on catalepsy produced by haloperidol, a neuroleptic and potent cataleptogen. In addition, experiments were undertaken to study the catalepsy seen in animals treated with α agonists and antagonists alone.

1. The effect of drugs acting at α receptors on catalepsy induced by haloperidol 0.2 mg/kg.

Haloperidol at a dose of 0.2 mg/kg s.c. induced catalepsy of 3 or more seconds at the first trial, one hour after injection, in 28 out of 60 animals. On subsequent testing the intensity of catalepsy rose, such that 56 out of 60 animals maintained an abnormal posture for 3 or more seconds by 90 minutes after injection. The mean duration of posture retention at this time was 18 seconds. Further testing produced a greater increase in the intensity of catalepsy, which began to level off by 3 hours after injection. Naive mice were used for each experiment, as on re-use, animals were found to be more cataleptic on the first and second trials than naive mice.

1.1. The effects of α agonists.

Clonidine in very low doses (0.01 and 0.05 mg/kg) given s.c. 15 minutes before haloperidol, reduced the catalepsy produced by the neuroleptic, for up to 3 hours after injection (Fig 8.1.). A higher dose (0.5 mg/kg) produced a slight potentiation 1½ hours after haloperidol, followed by a decrease 2½ to 3 hours after injection. It was noted that animals appeared sedated after this dose of clonidine at 1½ to 2 hours after injection.

Guanfacin (0.05 and 0.1 mg/kg) markedly decreased the catalepsy produced by haloperidol, the higher dose being effective for at least 3 hours (Fig 8.2.).

Methoxamine 5.0 mg/kg produced a marked increase in the posture retention time for 1 to 2 hours after injection (Fig 8.3.), although 10.0 mg/kg had no effect. To determine whether this effect was due to peripheral or central mechanisms, methoxamine was also administered i.c.v. 45 minutes after haloperidol. Similar administration of saline caused one or two animals to maintain the abnormal posture for much longer than

usual on the first trial. On subsequent trials, however, such animals were no different from animals given saline s.c. Methoxamine 5 µg i.c.v. produced a very marked increase in posture retention time on first testing (Fig 8.4.), which then remained above control levels for at least 3 hours.

Noradrenaline s.c. had no effect on haloperidol catalepsy, but after central administration of 5 µg, produced a marked increase in catalepsy intensity on first testing (Fig 8.4.).

1.2. The effect of α antagonists.

Yohimbine at a dose of 1.0 mg/kg had no effect on haloperidol catalepsy. Higher doses however, (2.5 and 5.0 mg/kg) increased posture retention time for 1 to 2 hours after haloperidol (Fig 8.5.). Piperoxane 10.0 and 20.0 mg/kg had a similar effect , potentiating haloperidol catalepsy for 1 to 2 hours after injection (Fig 8.6.).

Prazosin markedly decreased posture retention time for up to 2 hours after injection (Fig 8.7.) at a dose of 1.0 mg/kg, while a higher dose (5.0 mg/kg) potentiated catalepsy. Phentolamine administered centrally at a dose of 5 µg increased the intensity of catalepsy 1 to 2 hours after injection, but had no effect when given s.c. (Fig 8.8.).

1.3. The effect of α antagonists on the potentiation of haloperidol catalepsy by methoxamine 5 µg i.c.v.

Animals were treated with either saline or an α antagonist 15 minutes before haloperidol and saline or methoxamine 5 µg i.c.v. 45 minutes after haloperidol. Methoxamine produced a marked increase in catalepsy intensity at all times after injection. Yohimbine 2.5 mg/kg potentiated catalepsy alone ($P > 0.05$) and also potentiated the increase due to methoxamine on first testing (Fig 8.9a). Prazosin slightly decreased the catalepsy produced by haloperidol at a dose of 1.0 mg/kg and markedly

reduced the potentiating effect of methoxamine (Fig 8.9b). Phentolamine 5.0 mg/kg slightly potentiated the effect of methoxamine, but completely prevented its effect when administered centrally at a dose of 5 µg, bringing catalepsy down to control levels (Fig 8.10.).

2. The effect of drugs acting at α receptors on catalepsy in mice not treated with haloperidol.

Many of the drugs investigated for their effects on haloperidol catalepsy were also administered to mice which were subsequently tested for catalepsy 8 times. Saline-treated mice were also included as most of the mice were found to exhibit some degree of catalepsy after 4 tests.

After injection of saline, mice did not stay on the bar for more than 2 seconds on the first and second testing. However, on the third test, 33% of mice remained in position for 3 or more seconds; this figure rose to 66% on the fourth test and by the seventh test, all mice remained on the bar for at least 3 seconds (Fig 8.11a), the average time being 22.1 seconds with a range of 3 - 84 seconds. The animals were similar in appearance to haloperidol-treated animals when remaining in the imposed posture, showing muscular rigidity and the maintainence of a ridge of skin on release of the scruff grip.

Clonidine at a dose of 0.01 mg/kg appeared to reduce the development of posture retention (Fig 8.11b), the average time animals remained in position being significantly lower than saline-treated controls (Fig 8.12a). A higher dose, 0.5 mg/kg, however, slightly increased the incidence of posture retention for 3 or more seconds (Fig 8.11c) and also increased its average duration on the first 3 trials.

Guanfacin 0.1 mg/kg markedly inhibited the development of posture retention, the average time being significantly lower than in saline-treated animals (Figs 8.11d and 8.12b).

Animals treated with methoxamine 5.0 mg/kg stayed on the bar much more readily, 66% of animals remaining in position for 3 or more seconds on the second trial, and all animals doing so by the fifth test (Fig 8.11e). However, the average duration of posture retention was lower than in controls with a much narrower range (Fig 8.12b).

Both yohimbine and piperoxane potentiated posture retention time. Yohimbine, however, was effective on the first trial, 66% of animals remaining in position for 3 or more seconds (Fig 8.11f) although the effect was of short duration (Fig 8.13a), while piperoxane did not have a marked effect until the second trial (Figs 8.11g and 8.13a).

Prazosin at a dose of 1.0 mg/kg slightly inhibited the development of posture retention (Figs 8.11h and 8.13b), while 5.0 mg/kg caused a potentiation both in the incidence (Fig 8.11j) and duration (Fig 8.13b) of posture retention.

The effects of both α agonists and antagonists were thus similar in direction to the effects which they produced on catalepsy induced by haloperidol.

3. Further investigation of the ability of animals to retain an imposed posture.

As described above, animals injected only with saline would retain the abnormal posture imposed upon them after sufficient trials. Experiments were carried out in order to determine whether this correlated with the number of trials or with the time after injection as follows: animals were all injected at time 0, the first group only were tested for catalepsy after 15 minutes; the second group were then tested at 30 minutes and the first group re-tested at this time. This procedure was continued with all groups, so that the last group were tested only once at 180 minutes after injection. It was found that there was no difference in posture retention time on

the first trial, whatever time after injection the animals were tested (Fig 8.15a), whereas animals tested only once at 180 minutes after injection had significantly less ($p < 0.01$) posture retention times than animals also tested at 180 minutes which had had at least two previous trials (Fig 8.15a). The posture retention time was found to be significantly correlated with the number of trials ($p < 0.01$, $r = 0.974$).

Animals which had been weighed and marked only i.e. had no injection were also tested for posture retention to determine whether the administration of saline may be responsible for the development of this phenomenon. These animals showed similar characteristics to the saline-treated animals in developing retention of the abnormal posture imposed. There were no significant differences between the two groups, although on the eighth trial, untreated animals had a lower mean posture retention time than saline-treated animals (Fig 8.14a)

Some of the animals used in this experiment were retained and retested by the same procedure 5 days later. Both groups of animals more readily retained the imposed posture on the first trial and the mean duration was increased over the first four trials (Fig 8.14b). There was, however, no increase in the final posture retention time when compared to the same parameter obtained with fresh animals. Again, some animals were retained and tested again, this time after 24 hours. There was a marked increase in the mean posture retention time in both groups of animals on all trials; and 80% of animals retained their posture for at least 3 seconds on the first trial (Fig 8.14c).

Since animals which had been injected with saline or not injected at all were found to retain an imposed abnormal posture on repeated testing, an experiment was carried out to determine whether haloperidol-induced catalepsy may also be

affected by the number of trials. The experiment was designed to be comparable to the experiments performed previously on haloperidol-treated animals, but otherwise was similar to that described above. In all cases, 80% of animals remained in position for at least 3 seconds on the first trial at whatever time after injection they were tested, thus were cataleptic compared to saline-treated animals. However, animals tested once only at 180 minutes after injection had significantly ($p < 0.05$) less catalepsy than animals also tested at 180 minutes which had had at least 3 previous trials (Fig 8.15b). Catalepsy intensity was significantly correlated with the number of trials ($p < 0.01, r = 0.965$), but did not vary on the first trial with the time after injection (Fig 8.15b).

DISCUSSION

A range of drugs acting on both α_1 and α_2 receptors were found to have marked effects on catalepsy induced by haloperidol. Those drugs which potentiated haloperidol catalepsy were also found to accelerate the development of 'catalepsy' in mice not treated with haloperidol, while drugs which decreased haloperidol catalepsy prevented this development.

Methoxamine was found to potentiate haloperidol catalepsy both after peripheral and central administration. Phentolamine prevented the effect of i.c.v. methoxamine when the two drugs were administered together, but was ineffective on peripheral injection, thus indicating that methoxamine was acting on central α receptors. Yohimbine which selectively blocks α_2 receptors did not affect the potentiation of catalepsy due to methoxamine, while prazosin, which blocks only α_1 receptors, prevented this effect. Methoxamine thus appears to produce potentiation of haloperidol catalepsy by stimulation of central α_1-adrenoceptors.

Noradrenaline, which is a non-selective agonist; and clonidine at a dose of 0.5 mg/kg, which may be sufficient to stimulate α_1 receptors, also caused potentiation. Lower doses of clonidine were, however, found to decrease haloperidol catalepsy, as was guanfacin. The latter drug has a higher selectivity for α_2 receptors than clonidine, thus it seems likely that this inhibitory action may be the result of α_2 receptor stimulation.

The effects of α antagonists were consistent with these results. Yohimbine and piperoxane, which are both selective for α_2 receptors, potentiated haloperidol catalepsy, while blockade of α_1 receptors by prazosin decreased it. The potentiation by a high dose of prazosin may be a consequence of the sedation produced by this dose. A similar mechanism may also be responsible for the potentiating effect of clonidine 0.5 mg/kg.

The effects of α antagonists on the action of i.c.v. methoxamine were also consistent with their effects alone.

The results suggest that activation of noradrenergic systems potentiates haloperidol catalepsy in the mouse, while reduction in noradrenergic function leads to inhibition of catalepsy. Stimulation of α_1 or blockade of α_2 receptors thus has a potentiating effect, while blockade of α_1 or stimulation of α_2 receptors is inhibitory. There appears to be a noradrenergic system, therefore, which has a facilitatory effect on neuroleptic catalepsy in this species. The effects of α agonists and antagonists on catalepsy in the absence of haloperidol were qualitatively similar to their effects as described above, thus supporting the suggestion of a facilitatory effect of NA on catalepsy.

Most other workers have studied catalepsy in the rat. The effect of noradrenergic systems on catalepsy appears to be less clear in this species, although many drugs have similar effects to those found in the mouse. Thus, other workers have also found low doses of clonidine to decrease haloperidol catalepsy (Al-Shabibi & Doggett,1978; Honma & Fukushima,1977; Weilosz et al.,1978), although Pycock et al.,(1977) found a higher dose (0.5 mg/kg) to potentiate catalepsy. Naphazoline and xylometazoline, which are both selective agonists of α_2 receptors, also inhibited catalepsy (Weilosz et al.,1978). Phenylephrine, which has very little action on α_2 receptors, did not affect haloperidol catalepsy in the rat, while methoxamine was only effective in producing inhibition at a dose of 100 μg (ibid). The order of potency which these workers found for inhibition of catalepsy thus supports the involvement of α_2 receptors in this effect.

The lack of any potentiating effect of methoxamine, such as was seen in mice, suggests that α_1 receptors may not be

involved in catalepsy in the rat. However, experiments using α antagonists do not bear this out. Al-Shabibi and Doggett,(1978) found PBZ, an antagonist of α_1 receptors, to potentiate catalepsy in the rat and to produce sedation and catalepsy when given alone. Such an effect is similar to that seen with a high dose of prazosin in mice. Phentolamine i.c.v. also potentiated catalepsy in rats (Honma & Fukushima,1979), as occurred here, although these workers also found peripheral administration of the drug to have a similar effect, hence phentolamine may act differently in the two species.

Yohimbine was found by Al-Shabibi and Doggett,(1978) to markedly reduce catalepsy in the rat. This effect is the opposite of that seen here in mice. These workers suggested that the effect may be due to increased NA release, via α_2 receptor blockade; and that PBZ potentiated catalepsy by α_1 blockade. This suggestion is thus the reverse of the hypothesis outlined above; and would require that a decrease in noradrenergic function lead to potentiation of catalepsy.

The effects of drugs which block the synthesis of NA and lesioning experiments appear to bear out this suggestion. FLA 63 or diethyldithiocarbamate (DDC), which decrease NA, but not DA, levels, may themselves induce catalepsy in the rat (Al-Shabibi & Doggett,1978) and potentiate neuroleptic catalepsy (Honma & Fukushima,1977). Lesions of the L.C. have also been reported to have a facilitatory effect on neuroleptic catalepsy in the rat (Pycock,1977; Kostowski et al.,1978), although Mason et al.,(1978b) suggest that the ascending dorsal noradrenergic bundle may not be responsible for this effect.

On the other hand, ventral bundle lesions have been found to reduce haloperidol catalepsy, whilst also reducing forebrain NA levels (Kostowski et al.,1978). It is possible, therefore, that an inhibitory function of the dorsal bundle may

predominate in rats, while the facilitatory effect mediated by the ventral bundle may be more important in the mouse. Preliminary studies using DDC have indicated that this drug does inhibit catalepsy in the mouse.

It thus appears that in the mouse a classical noradrenergic synapse with α_2 receptors located presynaptically and α_1 receptors postsynaptically, may mediate the effects of NA on catalepsy. Such a system may also be present in the rat and mediate the effects of α_2 agonists on catalepsy. Alternatively, the α_2 receptor sensitive to clonidine may be located postsynaptically. A postsynaptic inhibitory α_2 receptor would account for the effects of lesions of the dorsal bundle and NA synthesis inhibition in this species. However, in addition, an α_1 receptor appears to be involved, since PBZ produces catalepsy, while methoxamine inhibits it. Also, the effect of yohimbine can only be explained by a presynaptic α_2 receptor at an inhibitory synapse. The similarity in direction of effect of both clonidine and yohimbine suggests that the two drugs must act at functionally opposite receptors. However, the biochemical effects of these drugs on dopaminergic activity suggest that opposite effects should be seen.

Both yohimbine (Anden & Grabowska,1976) and methoxamine (Weilosz et al.,1978) increase DA turnover in rat brain, which may account for their inhibitory effects on catalepsy in this species. Clonidine, however, reduces DA turnover via α_2 receptor stimulation (Anden & Grabowska,1976). which would be expected to have a potentiating effect on catalepsy. Thus, if NA does have an inhibitory effect on catalepsy in the rat, as has been suggested (Honma & Fukushima,1977), clonidine must stimulate α_1 receptors to achieve its effect. This seems unlikely in view of the high selectivity of this drug for α_2 receptors and the effects of other α agonists mentioned above (Weilosz et al., 1978).

The effects of noradrenergic activity on catalepsy in the rat thus appear to be complex and can not be explained by a simple inhibitory effect as suggested by Honma and Fukushima (1977). The rat is much less susceptible to the cataleptogenic effect of haloperidol than the mouse and in addition does not tend to submit to the effects of training as other species (see below). Hence there appear to be marked differences in effects on catalepsy between rats and mice.

One of the most important differences between the species is the effect of yohimbine. This drug inhibited catalepsy in the rat while producing stereotyped behaviour (Al-Shabibi & Doggett,1978). Similar stereotypy was also seen in the mouse (Chapter 3), but the drug also potentiated haloperidol catalepsy and accelerated the development of catalepsy on training. Yohimbine also caused abnormal posturing behaviour, both in rats (Papeschi et al.,1971) and mice (Chapter 3).

Both the stereotypy and posturing may bear some relationship to 'fear'. The stereotyped behaviour consisted mainly of short bursts of running, grooming and sniffing, thus differed from amphetamine-induced stereotypy in that no head-searching or gnawing occurred. In addition, animals did not show the marked increase in activity seen after low doses of amphetamine. The short bursts of running and grooming in yohimbine-treated animals may be expressions of 'fear', interspersed with periods of 'freezing', where the 'fear' becomes so great as to induce a type of 'panic reaction' similar to catatonia in humans (Gallup & Maser,1977).

Piperoxane and methoxamine also induce a similar behavioural syndrome including a reduction in exploratory activity, which has been interpreted as a measure of 'fear' (see Chapter 9). All these drugs potentiate haloperidol catalepsy and the development of catalepsy in drug-free mice, while

clonidine, which has been found to possess anxiolytic-like activity, inhibits both types of catalepsy. There is a possibility that the facilitatory effect of NA on catalepsy in the mouse is merely a reflection of the effects of noradrenergic systems on arousal, which mediates the effects of drugs on catalepsy. Thus an inhibitory noradrenergic system similar to that found in the rat may also exist in the mouse, but may be masked by effects on arousal and 'fear'.

The phenomenon of catalepsy in drug-free animals may bear some resemblance to tonic immobility ("animal hypnosis") since both involve muscular rigidity and decreased responsiveness to external stimuli with no loss of consciousness. Tonic immobility also appears to be a 'fear reaction' (Gallup & Maser, 1977) and is potentiated by electric shock, especially if inescapable, or a cue previously paired with shock i.e. conditioned fear. Tranquilisers decrease the duration of TI and make its induction difficult, although low doses of chlorpromazine may potentiate the reaction.

Gallup and Maser have suggested that TI may be used as a model for human catatonia, since the two phenomena resemble each other in many ways. The inhibitory effect of chlorpromazine and also of imipramine, both of which are used to treat catatonic behaviour in man, supports the similarity. Catalepsy in drug-free mice may thus also resemble human catatonia, although it bears more similarity to neuroleptic-induced catalepsy, and thus may also parallel parkinsonian side effects of neuroleptics.

The effects of drugs on this catalepsy are similar to those found on neuroleptic catalepsy in mice; thus not only do drugs acting at α-adrenoceptors have similar effects, but atropine and apomorphine are able to reduce catalepsy, while naloxone is ineffective (Brown & Handley, 1980). A further similarity

between the two types of catalepsy is that both are increased by repeated testing. This constitutes a major difference both from TI and possibly from other types of catalepsy. TI is not a learned phenomenon; and, in fact, handling, taming and familiarisation antagonise immobility (Gallup & Maser,1977).

Stanley and Glick (1976) and Moleman et al.(1978) report that haloperidol-treated rats become more cataleptic on repeated testing, while Costall et al.(1978) appear to find no such effect. In rats treated with a vehicle only, Ezrin-Waters et al.(1976) did not find any training effect upon repeated testing, nor was there any increase in catalepsy in morphine-treated rats (Moleman et al.,1978). It would thus appear that the invovement of training in catalepsy may be dependent on species, strain and the type of method used for inducing and measuring catalepsy. It is interesting that the rat has been found to be somewhat resistant to the induction of TI (Klemm, 1971), although again strain may affect the phenomenon (McGraw & Klemm,1973).

The similarities between training catalepsy and neuroleptic catalepsy suggest that noradrenergic modulation of dopaminergic activity may be the basis of the effect. Noradrenaline has been implicated in learning mechanisms, although Mason and Fibiger (1979c) have shown that many tasks may still be learned in the absence of forebrain NA. The biochemical basis of TI still remains to be elucidated, although 5-HT appears to be involved in TI in chickens (Gallup & Maser,1977), but not in rabbits (Hatton et al.,1978). The involvement of NA in TI is even less clear, as adrenaline potentiated TI, but reserpine had no effect (Gallup & Maser,1977).Fear appears to be involved in this phenomenon (ibid), thus adrenaline may act by producing peripheral signs of fear. However, morphine, which is known to have anxiolytic properties, has been shown to potentiate TI,

thus the model does not appear to be a simple consequence of fear.

Gallup and Maser suggest that TI may represent an evolved defence against predators and have shown that selective breeding may produce animals with high or low susceptibility to TI.Such breeding may, however, be similar to that undertaken by Broadhurst (1959), who produced rats with differing emotionalities. Indeen, chickens which showed prolonged immobility also had higher emotionality scores than chickens which showed brief TI (Gallup & Maser,1977). During initial experiments on catalepsy, it was noted that mice obtained from a breeder were more cataleptic than home bred mice. This may reflect similar differences in emotionality between mice of the same strain.

It thus appears that the strain and species of animal used may affect both TI and training catalepsy, possibly by the involvement of 'fear'. Procedures which increase 'fear' potentiated TI, while drugs which appear to induce 'fear' accelerated the development of training catalepsy. The similarity between these effects and the effects of drugs on haloperidol catalepsy in mice supports the suggestion that 'fear' may also be involved in this phenomenon. Such an effect may not occur in rats, since the involvement of NA in 'fear-related behaviour' in this species has not as yet been confirmed (Chapter 9). The potentiation of neuroleptic catalepsy by benzodiazepines in rats (Keller et al.,1976) tends to suggest that 'fear' is not a contributory factor in catalepsy in this species, although a direct action involving GABA may be involved in the effects of these drugs. The involvement of 'fear' in catalepsy in mice remains to be confirmed. However, the potentiating effect of drugs which appear to markedly increase arousal seems unexpected in view of the additional potentiation produced by sedative doses of clonidine and prazosin. Thus 'fear' produced by

increased arousal, may be instrumental in mediating the effects of yohimbine, piperoxane and methoxamine on catalepsy.

