THE EFFECTS OF SOME DRUGS WHEN INJECTED DIRECTLY INTO THE BRAIN

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ABSTRACT

A review has been made of the properties of agents with possible transmitter function, and of various methods used to administer agents directly into the brain.

In particular, a method is described for the administration of agents directly into the cerebral ventricles of conscious rats. Using this method, a number of possible transmitters have been administered and their effects upon the behaviour of rats described. Also, the effects of these agents and the effects of some of their antagonists on body temperature are compared with the effects of morphine given intraventricularly. It is possible that morphine mediates its effects on body temperature through alterations in central levels of acetylcholine (ACh) and noradrenaline (NA).

The effects of possible transmitters given intraventricularly on the nociceptive threshold of conscious rats have also been studied, as have their effects upon the anti-nociceptive effects of morphine. Morphine is potentiated by intraventricularly injected 5-hydroxytryptamine (5-HT) and antagonised by intraventricular NA. A previously observed effect, that reserpine antagonises the anti-nociceptive effect of morphine, has been confirmed. This effect of reserpine was reduced by the intraventricular injection of 5-HT, but not NA. The effect of reserpine on morphine may be due to a central depletion of 5-HT rather than of NA. Experiments with cholinergic agents and both narcotic and narcotic-antagonist agents suggest that analgesics of both types depend upon a cholinergic link within the

central nervous system; in contrast, tryptaminergic and adrenergic links appear to be important only in the narcotic agents. Intraventricular infusion experiments suggest that 5-HT and NA may be involved in the genesis of morphine tolerance and/or dependence. A few experiments have involved the placement of agents in more discrete areas of the brain, in an attempt to localise their effects. A number of previously recorded effects were seen again in this study, for example hyperphagia and body temperature changes. In contrast, effects upon the nociceptive threshold were limited.

The involvement of NA, 5-HT and ACh in the mediation of temperature regulation, and in altering the nociceptive threshold, has been discussed; so too has the interaction of these agents with morphine. A possible basis for tolerance and/or dependence in the rat is outlined.

C O N T E N T S

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S E C T I O N O N E

INTRODUCTION

INTRODUCTION

CHAPTER ONE

SUBSTANCES WITH TRANSMITTER FUNCTION

The region where two neurons come into close juxtaposition is called a synapse, and the nature of transmission across the synapse has been the subject of considerable controversy. The chemical transmitter theory was finally accepted for peripheral synapses in the late 1930s and for the central nervous system (CNS) as recently as 1950/51 (see Eccles, 1964).

(a) Peripheral

The role of acetylcholine (ACh) as a transmitter at the neuromuscular junction is now unequivocally established (Dale, Feldberg & Vogt 1936; Brown, Dale & Feldberg 1936; Crossland 1967). Paton (1958), in reviewing transmission in the nervous system, listed the most important evidence necessary to demonstrate chemical transmission:

1) The transmitter must be released in a pharmacologically identifiable form and the rate of release is increased when the nerve is stimulated.
2) The action of the transmitter must reproduce the effects of normal nervous transmission.
3) The action of the transmitter and that of normal transmission must be blocked by the same competitive blocking agents.
4) The transmitter must be stored at nerve endings and be identified in extracts.
5) When the local store is depleted normal transmission must not occur.

6) The transmitter must be destroyed by an enzyme that limits its action.

7) Drugs which prevent the release of the transmitter must inhibit transmission.

None of these pieces of evidence is sufficient by itself, but taken together they make a convincing case (Gaddum, 1962).

The majority of these criteria are met for ACh at the neuromuscular junction, at autonomic ganglia and at the terminals of parasympathetic nerves (Crossland, 1967). At the terminals of the sympathetic nerves similar criteria have been shown for noradrenaline (NA) (Peart, 1948; Euler, 1946). There is little evidence for substances such as 5-hydroxyptryptamine (5-HT) and dopamine (DA) having such a role in peripheral systems. Neuronal synapses in the CNS are more complex than in the periphery. They do not necessarily operate an 'all or none' response as in the neuromuscular junction (Bradley, 1968), which complicates attempts to study the way they react to potential transmitters.

(b) Central

ACh, DA, 5HT and NA are the agents most widely considered for roles as transmitters in the CNS, though increasing attention is being given to certain amino acids which may modify transmission (Nelson & Walaszek, 1968). Some evidence for the involvement of the former agents follows:-

Acetylcholine

There is strong though circumstantial evidence that ACh is a transmitter in the CNS.

In peripheral systems, one of the enzymes responsible for the manufacture of ACh (choline acetyltransferase) is at a

high level when mediation is by ACh and low where it is not (Hebb & Silver, 1956). Feldberg & Vogt (1948) found this enzyme to be widely distributed in the CNS, and regions of the brain with many, and regions with a few such neurons were demonstrated. Highest concentrations were found in the anterior horns and anterior roots of the spinal cord, and the caudate nucleus.

The enzyme responsible for the breakdown of ACh, acetylcholinesterase is found at both presynaptic and postsynaptic sites in the CNS. It is reasonable to assume that the distribution of these enzymes in the CNS reflects the distribution of cholinergic neurons in the CNS (Crossland 1967). By tracing neurons histologically it has been shown that the reticular activating system consists mainly of cholinergic neurons (Shute & Lewis, 1961; 1967).

Release of ACh from the brain surface has been demonstrated by MacIntosh & Oborin (1953) and from deeper structures by means of a push-pull cannula (McLennan, 1964) and by perfusing the lateral cerebral ventricles (Portig & Vogt, 1966). By means of subcellular fractionation of brain, bound ACh has been found in fractions containing isolated synaptic vesicles and in the fractions containing disrupted nerve endings (De Robertis, 1964; Whittaker, 1964).

Microelectrophoretic application (see Chapter 3) of ACh to the CNS has been investigated. Renshaw cells in the spinal cord are ~~chlor~~Chol.inoceptive and are excited by ACh (Curtis & Eccles, 1958) and closely mimic the effects of synaptic excitation. It is generally accepted that the synapse between collaterals of motor axons and Renshaw cells of the anterior horn are

cholinergic (Bradley, 1968). ACh has been applied to neurons in the cortex (Krnjevic & Phillips, 1964), the thalamus (McCance, Phillips & Westerman 1966), the brain stem (Bradley & Wolstencroft, 1965; Bradley, Dhawan & Wolstencroft, 1966), the hypothalamus (Bloom, Oliver & Salmoiraghi, 1963) and in the cerebellum (Crawford, Curtis, Voorhoeve & Wilson, 1966). Varying quantities of neurons sensitive to ACh have been found in each area; some neurons were excited and some depressed.

Noradrenaline

NA is not so widely distributed in the CNS as ACh. It is preferentially distributed in the brain stem (Vogt, 1954; Carlsson, 1959). Fluorescence microscopy has shown it to be present in neurons scattered throughout the brain, the main concentration being in the hypothalamus (Carlsson, Flack & Hillarp, 1962; Flack, 1964, Dahlstrom & Flack, 1964). Studies of the distribution of NA in the subcellular fractions of brain homogenates have shown it to be present in nerve endings (De Robertis, 1964; Whittaker, 1964).

Electrophoretic administration of NA to neurons in a number of central areas has shown most responsive cells to be depressed, but a number are consistently facilitated (Salmoiraghi, 1966). Injection of NA into the hypothalamus or into the cerebral ventricles causes changes in body temperature and it is possible that noradrenergic neurons play a part in the regulation of body temperature (Feldberg & Myers, 1964). NA and ACh have also been implicated in the hypothalamic and extrahypothalamic mechanisms concerned with food and water intake (Grossman, 1969a).

Dopamine (3-hydroxytyramine)

The presence of DA in the brain was first shown in 1958 (Carlsson, Lindqvist, Magnusson & Waldeck). It has long been known to be a precursor of NA, yet it has now been found in large quantities in the extra-pyramidal system of the brain where there is little or no NA (Carlsson, 1959; Hillarp, Fuxe & Dahlstrom, 1966). Experimental evidence suggests that there are fine dopaminergic nerve terminals in the Corpus striatum and that they constitute 16% of all axons there (Vogt, 1969). The striatal DA-containing nerve terminals appear to arise from cell bodies in the substantia nigra (Bradley, 1968). Certain forms of Parkinson's disease are associated with disturbances in the brain levels of DA, suggesting that a system employing DA-mediated fibres may be disturbed in this disease. (Vogt, 1969; Crossland, 1967; Ernst, 1969).

Using a push-pull cannula, DA has been shown to be released from the putamen in response to electrical stimulation of neurons in the thalamus (McLennan, 1965), and stimulation of the Substantia nigra of the cat causes a small erratic release of DA into the perfused ventricles. However the release of the DA metabolite homovanillic acid (HVA) is increased. Further, increasing anaesthesia decreases the rate of release of HVA. These facts suggest a dopaminergic nigro-striatal pathway (Portig & Vogt, 1969).

On electrophoretic application, DA has been found to be mainly inhibitory in the cuneate and gracilis nuclei of the cat

(Steiner & Meyer, 1966), and was inhibitory on about 25% of those sensitive in the caudate nucleus (Bloom, Costa & Salmoiraghi, 1965).

5-Hydroxytryptamine (5-HT)

The presence in the brain of 5-HT was first reported in 1953 by Twarog and Page. Its distribution in the CNS is similar to that of NA, the largest amounts are found in the thalamus, hypothalamus and mid brain (Garattini & Valzelli, 1965). The existence of 5-HT containing efferent neurons in which 5-HT could be the transmitter have been demonstrated (Dahlstrom & Fuxe, 1965). Differential centrifugation of brain homogenates indicates its concentration in presynaptic terminals (Whittaker, 1964). Microelectrophoretic application indicates a predominantly inhibitory action, similar to that found with other monoamines (Krnjevic & Phillips, 1963). Only very small amounts of 5-HT are released from the cortex in response to electrical stimulation (Eccleston, Randic, Roberts & Straughan, 1969), and the caudate nucleus (Feldberg & Myers, 1966; Portig & Vogt, 1969). The quantity released tends to fall over a period of time, and there is no evidence that 5-HT is released in the course of neuronal activity in the vicinity of any of the cells on which it is known to be active, (a feature difficult to explain if it is a transmitter substance). However, use of the drug p-chlorophenylalanine, which depletes tissues of 5-HT suggests that 5-HT is involved in central neuronal activity (Vogt, 1969).