The effects of α agonists and antagonists on catalepsy in the mouse may therefore involve both increased or decreased arousal and/or a facilitatory noradrenergic system. The results suggest that there are marked differences in this phenomenon between species; and that, in the mouse, at least, repeated testing may produce a state resembling catalepsy in animals not treated with neuroleptics. Thus, unless only a single test is used, control groups should be included in all experiments involving catalepsy.

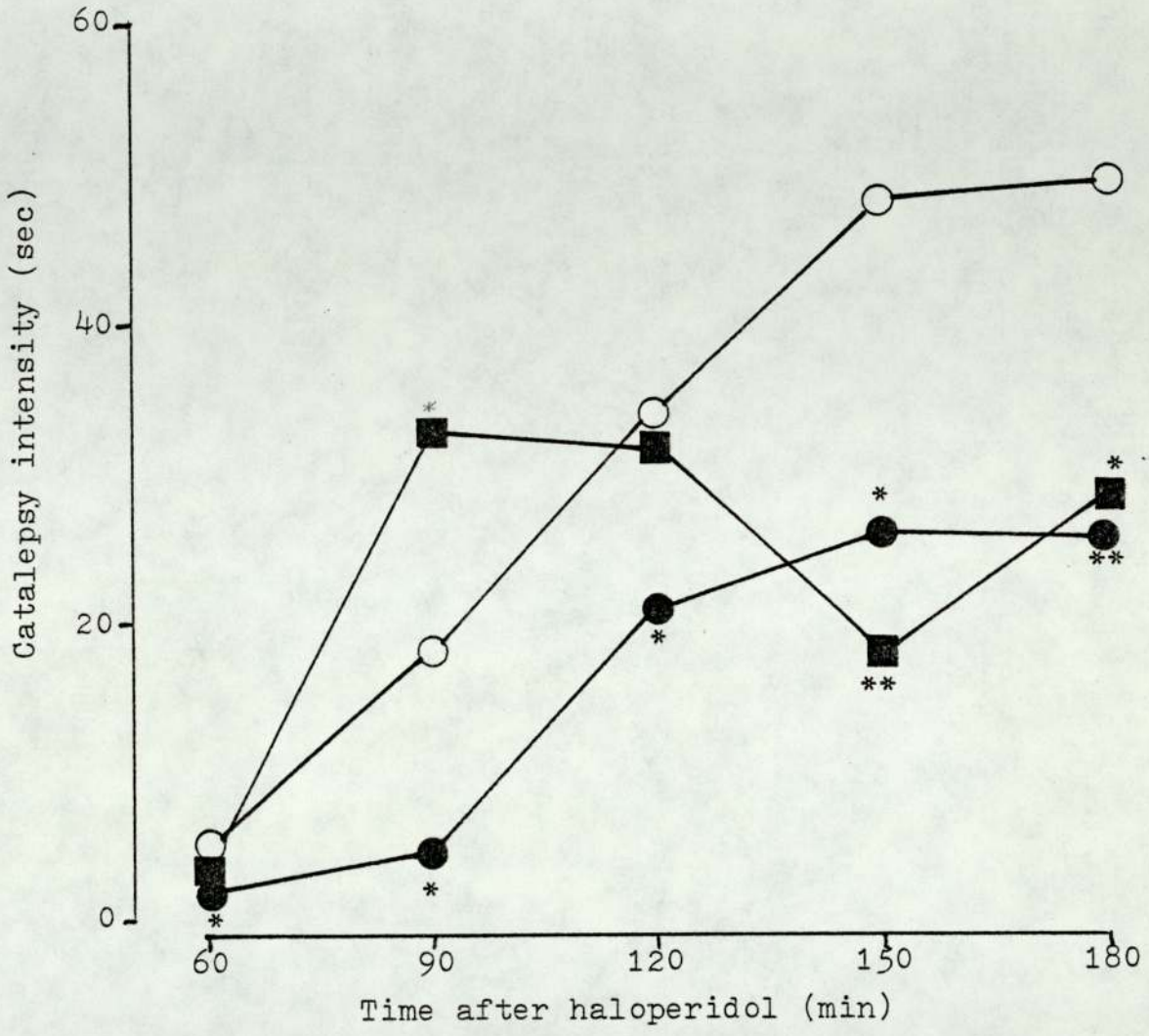


Fig 8.1. The effect of clonidine on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were treated with saline (○), clonidine 0.01 mg/kg (●), or clonidine 0.5 mg/kg (■) 15 minutes before haloperidol and tested for catalepsy by placing the forepaws on a 4 cm high bar.

* $p < 0.05$, ** $p \leq 0.01$ difference from saline-pretreated animals (Mann Whitney 'U' test).

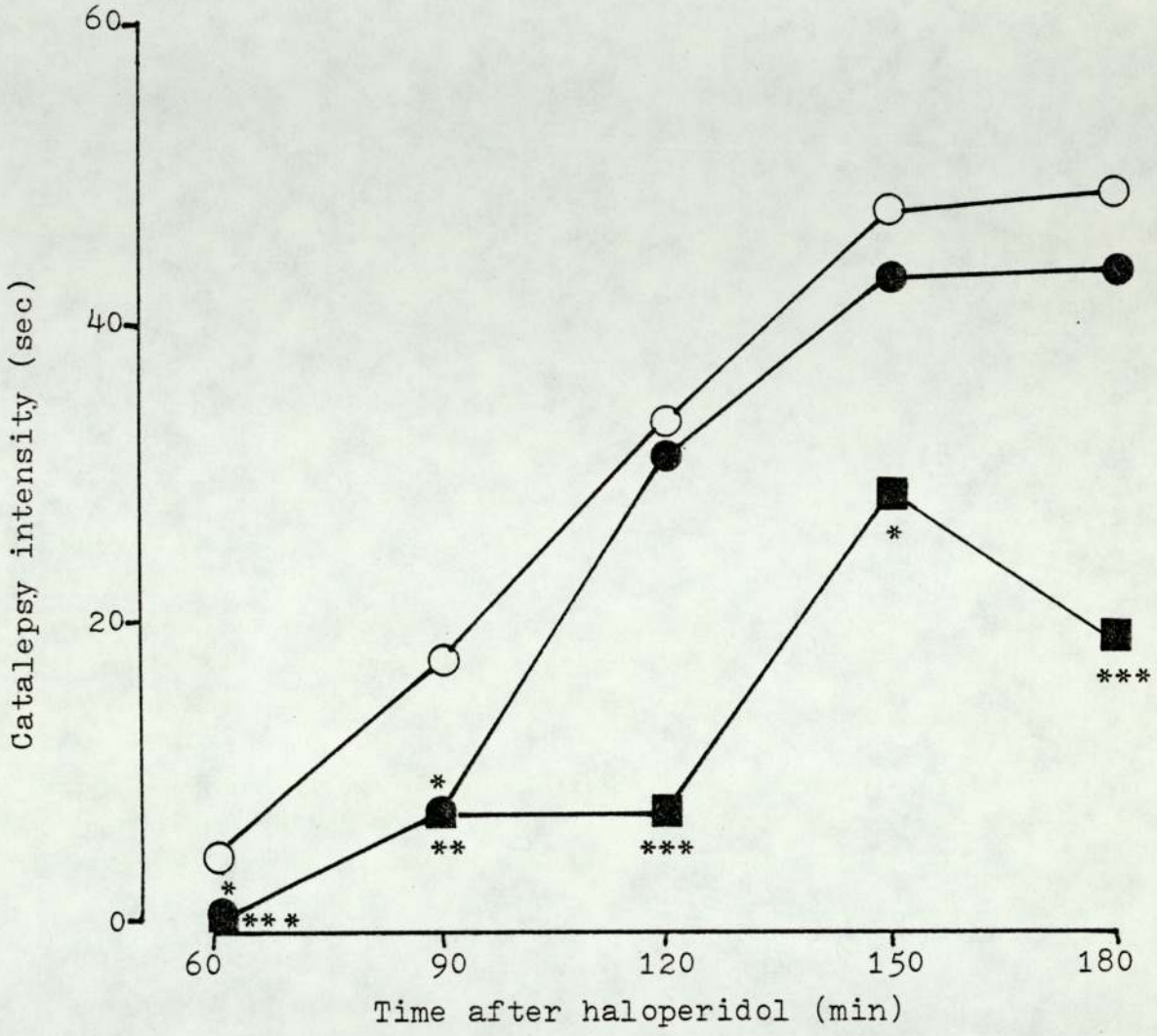


Fig 8.2. The effect of guanfacin on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were treated with saline (○), guanfacin 0.05 mg/kg (●) or guanfacin 0.1 mg/kg (■) 15 minutes before haloperidol and tested for catalepsy by placing the forepaws on a 4cm high bar. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from saline-pretreated animals (Mann Whitney 'U' test).

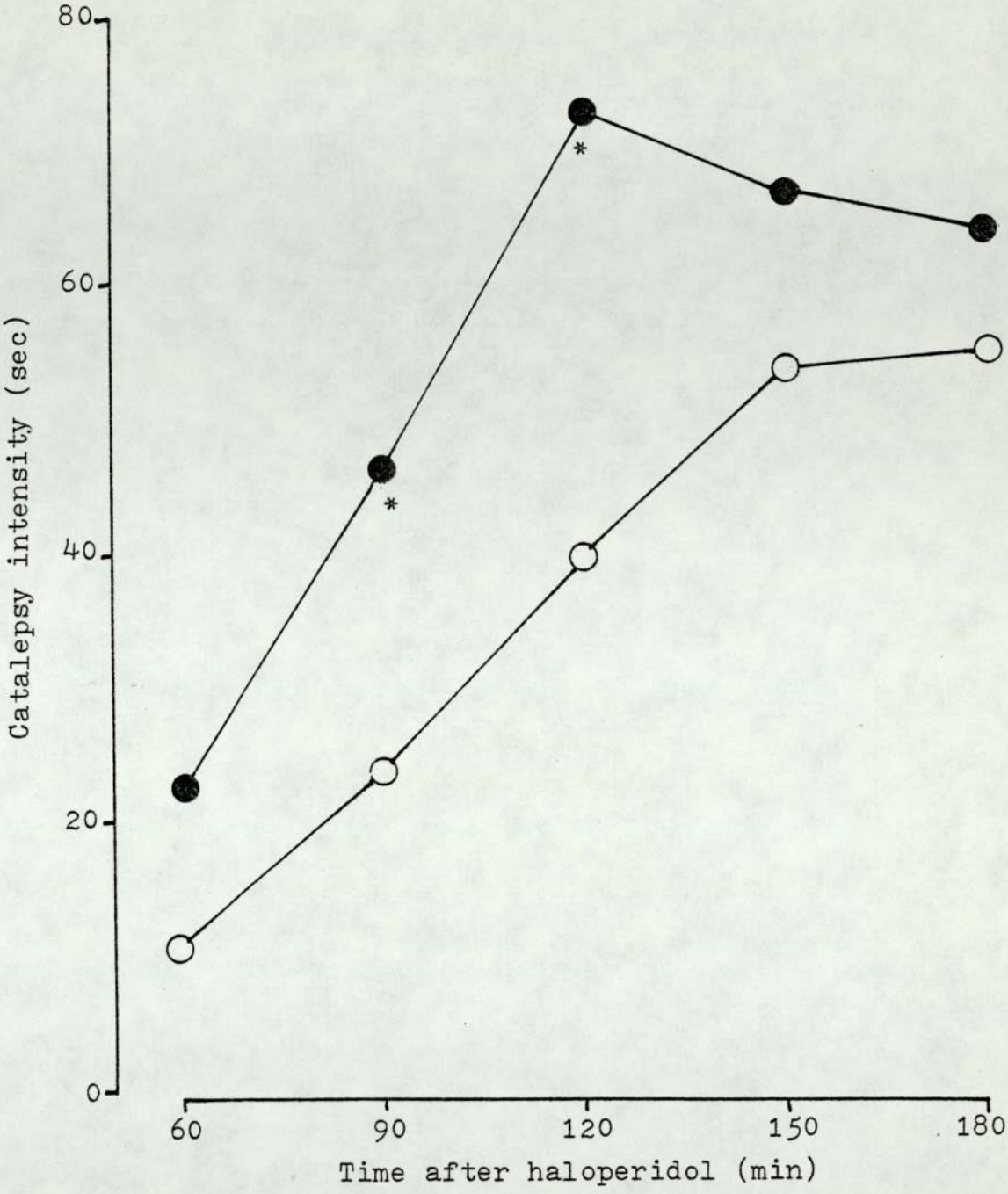


Fig 8.3. The effect of methoxamine on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were treated with saline (○) or methoxamine 5.0 mg/kg (●) and tested for catalepsy by placing on a 4cm high bar.

* $p < 0.05$ difference from saline treated animals (Mann Whitney 'U' test).

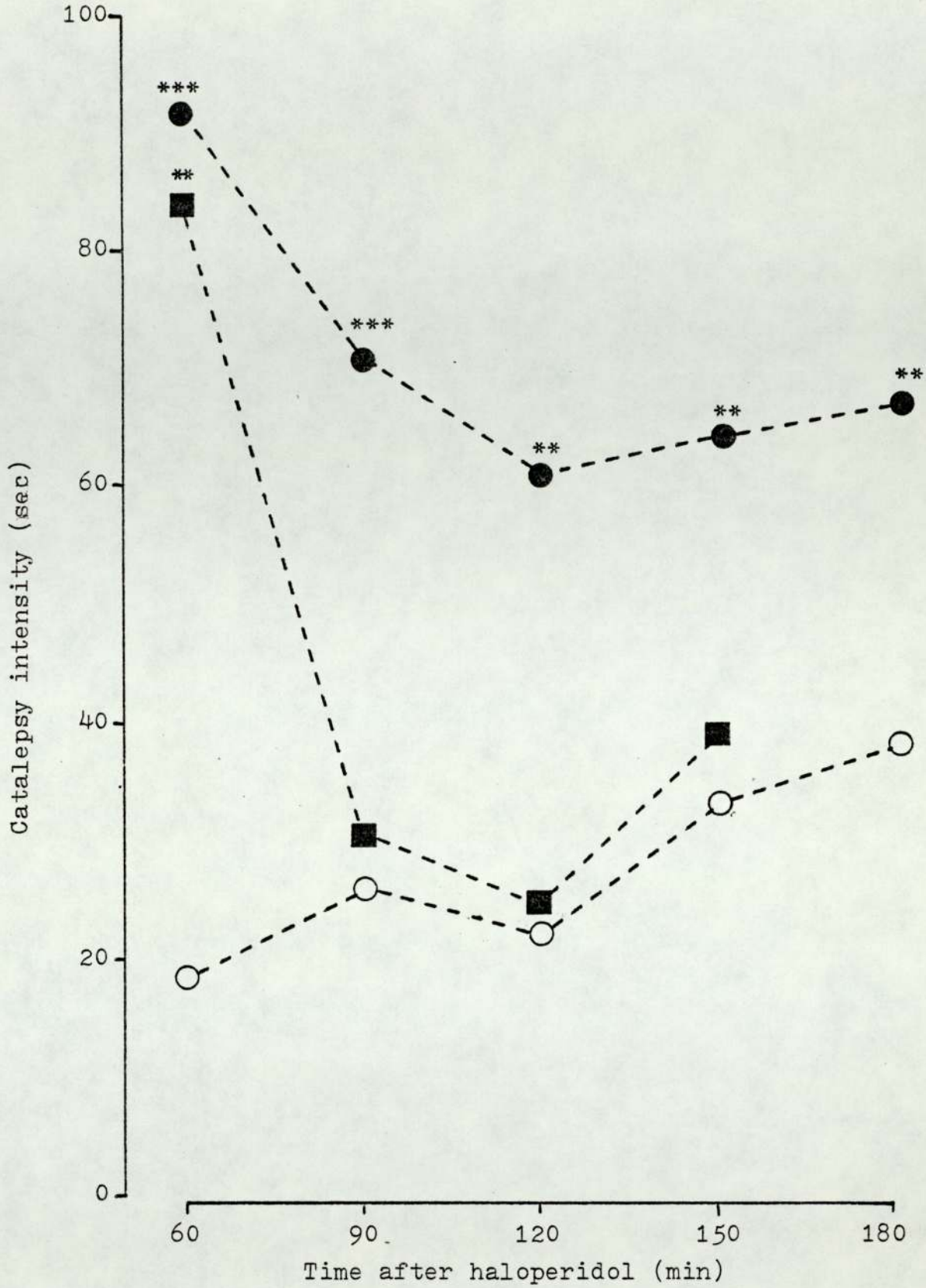


Fig 8.4. The effect of noradrenaline and methoxamine injected i.c.v. on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were treated with saline (○), NA 5.0 µg (■) or methoxamine 5.0 µg (●) i.c.v. 45 minutes after haloperidol. ** $p < 0.01$, *** $p < 0.001$ difference from saline (Mann Whitney 'U' test).

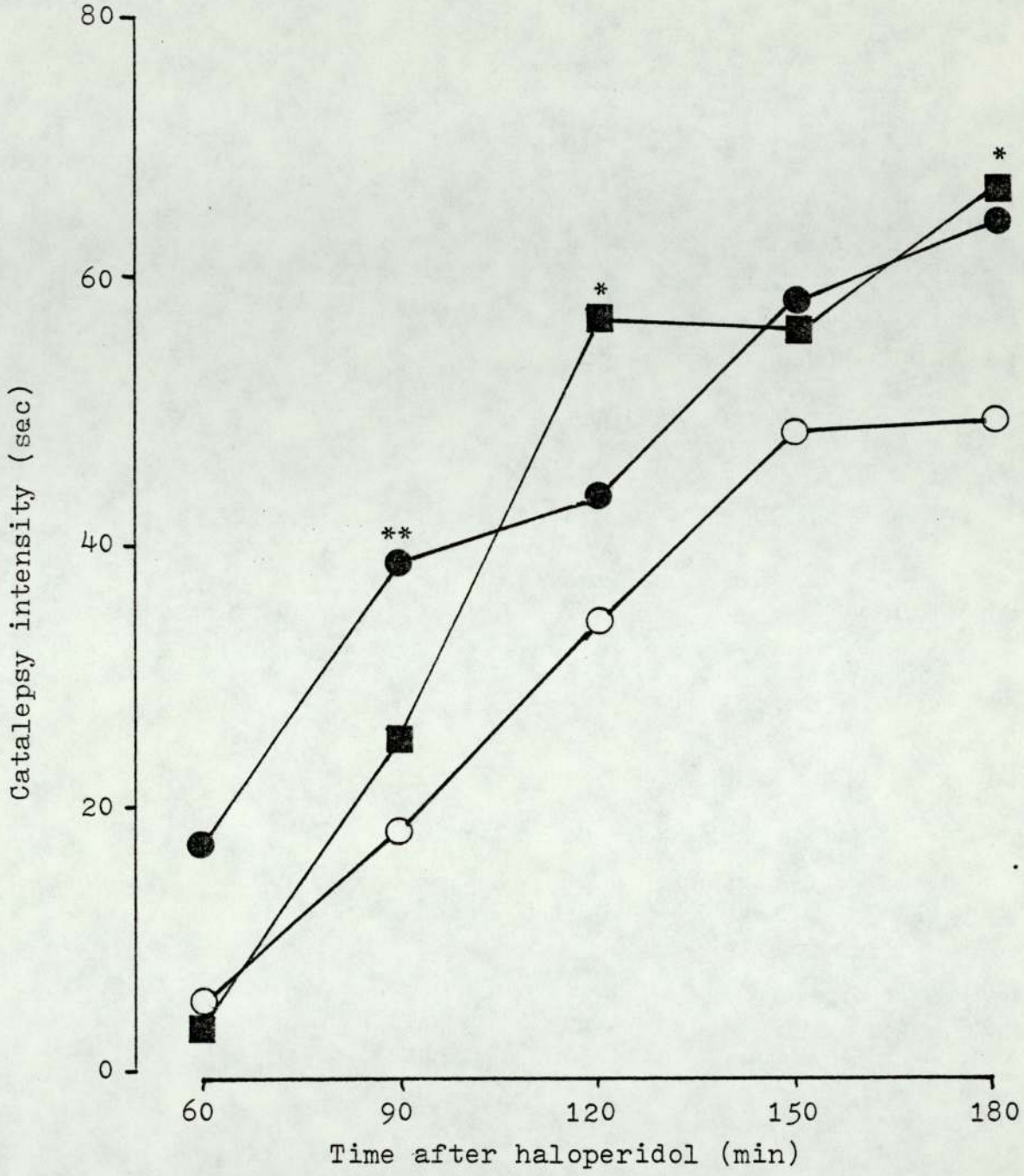


Fig 8.5 The effect of yohimbine on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were pretreated with saline (○), yohimbine 2.5 mg/kg (●) or yohimbine 5.0 mg/kg (■), 15 minutes before haloperidol and tested for catalepsy by placing the forepaws on a 4cm high bar. * $p < 0.05$, ** $p < 0.01$ difference from saline treated animals (Mann Whitney 'U' test).

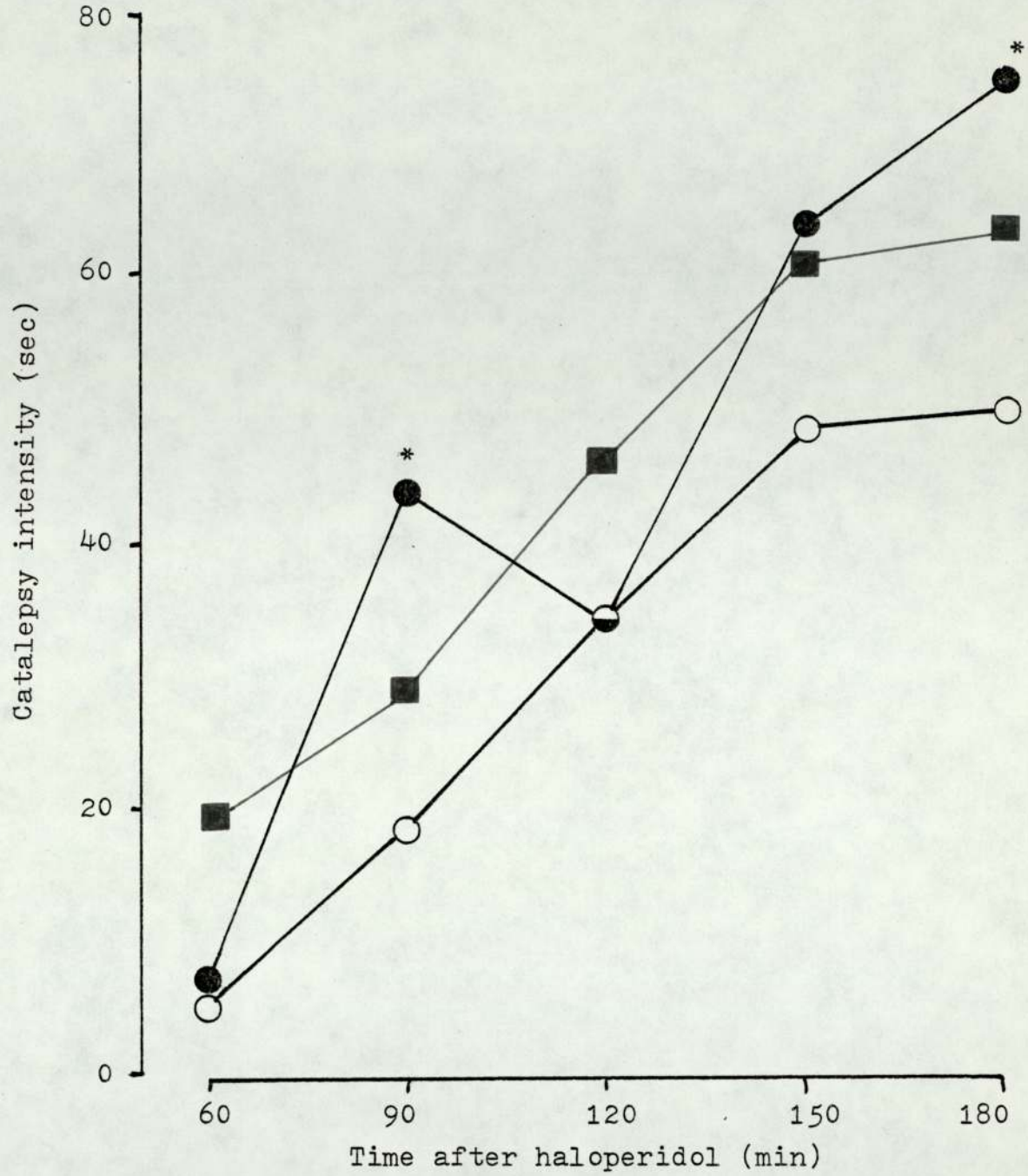


Fig 8.6. The effect of piperoxane on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were pretreated with saline (○) or piperoxane 10(●) or 20 (■) mg/kg 15 minutes before haloperidol and tested for catalepsy by placing the forepaws on a 4cm high bar.

* $p < 0.05$ difference from saline treated animals, (Mann Whitney 'U' test).

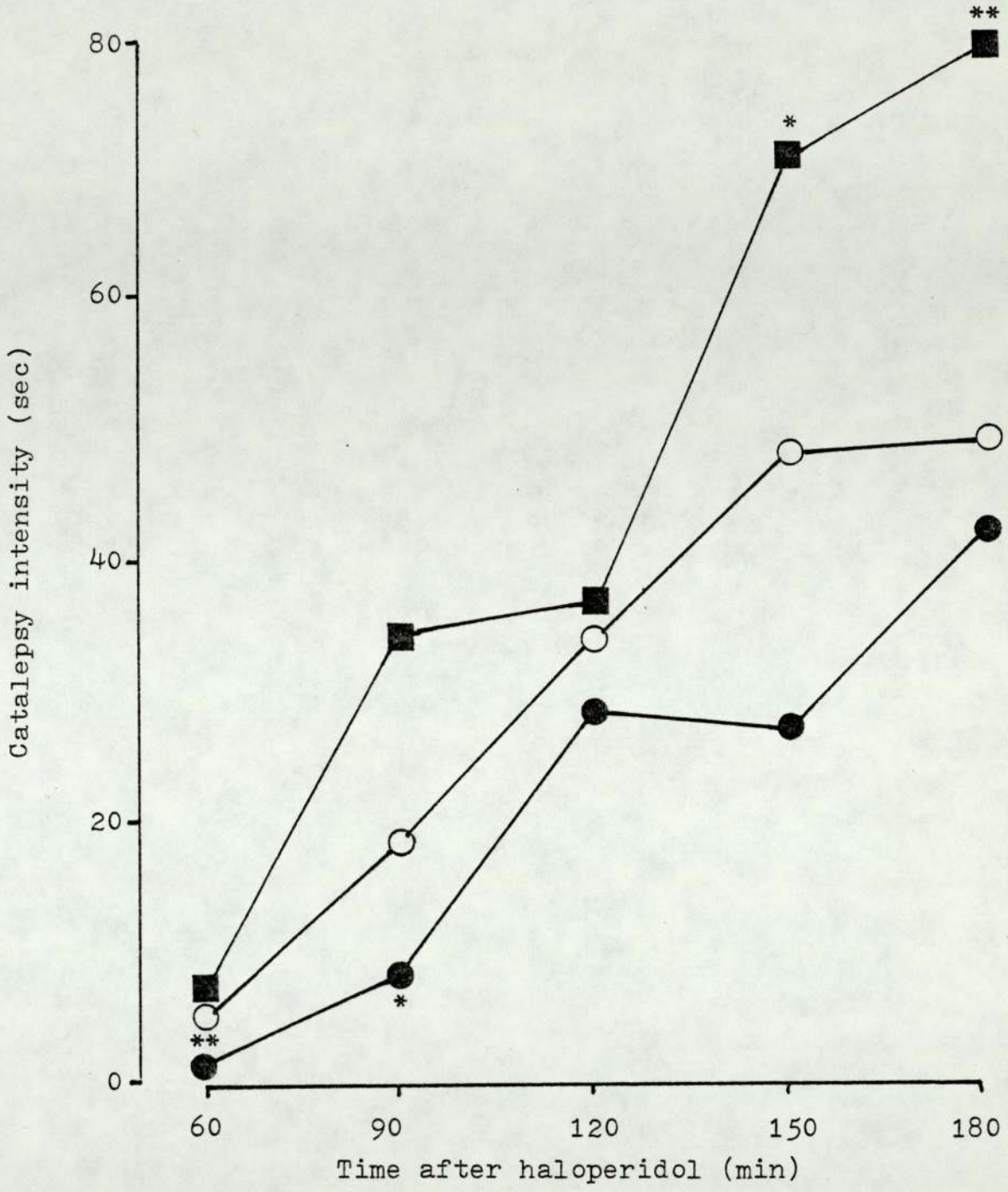


Fig 8.7. The effect of prazosin on catalepsy induced by haloperidol 0.2 mg kg s.c.

Animals were pretreated with saline (○), prazosin 1.0 mg/kg (●) or prazosin 5.0 mg/kg (■) 15 minutes before haloperidol and tested for catalepsy by placing the forepaws on a 4cm high bar. * $p < 0.05$, ** $p < 0.01$ difference from saline-treated animals (Mann Whitney 'U' test).

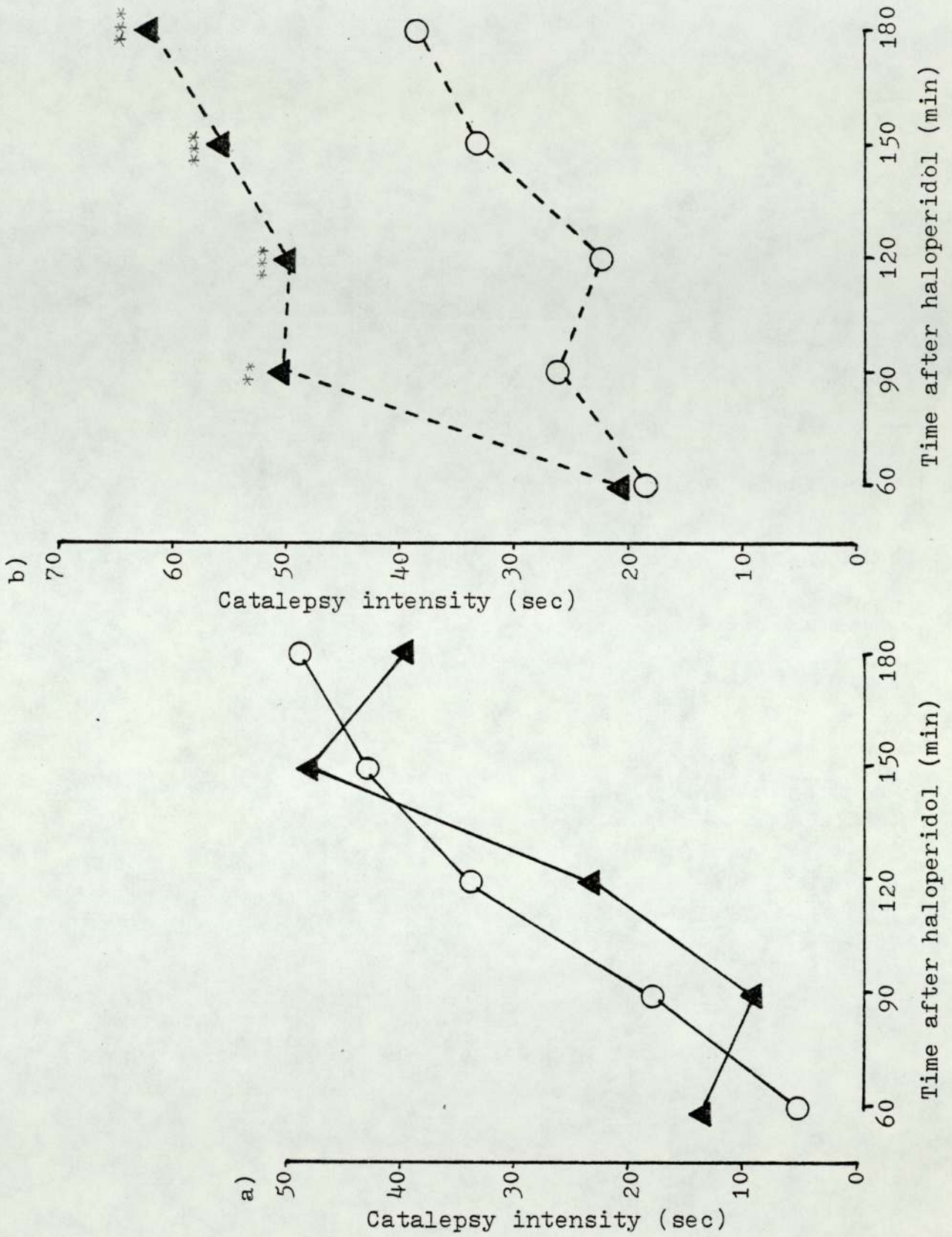


Fig 8.8. The effect of phentolamine injected a)s.c. and b)i.c.v. on catalepsy induced by haloperidol 0.2 mg/kg s.c.

Animals were treated with saline (○) or phentolamine (▲) 5 mg/kg s.c. (solid line) or 5 µg i.c.v. (dashed line).

** $p < 0.01$ *** $p < 0.005$ difference from saline (Mann Whitney 'U' test).

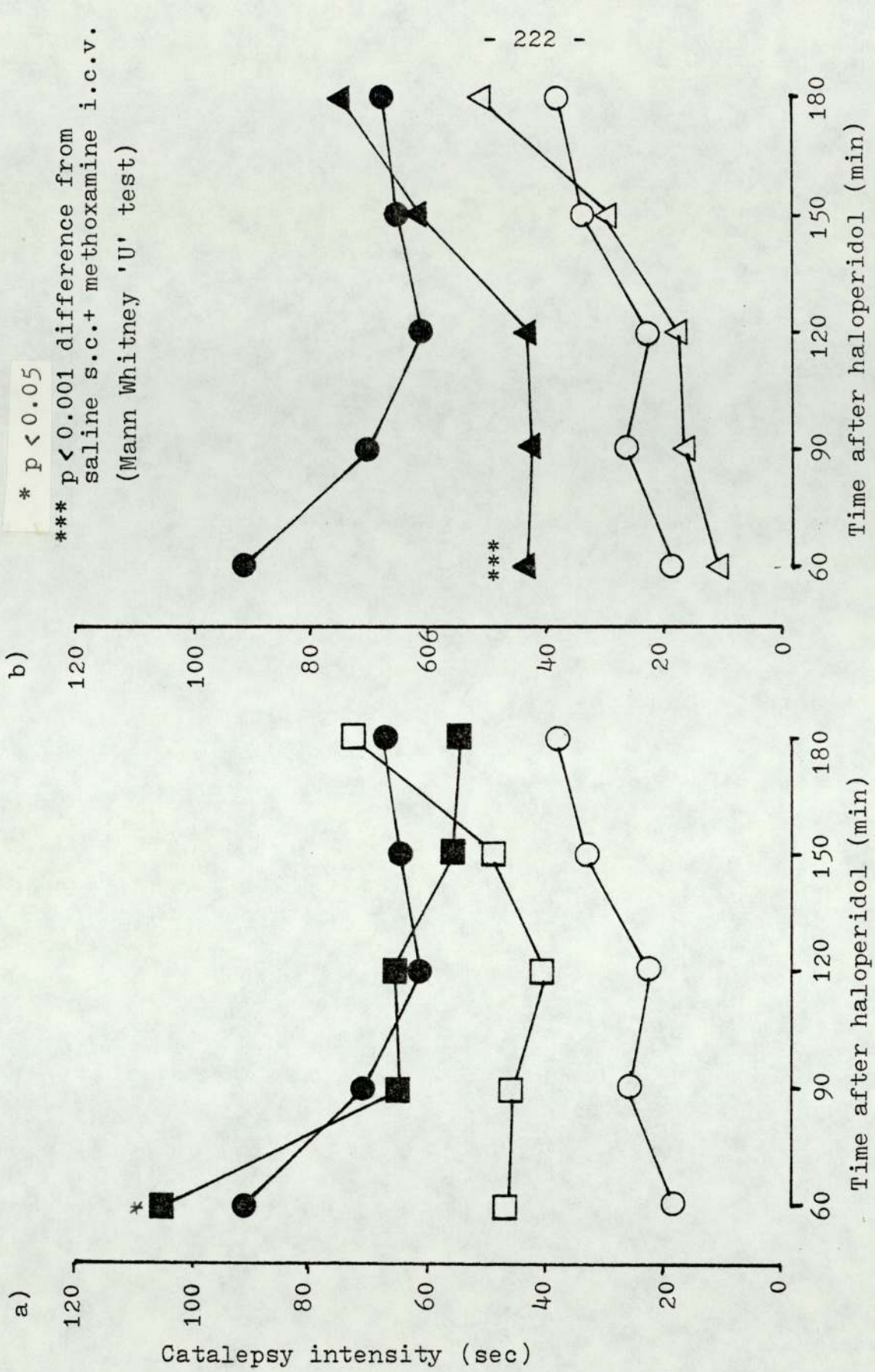


Fig 8.9.The effect of a)yohimbine 2.5 mg/kg and b) prazosin 1.0 mg/kg on catalepsy induced by haloperidol and its potentiation by methoxamine 5 μg i.c.v.

(○) saline s.c.+ saline i.c.v.; (●) saline s.c.+ methoxamine 5 μg i.c.v.; (□) yohimbine s.c.+ saline i.c.v.; (■) yohimbine s.c. + methoxamine i.c.v.; (△) prazosin s.c.+ saline i.c.v.; (▲) prazosin s.c.+ methoxamine i.c.v.

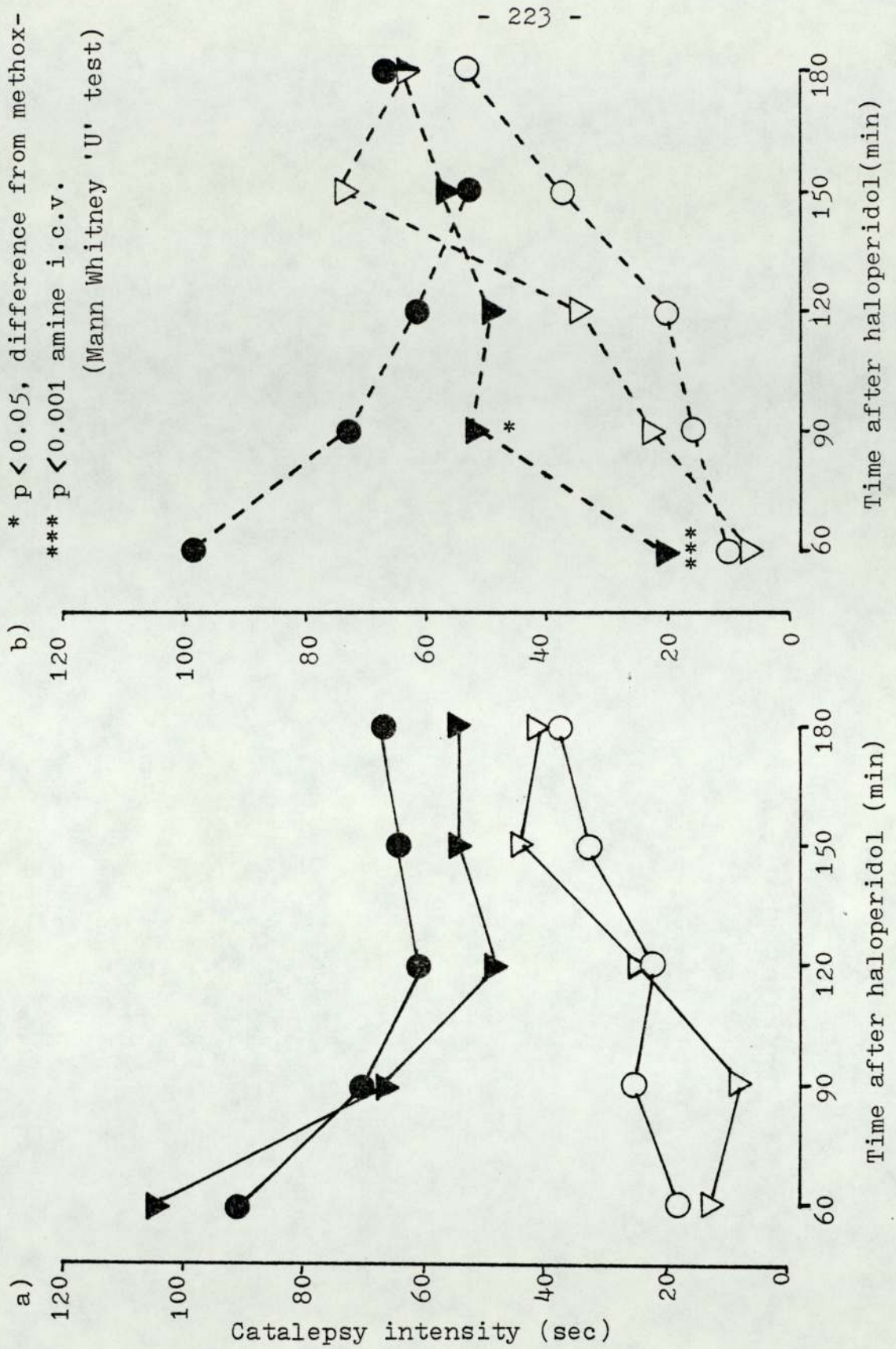


Fig 8.10. The effect of phentolamine administered a) s.c. and b) ic.v. on catalepsy induced by haloperidol and its potentiation by methoxamine 5 μg i.c.v.

(○) saline s.c.+saline i,c,v. (●) salines.c.+methoxamine i.c.v.; (▼) phentolamine 5 mg/kg s.c.+ methoxamine i.c.v. (▽) phentolamine s.c.+ saline i.c.v. (solid lines): (▽) phentolamine 5 μg i.c.v.; (▼) phentolamine i.c.v.+ methoxamine i.c.v. (dashed lines).