α-aminobutyric acid (GABA) and glutamic acid

There is considerable evidence that certain aminoacids, notably GABA and L-glutamic acid, are involved in central transmission in

vertebrates (Bradley, 1968). On the somato-sensory pathway in the cuneate nucleus, ACh, catecholamines, 5-HT, histamine and substance P all have very weak actions, and GABA and L-glutamate may be the inhibitory and stimulatory transmitters in this area (Galindo, Krnjevic & Schwartz, 1967). There is no evidence against GABA being the inhibitory transmitter in the cortex (Krnjevic & Schawartz, 1966), and recently it has been demonstrated that GABA is released from brain slices in response to electrical stimulation (Mitchell, Neal & Srinvasan, 1969) and from the surface of the brain by stimuli that produce synaptic inhibition (Mitchell & Srinvasan, 1969).

Other Substances

An excitatory factor has been identified in the cerebellar and cerebral cortex as ergothionine, (Crossland, Woodruff & Mitchell, 1964). Direct application to some neurons in the brainstem has stimulated activity in these neurons (Avanzino, Bradley Corris & Wolstencroft, 1966).

Substance P is a depressant polypeptide with many characteristics of a potential transmitter, but there is little evidence to suggest such a role (Bradley, 1968; Crossland, 1967). Similarly, adenosine triphophate (ATP) has been suggested, but its actions on iontophoretic application are probably due to its chelating properties (Galindo, Krnjevic & Schwawartz, 1967).

Histamine is present in the brain, concentrated mainly in the hypothalamus (Green, 1964; Adam & Hye, 1966), and has been isolated in small nerve endings and synaptic vesicles of the rat brain (Kataoka & De Robertis, 1967). It has been considered as a central transmitter (Gaddum, 1963). However at present there is

insufficient evidence to prove it a transmitter.

Prostaglandins (long-chain, unsaturated fatty acids) have been identified in the brain. They have effects on neurons in the brain stem when applied iontophoretically (Avazino, Bradley & Wolstencroft, 1966) and are released at the surface of the cerebral cortex of cats (Ramwell & Shaw, 1966). There is considerable evidence that glycine may be an inhibitory transmitter in the spinal cord (see: Hebb, 1970).

Summary

At the neuromuscular junction evidence for the transmitting action of ACh is proven. It is generally accepted that NA is the transmitter at sympathetic nerve endings, but for the present 5-HT and DA are not thought to be peripheral transmitters. In the CNS, all evidence for transmitters is circumstantial. In some cases, as with ACh, NA and 5-HT, the evidence is stronger than it is with others. Yet due to the complexity of the CNS the exact site and mode of action of the proposed substances cannot be established with the techniques at present available.

CHAPTER TWO

THE BLOOD-BRAIN BARRIER

For a number of substances, passage from the blood to the CNS is extremely slow; passage is slow in comparison with the rates at which these substances may pass from the cerebrospinal fluid (CSF) into the parenchyma of the CNS. Ehrlich (1885) first observed that certain aniline dyes, when injected into the blood stream, stained all tissues except those of the CNS. Subsequent work showed that this restraint or barrier existed for a great number of substances (Winterstein, 1961). For many years, this barrier was believed to be exclusive to the CNS (Dobbing, 1961), but it is now realised that this is not so (Levin & Scicili, 1969). The exact nature of the barrier aroused interest in the CSF; some considered all exchange of substances between blood and CNS to take place through the CSF (Von Monakow, 1921). However in 1941 Freidman considered that the choroid plexus, meningeal vessels and CSF were not concerned in the exchange of substances between blood and CNS, but the barrier was more likely to be connected with the phenomenon of capillary permeability. More recently it has become evident that the barrier phenomenon is a summation of many factors.

The barrier could be located at 3 principal sites:

a) At the blood-CSF boundary. The bulk of CSF is probably produced from the blood at the choroid plexus. However about 20% is produced at the ependymal walls lining the ventricles (Pollay & Curl, 1957).

b) Between the blood and the CNS tissue. An interface is found at the level of the cerebral capillaries. About 50% of the capillary surface is covered in pericytes found in the basement membrane of the capillaries. Also the perivascular glia (vascular end feet), possibly astrocytic in nature, cover 85% of the vascular surface (Farquhar & Hartman, 1956; Maynard, Shultz & Pease, 1957). It has been suggested that the astrocytes themselves are an important element in the blood-brain barrier and that they must be traversed by all substances entering and leaving nervous tissue. This is further supported by the coverage of neuron surfaces by similar glia processes (Elliott, 1963). Further, electron microscopy has indicated the space between cells in the CNS to be only 150-200 Å wide, giving rise to the theory that substances were excluded from the brain because there is little extracellular space and no water for them to dissolve into (Edstrom, 1958; 1964; Davson, Kleeman & Levin, 1963).

c) At synapses. The use of microelectrophoretic methods to apply drugs to individual neurons in the CNS has revealed the possibility of a synaptic barrier surrounding the axonal endings and synaptic areas of the postsynaptic neuron (Curtis & Eccles, 1968).

When a substance passes from the blood to the depths of a neural tissue it has to pass certain potential barriers:

1) through the capillary wall
2) through the extracellular space of the tissue and
3) uptake by cells.

The two major functions by which this transit can occur are: passive diffusion, and active transport. A number of other

factors, e.g. metabolism and other structures, are also involved.

Passive diffusion

A number of authors have shown that the blood-cerebrospinal fluid, and the blood-parenchyma barriers behave as lipoid membranes towards foreign organic compounds (Brodie & Hogben, 1957; Bútler, 1942; Shanker, 1961; Shanker, 1962). The rate of penetration into the brain has been correlated with the lipid-to-water partition coefficiants of drugs at pH 7.4 (Mayer, Maickel & Brodie, 1959).

The blood-CSF membrane has been demonstrated to be permeable to the unionised form of weak organic bases and acids, and relatively inpermeable to the ionised form (Rall, Stabenau & Zubrod, 1959). Therefore increased ionisation at a physiological pH will decrease the fraction of unionised compound available for diffusion (Rall & Zubrod, 1962). The degree of ionisation also alters the distribution of weak organic electrolytes. Weak acids are partly barred from areas of lower pH and concentrated in areas of high pH. (Milne, Scribner & Crawford, 1958).

The binding of a drug to plasma proteins can effect its entry into the CNS. CSF is protein free and the reason why trypan blue and other dyes do not enter the CSF is probably related to their firm binding to plasma proteins (Rall & Zubrod, 1962).

Thus compounds that are poorly lipid-soluble, ionised and are protein bound have a very slow entry to the CSF.

The CSF is returned to the blood at the arachnoid villi and dissolved substances are consequently returned to the

peripheral circulation. This creates concentration gradients in the extracellular fluid of the CNS. A knowledge of the size of the extracellular space is essential to an understanding of the processes involved in the exchange of substances between blood and brain (Woodward, Reed & Woodbury, 1967). Various authors, while investigating the size of the space, found that the blood and the CSF act as sinks (or drains) into which substances flow when reaching the nervous parenchyma (Woodward, Reed & Woodbury, 1967; Olendorf & Davson, 1967; Bito, Bradbury & Davson, 1966). A substances supplied to the parenchyma via the blood will diffuse into the CSF, and one supplied by the CSF will diffuse to the blood. However a compound reaching the CSF may not diffuse freely to the blood because of the brain capillary barrier (Levin & Scili, 1969).

However the concept that the blood-brain exchange depends purely on the physical properties of the substances concerned is not now regarded as a complete explanation of the passage (or lack of it) through the barrier (Davson, Kleeman & Levin, 1963; Bito & Davson, 1966; Bito, 1969).

Active Transport

Active transport (carrier mediation) has been proposed as the mechanism of barrier permeation (Fishman, 1964; Bidder, 1966; Blasberg & Lajtha, 1966; Bradbury & Davson, 1964; Crone, 1965). The entry of certain substances into the CNS appears to depend on their ability to combine with carrier molecules at the level of the capillary wall or perivascular glia. It has been suggested that physical diffusion is only significant in pathological conditions (Eidelberg, Fishman & Hams, 1967).

Active transport of substances from the CSF to the blood occurs within the choroid plexuses. It has been shown for, diodrast (Pappenheimer, Heisey & Jordan, 1961; p-aminohippuric-acid (Davson, Kleeman & Levin, 1962) iodine (Pollay & Davson, 1963), quaternary ammonium compounds (Schanker, Prockop, Schoy & Sisodia, 1962), thiolyanate (Pollay & Curl, 1967) bromide (Bito, Bradbury & Davson, 1966) and penicillin (Fishman, 1966). There is also some evidence that potassium (Cserr, 1965), 5HT (Tochino & Schanker, 1965) and NA (Glowinski, Kopin & Axelrod, 1965) are also actively transported out of the CSF. Thus in certain cases the sink action of the CSF can be attributed to the active efflux of substances by the choroid plexuses (Levin & Scicli, 1969).

Metabolism

The involvement of a substance in the metabolism of nervous tissue will have an influence on the steady state distribution of that substance between plasma and CSF and between plasma and extracellular fluid (Davson, Kleeman & Levin, 1963). Failure to produce pharmacological actions in the CNS does not necessarily mean that the entry of the drug has been impeded, particularly when it is known to be rapidly metabolised (Dobbing, 1961).