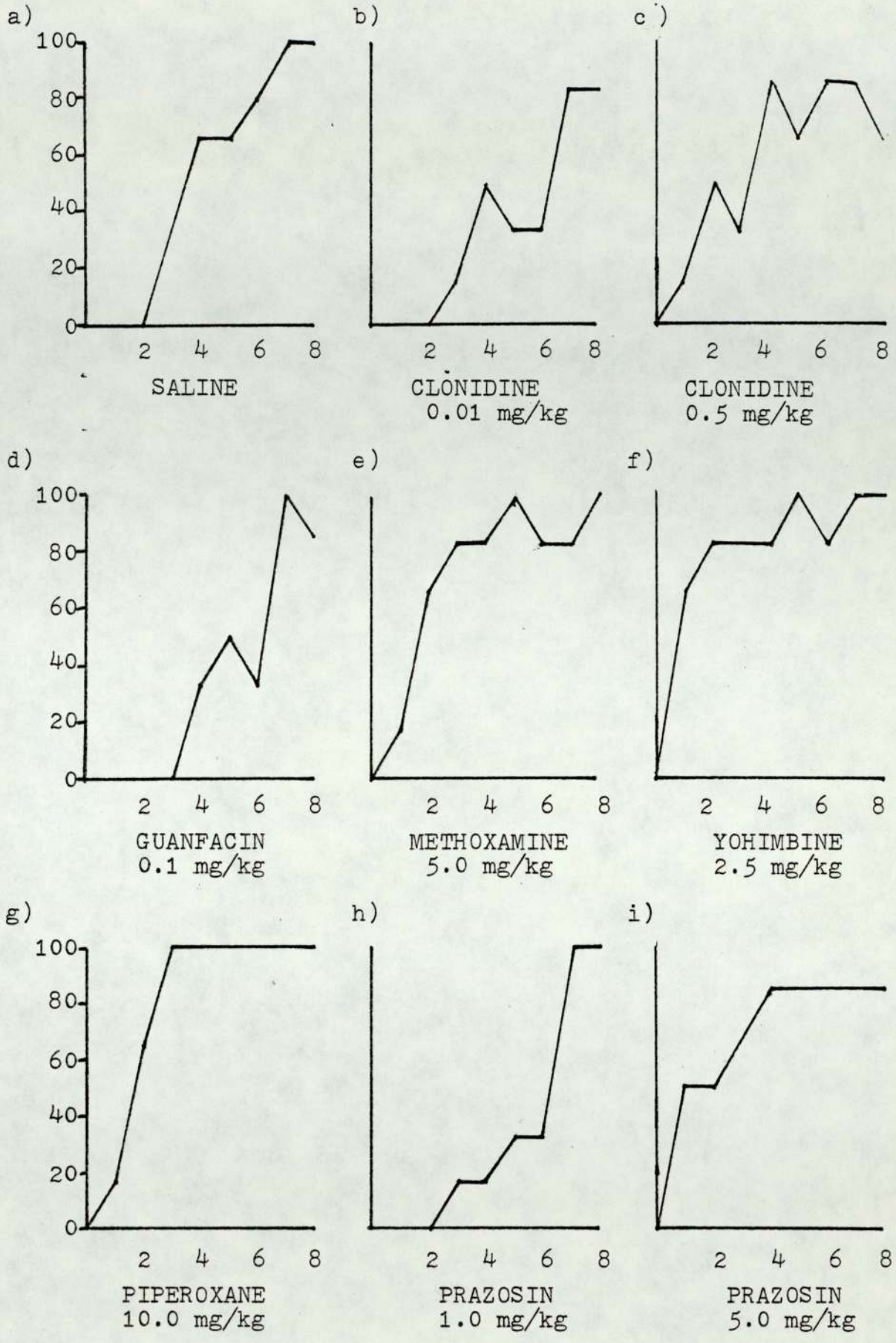


Fig 8.11. The effect of various α agonists and antagonists on the incidence of posture retention in mice on repeated testing.

Ordinate - number of trials for catalepsy, abscissae - percentage of animals remaining in abnormal posture for 3 or more seconds.

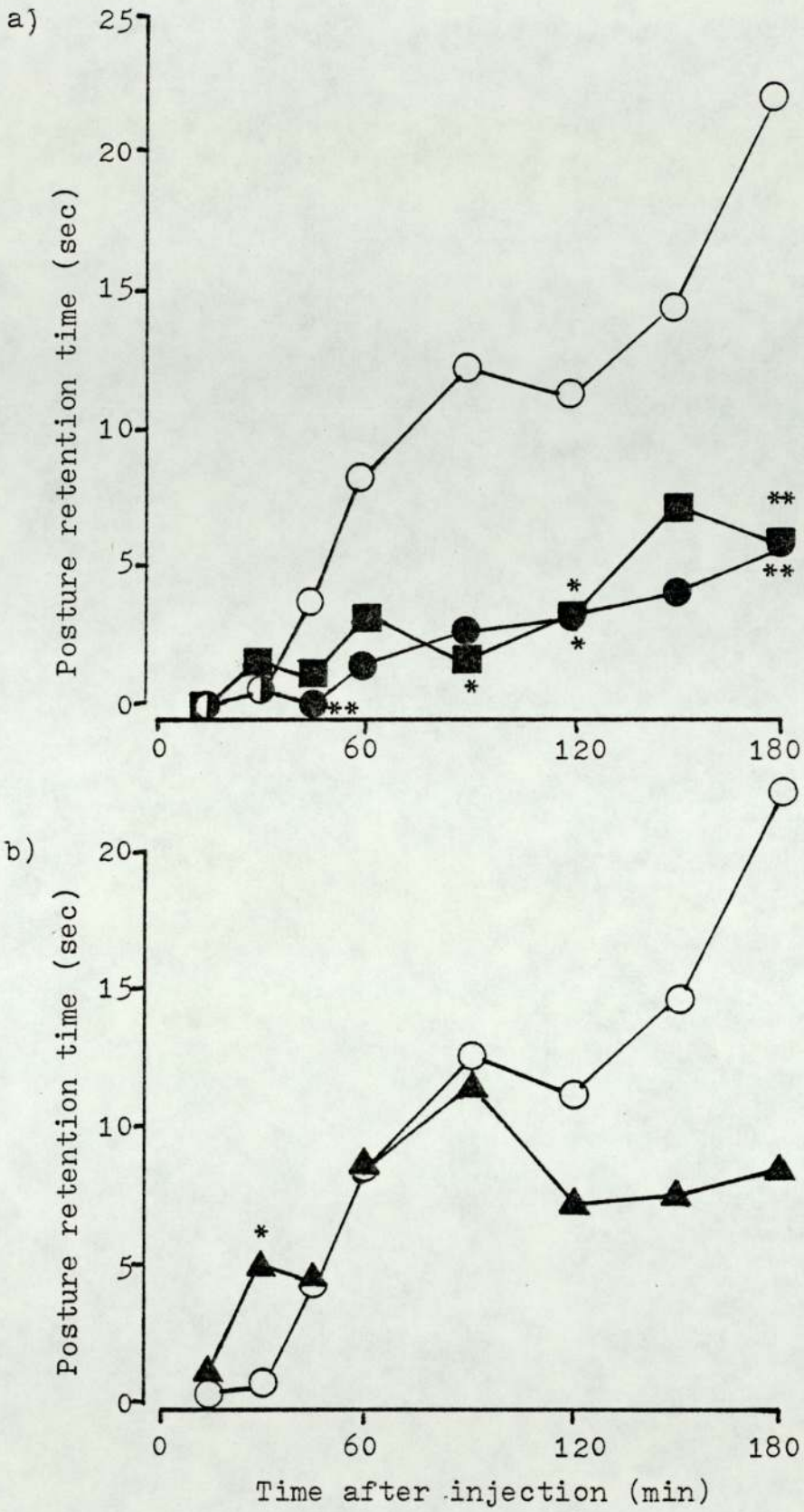


Fig 8.12. The effect of α agonists on posture retention time on repeated testing.

(○) saline; (■) clonidine 0.01 mg/kg; (●) guanfacin 0.01 mg/kg; (▲) methoxamine 5.0 mg/kg. Animals were treated with the α agonist 15 mins before the first trial for posture retention. *p 0.05; **p 0.01 difference from saline-treated animals (Mann Whitney 'U' test).

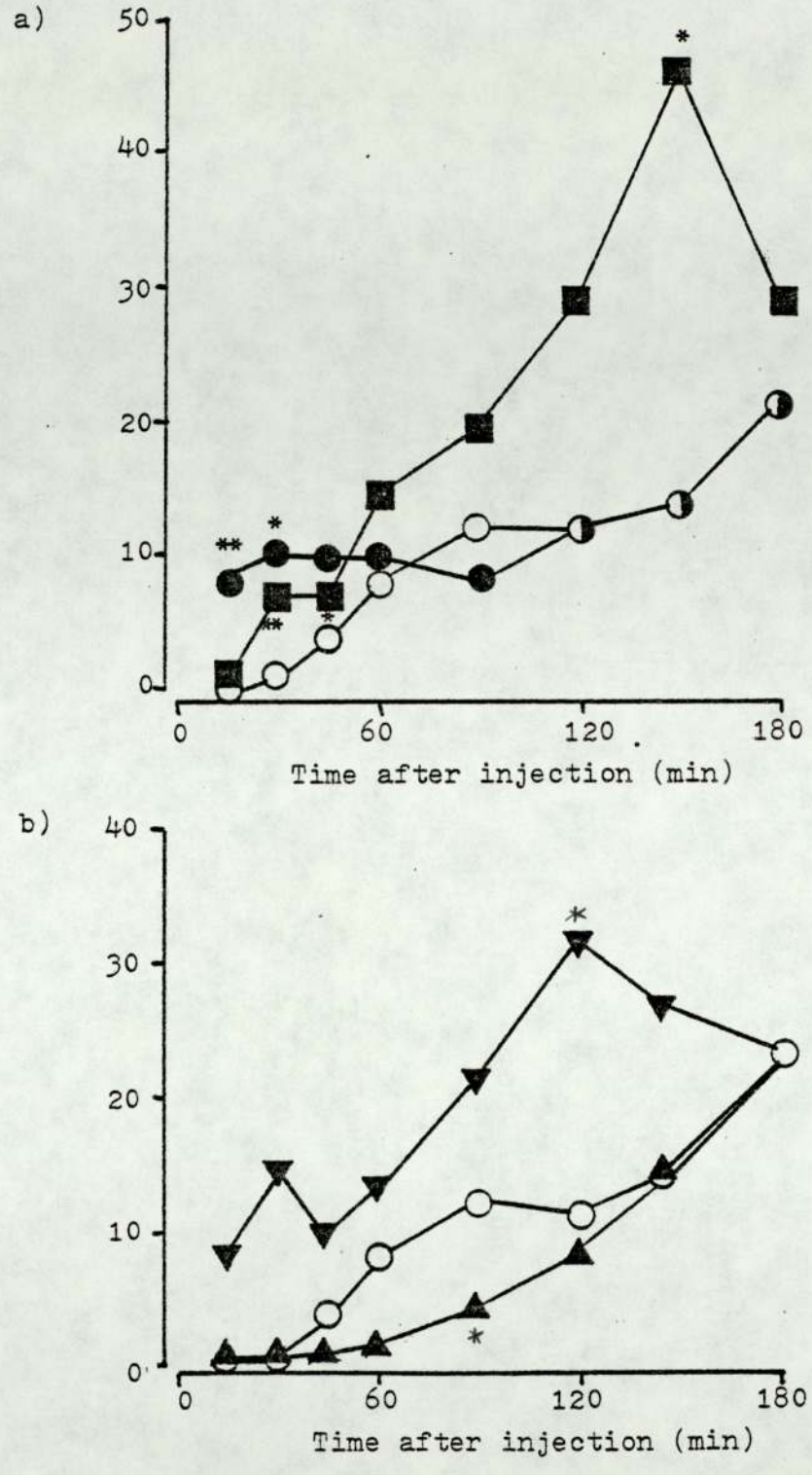


Fig 8.13. The effect of α antagonists on posture retention time on repeated testing.

(○) saline; (●) yohimbine 2.5 mg/kg; (■) piperoxane 10.0 mg/kg (▲) prazosin 1.0 mg/kg; (▼) prazosin 5.0 mg/kg. Animals were treated with the α antagonist 15 mins before the first trial for posture retention. *p 0.05, **p 0.01 difference from saline-treated animals (Mann Whitney 'U' test).

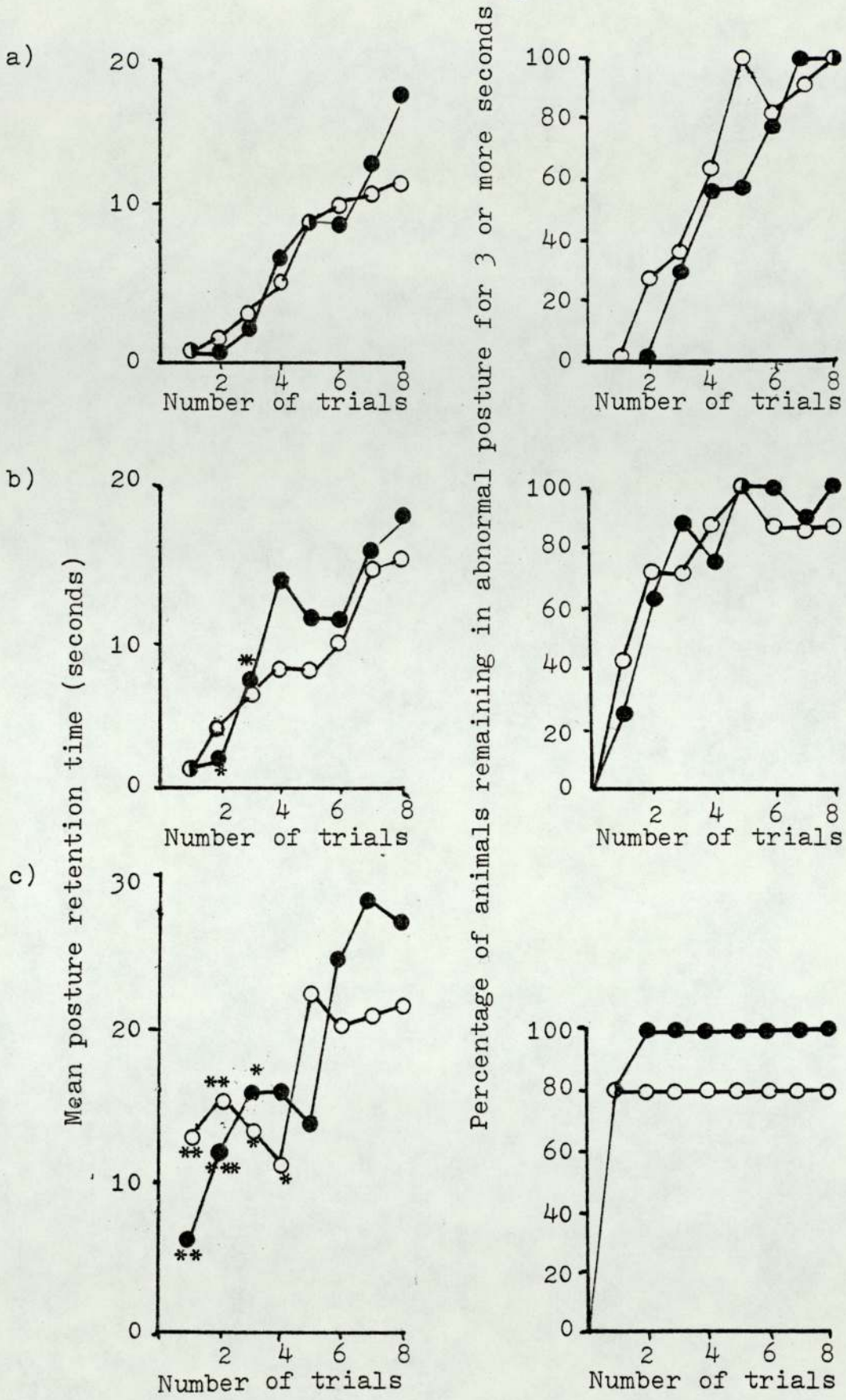


Fig 8.14. The effect of repeated testing on posture retention time in (●) saline-treated and (○) untreated mice; a) initial test, b) repeated after 5 days, c) repeated after a further 24 hours.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from initial test (Mann Whitney 'U' test).

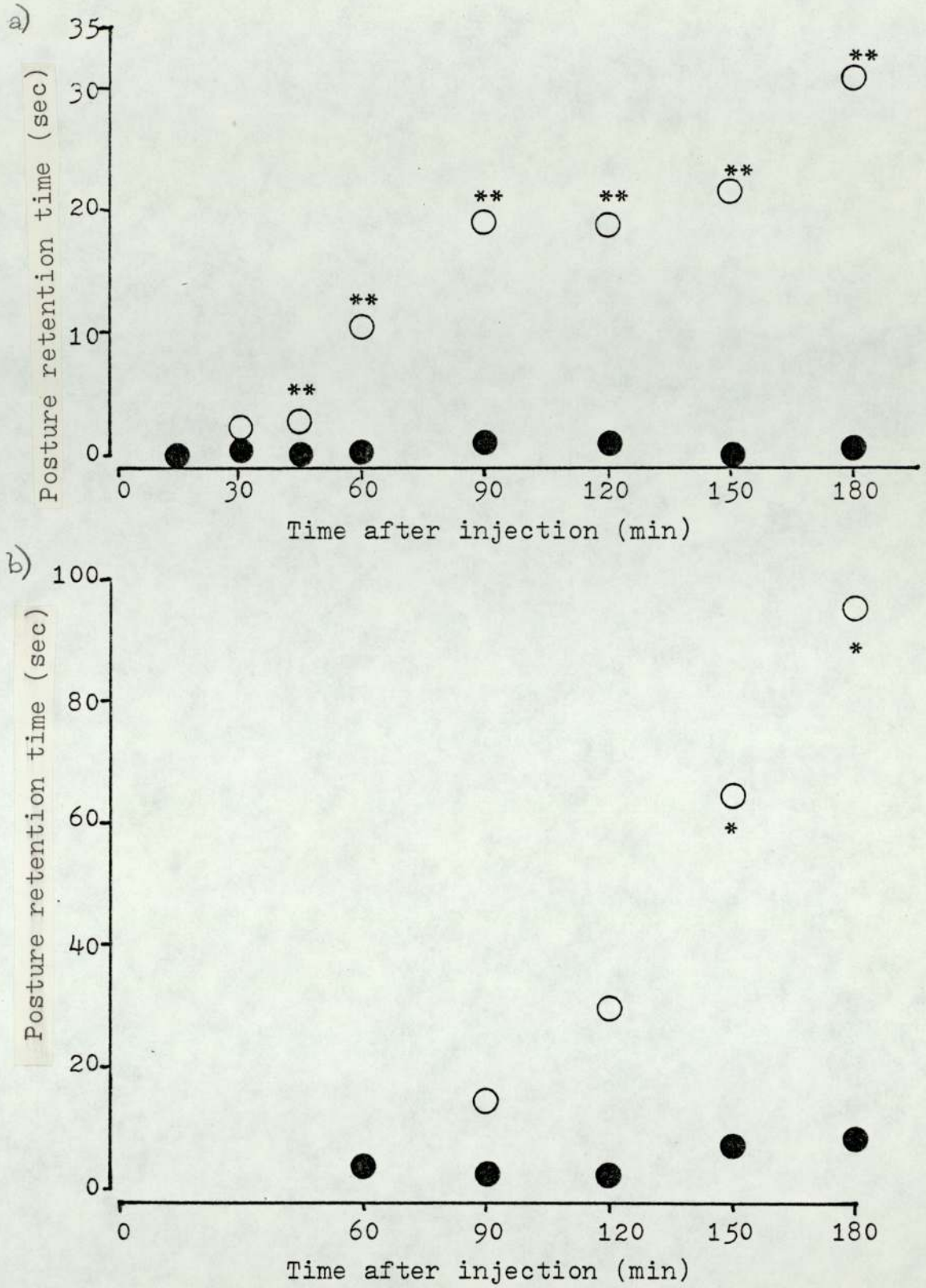


Fig 8.15. The relationship of posture retenion time to the time after injection and the number of trials in a) saline-treated and b) haloperidol-treated mice.

(●) animals tested only once, (○) animals tested at all previous times in addition.

* $p < 0.05$, ** $p < 0.01$ difference from animals tested only once at the corresponding time after injection (Mann Whitney 'U' test).

CHAPTER 9

INVESTIGATION OF THE "ANXIETY-PRODUCING" EFFECT OF YOHIMBINE

CHAPTER 9.

INTRODUCTION

Yohimbine has been shown to decrease spontaneous locomotor activity in rats (Papeschi et al.,1971). A slight transient decrease in activity in mice was seen in the present study (Chapter 5), but this was followed by increased activity. The drug also attenuated the decrease in locomotor acivity due to clonidine (Chapter 5; Clineschmidt et al.,1979), hence is unlikely to reduce activity due to a sedative action, as some potentiation of sedation would then be expected. Al-Shabibi and Doggett (1978) found yohimbine-treated rats to show periodic hyperactivity and stereotyped sniffing, although yohimbine was able to inhibit clonidine-induced potentiation of d-amphetamine gnawing (Thomas & Handley,1978). A very high dose of yohimbine, 20 mg/kg, in rats produced ataxic gait and a decrease in motor activity (Papeschi et al.,1971). Animals were not however sedated, since they were overreactive to external stimuli and showed no degree of ptosis.

During observation of yohimbine-treated mice (Chapter 3), it was noted that they had increased startle, touch and tail pinch responses, very brisk flexor and pinna reflexes and were hyperreactive to noise or movement. Although it is not possible to ascribe emotions to animals, the mice appeared to be in a state of constant fearfulness both of the operator and of each other. They had a markedly decreased spontaneous activity and decreased reactivity to a novel environment. Subsequent use of the terms 'fear' or 'fear-related behaviour' is indicative of the behaviour of animals as described here. Similar effects were produced by piperoxane, methoxamine and very high doses of clonidine, all of which increase noradrenergic function. The possibility that this behavioural effect of yohimbine may be due to central α_2 adrenoceptor blockade causing an increase in noradrenergic activation was therefore

investigated.

A suitable method for quantification and investigation of this effect of yohimbine would be one which was able to distinguish effects of anxiolytic, sedative and anxiety-producing drugs. Several methods are used to study anxiolytic actions of drugs, such as punished responding (Stein et al.,1973), Geller conflict test (Geller et al.,1962), exploratory activity (Christmas & Maxwell,1970) and food preference tests (Cooper & Crummy,1978). Most of these tests involve some kind of conflict of drives, which presumably results in anxiety. Anxiolytic drugs are able to restore inhibited responses to normal. The most simple type of test involves exploratory activity. Here the animal is placed in a strange environment which produces two conflicting drives: the drive to explore and the fear of exploration (Montgomery,1955). Early work in rats using a circular enclosure divided into sectors showed that anxiolytic drugs could increase the number of lines crossed in a given period (Christmas & Maxwell,1970). Similar experiments were performed by Marriott & Smith (1972), who used a square enclosure, the base of which was made up of metal plates separated by 3mm gaps. Both this and the use of a hole board (Nolan & Parkes,1973) would be expected to slightly increase the 'fear' drive due to the unknown nature of the area beneath the floor. Benzodiazepines increased exploration in both studies, while previous exposure to the novel environment decreased activity. Anxiolytic drugs were less able to increase activity in previously exposed animals, suggesting that their action was on a component only present in animals when faced with a novel situation, possibly the 'fear' drive. A method similar to that of Marriott & Smith was used here to investigate the possibilty of a 'fear-producing' effect of yohimbine, which may involve central α adrenoceptors.

1. The effects of drugs acting on α receptors on plate-crossing.

The parameters which were used to compare drug-treated animals to controls were chosen after an analysis of a series of results taken from saline-treated controls. The time taken for the mouse to make the first crossing was used, since this would be expected to be most sensitive to any change in the emotional state of the animal. The time taken to make five crossings was also measured, since this was found to be fairly consistent in control animals. Both the number of crossings in 60 and in 90 seconds were measured, as the majority of exploration in controls took place between 15 and 60 seconds (Fig 9.1), after which time the rate of plate-crossing decreased, presumably due to some familiarisation with the environment. Control animals took approximately 10 seconds to make the first crossing and 40 seconds to make five crossings. The total number of crossings was usually between 8 and 10 in 60 seconds and between 11 and 14 in 90 seconds. Intracerebroventricular injection of saline did not affect the exploratory activity in any way.

1.1. The effect of yohimbine.

Doses of 0.5 to 5.0 mg/kg had a marked effect on the plate-crossing activity of mice (Fig 9.1 and Table 9.1.). There was a dose-dependent increase in the latency to first and fifth crossings, although the majority of crossings were still made in the first 60 seconds. The total number of crossings was also reduced in a dose-dependent manner. Both the number in 60 and in 90 seconds was significantly reduced at 2.5 and 5.0 mg/kg. The animals had an increased startle response in their home cage and resisted handling. They did not appear sedated, in fact had a higher activation score than controls (Table 9.6), but once placed in the box, sniffed their immediate surrounding area and showed typical approach-avoidance

behaviour, venturing to the edge of the plate, then shrinking away. Many animals treated with 5.0 mg/kg did not make one crossing throughout the 90 seconds. Piloerection and exophthalmos were also present at this dose and many animals had loose frequent diarrhoea. No control animals dropped faecal boli, thus this could not be used as an additional measure of 'fear'. In addition to sniffing more than controls, a larger proportion of yohimbine-treated animals spent some time grooming in the box. This may be an indication of the emotional state of the animal, since it did not appear stereotyped in nature.

1.2. The effects of other α-adrenergic antagonists.

Piperoxane 0.5 to 5.0 mg/kg had a similar effect to that of yohimbine on plate-crossing activity (Table 9.1). The effects were, however, much less marked and did not appear to be related to the dose given.

Prazosin 0.25 to 2.5 mg/kg had no significant effect on plate-crossing (Table 9.1). A slight increase in the number of crossings did occur with 0.25, 0.5 and 1.0 mg/kg, but none of these was statistically significant.

1.3. The effects of α-adrenergic agonists.

Clonidine 0.025 to 0.1 mg/kg produced a dose-dependent decrease in the latency to the first and fifth crossings (Table 9.2). The two lower doses also increased the total number of crossings in 60 and 90 seconds. The animals had piloerection and exophthalmos, but appeared somewhat sedated in their home cage, displaying little spontaneous activity. Activation scores were less than in control animals (Table 9.6). Slightly higher doses of clonidine, 0.25 and 0.5 mg/kg, had no effect on plate-crossing apart from a slight increase in the latency to the fifth crossing. These animals again appeared sedated (Table 9.6), but did not become as alert when placed in the new environment as did animals given the lower doses.

They had a slow gait, sometimes slightly ataxic and tended to remain still in one place for up to 15 seconds at a time.

Methoxamine 5 and 10 mg/kg caused a slight decrease in the total number of crossings. These animals again had piloerection and exophthalmos. Some also had an increased startle response and a raised body position. They did not, however, appear sedated when placed in the box, in fact were actively sniffing the floor and walls. After i.c.v. injection, methoxamine, 1 to 10µg, produced a marked decrease in plate-crossing and piloerection, exophthalmos and vasodilatation. Several animals also had diarrhoea. Many 'froze' when placed in the box, some for the whole 90 seconds, whilst others appeared very jumpy and spent much time sniffing the box.

2. The effects of drugs known to have an anxiolytic action on plate-crossing.

Various drugs with diverse pharmacological spectra but which have been found to have an anxiolytic action, either in animals or in man, were tested using this paradigm to investigate whether such an effect could be demonstrated here.

2.1. Diazepam.

Doses of 0.5 and 1.0 mg/kg caused a marked increase in the total number of crossings, especially after 60 seconds.(Table 9.3). Animals tended to sniff around before making the first crossing, hence the effect on this parameter was not so marked. Mice then became very active, rushing round the box, but with little sniffing and rearing, then decreasing exploration again. These animals were not hyperactive in the home cage, however (Table 9.6). The difference in the time from the first to the fifth crossing was thus much smaller than in controls. Higher doses, 2.5 and 5.0 mg/kg, caused a marked increase in the latency to the first and fifth crossings. The higher dose also decreased the total number of crossings.

Animals treated with this dose were ataxic and sedated (Table 9.6), and one or two appeared indifferent to the new environment.

2.2. Chlordiazepoxide.

A dose of 1.0 mg/kg had no effect on plate-crossing (Table 9.3), although 2.0 and 4.0 mg/kg produced an increase in the number of crossings, whilst having no effect on the time taken to make the first crossing. This drug was less potent than diazepam in this test since the maximal increase in plate-crossing occurred at 2.0 mg/kg and was not as marked as with diazepam, 1.0 mg/kg. The appearance of the animals was similar to those treated with diazepam, although ataxia was not present even at 4.0 mg/kg.

2.3. Amylobarbitone.

A low dose of amylobarbitone (7.5 mg/kg) had no effect on plate-crossing; 15.0 mg/kg, however, decreased the latency to the first and fifth crossings and slightly increased the total number of crossings.(Table 9.3). Higher doses, 30.0 and 60.0 mg/kg, increased the total number of crossings and 30 mg/kg also decreased the latency to first crossing. The time which elapsed before the first crossing, however, was increased by both doses, although this was most marked with 60.0 mg/kg. Animals treated with doses of 30.0 and 60.0 mg/kg developed marked ataxia 5 to 10 minutes after injection, which had disappeared when they were placed in the box at 30 minutes. They showed a marked increase in activity in the home cage (Table 9.6); and when placed in the box, displayed similar behaviour to animals treated with diazepam.

2.4. Morphine.

Morphine produced similar results to those obtained with diazepam (Table 9.3). Doses of 0.5 and 1.0 mg/kg increased the number of crossings, while decreasing the latency

to both the first and fifth crossings. These animals had slight piloerection and spent much time in the box sniffing and rearing i.e. typical exploratory behaviour. Animals treated with 2.0 mg/kg also explored the box, but there was no increase in plate-crossing compared to controls. A higher dose of 4.0 mg/kg markedly decreased the total number of crossings, without having any effect on latency to first or fifth crossing. These animals appeared sedated with very little spontaneous activity in the home cage, and a slow gait with periods of inactivity when placed in the box.

2.5. Propranolol.

Despite reports of anxiolytic activity in man, this drug, in doses of 1.0 and 2.0 mg/kg did not have any such activity, as determined by an increase in plate-crossing, in mice. Animals did not appear sedated or show a decrease in exploratory activity.

3. Investigation of the effect of yohimbine.

Yohimbine produced signs of peripheral sympathetic activation, such as piloerection and exophthalmos, which may be due to peripheral presynaptic α_2 receptor blockade. The possibilty exists that these signs, along with tachycardia which may also be produced, may contribute to the awareness of 'fear' and thus to the ensuing behaviour. It was therefore necessary to investigate whether the decrease in exploratory activity due to yohimbine was peripheral in origin, by blocking peripheral sympathetic activation. Neither phentolamine nor practolol when given before yohimbine 5 mg/kg had any effect on the decrease in plate-crossing produced. (Table 9.4) Phentolamine 1 mg/kg did, however, prevent the onset of vasodilatation, piloerection and exophthalmos.

Several of the anxiolytics investigated were used in an attempt to prevent the inhibitory effect of yohimbine on

plate-crossing. Clonidine was also used as it had been found to have a similar effect to that of the anxiolytics (Fig 9.2) and in order to determine whether α_2 receptors were involved in yohimbine's action. The effects of the drugs studied are shown in Fig 9.3. Diazepam 0.5 and 1.0 mg/kg attenuated the decrease in plate-crossing due to yohimbine 5 mg/kg, the maximal effect being seen at 1.0 mg/kg. A higher dose produced ataxia and the animals appeared sedated; and a further decrease in plate-crossing was seen. Morphine 1.0 mg/kg completely reversed the effect of yohimbine, as did propranolol 1.0 mg/kg, although a higher dose of the latter drug was less effective. Clonidine had no significant effect on the decreased plate- crossing, in doses of 0.25 and 0.5 mg/kg s.c. A dose of 1.0 mg/kg produced limb splay and ataxia and further decreased plate-crossing. Prazosin reversed the exophthalmos and piloerection produced by yohimbine, but none of the doses used, 0.5 to 5.0 mg/kg, attenuated the decrease in plate-crossing. Animals still had a markedly increased startle response, spent most of their time in the box sniffing and appeared extremely 'fearful'. All the doses of prazosin further decreased the total number of crossings although this was only significant at 2.5mg/kg.

The possible involvement of β-adrenoceptors in the action of yohimbine was investigated, since propranolol was effective in preventing the effect. Isoprenaline, 5 to 40 µg after i.c.v. injection, produced a dose-dependent decrease in the total number of crossings and an increase in the latency to both first and fifth crossings (see p 255). Animals treated with isoprenaline appeared to have difficulty in respiration, had an increased startle response and displayed short periods of immobility when placed in the box. They did not however, appear sedated or exhibit ataxia. Propranolol 1.0 mg/kg significantly attenuated the effect of isoprenaline 20µg on plate-

crossing (Table 9.5), bringing the number of crossings to control levels. The respiratory difficulty shown by isoprenaline-treated animals was also prevented by propranolol. Diazepam 1.0 mg/kg had no inhibitory effect on the action of isoprenaline, in fact, there was a slight further decrease in plate-crossing.(Table 9.6). Animals treated with this drug combination appeared sedated and several had hind limb splay. A dose of yohimbine, 1.0 mg/kg, which did not reduce plate-crossing alone, markedly potentiated the effect of isoprenaline (Table 9.5).

The possible involvement of opiates in the effect of yohimbine was also investigated further, since morphine was able to prevent this effect. Morphine alone had produced an increase in plate-crossing, at a dose of 1.0 mg/kg. Naloxone 1.0 mg/kg was found to prevent this effect of morphine (Fig 9.4), thus demonstrating opiate receptor involvement. Yohimbine 5.0 mg/kg also prevented morphine's anxiolytic effect, suggesting that α receptors may also be involved. Clonidine's facilicating effect on plate-crossing was reversed by naloxone 1.0 mg/kg, a dose which had no effect alone.

DISCUSSION

The method used was able to show the effects of anxiolytic drugs on behaviour in a novel situation. Low doses increased exploratory activity, while high doses caused a decrease due to sedation. The effect of yohimbine was to decrease exploration, but this was found to differ from the sedative/anxiolytic profile seen with the other drugs, as no dose produced an increase in activity. This method thus appears to be useful not only for measuring anxiolytic actions, but also for anxiety-producing and sedative effects. It has the advantage of being rapid and easy to perform, requiring no training, although conditions must be kept constant to eliminate as many variables as possible, since any method of measuring an emotional state is sensitive to many external factors.

Yohimbine produced a dose-dependent decrease in plate-crossing activity. This was shown not to be due to peripheral sympathetic activation leading to awareness of the autonomic signs of 'fear', since blockade of peripheral effects by phentolamine and practolol had no effect on the action of yohimbine. It is also unlikely that the decrease was due to sedation produced by yohimbine, as observation of the animals did not detect any sedative effects. The activation score for yohimbine-treated animals, in fact, showed them to be more aroused than control animals. In addition, if yohimbine were producing a decrease in activity by a sedative action, the combination of yohimbine with sedative drugs would be expected to further decrease the activity at all doses. Such an effect was seen with prazosin, which, although shown to be sedative in several tests (Chapter 4), had no effect on plate-crossing alone and did not sedate yohimbine-treated animals. Diazepam and morphine, on the other hand, reduced the effect of yohimbine at doses which increased activity alone; clonidine

however, was ineffective at doses which did not reduce plate-crossing alone. Higher doses of these drugs, at which sedative effects were beginning to predominate, did produce a further decrease in plate-crossing in yohimbine-treated animals. The effect of yohimbine was also reduced by propranolol, a drug which has been reported clinically to have anxiolytic actions due to its blockade of β-adrenergic receptors (Conway et al., 1978). It seems possible, therefore, that yohimbine may produce or enhance the state of 'fear' of an animal placed in a novel environment, an effect which is attenuated by drugs known to have an anxiolytic action. Clonidine produced a similar effect on plate-crossing to the known anxiolytics diazepam, amylobarbitone and morphine; and also reduced the effect of yohimbine. It is possible, therefore, that clonidine also possesses anxiolytic activity as suggested by Redmond (1977).

Both clonidine and yohimbine have been shown to have actions at α_2-adrenoceptors; they have opposite effects on the release of NA from brain slices (Chapter 2) and on brain NA turnover (Anden & Grabowska,1976). It is likely, therefore, that the behaviourally opposite and antagonistic actions of clonidine and yohimbine are the result of changes in central noradrenergic activity brought about by opposing actions on α_2 receptors. The small reduction in the effect of yohimbine by clonidine may be due to the very high dose of yohimbine used: compare, for example, the ID_{50} dose against clonidine 1.0 mg/kg in the pinna reflex (0.85 mg/kg). Morphine, however, has no direct actions on adrenoceptors (Farsang & Kunos,1979), while diazepam is unlikely to have any direct effect on noradrenergic neurones (Lidbrink & Farnebo,1973), although both drugs may decrease NA turnover (Blasig,1978; Corrodi et al.,1971). Diazepam has also been found to block stress-induced increase in NA turnover in rat cortex (Corrodi et al.,1971) and also

the increase produced by piperoxane 5.0 mg/kg or yohimbine 0.5 mg/kg (Fuxe et al.,1975). Propranolol has also been shown to decrease NA turnover in the rat cortex (Conway et al.,1978). It is therefore possible that all the drugs which were able to diminish the effect of yohimbine may do so by decreasing the high NA turnover which would be induced by this drug.

The marked effect of propranolol in preventing the decrease in plate-crossing by yohimbine and the lack of effect of practolol suggest that, at least in this test situation, propranolol's anxiolytic action is of central origin. This is in contrast to the conclusions of other workers who suggest that both propranolol (Granville-Grossman & Turner,1966) and practolol (Bonn et al.,1972) may alleviate autonomically-mediated peripheral symptoms to produce an anti-anxiety effect. D- propranolol, which has very little β blocking activity, has no effect on anxiety (Conway et al.,1978), thus β receptors, whether peripheral or central, are responsible for propranolol's anxiolytic action. Central effects of β blockers have been reported; several drugs of this type have been found to antagonise 5-HT (Weinstock et al.,1977) and propranolol does produce sedation both in animals and man (Granville-Grossman & Turner, 1966), although only at high doses. Propranolol has also been found to potentiaie the anxiolytic effect of chlordiazepoxide in a conflict test (Sepinwall et al.,1973), while Farhoumand et al.,(1979) suggested that oxprenolol exerted a similar central action to that of lorazepam, since both drugs decreased alertness and concentration and also decreased skin conductance after stress.

Propranolol is unlikely to exert its anxiolytic action by decreasing the activity of noradrenergic neurones directly, since sotalol, a similar compound, does not alter the spontaneous firing of these neurones in the L.C. (Cedarbaum &

Aghajanian,1976). Beta-adrenoceptors are involved, however, as isoprenaline was found to decrease plate-crossing, an effect which was reversed by propranolol. Isoprenaline has been shown to further decrease punished responding in rats when administered into the amygdala (Margules, 1971), an area which has long been thought to be associated with 'fear' (Milner,1970; Schallek et al.,1979). The mutual potentiation of yohimbine and isoprenaline on plate-crossing activity and the antagonism of both by propranolol suggests that the two former drugs are acting on the same neuronal pathway. This pathway could therefore involve a noradrenergic innervation of the amygdala; and both stimulation of these neurones or direct stimulation of amygdaloid neurones may result in decreased exploratory activity. The observation by Cedarbaum & Aghajanian (1976) taat isoprenaline decreases the rate of spontaneous firing of L.C. neurones does not conform to the hypothesis that decreased firing mediates anxiolytic effect. However, both sotalol and piperoxane only slightly reversed this action of isoprenaline and the authors suggested that the receptor involved may not differ from the clonidine-sensitive receptor. Thus isoprenaline would be expected to have greater affinity for the β receptor involved in the potentiation of anxiety, than for the α_2 receptor involved in its decrease.

The decrease in plate-crossing activity produced by methoxamine suggests that an α_1 receptor may also be involved in this effect. However, prazosin was unable to decrease the effect of yohimbine; and, in fact, appeared to potentiate the latter drug, but not by producing sedation. The role of α_1 receptors in mediating 'fear-related behaviour' is thus unclear, although peripheral mechanisms may be involved, since methoxamine also decreased plate-crossing after peripheral injection.

Diazepam is thought to exert its anxiolytic, anti-convulsant and sedative effects by the potentiation of the inhibitory actions of GABA (Schallek et al.,1979). It may therefore reduce the effect of yohimbine by an indirect decrease in noredrenergic activity via GABA. Benzodiazepines (BDZ) have also been found to bind specifically to membranal sites (Squires & Braestrup,1977), which are concentrated mainly in the frontal cortex and midbrain, although they are also present in striatum, cerebellum, medulla and spinal cord (Placheta & Karobath,1979). These sites are associated with GABA binding sites and BDZ's are assumed to produce some interaction between the two. Clinical anxiolytic activity, anticonvulsant activity and the ability to increase punished responding in rats all correlate with BDZ, rather than GABA, binding sites (Tallman et al.,1980). BDZ receptor binding has been found to be greater in Maudsley non-reactive rats than Maudsley reactives (Robertson et al.,1978). Electric footshock or conflict has been shown to produce a rapid decrease in BDZ binding in frontal cortex in normal rats (Tallman et al.,1980). Thus it is possible that stress releases an endogenous anxiety-producing compound of which BDZ's are antagonists. The possibilty exists that yohimbine's effect may be mediated via such a compound.

The ability of morphine to reverse the inhibition of plate-crossing activity by yohimbine is only one of several interactions shown to exist between drugs acting at opiate and α-adrenoceptors (Chapter 6). Naloxone reverses the antihypertensive effect of clonidine (Farsang & Kunos,1979) and also the inhibition of contraction in the co-axially stimulated guinea pig ileum (Mithani,1980). The central analgesic action of clonidine is not reversed by naloxone (Chapter 6), although a local effect of morphine on the gut is sensitive to piperoxane

(Bentley,1978). In addition, clonidine is able to reduce symptoms of morphine withdrawal both in rats (Vetulani & Bednarczyk,1977) and clinically in man (Gold et al.,1978). Schwartz (1979) has suggested that opiate receptors may be present on cortical and cerebellar neurones originating from the L.C., as well as on L.C. cell bodies. He has proposed that hypersensitivity of noradrenergic receptors, arising from disuse due to inhibition of neuronal activity by morphine, is the basis of both tolerance and physical dependence produced by this drug. Withdrawal of morphine would thus lead to an increase in transmitter release, which can then be abolished by clonidine. This theory is supported by the work of Aghajanian (1978), who produced tolerance to the suppressant effect of morphine on L.C. neurones by continuous direct applicaion of morphine for 5 days. The firing rate returned to normal over this period. Naloxone produced an increase in firing, which was interpreted as the withdrawal response. Acute application of morphine was unable to depress firing in these neurones; clonidine was, however, still able to do so. NA turnover has also been found to increase during naloxone-induced withdrawal from morphine (Crawley et al.,1979), an effect which is reversible by clonidine. Stimulation of L.C. neurones (Goldet al.,1978) and piperoxane (Aghajanian,1978) both lead to symptoms similar to those seen in opiate withdrawal. Morphine has been shown to block the effects of L.C. stimulation (Gold et al.,1978) and of yohimbine on behaviour (this study). It thus appears that the morphine withdrawal syndrome may involve both 5-HT (Chapter 7) and noradrenergic activation; and that clonidine is able to reduce both components of withdrawal. Reciprocal experiments in which naloxone was found to reverse the anxiolytic-type action of clonidine

and yohimbine reversed the effect of morphine , suggest that the same neurones are involved in the action of all these drugs, although there is no competition for receptors (Farsang & Kunos, 1979; Aghajanian,1978).