One example of an enzymic barrier has been described. It is well established that there is a resistance to catecholamines and 5HT reaching the brain parenchyma from the circulation (Axelrod, Weil-Malherbe & Tomchick, 1959; Samorajski & Marks, 1962; Weil-Malherbe, Whitby & Axelrod, 1961). A similar effect has been shown for dopamine. Recently the brain capillaries, but not the peripheral capillaries have been shown to contain DOPA

decarboxylase, which functions as an enzyme-based trapping mechanism for L-3,4,dihydroxyphenylalanine (L-DOPA) (Owman & Rosengren, 1967; Bertler, Flack, Owman & Rosengren, 1966). Systematically-injected DOPA is found as dopamine in the endothelial cells of brain capillaries, and not in the surrounding brain cells. However the administration of a DOPA-decarboxylase inhibitor (e.g. RO-4-4602) destroys the barrier and systematically-administered DOPA is found as dopamine in the brain cells and not in the capillary endothelial cells. The intensity of this barrier varies from area to area in the brain (Constantinidis, De La Torre, Tissot & Geissbuhler, 1969). The majority of peripheral organs do not contain decarboxylase though some in the kidney do behave in a similar manner. This would suggest that cerebral capillaries are peculiar in comparison with peripheral capillaries, with respect to their barrier properties.

Extra-blood-brain-barrier Structures

The area postrema, the dorsal crest, the subcommisural organ (all of which line the ventricular cavities) and also the subfornical organ and the supraoptic crest, can all be separated from the cerebrum proper in that they accept intravenously-injected compounds and are evidently located "outside" the blood brain barrier. They appear not so much to be a means by which substances can enter the brain and act on central structures, but rather to be part of the barrier itself. They could also constitute a receptor area for a number of chemicals (Koella & Sutin, 1967).

Summary

The term blood-brain barrier implies a total barrier effect on substances entering or leaving the CNS. It is made up of a

complex of factors including capillary permeability, transport at capillaries and choroid plexuses, the flow of the CSF and metabolic processes in the CNS.

It is essential to overcome the blood-brain barrier if a compound is to gain access to the CNS. This can be achieved in one of two ways: either the physio-chemical characteristics of the compound are such that on administration to the peripheral vasculature it will pass the barrier, or the compound can be presented directly to the CNS tissue. Presentation directly to the CNS has the advantages of avoiding the main peripheral actions a compound may have, of allowing the use of minute amounts of agents, of ensuring contact with the CNS tissue and allowing administration to localised areas in the CNS.

CHAPTER THREE

ADMINISTRATION OF DRUGS DIRECTLY TO CENTRAL SITES

Historical

For many years little interest was taken in the CSF, and as late as 1920 many held the following view: "The CSF is preeminently a fluid for the hydraulic suspension of the brain and cord, its function is essentially hydrostatic. It possess no function, and plays no role in nutrition. Attempts at medication of the brain and cord through the subarachnoid space are unscientific .... the beneficial effects hitherto ascribed .... are due entirely to incidental spinal damage." (Dercum, 1920).

The study of the passage of compounds through the blood-brain barrier made it essential to administer substances directly into the cerebral ventricles. Dyes were initially used though it later became of interest to see if compounds that were without effect on conventional administration via the peripheral vascular system, had any effect when applied directly to the CNS (Winterstein, 1961).

As early as 1875 it had been observed that subdural injection of morphine into pentobarbitone-anaesthetised dogs, caused excitement and an apparent reversal of the anaesthesia (Bernard, 1875). Also, towards the end of the 19th century, a number of compounds had been injected into the CSF, normally in the spinal region. Thus in 1899 lumbar anaesthesia was introduced by subdural injection of cocaine (Bier, 1899).

Between 1910 and 1916, Dixon and Halliburton performed a number of studies on the formation of CSF, and the effects of drugs upon this formation (Dixon & Halliburton, 1910, 1913, 1912, 1916). In 1912,

they were the first to describe the rapidity with which drugs were absorbed from the CSF. In 1916, in a more extensive study, they compared the rapidity of action following injection into the cisterna magna, with that after an intravenous injection, using atropine, adrenaline and nicotine, and found the effects to be similar by both routes. The work of Becht (1920) suggested that some of the effects seen by Dixon and Halliburton were due to the compounds leaking from the CSF into the peripheral circulation (see Winterstein, 1961). In 1931 Cushing described the effects of pituitary extracts, atropine, pilocarpine and tribromoethanol which had been injected into the ventricles of conscious humans (after they had undergone various forms of brain surgery). In 1934 Dikshit (1934a) described a method for injection into the cerebral ventricles of anaesthetised cats, in which the head was held and the skull trephined before injections were made. Later Dikshit (1935) extended the method to cats prepared previously, injections being made into the ventricles of conscious animals. In 1947 Lackey described experiments in which a needle for injection was placed directly into the cisterna magna of conscious dogs. He maintained that the procedure could be carried out without the animals struggling.

Until 1938, when Adam, McKail, Obrador and Wilson introduced a method of perfusing the cerebral ventricles, substances had been introduced either by direct application to the surface of the brain, or with an injection needle into the spinal cord, the cisterna magna or lateral ventricles. The animals were usually anaesthetised (Winterstein, 1961). Yet as long ago as 1918, Batelli described a method remarkably similar in principle to that of Grossman (1960).

The first systematic examination of the effects of compounds administered to the brains of conscious animals was started by Feldberg and Sherwood in 1953.

Methods Available

The principles of direct administration of compounds to central nervous tissue, are essentially the same as those employed in the studies mentioned in the brief historical survey. The methods used today are mainly refinements of those older methods.

a) Methods Mainly Used in Anaesthetised Animals

In 1914 Bikeles and Zbyszweski applied drugs directly to the surface of the brain by exposing the brain surface and applying filter papers soaked in solutions of the required drug. A more controlled method is to expose the cerebral cortex and place on the surface cups that are open at both ends. These cups can be filled with Ringer's solution or artificial CSF in order to collect for assay any substance that may be released, such as ACh (MacIntosh & Oborin, 1953; Mitchell, 1963). Drugs can be simply applied by pumping through the cup artificial CSF that contains the required concentration of drug. Using this method, and employing a KCl-filled glass microelectrode to study electrical changes in the cortex, a cholinergically-activated noradrenergic inhibitory system has been postulated and mapped out in the rat somatosensory cortex (Malcolm, Saraiva & Spear, 1967).

For collecting samples of released substances from, or the application of a fixed concentration of solutions to, deep structures, the push-pull cannula can be employed (Gaddum, 1961). This is a device in which solutions run through a fine needle into a tissue and

are collected in a wider tube surrounding the needle, so that a small area can be continually bathed. A modification of the method has since been used to transfer hypothalamic chemical factors between conscious animals (Myers, 1967; Myers, 1969).

To study the uptake of substances from the cerebral ventricles, animals can be anaesthetised, placed in a steriotaxic apparatus and the substance injected or perfused. When substances are injected into the lateral ventricles they pass from there into the third ventricle and from there into the fourth ventricle, then through the foramen of Luschka into the subarachnoid space around the brain stem (Feldberg & Fleischhauer, 1965). A number of special perfusion techniques have been developed to localise the area perfused (see Feldberg & Fleishhauer, 1965; Feldberg, 1963). The simplest such localisation is the exclusion of the subarachnoid space by collecting effluent from a cannula placed through the opened cisterna, along the floor of the fourth ventricle and into the middle of the aqueduct (Bhattacharya & Feldberg, 1956). Altering the position and numbers of the cannulae can further restrict the perfused area (Carmichael, Feldberg, Fleischhauer, 1964).

The effects of intraventricular injection can be observed in conscious animals, if the injection if made while the animal is lightly anaesthetised and is subsequently allowed to recover. Fuxe and Understeadt (1966) anaesthetised rats with fluothane-N_2O-O_2 which gives a very rapid recovery. They gave intraventricular injections of catecholamines, allowed the animals to recover consciousness and then studied the uptake of these amines by neurons surrounding the ventricles.

In 1953 a technique was developed for the administration of very small quantities of drugs in the immediate vicinity of nerve cells,

while recording their activity (Nastuk, 1953). The technique consists of the injection of ions by electrical currents from a micro-pipette. Originally a 2 barrelled micropipette was used for application to muscle end-plates, but it was found to be unsatisfactory for use in the CNS, as it is not normally possible to identify the neuron stimulated. In order that the same neuron is stimulated when various drugs are applied, a more complicated cannula is required. Now as many as six extracellularly-located micropipettes fixed together, can be used for administration, while spike potentials are simultaneously recorded (Votava, 1967). The technique has been used extensively (Curtis, 1964 & 1965; Salmoiraghi, 1964; Bradley & Wolstencroft, 1965; Krnjevic, 1965). The amount of drug released from these cannulae ranges from 10-100 pica-moles following a current of 100 μA. The method has the merit of bypassing the major diffusional and enzymatic barriers and of greatly limiting the number of potential sites of action (Salmoiraghi, 1966).

b) Methods Used Mainly in Conscious Animals

In 1953 Feldberg and Sherwood introduced the principle of permanently fixing a cannulae guide to the skulls of cats, so that a cannula could be introduced through a rubber protective membrane into the brain of the conscious animal, causing no pain or distress to the animal. Thus interactions with other drugs, such as anaesthetics, were avoided, and the behavioral effects of centrally-administered drugs could be studied without the results being influenced by pain or fear responses. Similar cannulae for injection into the ventricular system have been designed for the cat (Crowley, 1967), the monkey (Feldberg, Hellion & Lotti, 1967; Myers, 1969), the rabbit (Hassleblatt & Sproull, 1961; Cooper, Cranston & Honour, 1965; Banergee, Burks, Feldberg &

Goodrich, 1968), the dog (Haley & Weinberg, 1955; Feldberg, Hellion & Myers, 1966; Bartlestone, Reilly & Wang, 1958), the sheep (Ruckebusch, Grivel & Paplace, 1965; Bligh, 1966), the goat (Pappenheimer & Heisey, 1963; Andersson, Jobin & Olsson, 1966), the ox (Findlay & Robertshaw, 1967) and the rat (Feldberg & Lotti, 1967; Hayden, Johnson & Maickel, 1967; Khavari, Feider, Warburton & Martin, 1967; Decima & George, 1967; Booth, 1968; Myers, 1963; Myers, Cassaday & Holman, 1967). A method for injecting compounds into the ventricles of the conscious mouse without the use of an anaesthetic or cannula guide has been described by Haley & McCormick (1957) and modified by Brittain & Handley (1967).