Morphine and clonidine may thus reduce NA turnover by stimulation of receptors on L.C. neurones, leading to a decrease in firing rate. Could this be the basis of the anxiolytic-like effect exerted by both drugs? Gray et al.,(1975) suggested that amylobarbitone, chlordiazepoxide and α-mpt all exerted their effect on the hippocampal theta rhythm of the EEG by a selective impairment of noradrenergic input to the septum via the dorsal bundle; and that lesions of this pathway should have a similar behavioural effect to that of the BDZ's. The increase in turnover of cortical NA which occurs after stress (Corrodi et al.,1971) has been shown to originate from the L.C. via this pathway (Korf et al.,1973). Redmond and co-workers also maintain that the dorsal noradrenergic pathway, originating in the L.C. is involved in fear and anxiety (Redmond, 1977; Davis et al.,1979).

In the stump-tailed monkey, electrical stimulation of the L.C. area produced behavioural and physiological changes which simulate 'natural fear', while lesions of this area lead to elimination of such changes (Redmond,1977). Piperoxane increased the rate of spontaneous firing of L.C. neurones (Cedarbaum & Aghajanian,1976), probably by blockade of α_2 adrenoceptors; and also produced a similar effect in monkeys to that of L.C. stimulation (Redmond,1977). Yohimbine would be expected to have similar actions, both on L.C. firing and behaviourally; and, in fact, both yohimbine and piperoxane have been reported to produce anxiety in man (Redmond,1977). A reduction in the firing rate of L.C. neurones would therefore be expected to have an opposite effect i.e. reduce anxiety.

Thus clonidine and morphine may both act in this way. GABA also reduces spontaneous firing from the L.C. region (Cedarbaum & Aghajanian,1976), thus diazepam may also produce its anxiolytic effect in this way, by potentiation of GABA.

The involvement of the L.C. in fear and anxiety is however, by no means certain. The effects of stimulation and electrolytic lesions may not be confined to the L.C. region. Other adjacent structures may be affected, or effects on other systems which occur as a consequence of L.C. lesions may be the cause of the behavioural changes. Stimulation of the L.C. region does not exclusively affect noradrenergic systems, as intra-cranial self-stimulation with electrodes in the L.C. is not abolished by destruction of dorsal bundle noradrenergic fibres.(Clavier et al.,1976). The species used by Redmond has in fact been shown to possess serotonergic neurones in the L.C. (Sladek & Walker,1977), while 5-HT depletion has also been found to have a similar effect on exploratory activity to that of NA depletion (Hanigan & Scudder,1977).

Mason and co-workers maintain that electrolytic lesioning may affect other neurones, while the drugs used by Redmond et al. are not sufficiently specific to noradrenergic neurones (Mason & Fibiger,1979a) and dispute the involvement of the L.C. in fear-related behaviour. These workers suggest that, if decreasing the activity of dorsal bundle neurones reduces 'fear', then it should also reduce learning of fear-motivated tasks, such as active and passive avoidance. To overcome the lack of specificity mentioned above, lesions of the L.C. were made by using 6-hydroxydopamine (6-OHDA), a neurotoxin which selectively destroys noradrenergic neurones. Such lesions did not reduce the ability of animals to learn avoidance tasks (for review, see Mason & Fibiger,1979a). However, such learning may not be based on fear as Mason suggests; an animal does not have to

fear a shock to wish to avoid it. In addition, the procedures used by Mason involved avoidance; inescapable shock, such as that used by Redmond would be much more likely to elicit fear. Also the BDZ's, which would be expected to decrease fear, far from reducing performance in avoidance tests, have been shown to enhance it (Gray 1977). A test which is more likely to be sensitive to fear - the conditioned emotional response - has been used by Mason and Fibiger (1979b). This paradigm has a similar basis to the punished responding used by Stein et al., (1973), which was sensitive to anxiolytic effects of BDZ's. Animals with a reduced capacity for fear would be expected to show an increased responding in the CER test, while responses in normal rats would be suppressed. Lesions of the dorsal bundle did not increase responding, in fact, a decrease was found on extinction days. This latter effect has been found in many of the paradigms used by Mason and Fibiger. Tremmel et al. (1977) suggest that this effect may be due to an increase in non-reward due to lack of NA, thus leading to a lack of frustration. Hence it may be an emotional effect, but is unlikely to involve decreased fear. Neophobia, or the reluctance to sample novel solutions in a new environment, has also been investigated as a means of detecting reductions in fear. Rats with dorsal bundle lesions showed a decreased consumption of novel solutions than did control rats (Mason et al.,1978a), which suggests that lesions may increase, rather than decrease fear. Chlordiazepoxide, however, which would be expected to increase consumption of novel food and drink, only produced an increase in total food consumption, not in novel foods (Cooper & Crummy,1978), hence this test may not be sufficiently sensitive to detect changes in the emotional state of the animal.

The measurement of exploratory activity in a novel environment has also been used by Mason & Fibiger(1979a), hence similar results to those found here would be expected if the dorsal noradrenergic pathway is invloved in such activity. The number of alleyways in a maze entered was measured; and after the first three minute period, when no significant increase was seen, lesioned rats showed a marked increase in exploration. This is in general agreement with the results obtained here for anxiolytic drugs on plate-crossing activity. There was an increase in total activity, but the latency to the first crossing was not markedly affected. It was only after the initial crossing that exploration was higher than in controls. An increase in the time spent in contact with a novel object by L.C. lesioned animals may also be interpreted as a decrease in fearfulness, although Mason & Fibiger maintain that this is a further example of a decrease in extinction.

The work of File and co-workers using a social-interaction test of anxiety has shown that lesions anterior and lateral to the L.C., which decrease cortical and hypothalamic NA(Crow et al.,1979), have no effect on social interaction. Latency to drink in a novel environment was similarly unaffected by these lesions (File et al.1979)but lesioned animals in their home cage were found to be more aggressive towards an intruder than controls (File et al.,1979).

These and the results obtained by Mason described above have led to the suggestion (Mason & Fibiger,1979a) that anxiety is not dependent upon the dorsal noradrenergic system in the rat. However, Davis et al.,(1979) have found that clonidine, which has been shown to act on central noradrenergic systems, was able to decrease the potentiated startle response in this species. This paradigm, which has a similar basis to the CER, involves the association of a cue (light) with an

inescapable shock and subsequent pairing of this cue with an acoustic stimulus (tone), leading to potentiation of the startle produced by the tone. It has been found to be sensitive to the anxiolytic action of BDZ's (Davis,1979a), sodium amylobarbitone (Chi,1965) and morphine (Davis,1979b). Piperoxand and yohimbine increased potentiated startle response while propranolol and WB4101 decreased it slightly. All of these results fit into the theory that stimulation of the dorsal noradrenergic bundle produces anxiety. However, as stated earlier, the use of drugs may not be specific and actions on other neuronal systems cannot be excluded. Hence a report that lesions of the L.C. decrease the startle response to a puff of air may be of interest (Geyer et al., 1976, quoted in Redmond & Huang,1979). The L.C. thus seems to be involved in anxiety in the rat as well as in the monkey. The lack of effect of L.C. lesions in some tests designed to study fear may be a reflection of the emotionality of the rat strains used, since differences have been found in behavioural measures of anxiety, even in rats of the same strain from different sources (File & Velluci,1979).

It thus seems possible that noradrenergic neurones originating in the L.C. are involved in the drug effects described in these experiments. The amygdala may also be involved in some of these effects, since this area has been implicated in the anxiolytic effect of diazepam (Nagy et al., 1979; Umemoto & Olds,1975): and isoprenaline may also produce 'fear' by stimulation of amygdaloid neurones. A neuronal pathway may thus be postulated, involving the noradrenergic innervation of the amygdala from the L.C. (Jones & Moore,1977), stimulation of which results in an increase in 'fear-related behaviour', probably via subsequent pathways from the amygdala to the cortex. Direct stimulation of these latter neurones would have

a similar behavioural effect, which would not be antagonised by decreased activity in the noradrenergic pathway. The lack of antagonism of the effect of isoprenaline by diazepam may thus be explained. Further experiments to investigate the type of β-adrenoceptor involved and the role of α_1-adrenoceptors would help to clarify the situation. However, specific lesioning experiments are also necessary to establish the role of both NA and the L.C. in 'fear-related behaviour'.

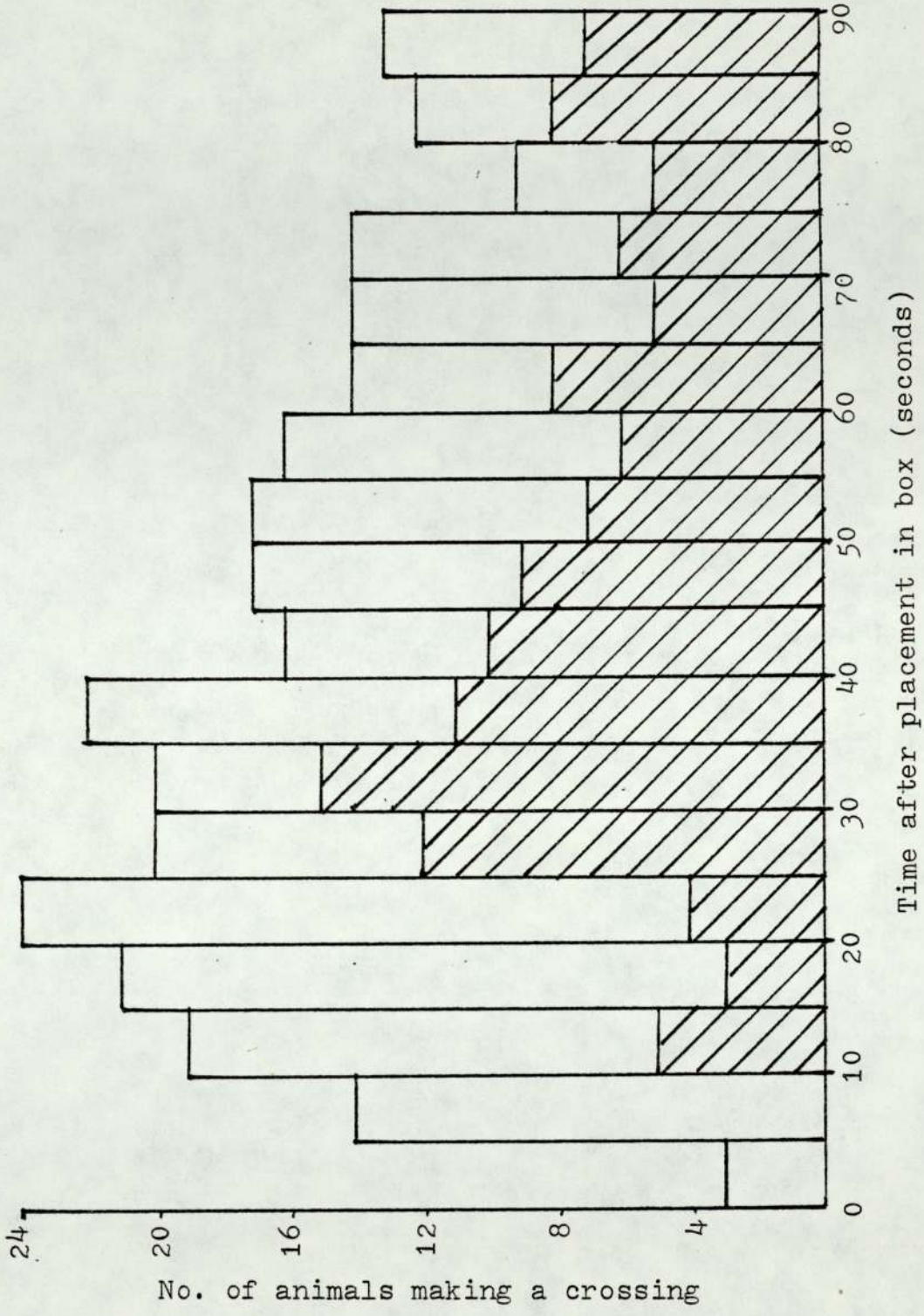


Fig 9.1. Variation in plate-crossing over a 90 second period.

Data taken from 20 saline-treated (blank columns) and 20 yohimbine-treated (hatched columns) animals injected 30 minutes before placement in the box.

DRUG AND DOSE mg/kg s.c.	Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 60 sec	Number of crossings in 90 sec
YOHIMBINE				
0	13.0	40.2	7.66	11.9
0.5	18.4	42.0	7.0	11.2
1.0	19.6	49.7	6.8	10.3
2.5	20.8	57.5 *	5.4 *	8.8 *
5.0	31.0 **	68.0 **	3.6 **	6.2 **
PIPEROXANE				
0	10.58	35.3	9.4	14.08
0.5	11.6 *	36.8	9.0	13.4
1.0	9.6	34.4	7.6	10.6 *
2.5	20.4	49.2	6.0	11.0
5.0	16.4	43.2	7.4	10.4 *
PRAZOSIN				
0	10.38	30.1	9.7	14.2
0.25	10.5	31.3	9.5	15.6
0.5	12.0	32.6	9.73	13.3
1.0	11.0	32.8	11.4	15.6
2.5	15.0	36.8	9.6	13.8

Table 9.1. The effect of α-adrenoceptor antagonists on plate-crossing in a 90 second period, measured 30 minutes after injection.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from saline controls (Mann Whitney 'U' test).

DRUG AND DOSE	Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 60 sec	Number of crossings in 90 sec
CLONIDINE mg/kg s.c.				
0	10.75	35.3	8,5	12.72
0.025	7.8	35.2	10.0	15.6 *
0.05	6.9 *	28.2	10.4 *	15.3 *
0.1	5.6 *	26.8	9.4	13.6
0.25	12.8	34.6	8.6	12.0
0.5	11.6	44.4	8.4	11.6
METHOXAMINE µg i.c.v.				
0	8.44	32.35	10.77	14.54
1.0	10.26	38.6	9.33	13.26
2.5	20.2 *	49.3	6.28 *	9.2
5.0	17.1	42.7	8.4	11.3
10.0	35.6 ***	54.9 **	5.4 **	6.3 ***

Table 9.2. The effect of α-adrenoceptor agonists on plate-crossing in a 90 second period, measured 30 minutes after injection.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from saline control (Mann Whitney 'U' test).

DRUG AND DOSE mg/kg s.c.	Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 60 sec	Number of crossings in 90 sec
Diazepam				
0	10.46	39.4	7.7	11.6
0.5	9.2	30.6	12.4 **	18.0
1.0	6.2	19.8 ***	14.0 ***	17.4 ***
2.5	39.4	50.4	11.0	15.0
5.0	54.8 **	71.0 **	4.0	6.8
Chlordiaze-poxide 0	8.3	30.0	10.5	14.94
1.0.	9.7	28.8	10.5	15.0
2.0	8.2	25.2	12.8 *	19.0 *
4.0	10.8	32.5	12.0	17.8
Amylobarb-itone 0	13.0	42.2	7.5	11.08
7.5	11.0	39.0	8.0	11.6
15.0	9.8	31.2	9.8	15.4 *
30.0	15.0	31.8 *	11.2	17.1 ***
60.0	23.8 *	45.2	8.8	15.0
Morphine				
0	10.16	34.6	8.72	12.55
0.5	10.4	35.0	10.0	14.0
1.0	7.2	26.9	10.9 *	14.5 *
2.0	9.6	32.4	9.1	13.1
4.0	12.2	39.2	6.8 *	8.6 **

Table 9.3. The effect of drugs which have anxiolytic activity in man on plate-crossing in a novel environment in mice.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from saline-treated controls (Mann Whitney 'U' test).

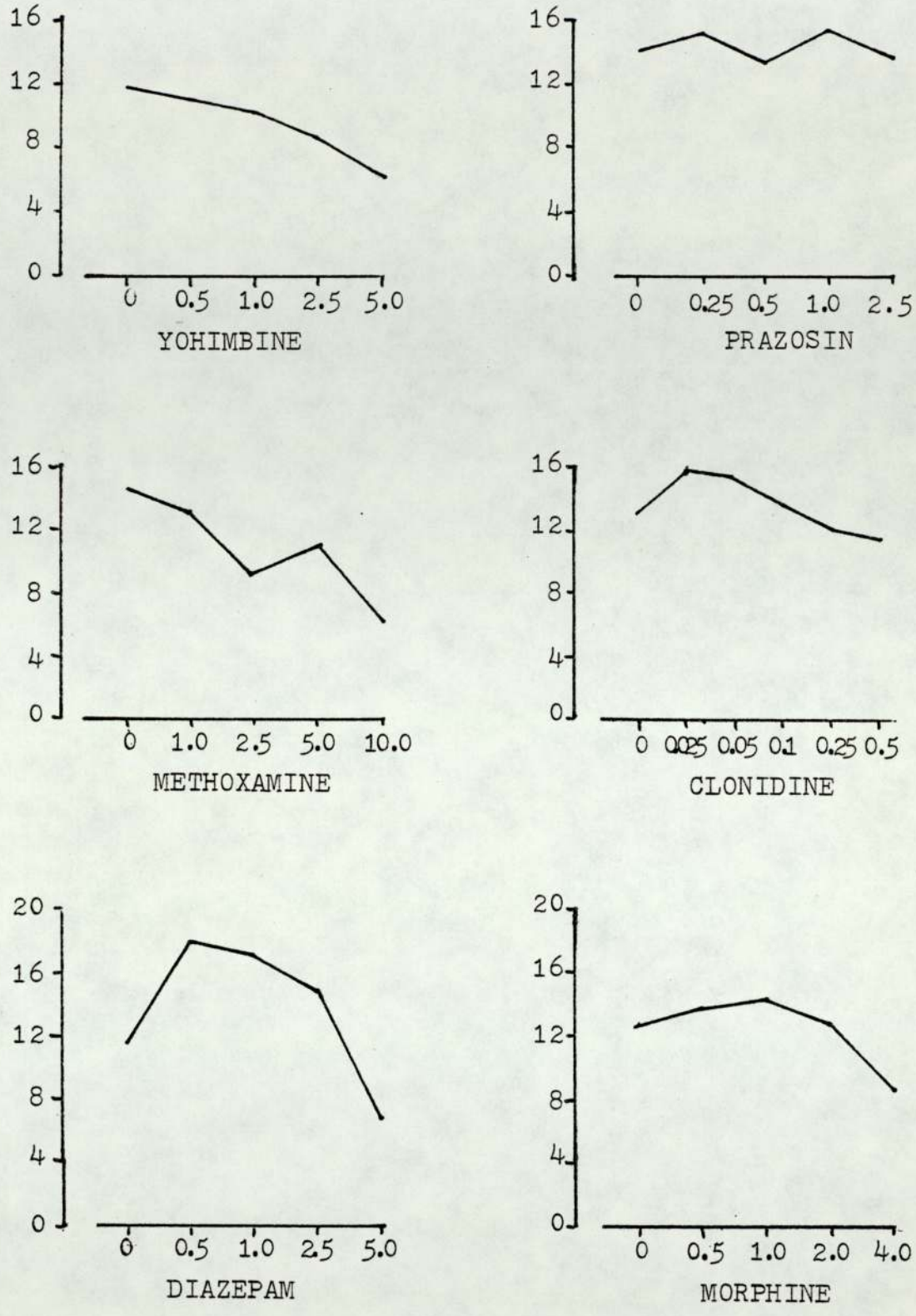


Fig 9.2. The effect of different types of drugs on the number of plate-crossings in 90 seconds. Ordinate = no. of crossings, abscissa = dose of drug in mg/kg s.c. except methoxamine - dose in µg i.c.v.

Animals were placed in the box 30 minutes after injection.

DRUG AND DOSE	Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 60 sec	Number of crossings in 90 sec
Saline	10.79	39.41	8.09	12.27
Yohimbine 5 mg/kg	27.4	75.4	2.0	4.8
Practolol 1 mg/kg	5.8	29.8	10.8	15.8
Yohimbine + Practolol	35.16	78.5	2.66	3.83
Saline	10.79	39.41	8.09	12.27
Yohimbine 5 mg/kg	24.6	74.6	3.4	5.8
Phentolamine 1 mg/kg	12.8	36.4	9.4	13.0
Yohimbine + Phentolamine	44.83	79.16	2.6	4.0

Table 9.4. The effect of practolol and of phentolamine on plate-crossing in a novel environment and its inhibition by yohimbine.

Animals were treated with either phentolamine or practolol 15 minutes before yohimbine or saline and were placed in the box 30 minutes later.

Saline	8.13	29.43	9.13	11.91
Isoprenaline 5 μg	12.0	42.40	7.25	11.5
10 μg	18.3	51.6 *	6.90	10.6
20 μg	16.3 *	54.6 *	6.06 *	8.2
40 μg	28.4 **	60.4 **	4.8 **	8.4

The effect of isoprenaline injected i.c.v. on plate-crossing.

Animals were placed in the box 30 minutes after injection.

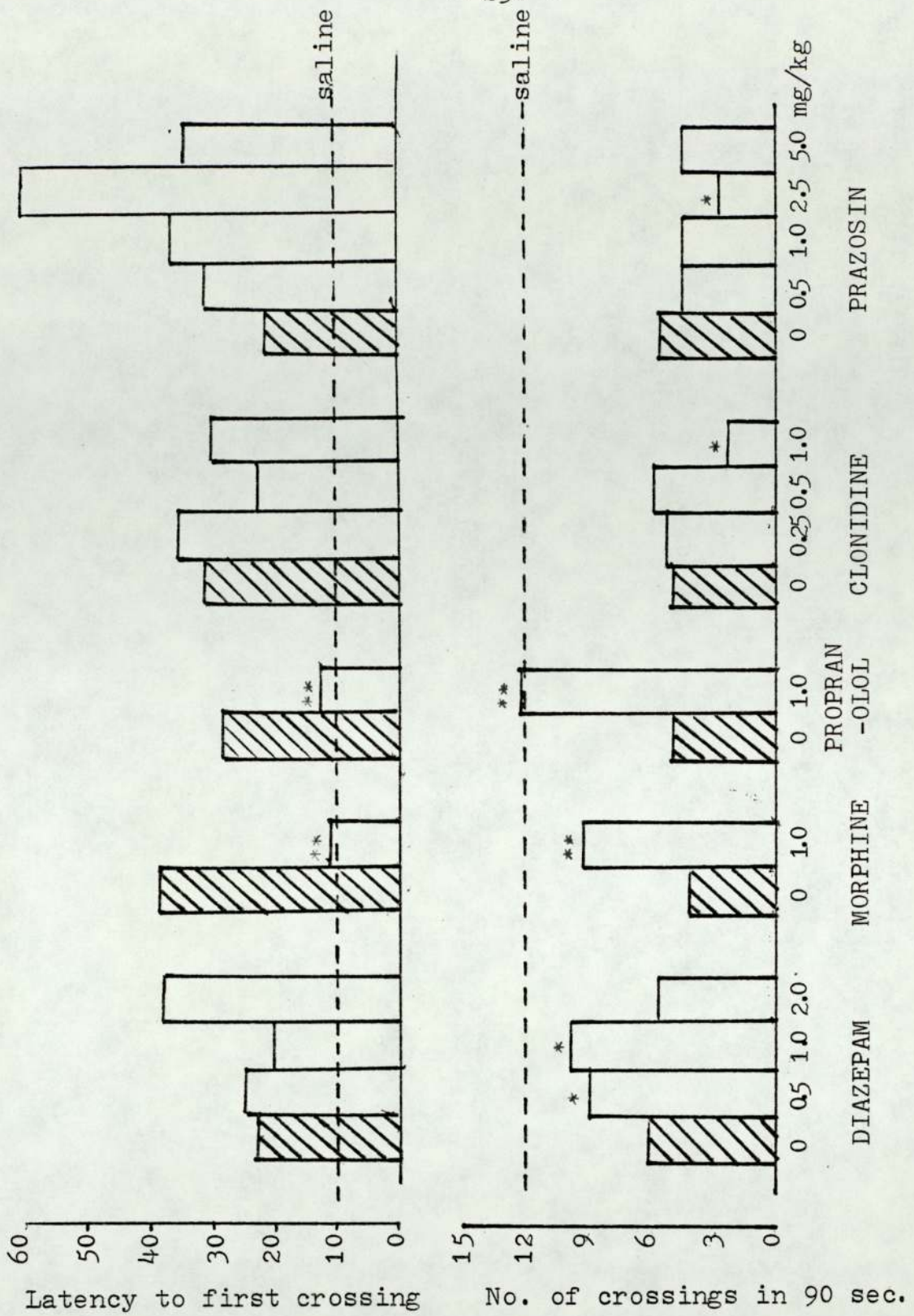


Fig 9.3. The effect of various drugs on the decrease in plate-crossing produced by yohimbine 5.0 mg/kg s.c.

All drugs were administered s.c. 15 minutes before yohimbine and animals were placed in the box 30 minutes later.

* $p < 0.05$; ** $p < 0.01$ difference from yohimbine + saline (Mann Whitney 'U' test).

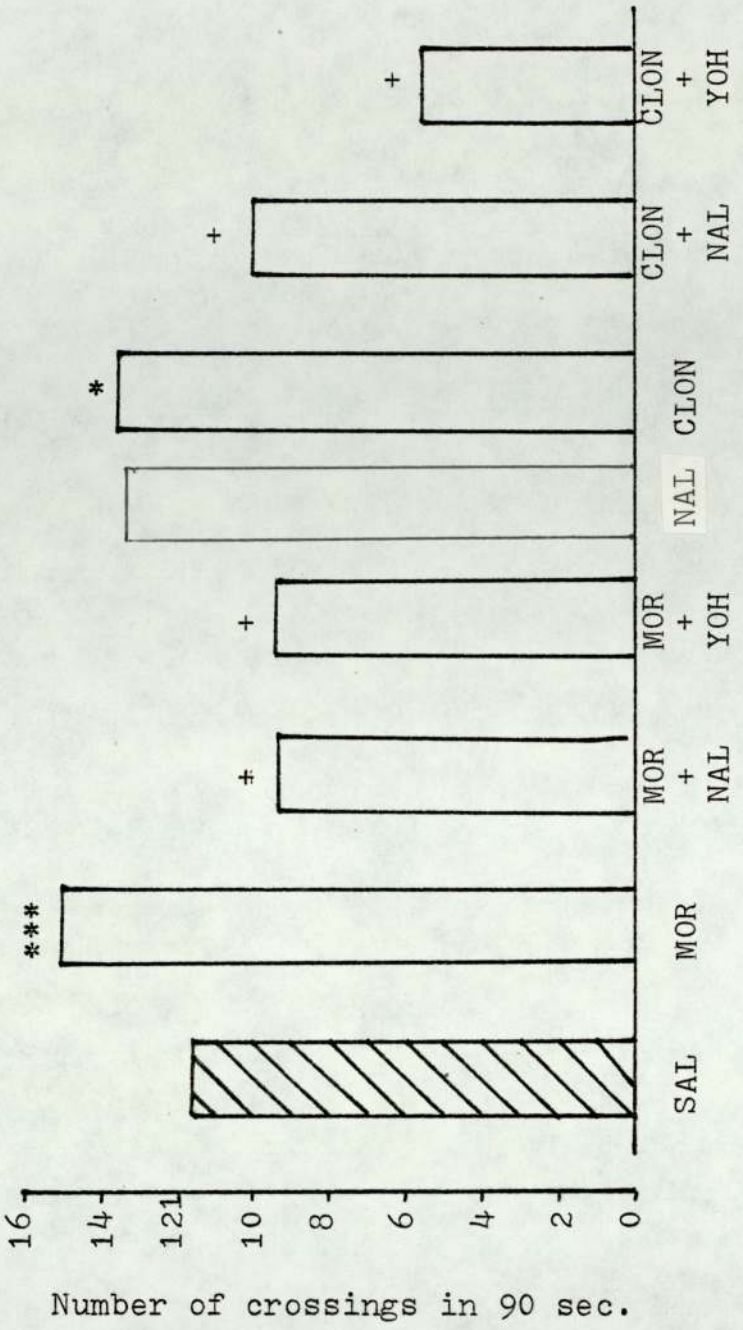


Fig 9.4. The effect of naloxone and yohimbine on the increase in plate-crossing produced by morphine and clonidine.

SAL - saline; MOR - morphine 1.0 mg/kg; NAL - naloxone 1.0 mg/kg; YOH - yohimbine 5.0 mg/kg; CLON - clonidine 0.05 mg/kg.

* $p < 0.05$; *** $p < 0.001$ difference from saline:

+ $p < 0.01$ difference from agonist alone (Mann Whitney 'U' test).

The antagonist was injected 15 minutes before the agonist and animals were placed in the box 30 minutes later.

DRUG AND DOSE	Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 60 sec	Number of crossings in 90 sec
Saline s.c. + Isoprenaline 20 µg i.c.v.	28.65	51.4	7.45	10.2
Propranolol 1 mg/kg s.c. + Isoprenaline 20 µg i.c.v.	12.65 *	37.6	9.15 *	12.65 *
Diazepam 1 mg/kg s.c. + Isoprenaline 20 µg i.c.v.	39.8	55.0	5.2	6.7
Yohimbine 1 mg/kg s.c. + Isoprenaline 20 µg i.c.v.	31.8	77.6 *	3 .0	4.2

Table 9.5. The effect of propranolol, diazepam and yohimbine on the decrease in plate-crossing in a novel environment produced by isoprenaline 20 µg administered i.c.v.

Animals were given saline or one of the drugs studied 15 minutes before isoprenaline and were placed in the box 30 minutes later.

* $p < 0.05$ difference from animals treated with saline s.c. + isoprenaline 20 ug i.c.v. (Mann Whitney 'U' test).

ACTIVATION SCORES (each score is the mean of 3 animals)

DRUG AND DOSE	Ptosis 0 - 4	Body position 0 - 4	Activity 0 - 8	Startle response 0 - 4	Transfer arousal 0 - 8	Visual placing 0 - 4	Muscle tone 0 - 4	TOTAL Max. 36
Saline	3.83	2.25	1.33	2.0	5.0	2.0	2.0	18.41
Yohimbine 2.5 mg/kg	3.66	2.08	0.5	3.0	4.66	2.0	3.66	19.56
Yohimbine 5,0 mg/kg	3.5	1.75	0.5	3.0	4.0	2.0	2.66	17.41
Clonidine 0.25 mg/kg	4.0	1.92	0.0	1.66	2.66	1.66	2.66	14.56
Clonidine 0.5 mg/kg	4.0	2.0	0.33	1.66	2.33	1.66	1.33	13.31
Diazepam 1.0 mg/kg	3.83	2.08	1.5	2.0	5.66	2.0	2.33	19.40
Diazepam 2.5 mg/kg	3.83	2.0	1.0	1.66	5.33	1.33	1.0	16.15
Diazepam 5.0 mg/kg	2.0	1.75	0.0	0.66	3.33	0.83	1.0	9.57
Amylobarbitone 30.0 mg/kg	4.0	2.25	3.0	2.33	6.33	1.66	2.0	22.57
Amylobarbitone 60.0 mg/kg	4.0	2.0	3.33	2.33	5.66	1.66	1.66	20.66

Table 9.6. Activation scores for drugs which affect plate-crossing in mice.
Animals were observed 30 minutes after injection.

GENERAL DISCUSSION

Peripheral in vitro experiments with a range of α-adrenoceptor agonists and antagonists showed that many of these drugs possessed selective actions at either pre- (α_2) or post-synaptic (α_1) receptors. The results support the existence of two different types of α receptor, which differ in their affinity for drugs. It has been shown (Farnebo & Malmfors,1971; Starke et al.,1974) that α_2 receptor agonists produce their inhibitory effects by reducing the amount of NA released on stimulation. These drugs were also found to have a similar effect on central neurones, while α_2 antagonists increased NA release, suggesting that α_2 receptors are also present in the CNS.

Radioactive ligand binding studies have also demonstrated that central neurones possess receptors which pharmacologically resemble peripheral α_2 receptors, in addition to those resembling α_1 and β receptors (Tanaka & Starke,1980; U'Pritchard & Snyder,1979). Behavioural studies in mice were undertaken in order to study whether drugs found to show selectivity peripherally between α_1 and α_2 receptors may have different behavioural effects; and to determine whether certain behaviours could be ascribed to α_1 or α_2 receptors. In addition, it was hoped that the behaviours studied could be used to assess drug potency at the two types of receptor and selectivity between the receptors and possibly provide methods of screening for novel compounds at central α receptors.

In general, agonists which had been found to be selective peripherally for α_1 or α_2 receptors had opposite effects on observed behaviour. Animals trested with clonidine, in most of the doses studied, appeared sedated. They were less responsive to external stimuli than control mice, were inactive and passive. Similar behaviour was produced by other selective α_2 agonists: guanfacin and guanabenz. Methoxamine, on the other hand, which had been found to selectively stimulate α_1 receptors

peripherally, produced a syndrome involving hyperalertness, hyperreactivity to external stimuli and hyperalgesia. A very high dose of clonidine (5 mg/kg) produced similar behavioural effects, which may have been due to α_1 receptor stimulation. Although clonidine was found to be a selective agonist of α_2 receptors, it was also a potent α_1 agonist peripherally.

In order to assess the possibility of a particular behavioural effect being due to the stimulation of either α_1 or α_2 receptors, criteria were set up as follows:

(i) the range of α agonists studied should show similar relative potencies to those found peripherally for either the α_1 or the α_2 receptor, although differences in the ability of the drugs to penetrate the CNS may affect this;

(i) antagonists selective for the proposed type of receptor should prevent or reverse the effect of a suitable agonist, while antagonists selective for the other type of receptor should be less effective or potentiate the effect of the agonist;

(iii) effects which may be produced by antagonists when given alone should be compatible with the proposed receptor involvement.

Using these criteria, experiments showed that sedation as assessed by potentiation of chloral hydrate sleep or reduced ability to remain on an accelerating Rotarod was probably due to α_2 receptor stimulation, although certain effects were not entirely compatible with this suggestion. For example, yohimbine was sedative in both tests and was unable to reduce the effect of agonists on sleeping time. Clonidine also inhibited motor activity, the pinna reflex, 5-HT-induced head twitches and haloperidol-induced catalepsy and produced analgesia and an anxiolytic-like effect. All of these effects were found to involve α_2 receptor activation. Since α_2 receptors are also involved in producing sedation, there is a possibility

that some of these depressant effects could be consequential to sedation. However, the very low doses of clonidine which were able to inhibit 5-HT and motor activity, relative to those causing sedation, suggests that these may be more specific effects. In addition, low doses produced an increase in plate-crossing in a novel environment and inhibited catalepsy, a behaviour which sedation would be expected to potentiate.

Methoxamine-induced potentiation of haloperidol catalepsy and apomorphine-induced activity in reserpinised mice were found to be due to stimulation of α_1 receptors. High doses of methoxamine were able to inhibit the pinna reflex and 5-HT-induced head twitches, thus despite the high peripheral selectivity for α_1 receptors, this drug was able to stimulate α_2 receptors centrally at high doses. Methoxamine also inhibited motor activity, although experiments were not undertaken to determine whether this was due to α_2 receptor activation. It seems possible that α_1 receptors may be involved, however, since methoxamine also reduced plate-crossing activity, which was the opposite effect to that seen with clonidine.

In general, selective antagonists, when given alone, produced opposite behavioural effects from their appropriate agonists. Thus α_2 antagonists, yohimbine and piperoxane, produced hyperalertness, while prazosin, an α_1 antagonist, was sedative. Yohimbine, in addition, appeared to induce 'fear-like behaviour' on observation, which was interpreted as a possible basis for the reduction in plate-crossing which this drug produced. This may also have contributed to the inhibition of motor activity also seen after yohimbine, since it did not produce any apparent signs of sedation. Thus, despite the fact that this was not the opposite effect to clonidine, it may have a different basis.

Prazosin alone produced a syndrome similar to

that seen with clonidine, although it did not produce analgesia or inhibit the pinna reflex and had a less marked effect on plate-crossing. Thus it appears that, in these behaviours, the effects of α_2 agonists are not mirrorred by α_1 antagonists, as would be expected if similar synaptic arrangements to those generally found at α-adrenoceptor synapses peripherally were involved.

In the CNS, because of the complexity of inter-neuronal connections, such 'classical' synapses cannot be assumed to mediate all noradrenergic effects. However, the results do suggest that regulatory α_2 receptors facing effector α_1 receptors may be involved in some behavioural effects seen here. In motor activity experiments, for example, α_2 receptor stimulation inhibited activity, while α_1 stimulation, under certain conditions, increased it. Similarly with the state of activation as assessed by observation, drugs which inhibited noradrenergic function, such as α_2 agonists and α_1 antagonists, reduced the activation score, while α_2 antagonists and α_1 agonists increased it. Both α_1 and α_2 receptors were also found to be important in the noradrenergic modulation of dopaminergic and serotonergic neurones. Agonists of α_1 and α_2 receptors had opposing effects on haloperidol-induced catalepsy, while α_2 antagonists produced similar potentiating effects to α_1 agonists and α_1 antagonists were inhibitory. A similar situation was seen with the 5-HT-induced head twitch, although the potentiating effect of methoxamine only occurred in the presence of an α_2 receptor antagonist.

In situations where α_1 receptors did not appear to be involved, two possibilities exist. Firstly, the response to released NA may be mediated postsynaptically by β receptors. This type of synapse may be involved in plate-crossing, since propranolol was able to reverse the inhibitory effect of

yohimbine, while prazosin was not. A second possibility is that the inhibitory α_2 receptor may itself be located postsynaptically, thus no α_1 receptor need be involved. This appears to be the case in clonidine-induced inhibition of pinna reflex, since neither α_1 nor β blockers produced any effect.

In accordance with the aims of the project, certain behavioural effects were selected, which could be used to study drug potency at α_1 and α_2 receptors separately, in order to assess whether drug selectivity was retained *in vivo*. Effects which do not appear to involve an α_1 receptor are particularly suitable for assessing the potency of drugs at α_2 receptors. Inhibition of the pinna reflex involves such a situation, thus the potency of α agonists in producing such inhibition should be equivalent to their potency as α_2 receptor agonists. The potency order for pinna reflex inhibition was:

clonidine > guanfacin = guanabenz > NA = oxymetazoline > methoxamine

with potency ratios:

1 : 4 : 4 : 6 : 6 : 34.

Guanabenz and oxymetazoline were not studied in *in vitro* experiments. Doxey (1979) has, however, investigated oxymetazoline and found it to be equipotent with clonidine in inhibiting the twitch response of the rat vas deferens under similar conditions. Thus the order of potency of α agonists in stimulating peripheral α_2 receptors is

clonidine = oxymetazoline > guanfacin > NA > methoxamine

with potency ratios:

1 : 1 : 2.4 : 1940 : 2315.

The potency of oxymetazoline in stimulating α_2 receptors *in vitro* was found by Doxey to be reduced *in vivo*, where selectivity for α_1 receptors was seen. This may also be the case here, since oxymetazoline has been shown to produce

the behavioural effects of α_1 agonists and such selectivity would render the drug less effective as as α_2 agonist, relative to clonidine. Ability to penetrate the brain will also be of importance in determining the relative potencies of drugs *in vivo*. Guanabenz and guanfacin were both less potent as α_2 agonists *in vivo* than clonidine, despite reported higher selectivity for these receptors. Other workers have shown that both drugs are 10 times less potent than clonidine as antihypertensives (Scholtysik et al.,1975; Barber & Reid,1978). Guanfacin was also shown (Barber & Reid,1978) to have a slower onset and longer duration of action than clonidine. This was seen in the pinna reflex studies and may in part be due to a delay in reaching the CNS. Jarrot et al.(1979) showed that neither guanfacin nor guanabenz had lower affinity for central α receptors than clonidine, thus the lower potency in inhibiting the pinna reflex may reflect a poorer ability to pass the blood/brain barrier and/or lower intrinsic activity.

These drugs have also been reported to be less sedative than clonidine (Scholtysik et al.,1975; Baum et al., 1970). This was also seen in the present study, although both guanfacin and guanabenz were only 4.5 times less potent than clonidine in potentiating chloral hydr te sleeping time. Baum et al.(1970) found guanabenz to be 10 times less sedative, while guanfacin was reported to be 100 times less sedative than clonidine in dogs (Scholtysik et al.,1975).

Antagonists of α_2 receptors were studied *in vivo* by their ability to reverse the effects of α_2 agonists on the pinna reflex. The potency order against clonidine was found to be:

piperoxane > yohimbine >> prazosin

with potency ratios of:

1 : 4.25 : 71.

This compares favourably with the peripheral potency of the

antagonists in reversing the inhibitory effect of cocaine on the twitch response of the rat vas deferens. The same potency order was found here, with potency ratios of:

1 : 3.1 : 1200.

However, it should be noted that piperoxane was 10 times less potent against clonidine than against cocaine *in vitro*, thus the potency order may differ; and also, yohimbine was more potent than piperoxane in reversing the effect of guanfacin, oxymetazoline and methoxamine on the pinna reflex.

It appears that, in general, drugs display similar relative potencies *in vivo* to those found *in vitro*. However, in general, drugs which were found to have little effect on the rat vas deferens i.e. were selective for α_1 receptors, have a higher relative potency in their effects on the pinna reflex, with the exception of oxymetazoline, discussed above. Thus NA, methoxamine and prazosin were much more potent *in vivo*, relative to other drugs, than *in vitro*. The order of magnitude for this difference is not constant, thus receptor accessibility is unlikely to be a controlling factor in the poor ability of these drugs to act on peripheral α_2 receptors. It is also possible that differences in peripheral and central α_2 receptors may be important here. Methoxamine has also been shown to have α_2 effects in other behavioural tests and an α_2 stimulant effect has also been seen in rats (Drew et al.,1979). Thus a lack of α_2 activity *in vitro* may not be reflected *in vivo*, while the reverse i.e. potent *in vitro* effects not seen *in vivo*, has also been shown to occur (Doxey,1979).

Similar comparison of drug effects on central and peripheral α_1 receptors was necessarily less accurate, since time only permitted sufficient experimentation to determine the suitability of a test situation for studying α_1 receptors, and did not allow for studies on several dosages. The potentiation

of apomorphine-induced activity in reserpinised mice is particularly suitable, since actions at presynaptic α_2 receptors are unable to affect the results, as depletion of transmitter renders such actions ineffectual. Since full data involving several doses of each agonist was not obtained, relative potency cannot be assessed with any high degree of accuracy. However, from the data which was obtained, a tentative potency order may be derived as follows:

clonidine $\geq$ oxymetazoline > guanabenz > methoxamine.

In vitro studies show relative potency as agonists in the isolated rat anococcygeus muscle to decline in the order:

oxymetazoline > clonidine >> methoxamine.

The relative decrease in potency of oxymetazoline in vivo is surprising in view of its higher selectivity for α_1 receptors in vivo, mentioned above. However, the use of data from only one dose is not sufficient on which to base suggestions relating to relative in vivo potencies.

A comparable potency order for α_1 receptor antagonism can not be made using this behavioural effect, since yohimbine was found to affect apomorphine-induced activity, as well as its potentiation by clonidine. A similar effect was found using phentolamine by Anden and Strombom (1974), thus this may be an unsuitable method for studying α_1 antagonists. Possibly blockade of methoxamine potentiation of haloperidol catalepsy may be used. Again, in the present study, only one dose of each drug was used and the poor ability of phentolamine to penetrate the CNS renders comparisons difficult. However, it would appear that a similar potency order to that seen peripherally in vitro may be deduced, namely:

prasosin > phentolamine > yohimbine.