These methods were devised primarily for giving intraventricular injections to animals. By modifications of the infusion pump system and the use of 50 or 100 µl syringes, volumes as little as 0.02-1.0 µl can be discharged into cerebral structures of conscious animals. As with the intraventricular cannulae, a cannula guide is first implanted in the skull of the animals. Injections can be made upon recovery from the operation (Feldberg & Myers, 1965; Myers, 1964; Fisher, 1964).

A further technique for applying drugs to cerebral structures involves the use of crystalline substances. A double cannula system, consisting of two concentrically mounted syringe needles is permanently mounted in the region under study. On recovery of the animal from the implantation procedure, the inner cannula is removed, cleaned and minute amounts of crystalline chemicals deposited in the tip. The inner cannula is then replaced and the chemicals slowly dissolve and diffuse into the tissues (Grossman, 1960 & 1962).

When a drug is given by the intraventricular route localisation of action to a given structure cannot be precisely specified. However to obtain a general idea of the particular central effect of a

compound, without concern for localisation, the intraventricular route is desirable, if only for its simplicity. For local specificity the chemicals must be infused directly into CNS structures.

The techniques of implanting crystals and injecting solutions both have advantages and disadvantages. Injections of solutions greater than 2 µl into the brain substance produce lesions and hemorrahage, which can themselves produce an effect upon the animal (Rech & Domino, 1959), or the solutions can spread up the cannula tract, making localisation difficult (MacLean, 1957). Also rapid injection tends to produce mechanical pressure on the tissue immediately surrounding the tip of the implant, which may result in neural stimulation or depression (Grossman, 1969b). However using dye diffusion methods it has been shown that if volumes of 0.5-1.0 µl are injected into rats at a rate of 1 µl in 43 seconds, lesions are not produced and the drug can be localised (Myers, 1966).

Inserting crystals into the brain tissue, one cannot control for such factors as pH, osmolarity, temperature, high concentration, and the rate of dissolution, and it is difficult to measure exactly, the amount of drug implanted (Myers, 1963 & 1964). However placing a crystal inside the inner and shorter of two cannulae, results in a more gradual diffusion of the drug to a much more restricted area, and the technique is more simple and less time-consuming than the injection of minute quantities of fluid. The latter is very difficult to accomplish with accuracy, whilst the crystal method is invaluable for the application of poorly soluble substances such as estrogenic steroids (Myers, 1969).

Since both methods have advantages and disadvantages it has been suggested that both should be used in a fully controlled study (Fisher,

1969). They should also be used to localise effects following studies involving intraventricular injections.

CHAPTER FOUR

KNOWN PHARMACOLOGICAL PROPERTIES OF AGENTS INJECTED CENTRALLY

A large number and variety of agents have been applied directly to the brain (see reviews: Winterstein, 1961; Marczynski, 1967; Feldberg & Fleischhauer, 1965). In a number of cases considerable differences in the activities of a particular agent are recorded by various Authors. These differences can be attributed to either variations in technique or to a very definite species difference. A few substances with possible transmitter functions in the CNS, and 2 drugs affecting these transmitter will be considered here, with special emphasis on these differences.

Adrenaline and Noradrenaline

In 1914 Bass observed that an injection of adrenaline (Ad) under the dura of dogs produced a sleep-like state lasting 2-4 hours. Analgesia, sleep, anaesthesia and hypoglycaemia were observed on injection of Ad intracisternally into dogs (Leimdorfer, Arana & Hack, 1947; Leimdorfer & Metzner, 1949) and a condition indistinguishable from light pentobarbitone anaesthesia has been produced in the cat, by intraventricular injections of Ad and NA (Feldberg & Sherwood, 1954), and in sheep, (Palmer, 1959). In rats intraventricular injection of Ad has produced depression with reduced reflex and motor activity and in some animals signs of light hypnosis (Marseillan & Corrado, 1961), although the anaesthesia-like conditions described by Leimdorfer and Feldberg in the cat, were not observed by these authors, nor by ourselves (see section 3, chapter 1) in the rat.

In the cat periods of inactivity and apparent analgesia were accompanied by EEG desynchronisation suggesting that the animals were not unconscious (Rothballer, 1959), though it is possible that the sleep seen was 'Paradoxical sleep'. In rabbits intraventricular NA produced synchronisation followed by desynchronisation (Matsuda, 1969), while in cats injections of Ad placed directly into the brain stem reticular formation produced behavioral arousal and electrical desynchronisation (Cordeau, Moreay, Beaulness & Laurin, 1963).

Direct adrenergic stimulation of the hypothalamus of cats, by placing crystals of NA or injecting small volumes of solutions, produced sleep and a reduction in the response to nociceptive stimuli, yet similar stimulation of the dorsomedial thalamus produced exploratory behaviour similar to that of a playful kitten (Myers, 1964). The combination of effects seen on injection of a solution of Ad into the lateral ventricles could be due to the wide spread distribution of the substance. Ad injected into the ventricles of the cat probably penetrates 2-3 mm into the walls of the 3rd ventricle and aqueduct, so that the sedative action is presumably due to an action on structures lining the cavities (Marley, 1966).

Injections of small doses of NA (0.01 μg) into the lateral ventricles of cats produced cardio-acceleration and pressor responses that could be blocked by intraventricular injection of the -adrenergic blocking agent pronethalol (Gagnon & Melville, 1967). However high doses of NA (40-80 μg) caused sinus bradycardia and a slight depressor response which may have been due to a general central depression, thus the cardiovascular effect seen on intraventricular injection of NA is dependent on the dose employed (Share & Melville, 1963; Gagnon & Melville, 1966).

A further effect of applying catecholamines to central sites is the production of hyperphagia (Grossman 1962a;b; Wagner & De Groot, 1963; Miller, Gottesman & Emery, 1964; Coury, 1967; Booth, 1967, 1968; Grossman, 1969a). However intraventricular injections of these substances do not produce eating in the cat (Feldberg & Sherwood, 1954; Myers, 1969a) or the monkey (Myers, 1969a). It has been observed that high doses of NA only produce sleep and coma in the rat and only low doses produce eating (Grossman 1969a). It is possible that the doses used in cats were too high to show such behaviour.

Species differences in response to monoamines applied to the hypothalamus are well established. Intraventricular injections of catecholamines in the cat (Feldberg & Meyers, 1964), the dog (Feldberg Hellion & Meyers, 1966) and monkey (Feldberg, Hellion & Lotti, 1967; Myers, 1967) causes a decrease in body temperature. In rabbits (Cooper Cranston & Honour, 1965) and sheep (Bligh, 1966; Rukebusch, Grivel & Laplace, 1965) they cause an increase. In the ox (Findaly & Robertshaw, 1967) and goat (Andersson, Jobin & Olsson, 1966) they have no effect. In the mouse NA puts temperature up then down (Brittain & Handley, 1967) and in the rat NA puts it up or down depending on the dose (Feldberg & Lotti, 1967 and ourselves - see section 3, chapter 2).

Acetylcholine

In 1935 Dikshit injected ACh into the ventricles of conscious cats. This produced a sleep-like state within 10-30 minutes, which lasted several hours. Drowsiness and confusion was seen by Silver and Morton (1936) in similar experiments, and also in man by Henderson and Wilson (1936). Feldberg and Sherwood (1954) noted vocalisation and/or retching, following by a dazed state resembling akinetic seizure.

However Sherwood, Ridley and McCulloch (1952), using very much larger doses than Feldberg and Sherwood, saw anger or fear-like responses following intraventricular ACh. Injection directly into hypothalamic sites in cats caused "fear-like responses" which included spitting, hissing and rage (Myers, 1964). A similar rage reaction sometimes associated with tremor, was seen on injection of ACh, carbachol and eserine into the anteriomedial hypothalamus (Varszegi & Decsi, 1967). The site causing drowsiness and sleep would appear to be in the brain stem reticular formation. Injection of ACh into this region in cats caused synchronisation of the EEG, followed by drowsiness and sleep, so long as the animals were in an environment suitable for sleep (Cordeau, Moreau, Beaulnes & Laurin, 1963). The behavioural effects of injecting cholinesterase inhibitors into the cerebral ventricles of cats are mostly in accordance with the expected effects of ACh accumulating in the CNS (Feldberg & Sherwood, 1954b; Palmer, 1959). Injections of 30-50 μg of ACh into the cerebral ventricles of the rat produced a state of depression and reduced reaction to external stimuli, followed by catatonia (Marseillan & Corrado, 1961). Depression and akinetic seizure were also the main features of intraventricular injection of ACh into mice (Haley & McCormick,1957).

Oxotremorine, when administered into the ventricles of mice is as potent as morphine as an analgesic. ACh however is only weakly active but is potentiated by physostigmine. Atropine significantly reduced the activity of oxotremorine whilst methylatropine was inactive (Handley & Spencer, 1969).

Dikshit (1934a) injected ACh into the ventricles of anaesthetised cats and showed an inhibitory effect on respiration, similar to that produced by vagal stimulation. He also showed

(Dikshit, 1934b) that similar injections of ACh, caffeine and nicotine caused cardiac irregularities and a rise in blood pressure. Suh, Wang & Lim (1935) confirmed this in dogs, finding that the increased in blood pressure could be blocked by ergotoxine but not by atropine or vagotomy. In contrast Bhawe (1957), again in cats, found that intraventricular injection of ACh normally caused a decrease - and only occasionally an increase - in blood pressure, and that these effects could be blocked by atropine. Yet Armitage and Hall (1967a) gave intraventricular injections of ACh into chloralosed cats up to 1 mg and saw only slight effects - which were depressor. However carbachol and nicotine caused a profound decrease in blood pressure, which they suggested was caused by a release of ACh. Perfusion of tubocurarine from the lateral ventricle to the aqueduct in cats caused a rise in blood pressure which may be been due to an action on the walls of the 3rd ventricle (Carmichael, Feldberg & Fleischhauer, 1964). It was suggested that nicotine and carbachol elicit their effects on the cardiovascular system in the same area (Armitage & Hall, 1967b).