When the above data is studied in terms of ratios of α_1 and α_2 receptor activity, comparison to in vitro studies

becomes even more tentative. However, the ability of drugs to penetrate the CNS should not affect this perameter, since it is derived from studies employing the same methods of drug administration. For α agonists, selectivity ratios are similar in direction to those seen *in vitro*, although the magnitude changes considerably. It is important to remember, however, that only one dose was used in the determination of α_1 receptor activation.

DRUG	ED_{50} for pinna reflex inhibition	Dose potentiating motor activity	Ratio α_2/α_1	Ratio *in vitro*
Clonidine	0.275 mg/kg	1.0 mg/kg	3.63	18.8
Guanabenz	1.12 mg/kg	5.0 mg/kg	4.54	97.0+
Oxymetazoline	20.0 mg/kg	2.5 mg/kg	0.12	4.0*
Methoxamine	15μg i.c.v.	10μg i.c.v.	0.66	0.15

+ from Doxey & Hersom,(1980) using rat anococcygeus muscle *in vitro*

* from Doxey (1979). Data from peripheral in vivo studies however, gave a ratio for α_2/α_1 receptors as 1.0.

Table 10.1.

Relative potencies of α agonists for α_1 and α_2 receptors *in vitro* and *in vivo*.

The ratios suggest that the α agonists retain their selectivity for α_1 or α_2 receptors found to occur peripherally in tests involving central α receptors *in vivo*. Thus clonidine and guanabenz were selective for α_2 receptors both peripherally and centrally, guanabenz being slightly more selective, although less potent than clonidine. Oxymetazoline was found to be selective for α_1 receptors *in vivo*, although displaying marked α_2 activity peripherally *in vitro*, while methoxamine, although retaining α_1 receptor selectivity centrally, was also found to stimulte α_2 receptors.

Similar tentative comparisons may be made for α antagonists, using reversal of clonidine-induced pinna reflex inhibition for α_2 activity and either inhibition of clonidine potentiation of apomorphine-induced activity in reserpinised mice or inhibition of methoxamine potentiation of haloperidol catalepsy for α_1 activity.

DRUG	ID_{50} reversal of clonidine-inhibited pinna reflex	Dose inhibiting methoxamine potentiated catalepsy	Ratio α_2/α_1	Ratio <u>in vitro</u>
Yohimbine	0.85 mg/kg	2.5 mg/kg	3.0	112.0
Piperoxane	0.20 mg/kg	5.0 mg/kg*	25.0	94.0
Prazosin	14.20 mg/kg	1.0 mg/kg	0.07	0.0005

* dose inhibiting clonidine potentiation of activity.

<u>Table 10.2.</u>

<u>Relative potencies of α antagonists for α_1 and α_2 receptors in vitro and in vivo.</u>

Again the ratios suggest that selectivity is retained for central α_1 or α_2 receptors, although the magnitude differs considerably. It thus appears that α receptors present in the brain may be similar in their pharmacological characteristics to those found in the peripheral nervous system. Results obtained using ligand binding suggest that ^{3}H-clonidine binds to $\alpha_2$type receptors; and agonists have similar affinities for this site to their relative potencies on α_2 receptors in the vas deferens (Kapur et al.,1979). Similarly, drug potency at the α_1 receptor in the vas deferens correlates with their affinity for ^{3}H-WB4101 binding sites in the brain (ibid).

The similarity in potency order of agonists and antagonists in several tests involving central neurones, namely

release of ^{3}H-NA from brain slices, sedation, analgesia and effects on the pinna reflex additionally suggest that the α_2 receptors involved in all these effects are pharmacologically similar. Relative potencies of α agonists in these tests are shown in the following table. Comparable results for α antagonists are not shown, as the potency ratios are largely uninformative, due to the poor ability of the antagonists to reverse the analgesic and sedative effects of clonidine.

DRUG	Sedation (sleeping time)	Pinna reflex inhibition	Analgesia	Inhibition of NA release
Clonidine	1.0	1.0	1.0	1.0
Guanabenz	4.5	4.0	5.2	-
Guanfacin	4.5	4.0	5.7	1.0
Noradrenaline	-	36.3	10.0	-
Oxymetazoline	42.5	36.3	-	-
Methoxamine	500.0	90.0	100.0	50.0

Potencies of α agonists, relative to clonidine, in several tests involving central α-adrenoceptors.

Of the behavioural effects involving α receptors which have been studied, pinna reflex inhibition and its reversal are the most suitable means of testing drug potency at central α_2 receptors. Measures of activity, sedation and analgesia were less suitable, since α_1 receptors could also be affected. Inhibition of the 5-HT head twitch or haloperidol catalepsy could also be used to measure α_2 agonist potency. However, the involvement of a second drug and the sensitivity

of the methods to environmental factors render them less suitable than pinna reflex inhibition. Potentiation of apomorphine-induced activity in reserpinised animals may be used as a measure of α_1 receptor activation, although the large number of drugs neceasary may lead to difficulties in interpretation. The method is less suitable for studying α_1 receptor blockade, since it was found that yohimbine inhibited activity induced by apomorphine alone, as does phentolamine (Anden & Strombom,1974). Elucidation of the mechanism of this effect may enable modification to improve the usefulness of the model for α_1 antagonists.

None of the methods used to study sedation, including motor activity, were found to be entirely suitable for the investigation of sedative side effects of α_2 agonists. Chloral hydrate- induced sleep was found to be extremely variable, despite rigid control of possible interfering factors. However, provided suitable controls were included, and the higher dose of the hypnotic used, the method could be useful. The accelerating Rotarod is perhaps less suitable, since many effects could contribute.to an inability to remain on the rod, although in this and other studies (Drew et al.,1979), the method has produced orders of potency which correspond to those seen using other methods. Motor activity was particularly unsuitable, since all drugs tested markedly inhibited activity, with no differences in relative potency, despite apparent increases in alertness after certain drugs. The observational method studied also has disadvantages in its lack of objectivity and occasional increases in certain perameters in otherwise apparently sedated animals.

The effects of α agonists and antagonists on alertness and activity are particularly interesting, since the two may be related and first impressions may suggest that an increase or decrease in one would be concomitant with a similar

effect in the other. However, the relationship between alertness and motor activity does not appear to be straightforward and may involve complex interactions in the relationship of the animal to its environment.

Alertness is related to the level of arousal, which is a measure of the state of consciousness of an animal. However, consciousness is implicit in alertness, since this involves the degree of attention to sensory input from the environment. The drugs studied were able to alter the degree of alertness, which was measured by activation scores. Clonidine and prazosin reduced alertness; animals appeared drowsy and showed decreased reactivity to external stimuli. Methoxamine, piperoxane and yohimbine, on the other hand, appeared to increase alertness and lead to increased reactivity. Other workers have shown using EEG measurements that clonidine increases drowsiness (Kleinlogel et al.,1975), while piperoxane increase wakefulness (Fuxe et al.,1974).

The state of alertness may be expected to be directly reflected in measurements of activity. However, this was not found to be the case. Clonidine did indeed reduce activity, both in a familiar and a novel cage, which may be related to the degree of alertness of the animals. Very low doses, however, which did not appear to reduce alertness on observation of the animals, were able to depress motor activity. In contrast, these low doses increased plate-crossing in a novel environment. Differences in construction of the plate-crossing box, the fact that animals were placed in this box alone and the large difference in time scale may,in part, account for these apparent discrepancies.

One interpretation of the increase in plate-crossing is that low doses of clonidine reduced 'fear' of the novel environment without decreasing alertness. Previous work

(Marriott & Spencer,1965) has shown that on repeated exposure to an initially novel environment, exploratory activity is reduced and anxiolytics are no longer effective in increasing activity. Thus it is possible that clonidine may reduce 'fear' of the environment and allow high initial exploration lasting only several minutes, after which activity is depressed. Higher doses of clonidine, which did reduce alertness on observation, were found to have no potentiating effect on plate-crossing, but produced a marked and sustained inhibition of activity measured using an activity meter. This would thus be compatible with a reduction in alertness leading to a decrease in attention to the environment.

Drugs which were found to increase alertness on observation did not produce an increase in activity in either a familiar or a novel environment; and, in fact, reduced plate-crossing activity. This could be interpreted as opposite to the effect of clonidine i.e. an increase in 'fear'. It can be visualised that increasesin alertness could lead to heightened awareness of situations known to the animal to be potentially dangerous, such as the presence of an experimenter or an unknown environment. This in turn could lead to 'fear' of such situations and the behaviour seen in animals treated with these drugs was compatible with such a hypothesis.

The drugs which did increase alertness, particularly yohimbine, appeared to increase the reactivity of animals to situations not normally seen to produce such reactions, for example, the movements of other mice or slight noises. Indeed, yohimbine-treated animals often startled without any apparent cause. This may be related to the 5-HT stimulant effect of yohimbine (Sanghvi & Gershon,1974), since 5-HT is thought to be important in the production of hallucinations (Aghajanian,1977). It seems possible that 'fear' is only a

consequence of increased alertness when external stimuli are present which allow its induction. Thus, in the absence of such stimuli, drugs such as yohimbine may merely increase alertness. However, this is very difficult to assess without the facility of closed-circuit television, since even the presence of an experimenter seems likely to be 'fear-inducing'.

Yohimbine was found to interact with several other classes of drugs in its effects on plate-crossing, namely morphine, diazepam and propranolol. This suggests that, to produce its effect, yohimbine either acts on a noradrenergic system which may be modulated by other systems; or one of the other systems is of importance in plate-crossing activity and yohimbine modulates this, via α_2 receptors. It seems likely that the plate-crossing activity is related to anxiety or stress, since anxiolytics have such marked effects. Much work has accumulated which suggests that stress increases the turnover of NA and that, under conditions of severe stress, production cannot keep up with the increased utilisation (Aniseman,1978). While severe stress also increases DA and 5-HT turnover and produces a rise in ACh levels (ibid), these transmitters are not so markedly altered by stress as is NA. Endogenous opiates may also be increased after stress (Guillemin et al.,1977), although whether yohimbine may act by such a mechanism is,as yet, unknown. It thus seems likely that NA is involved in the effect of yohimbine on activity in a novel environment. Although work has shown that the increase in NA turnover after stress involves the L.C. (Korf et al.,1973), there is much controversy over the role of the L.C. in anxiety and fear (Mason & Fibiger,1979a; Redmond & Huang,1979).

Differences in species, strain and the method of assessing anxiety may be largely responsible for discrepancies between the effects of drugs, L.C. stimulation and L.C.

lesions on anxiety. Strain is important in the effect of stress on NA turnover, as is the type of stress, whether inescapable or not (Aniseman,1978), while differences in breeding are well known to affect emotionality (Broadhurst,1959; File & Velluci, 1979). Effects on complex behaviours, such as a social interaction test (Crow et al.,1978) or conditioned responding (Mason & Fibiger,1979c) may be difficult to interpret, while unexpected differences may be apparent even in simple tests. Thus, for example, plate-crossing and head-dipping in a hole board may seem comparable. However, to cross a gap, an animal must overcome its 'fear' of the unknown area beneath, while to repeatedly look over into a gap or a hole may reflect an increase in 'curiosity'; and, since a hole can be avoided while exploring, a hole-board may not potentiate the 'fear' drive as much as a plate-crossing box,

Although no method can categorically be said to measure the amount of 'fear' experienced by an animal, the fact that the method is sensitive to drugs known to be anxiolytic and anxiety-producing in man, suggests that measurement of 'fear' or anxiety is being achieved. In some of the models used to study the effects of L.C. lesions on anxiety, anxiolytic drugs have been found to be relatively ineffective or have not been studied. The model used here is sensitive to anxiolytic drugs and to yohimbine, which is reported to produce anxiety in man (Holmberg & Gershon,1961). It may thus prove useful for further investigation of noradrenergic involvement in 'fear-related behaviour'. Despite controversy over the role of the L.C. in anxiety and 'fear', it appears from the studies presented here that noradrenergic stimulation may, in the mouse, produce or potentiate 'fear', while reduction in noradrenergic activity leads to passivity and possibly anxiolytic-like effects. Whether the L.C. may be involved in these effects remains to be elucidated.

This effect of drugs which increase central noradrenergic activity may also be important in their effects on catalepsy induced by a DA receptor blocker or in untreated animals. Drugs found to produce apparent signs of 'fear' potentiated catalepsy, while drugs which showed an anxiolytic-like effect reduced it. However, the similarity in the effects of these drugs on catalepsy and 'fear' may be coincidental. Noradrenergic function does, however, have a marked effect on catalepsy, thus suggesting that NA may modulate the activity of dopaminergic neurones in the mouse. The drugs studied were also found to affect head twitches induced by 5-HT, thus NA may, in addition, modulate serotonergic neurones. An interaction with opiate receptors was also seen in the studies on plate-crossing, although clonidine analgesia does not appear to involve opiates.

Thus drugs acting at α-adrenoceptors were found to have widespread actions in the CNS and were able to modify effects involving other transmitters. Drugs which displayed selectivity for α_1 or α_2 receptors peripherally retained this selectivity for central α_1 or α_2 receptors. By using a wide range of drugs with varied selectivities, actions involving NA could be shown to be due to either α_1 or α_2 effects. Two methods were found which could be used to study α_1 and α_2 effects separately and thus provide a means of assessing drug selectivity *in vivo* in central neurones.

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PRELIMINARY NOTES

THE DEVELOPMENT OF CATALEPSY IN DRUG-FREE MICE ON REPEATED TESTING

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SUMMARY

When subjected to repeated testing for catalepsy, 24% of mice retained the imposed posture for more than 5 seconds at the 8th trial compared with 1.21% at the first. Median posture retention time of the 'trained' 24% was 0 seconds at the first trial, rising to 12 seconds at the 8th. During posture retention, 'trained' animals closely resembled mice treated with haloperidol in showing moderate rigidity with retention of imposed lateral configurations of the tail. Posture retention in 'trained' mice thus deserves to be called catalepsy. Preliminary experiments suggest that it is reduced by atropine and apomorphine but not by naloxone. The significance of the phenomenon is discussed.

Catalepsy, the acceptance and retention of imposed postures which require muscular effort, is seen in animals following a variety of drugs (e.g. Costall & Naylor, 1973). During studies on cataleptogenic drugs in these laboratories, we noticed that apparent 'catalepsy' developed in a proportion of saline-pretreated mice during repeated testing. The experiments reported here represent a preliminary investigation of this effect.

METHODS

Male TO mice (18-25g, 5-8 weeks) were housed in cages of 5 at least 24 h prior to experiment. Posture retention time (PRT) was measured as follows: the mouse was removed from the cage, gripped by the scruff and placed in the 'cataleptic' posture with forepaws on a 4 cm high wooden bar and hind-paws on the laboratory bench. PRT was measured from removal of the scruff grip (within 2 seconds of placement). The bar was arranged so that, during testing, mice faced a blacked-out window, away from experimenter and stop-clock. Great care was taken not to touch the tail during placement since even light tail-pressure potentiates catalepsy in the mouse (Ariyanayagam and Handley, 1975). The posture was terminated at 120 seconds to reduce fatigue effects at subsequent tests. Animals were returned to their home cages between tests.

For the initial investigation of the effect of repeated exposure to the test situation, naive mice were tested for posture retention at 15, 30, 45, 60, 90, 120, 150 and 180 mins after injection of saline (0.9% NaCl 10 ml/kg sc) or handling as if for injection. For subsequent examination of drug effects in 'trained' mice, animals were exposed to the above training schedule and those showing greater than 5 sec PRT at the 8th trial selected and divided into two groups of approximately equal mean PRT; one hour after the 8th trial, one group received saline sc and the other the drug under test. Further trials were then carried out at intervals after this injection. A further group of untrained mice was tested for catalepsy 60 min after haloperidol 0.2 mg/kg i.p. for comparison of the characteristics of the posture retention with those shown by drug-free trained mice.

RESULTS

Table 1 illustrates the development of posture retention on repeated exposure to the test situation. The frequency distribution of PRTs at each testing time failed to meet the symmetry requirements of a normal distribution at a significance probability of 0.05. Distribution-free statistics (Siegel, 1956) have therefore been used to describe the results. There was no significant difference in the development of posture retention between saline-treated and handled animals. From the pooled results, 1.21% of mice showed a PRT in excess of 5 seconds at the first trial compared with 24% on the 8th ($P < .001$, McNemar test for the significance of changes). Median PRT of this 'trained' 24% was

Trial Number:-	1	2	3	4	5	6	7	8
Minutes after injection or handling:-	15	30	45	60	90	120	150	180
	Percentage incidence of Posture retention†							
Saline (n=178)	0.6	4.5	9.6	15.3	15.7	14.6	22.6	23.6
Handling (n=70)	1.4	5.7	10.0	17.1	18.6	21.4	32.9	24.3
	Median Posture retention time of 'trainable' mice (seconds)††							
Saline (n=42)	0	2	3.5	6.5	8	9	11.5	13
Handling (n=17)	0	0	1.5	3	7	7	12	16

Table 1: The development of posture retention on repeated testing. Animals received 0.9% saline 0.01 ml/gm sc or were handled as if for injection.

† *Mice were considered to show posture retention if they remained in the test position for more than 5 seconds.*

†† *Mice showing a posture retention time in excess of 5 seconds at the 8th trial.*

There was no significant difference between saline-treated and handled mice at any trial either in incidence (Chi^2 test) or central tendency (Mann-Whitney U-test, 2-tailed).

0 seconds at the 1st trial and 12 seconds at the 8th. Maximum PRT was 7 seconds at trial 1 but 4 mice showed PRTs in excess of 120 seconds by trial 8. Of those mice failing to reach a PRT in excess of 5 seconds by trial 8, only 4.5% reached this value on any of the previous 7 trials. During posture retention, 'trained' animals closely resembled haloperidol-pretreated mice in showing moderate rigidity with retention of imposed lateral configurations of the tail. It was also noted that both 'trained' and haloperidol-treated animals tended to show retention of a ridge of skin after relaxation of the scruff-grip.

Median Posture Retention times (seconds)							
	Training - trial 8	min after injection 20	50	80	110	140	180
Saline	6.5	7	10	15	19	14	9
Apomorphine 10 mg/kg	7	4.5	3	2*	2*	1*	4.5
Saline	11.5	9	14	10	9	13	-
Naloxone 5 mg/kg	14	7	9	11	10	10	-
Saline	12	10	14	25	20	22	-
Atropine 4 mg/kg	10.5	10	4*	16	8	14	-
Atropine 10/mg/kg	13	4	6	3**	4**	8**	-

Table 2: Effect of drugs on median posture retention time in trained mice.

*Animals were trained as described under methods; drug or saline was injected 1 h after training trial 8. 10-15 animals per group. Significance of differences from saline control: *P <.05; **P <.02 (Mann-Whitney U-test, two-tailed)*

Apomorphine (10 mg/kg sc) and atropine (4-10 mg/kg sc) significantly reduced PRT in trained animals compared with matched saline-treated trained controls, but naloxone (5 mg/kg sc) was without effect (table 2).

DISCUSSION

Repeated testing induced the acceptance and retention of an abnormal posture in almost a quarter of mice tested, irrespective of whether animals had been saline-pretreated or handled only. The effect was therefore not due to the saline itself or to the injection procedure. Both incidence and intensity of posture retention increased with the number of trials up to 8; data from the control groups for the drug-treated animals suggests that a further increase in PRT can occur when the number of trials is extended beyond 8. The criterion of a PRT in excess of 5 seconds for determining the incidence of training-induced posture-retention was chosen in view of the almost negligible incidence of such values in untrained animals and also because of the difficulty of measuring accurately a PRT of less than 5 seconds duration. The posture retention induced by training closely resembled neuroleptic-induced posture retention and therefore appears to deserve the name 'catalepsy'. Preliminary experiments also indicated that it is antagonised by atropine and apomorphine but not by naloxone, suggesting a further resemblence to neuroleptic catalepsy. It should be noted that, in the mouse, the dose of apomorphine used here does not cause stereotypy and produces only a small and brief increase in locomotor activity (Handley & Thomas, 1978). The time course of the increase in locomotor activity is also very different from that of antagonism of catalepsy; locomotor activity having returned to control levels before the onset of significant antagonism of the training catalepsy.

The effect on catalepsy of repeated testing of control groups does not appear to have been reported previously in the mouse. In the rat, no training effect occurs when the test involves placing the forepaws on a bar (Ezrin-Waters et al., 1976; Stanley & Glick, 1976; Pycock, 1977), although it has been mentioned for a test involving vertical wire-netting (Papeschi & Randrup, 1973). Catalepsy is inducible by training in the guinea-pig bar-test (Naylor, R.J., Personal Communication).

It is possible to induce a state of 'tonic-immobility' in certain species by procedures involving restraint and/or visual fixation (Klemm 1971). In the present experiments, restraint and incentive for visual fixation were deliberately minimised. Nevertheless, it is possible that training-induced catalepsy is a variant of tonic immobility. The latter has been proposed as a model for human catatonic schizophrenia (Gallup & Maser, 1977). Training induced catalepsy may also prove to be a useful experimental model of human catatonia. It bears at least a superficial resemblance to the postural perseveration which can occur in this condition either spontaneously or on imposition of abnormal postures (Fish, 1967). Furthermore, repeated posture imposition evokes retention of progressively more bizarre postures in some patients (ibid). Tonic immobility however, is reduced by familiarisation with the test procedure (Gallup & Maser, 1977).

It would appear that a form of learning is involved in training-induced catalepsy and it is possible that the observed drug effects were exerted on this rather than the purely motor aspects of the phenomenon.

The results presented here also emphasise the importance of establishing control data on repetitive testing when selecting a test situation and species for experimentation on cataleptogenic drugs.

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