In 1962 Grossman (1962a and b) placed small crystals of ACh and carbachol into various hypothalamic areas of rats and found significant stimulation of their drinking behaviour. It is now accepted that cholinergic stimulation of a number of loci in hypothalamic and extra-hypothalamic areas causes increased drinking in satiated rats (Fisher, 1969). These drinking responses have not been observed in cats - possibly because they are swamped by the other behavioural effects (Miller, 1965). This drinking can be readily blocked by systematically-administered atropine, but methyl-atropine has a much smaller effect (Miller, 1965) - indicating the action to be due predominantly to a central effect.

Oxotremorine or carbachol injected into the hypothalamic thermoregulatory centres, caused a profound fall in body temperature in the rat which could be antagonised by atropine. Thus the action of cholinergic substances on the body temperature is muscarinic (Lomax & Jenden, 1966; Kirkpatric Jenden & Lomax, 1967). In contrast, intraventricular injection of ACh had no effect on body temperature in the rabbit (Cooper, Cranston & Honour, 1965) nor in the ox (Findlay & Thompson, 1968). A small dose of pilocarpine injected into the rostral hypothalamus of rats, caused a fall in body temperature. If the injection of pilocarpine was immediately preceded by a small dose of NA (i.e. a dose which alone caused a rise in temperature in some rats and a fall in others), then the pilocarpine fall was blocked. Thus catecholamines may modulate the cholinergic transmission that regulates body temperature in the hypothalamus of rats (Lomax, Foster & Kirkpatric, 1968).

Deposition of carbachol into various areas of the rat brain suggests that pathways involved in drinking and in causing hypothermia follow similar routes in the limbic system and diencephalon (Hulst & De Wied, 1967). In cats implantation of carbachol into those areas that case drinking initiated a fall in body temperature, and both the effects followed a similar time course (Miller, 1965).

5-Hydroxytryptamine

In cats small doses of 5-HT injected into the ventricles (10 μg) had no effect on the animals' behaviour. 75-500 μg had effects which gave the appearance of causing muscular weakness. There was also lip-licking, salivation, tremor and twitching of whiskers and eyelids (Feldberg & Sherwood, 1954; Kulkarni, 1967). This depression seen in

cats after intraventricular 5-HT could be antagonised by intraventricular injections of lysergic acid diethylamide (LSD), ergometrine, morphine, methadone and amphetamine (Gaddum & Vogt, 1956).

As with the catecholamines, and ACh, the effect of 5-HT on body temperature shows a wide species variation. In the cat (Feldberg & Myers, 1964), the dog (Feldberg, Hellion & Myers, 1967) and the monkey (Feldberg, Hellion & Lotti, 1967; Myers, 1967), there is a rise in body temperature. In the rabbit (Cooper, Cranston & Honour, 1965) and the sheep (Bligh, 1966), there is a fall - but the effect is usually small and by no means consistent. In the rat (Feldberg & Lotti, 1967), mouse (Brittain & Handley, 1967), ox (Findlay & Robertshaw, 1967, and goat (Andersson, Jobin & Olsson, 1966), there is a marked hypothermic effect. Other authors have recorded different effects - a predominently hypothermic effect on intraventricular injection into rats was noted by Kubikowski & Rewerski (1969) and a fall then a rise was found in cats (Kulkarni, 1967). This latter discrepancy has been explained on the basis of different methodology (Banergee, Burks & Feldberg, 1968).

Injection of small amounts of pyrogen (typhoid vaccine) (Villablanca & Myers, 1965) and leukocyte pyrogen (Jackson, 1967) into the anterior hypothalamus of cats results in a significant rise in temperature. There is a slight delay in the temperature rise, which suggests that either the pyrogen molecule is broken down before becoming effective or acts through the release of other substances - possibly biogenic amines. However a 90% depletion of the 5-HT in the brain stem of rabbits, using p-chlorophenylalanine, did not modify the febrile response to endotoxin (Des Prez & Oates, 1968). Present evidence therefore indicates that pyrogens exert their effects directly on the cells of the anterior hypothalamus, and that these

effects are not produced by interactions with endogenous monoamines (Feldberg, 1968).

Morphine

Intraventricular injection of morphine into conscious cats, has produced strong excitation (Gaddum & Vogt, 1956). Intraventricular injection into conscious cats, or perfusion of the 3rd ventricle of anaesthetised cats, with morphine, both caused shivering and an increase in body temperature. These effects could be prevented by previous intraventricular injections of nalorphine or ergotamine (Banergee, Feldberg & Lotti, 1968). Morphine injected into the cerebral ventricles of rabbits has produced a rise in body temperature sometimes preceded by a small fall. Catalepsy, rigidity and slowing of respiration accompanied this rise and the effects could be abolished by intraventricular pretreatment with nalorphine (Banergee, Burks, Feldberg & Goodrich, 1968b). Injections of 50 µg of morphine sulphate into the anterior hypothalamus of rats, has caused profound hypothermia (Lotti, George & Lomax, 1965). This effect has been arrested and reversed (to a hyperthermia) by the administration of nalorphine during the period of hypothermia (Lotti, Lomax & George, 1965). However Paolino and Bernard (1968) carried out similar experiments, only they used different ambient temperatures (5, 24 and 30°C). Using similar doses of morphine, they found that when injected into the hypothalamus, hypothermia and hyperthermia were produced with the animals in cold and hot environments respectively. Thus they proposed that the effect of morphine on body temperature is dependent upon ambient temperature. They also found that similar body-ambient temperature interactions were produced with systemic administration. Lotti et al suggested that the hypothermia they saw

was due to the lowering of the hypothalamic set point, for temperature control, while the results of Paolino & Bernard, indicate a total loss of ability to regulate temperature.

Since morphine is known to deplete hypothalamic noradrenaline (and adrenaline) (Vogt, 1954; Gunne, 1959), brain monoamines may function as mediators of the temperature responses to morphine (Paolino & Bernard, 1968; Banergee, Burks, Feldberg & Goodrich, 1968). Whilst it is conceivable that the effects are mediated by monoamines, it is equally possible that the effects are due to a direct effect of morphine on the cells of the hypothalamus.

Reserpine

In 1956 Gaddum and Vogt injected reserpine into the cerebral ventricles of cats. They found that a dose of 10 µg had no effect even after 3 hours, so they gave all further doses i.p. They found that the effects of i.p. reserpine were similar in many ways to those of intraventricular 5-HT, but were not sufficiently similar to suggest that the effects of reserpine were entirely due to 5-HT release. Dasgupta and Haley (1957) found that intraventricular doses of reserpine of 18-20 µg, caused relaxation of the nictitating membrane, narrowing of the palpebral fissure (ptosis), mild photophobia, diarrhoea, anorexia and tranquillization. This, apart from the anorexia, was essentially similar to the effects following intraperitoneal injections. With both doses normally agressive cats became docile, and fearful cats became friendly. In order to see if there was any relation between the 5-HT-releasing activity and the behavioural effects of reserpine, they gave sub-effective intraventricular doses of both 5-HT and reserpine into the same animal.

This was totally ineffective in reproducing the effects of 5-HT or reserpine given alone. They concluded that the effects of centrally administered reserpine were due to the effects of the drug per se. Injection of 500-750 µg of reserpine into the cerebral ventricles of cats, produced frequent defaecation and catalepsy (Banergee, Burks, Feldberg & Goodrich, 1968a).

In view of the ability of reserpine to impare the monoamines storage capacity of tissues, it is not surprising that it has effects on body temperature both when given systematically and intracerebrally. Cooper, Cranston & Honour (1967) showed that injections of reserpine into the cerebral ventricles of rabbits raise rectal temperature and deplete the hypothalamus of monoamines. On repeating such injections of reserpine every 14 hours the rise is no longer seen, though the intraventricular injection of NA still raises the body temperature. Similar experiments were carried out in cats (Banergee, Burks, Feldberg & Goodrich, 1968) and in the rat (Feldberg, 1968). These results support the contention that the temperature effects of reserpine are mediated via monoamines in the hypothalamus.

5. BASIS OF PROJECT

A number of agents with possible transmitter function in the brain have been reviewed. The effects of some of these agents when injected into both the cerebral ventricles and into various specific areas of the brain have also been reviewed. There are a number of conflicting reports about the effects of these substances when given by intracerebral injection. Much of the confusion in the literature is due partly to the fact that several different species have been involved in previous studies: the animals most frequently used for intraventricular studies have been the cat and dog, though a few studies involved the use of the rat.

Current information indicated that a further investigation into the effects of transmitter agents, injected into the cerebral ventricles of rats was warranted. Also there are a number of drugs believed to exert their effects in part or wholly by interfering with the normal activity of these transmitter substances. It was considered that intracerebral injection would be a useful method of investigating the effects of altered central transmitter levels on these agents.

In order to study the effects of drugs on behaviour and associated activities, it was essential to use conscious animals. ~~It was also desirable to study the actions of agents on other systems when the animals.~~ It was also desirable to study the actions of agents on other systems when the animals were free of anaesthetic and associated drug effects.

Thus an attempt has been made to establish a relatively simple technique for the injection of substances into the cerebral ventricles of the conscious rat. This technique was then used to re-investigate the effects of a few of the substances with central transmitter function when injected into the cerebral ventricles of rats. Further, a study has been made of the effects of altered central transmitter levels on the actions of some drugs administered peripherally.

S E C T I O N T W O

EXPERIMENTAL METHODS.

EXPERIMENTAL METHODS

1. ANIMALS

The animals used in the experiments reported in this thesis were male Wistar rats, 200-250 g in weight, supplied by Scientific Products Farms Ltd., Ash, Canterbury, Kent.

2. ANIMAL HUSBANDRY

After receipt, all animals were maintained in the animal house until their weight was between 250-300 g. The animals were fed a conventional 41B cube diet (supplied by Pilsburys Ltd.) and allowed tap water ad libitum to drink. They were kept in groups of 5 in polypropylene cages, 20 x 12½ x 6 inches (supplied by Associated Crates, Stockport). After cannulation they were placed individually in similar cages to the above, and moved to an air conditioned room. The average animal house temperature was 21°C and that of the air conditioned room 21 ± 1.0°C. A normal light/dark cycle was maintained. All experiments were performed in the air conditioned room, between 9.00 a.m. and 9.00 p.m., unless otherwise stated.

3. DEVELOPMENT OF A CANNULA FOR INJECTION INTO THE LATERAL VENTRICLES

a) Previous methods

A number of methods have been designed to permit solutions directly into the cerebral ventricles of animals. (For references see Section 1, Chapter 3). The majority of methods involve the construction of cannula guides of varying complexity except where the mouse is the experimental animal.

A method for direct injection into the conscious rat has been described (Valzelli, 1964). A needle is pushed through a

lateral fissure in the skull of a lightly anaesthetised rat, and a simple injection made. The method has the advantage of simplicity and speed, all surgical procedures being eliminated. The disadvantages include - a) interference of effects caused by the anaesthetic, b) the effects of shock in the animal, and c) entry to the brain is made laterally, making it difficult to be sure of entering the ventricles on every occasion.

Of the methods involving the implantation of a cannula guide, that described by Hayden, Johnson & Maickel, (1966) appeared to combine the advantages of simplicity of construction and use, lightness, and a shape least likely to cause discomfort to the animal. Therefore it was decided to try this method.

b) Construction

At first the cannula guides were constructed following the method of Hayden et al (1966) exactly. This produced a perspex block with the dimensions 12.7 x 6.35 x 6.35 mm ($\frac{1}{2}$ x $\frac{1}{4}$ x $\frac{1}{4}$ inches) and 5 mm of a 20 gauge needle protruded below this block. It was found that a block of perspex of this size was too large for simple fixing and its use involved an unnecessary amount of surgery. A number of block sizes were then tried and the best dimensions found were 6 x 7 x 6.35 mm.

The guides were produced in batches of 20. All the necessary holes were drilled in a sheet of perspex 34 x 31 x 6.35 mm deep. (1 mm being allowed for the width of cut of a circular saw).

Considering the construction of 1 cannula guide, the following sequence was observed.

i) A hole was drilled through the centre of the block using a drill 0.57 mm (0.0225 inch) in diameter.

ii) The hole was expanded with a 1.0 mm (0.040 inch) drill to a depth of 2 mm.

iii) On the side away from the 1.0 mm expansion the centre hole was expanded with a 3.2 mm ($\frac{1}{8}$ inch) drill to a depth of 3.0 mm.

iv) 20 gauge stainless steel tube (No. 0 hypodermic needle) was cut to a length of 7 mm and placed in the 0.57 mm hole and sealed into position with epoxy cement (Araldite) leaving a 5 mm length exposed. With later cannulae (see Section 3, Chapter 2) a 4 mm length was left exposed. It was necessary to heat the cannula to a temperature of 50°C when curing the epoxy. At this temperature the epoxy becomes fluid before setting and flows into the small space between the stainless steel and the perspex, affording a firm fixing and a patent seal.

v) After the epoxy had set, the blocks of 20 cannula guides were cut into the individual cannula guides. The edges of each cannula were then rounded and any excess epoxy removed, using a small file.

vi) The 3.0 mm deep cavity at the top of the guide was then filled with a cold setting silicone rubber solution (Silescol, SR 300, Esco Rubber Ltd.).

vii) The needle for injection was made from a 26 gauge (No. 20 hypodermic) needle. Each needle was cut to a length of 11.32 mm and given a very flat bevel (45°) by grinding on an Arkansas wet stone.

viii) Before implantation, each cannula guide was cleared of debris by passing a 26 gauge needle back and forth down the lumen.

ix) In later experiments (see Section 3, Chapter 2) a stilette was paced down the lumen of the cannula. 20 gauge stainless steel wire was cut to lengths of 14.32 mm and the ends were rounded off. The wire was then bent at an angle of 90°, 3 mm from one end (or 4 mm from one end when the 4 mm length of cannula guide protruded below the block). The longer arm of the wire then was placed down the lumen of the cannula guide and only removed immediately prior to injection. (Fig. 1 shows diagram of cannula and stilette).

c) Implantation of the Cannula Guide

Wistar albino rats of 250-300 g were used. After this stage in their development there is very little increase in the size of the skull, which might cause the cannula to be lost.

i) The animals were anaesthetised with a gaseous mixture, containing 20% oxygen; 80% nitrous oxide and halothane (Fluothane). For induction 3.5% halothane was used and the anaesthesia maintained on 1-2% in accordance with the needs of the animal.

ii) The animal was placed in the apparatus shown in Fig. 2, the head being held in the brass ear bars. This was found to be the most satisfactory method of holding the animal during the drilling and placing of screws and cannula guide.

iii) A mid-sagittal incision was made from just caudal to the eyes and rostral to the ears. The underlying tissues were cleared to one side and the skull cleaned with a 1% solution of Hibitane in 70% industrial methylated spirits. This served to both inhibit bleeding and give a clean, fat-free, dry surface, giving better adhesion of dental acrylic.

iv) Using a No. 2 round dental burr, a hole was drilled at the position described in section d.

v) 3 holes were drilled around this point to take small stainless steel screws (1.4 mm in diameter) which tapped themselves into the bone.

vi) Dental acrylic was placed around the needle at the base of the cannula guide. The guide was then held between forefinger and thumb, with the lower surface of the perspex horizontal to the surface of the skull, and the needle lowered into place.

vii) Dental acrylic was then built up round both the guide and the 3 screws.

viii) After the cement had hardened the edges of the incision were sutured with cotton, and the animal given intraperitoneally 48 mg (80,000 units) sodium penicillin G plus 40 mg streptomycin base. (Crystamycin Forte; Glaxo).

ix) After a minimum of 5 days recovery the animals could be used.

It was found necessary to keep the animals in individual cages, as in groups they tended to interfere with each other's cannulae, leading to episodes of violence and even death.

Fig. 3 is a photograph of an animal 2 weeks after cannulation.

d) Co-ordinates

In placing the cannula guides, it was decided to attempt to eliminate the use of a stereotaxic apparatus, thereby simplifying the method and saving time. A point 2.5 mm lateral and 0.9 mm caudal to the bregma was located and the No. 2 round burr hole for accepting the guide drilled. In order to locate the point dividers were used to scratch marks on the skull at the required distances. In later stages it was found that experience enabled the operator to gauge the point by eye with equal accuracy. (See Fig. 4).

The top of the lateral ventricles at the point drilled was found to be 4.5-5.0 mm below the surface of the skull. With a 5 mm length of guide needle protruding below the cannula guide, the tip lay just in the ventricles, and initially a 5 mm length of guide was used. For reasons explained in Section 3, Chapter 2, this was later altered to 4 mm. With this length of guide needle the tip lay just outside the ventricles. The approximate site of injection is shown in Fig. 5.

e) Dimensional trials

In order to check the accuracy of placing the cannula, two methods were employed. In the first instance a 10% solution of indian ink (Pelikan ink) in normal saline was injected into the ventricles. The animals were then killed at varying intervals after the injection and the brain dissected out. A Pelcool freezing microtome was employed to section the brain and the extent of spread of the ink examined, by inspection of the cut surface of the tissue.

A similar technique was employed using Pontamine sky blue as the indicator. Since this is a vital dye it was taken up by the protein of either side of the ventricles and gave a very definite indication of the spread of an injected solution. In order to demonstrate the spread of the dye, photographs were taken using a 4X orange filter. The filter cut down on the red end of the spectrum and intensified the blue, thus showing the blue dye as a more definite black in the photographs. (See Fig. 6).

4. CONSTRUCTION OF A CANNULA FOR IMPLANTATION OF CRYSTALS IN DISCRETE AREAS OF THE BRAIN

a) Principles

In order to attempt to localise some of the effects observed on injection of substances into the lateral ventricles, a cannula was designed to place small amounts of active substances into specific areas of the brain.

When injecting substances into the lateral ventricles, a relatively large lattitude for error can be allowed due to the comparatively large area of the ventricles in the lateral plane. With the previous cannula the depth of penetration was limited by the rubber plug. Depending on the pressure applied the depth of penetration could vary by small amounts. Also the lateral placing of the cannula could vary by small amounts due to the variation of the position of the bregma relative to brain structures (DeGroot 1959).

It was decided to use a technique similar to that described by Grossman (1962). Small quantities of crystalline substance were tamped into the end of a stainless steel tube. The tube was then placed in the area to be investigated and the substance allowed to diffuse into the surrounding tissues. This method eliminated the damage that could be caused by pushing the crystals out of the end of the tube.

To push a small tube containing crystals through the rubber plug could cause the crystals to become dislodged or a plug of rubber to be taken into the tube thus blocking it. Since the tube had to remain in place for some tine in a conscious animal the rubber plug would not give a sufficiently firm fixing. Therefore a cannula that screwed into the guide was designed.

There are cannulae described in the literature suitable for this kind of work, (e.g. Grossman 1962; Booth, 1968), but as before simplicity of both construction and use was desired.

b) Construction

As previously described for the lateral ventricular cannulae these cannulae were made in blocks of 20 from sheets of perspex

34 x 31 x 6.35 mm deep. The final dimensions of the blocks being 7.0 x 6.0 x 6.35 mm deep.

Considering the construction of 1 cannula the following sequence was observed.

i) A hole was drilled through the centre of the block using a drill 0.57 mm (0.0225 inch) in diameter.

ii) The hole was expanded with a 1.0 mm (0.040 inch) drill to a depth of 2 mm.

iii) Underneath, away from the 1.0 mm expansion, the centre hole was expanded with a 3.2 mm ($\frac{1}{8}$ inch) drill to a depth of 3.5 mm.

iv) On the same side as the 3.2 mm expansion and in the corner of the block, a 1.0 mm hole was drilled to a depth of 3.0 mm to accept a trochar during implantation.

v) The 3.2 mm hole was threaded with a 4 B.A. tap.

vi) 21 gauge stainless steel tube (No. 1 hypodermic needle) was cut to the required length and placed in the 1.0 mm hole and sealed in position with epoxy cement as previously described.

vii) The brass boss of a No. 20 hypodermic needle was then turned down to a diameter of about 3.5 mm on a small bench lathe, for a length of 40 mm, at the end proximal to the needle. A section distal to the needle was turned to a diameter of 5.0 mm for a length of 3.0 mm.

viii) The section of the boss turned to 3.5 mm was then threaded with a 4 B.A. dye.

ix) The modified No. 20 needle could then be screwed into the perspex block, and the end of this needle cut to the length required.

x) The open end of the No. 20 needle was then sealed with solder or silicone rubber. The advantage of the latter was that it could easily be removed to facilitate the cleaning of the lumen of the needle. (Fig. 7 shows a diagram of the cannula).

c) Implantation

Rats weighing 250-300 g were anaesthetised as previously described.

i) The cannula guide with the cannula screwed in place, was fixed on a trochar in the stereotaxic apparatus, and the apparatus zeroed to the tip of the tube.

ii) The animal was placed in the apparatus with the ear bars in the external auditory meatus and the nose bar just caudal to the incisor teeth. The nose bar was 5 mm above the level of the ear bars in accordance with the De Groot (1959) stereotaxic co-ordinates.

iii) The skull was prepared as before.

iv) Using the De Groot atlas of the rat brain the apparatus was adjusted to indicate the point on the skull to be drilled for implantation into the desired area.

v) A No. 20 round burr hole was drilled to accept the cannula and 3 other holes drilled round it to accept stainless steel screws.

vi) The cannula and guide were lowered into position and fixed with dental acrylic.

vii) The operation was concluded as previously described.

d) Administration of crystalline compounds

i) A small quantity of the compound to be administered was placed on a glass microscope slide. This was then compressed by placing another slide on top of the first and applying pressure.

ii) The cannula was removed from the guide, cleared of all debris, and dried. The tip of the tube was pushed once into the layer of compressed drug (tamping of the drug into the cannula).

iii) The cannula was then replaced in the guide tube and screwed home.

e) Dimensional trials

The co-ordinates used were dependent on the area to be investigated, and will be discussed in a later section.

To determine the accuracy of placement, crystalline pontamine sky blue was tamped into the end of the cannula. The brain was then dissected out, sectioned and the location of the cannula tip confirmed, by visual inspection of the cut surface.

5. RECORDING OF BLOOD PRESSURE IN ANAESTHETISED RATS

Animals were anaesthetised with pentobarbitone sodium 60 mg/kg. An incision was made in the neck of the animal and one carotid artery cannulated with polypropylene tubing (PP25 or PP30; Portex) filled with

heparinised saline. The tubing was connected to a blood pressure transducer and the pressure recorded on a 4 channel pen recorder. (Devices, using Sub.1C and DC.2C pre-amplifier and amplifier).

The animal was then placed in the head holding apparatus shown in Fig. 2. Since a non-gaseous anaesthetic was used, a rod was placed caudal to the incisor teeth to support the rostral end of the head. If the animal's head was not held in this way, transient changes in blood pressure were seen on administration of control intraventricular injections.

Connections were made to the transducer using disposable nylon 3-way taps. A 1 ml syringe was fitted to the side arm of the transducer, filled with saline containing heparin (0.001%). In the event of the cannula becoming blocked, or blood flowing back up the tube due to slight leakage, the system could be flushed out using the syringe.

In all these experiments the contralateral jugular vein was also cannulated with polypropylene tubing (PP25 or PP30), for the administration of intravenous injections.

6. RECORDING OF BLOOD PRESSURE IN CONSCIOUS RATS

Methods have been published for the recording of blood pressure in conscious rats (Still, Pradhan and Whitcomb, 1956; Popovic & Popovic, 1960; Weeks & Jones, 1960; Weeks & Davis, 1964; Thuranszky, 1966; Dawson, 1968, Fujita & Tedeschi, 1968). The methods of Weeks seemed to be excessively complicated and to necessitate extensive surgery to the animals. Those of Fujita (1968) and Dawson (1968), using the tail artery, could only be made to work in a very small proportion of the animals.

A method very similar to that of Popovic and Popovic (1960) was used. Cannulae were made from PP25 tubing. The tubing was cut to lengths of about 15 cm, one end being slightly sharpened. The cannulae were sterilised by soaking in a solution of Chlorhexidine (Hibitane 1%) in 70% industrial methylated spirits. They were then washed and filled with heparin (Pularin; heparin injection B.P.; 1,000 i.u./ml. One end of the tube was blocked by placing a tight fitting pin down the lumen.

Rats were anaesthetised with nitrous oxide, oxygen and halothane, placed on their backs and an incision made on the left hand side of the throat. The left hand carotid artery was exposed, the cannula pushed a short way down the artery and securely tied in with thread. A stainless steel tube 2 mm in diameter was then pushed under the skin from the back of the neck to the incision. The cannula was passed down this tube and then the steel tube withdrawn - leaving the cannula protruding from the stab wound in the skin caused by the tube. Both wounds were then sutured and the animal given the antibiotic treatment described in Section 2, part 3, paragraph VIII. With this preparation recordings of blood pressure could only be made for about 24 hours after cannulation due to occlusion of the carotid artery.

In order to overcome this effect the cannula was pushed down the left carotid artery into the aortic arch and thence into the aorta. By pushing the cannula from right to left across the animal's body entry into the aorta was ensured. If the cannula was not pushed to the left it very often went into the heart.

With this preparation recordings of blood pressure could be made for up to 3 weeks. However a large number of the animals pulled the cannula out of the aorta, in spite of it being securely tied into the tissues of the neck. The reason for this was that the surface of the

polythene was extremely smooth and slippery. In order to secure the cannula more securely, a bubble was placed in the polyethylene at about the point where it was tied into the tissue. The bubble was formed by heating the tube in a stream of hot air, whence the walls of the tube swelled, leaving the lumen the same size or larger than normal. The stream of hot air was generated by placing a micro-burner under a brass tube, $\frac{1}{2}$ inch in diameter and 12 inches long, inclined at an angle of 45^{o}. The temperature of the air leaving the tube was dependent on the size of the flame and its position on the tube.

This method proved extremely successful though a small proportion of the animals contrived to either bite the end off the tube, or to pull out the pin, which resulted in their death.

Prior to recording, the PP25 tubing was clamped with artery forceps, the pin removed and a length of PP60 tubing pushed over the exposed end. A pressure-tight joint was thus made. The PP60 tube was connected to a blood pressure transducer and blood pressure recordings were made as for the anaesthetised animals.

During recording the animal was kept in an individual cage with a wire mesh lid, through which the recording tubes could be led. It was advisable to have the animals thoroughly familiar with this environment and set up, and also to have handled them as much as possible before any experiments were carried out.

All these animals also had intravenous cannulae implanted, as described in the next section.

7. INTRAVENOUS INJECTION OR INFUSION OF SOLUTIONS INTO CONSCIOUS ANIMALS

Cannulae identical to those described for arterial blood pressure recording, were placed in the jugular veins of rats, so that the tip of the tube lay at the junction of the right superior and inferior vena cavae.

For intravenous injections, the PP25 was clamped with rubber covered forceps and, using a length of 26 gauge stainless steel tubing, a junction made with an extension length of PP25 tubing. Injections were made into this tube with a 1 ml syringe fitted with a No. 20 hypodermic needle.

For intravenous infusions, the jugular cannula was attached to a similar extension of PP25 tubing, connected to a 5 ml syringe. This syringe was fitted into a Palmer automatic injection apparatus set with a ram rate of 1 inch in 160 minutes. This gave an infusion rate of 1 ml per hour. A simple modification of the Palmer apparatus enabled 5 syringes to be operated at one time. The animals were kept in a metal box with 5 individual compartments. The tubes were held above the animals heads by passing them over a bar. A Plasticine weight on the tube (the other side of the bar) maintained a reasonably constant tension on the tube (See Fig. 8). No effects could be observed in animals infused with 1 ml per hour of pyrogen-free sterile physiological saline, for up to 8 hours.

8. INFUSIONS INTO THE CEREBRAL VENTRICLES OF CONSCIOUS RATS

Methods have been described for the long term infusion of the cerebral ventricles of the rat brain (Myers, Casaday & Holman, 1967;

Booth, 1968; Goodrich, Greehey, Miller & Pappenheimer, 1969). The methods all involve the use of differently designed cannula guides.

In the present experiments the previously described cannula guide for injections into the cerebral ventricles was used. Instead of using a simple syringe plus needle, a length of 26 gauge needle was cut from a No. 20 hypodermic needle and fixed to the end of a length of PP25 tubing. The end of the tubing was heated in a stream of hot air as previously described. This served to fix the needle firmly in the end of the tube and also to expand the end of the tubing to give a more substantial shoulder to be pushed against the rubber bung in the cannula guide. The length of 26 gauge tubing protruding from the end of the PP25 was the same as the length of the needle used for intraventricular injections (11.32mm).

1 ml glass syringes fitted with No. 20 hypodermic needles were placed in a Palmer automatic injection apparatus set with a ram drive of 1 inch in 60 minutes, giving a rate of infusion of 2.8 µl/min. This rate was chosen since the rate of production of rat cerebrospinal fluid is about 3 µl/min (Cserr, 1965). The PP25 tubing was attached to the 1 ml syringes and the rats placed in the same box used for the intravenous infusions.

The modification of the Palmer automatic injection apparatus enabled both intravenous and intracerebral infusions to be carried out simultaneously in the same animal, with 5 animals to a group. (Fig. 8 shows a photograph of this apparatus).

9. MEASUREMENT OF CORE TEMPERATURE IN CONSCIOUS RATS

Initially it was decided to record the oesophageal temperatures of the rats. To repeatedly push an oesophageal temperature probe

(Light Laboratories Ltd.) down the throat of a rat caused distress to the animals to the extent that it made them difficult to handle. Conversely to place the same probe into the rectum of the animals had considerably less effect on them and after two or three measurements had been made, the procedure was very well tolerated.

The oesophageal probe mentioned above was lubricated with liquid paraffin B.P. and placed at least 6 cm into the rectum (See Lomax, 1966). On repeated insertions in one animal at depths up to 5 cm, readings could vary by as much as 0.5°C. With repeated insertions in one animal to a depth of 6 cm, constant readings of body temperature could be obtained. When the probe was inserted to depths less than 5 cm, a reading lower than the true core temperature was obtained. This reading decreased with decreasing depth of insertion. With a depth of insertion greater than 6 cm the reading was constant, up to the maximum depth tried of 8 cm.

10. MEASUREMENT OF CHANGES IN THE NOCICEPTIVE THRESHOLD IN RATS

a) The foot pressure method

A method for measuring changes in the nociceptive threshold by applying pressure to the rats paw has been described by Randall and Selitto (1959).

A device for applying pressure to the rats paw is commercially available. (The Analgesy-Meter; Ugo Basile, Italy). A photograph of the apparatus is shown in Fig. 9. An electric motor M drives a worm screw A, which moves the weight B along the arm C. The arm C is pivoted at F. As the weight B moves along the arm the pressure under the Teflon cone E increases. The

animal's foot is placed on the block G and the cone placed on the upper surface of the paw. The electric motor is activated by the operator, using a foot pressure switch.

The end point was when the animal made its first definite attempt to remove the foot from the point of stimulus. If the attempt was violent the foot slipped easily from under the Teflon cone, without being damaged. The operator then released the pressure switch, and the final weight applied to the animal's foot was indicated on the scale S. The range of pressures could be varied considerably by the addition of extra weights to the basic sliding weight.

If weights greater than 500 g were consistently placed on the foot of a rat, damage occurred, the foot swelled and the nociceptive threshold changed. In obtaining a dose response curve for morphine, it was noted that if a treated animal did not react to a weight of 500-600 g, then in most cases very large weights had to be applied to get a response or alternatively there was no response at all. Therefore a maximum weight of 500 g was used in all experiments.

When using narcotic analgesics it was not necessary to use the inflamed paw, as originally described by the Authors of this method. When investigating non-narcotic analgesics, it was necessary to cause inflammation in the paw. This was produced by the injection of 0.1 ml of a 20% suspension of bakers yeast into the plantar surface of the paw. Tests for changes in the nociceptive threshold were conducted 2 hr after the yeast injection since at this time pain sensitivity had reached a maximum.

Only small numbers of animals were available, and therefore it was not possible to select groups in which the individual members had similar basal nociceptive thresholds. Therefore the analgesic index described by Cox, Ginsburg & Osman, (1968) was used, and this accounted for individual variations in basal nociceptive thresholds.

The analgesic index is calculated by

$$\Delta_{obs}/\Delta_{max},$$

where $\Delta_{obs} = P_{obs} - P_i$

and $\Delta_{max} = P_{max} - P_i$ (P_i, P_{obs} and P_{max}

are the pressure thresholds recorded before drug treatment, at times after drug administration and the maximum pressure, respectively). In all experiments where alteration in the nociceptive threshold was determined, the results were recorded as the analgesic index.

The standard error of the mean of each group of animals was calculated unless there were two or more animals in the group with analgesic indices greater than 1.0.

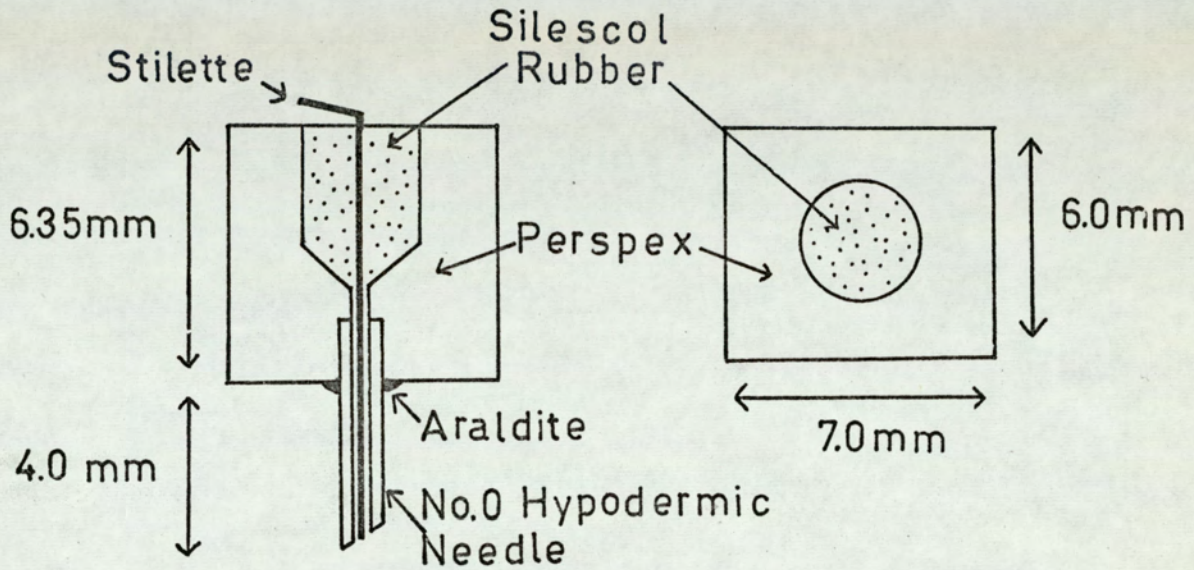

FIGURE 1 VERTICAL AND PLAN VIEWS OF THE CANNULA GUIDE IMPLANTED INTO THE LATERAL VENTRICLES OF RATS

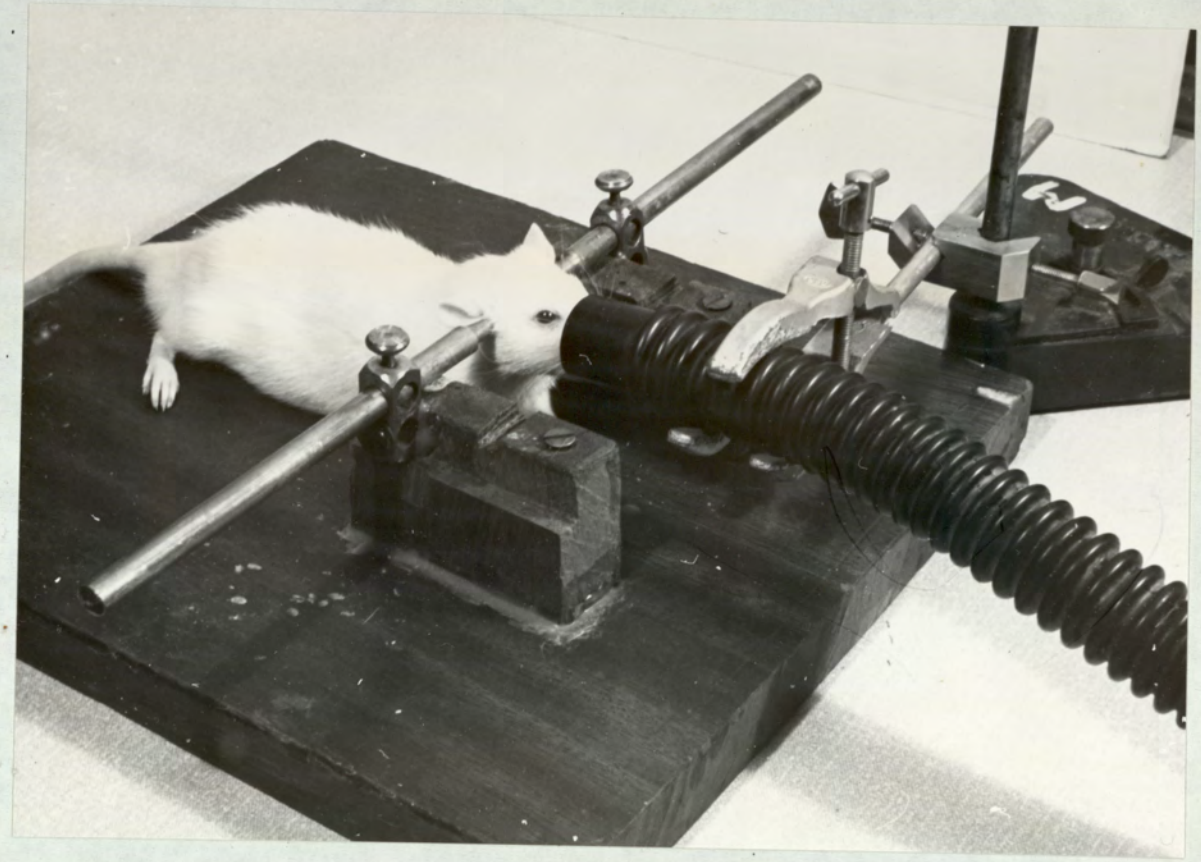

FIGURE 2 PHOTOGRAPH OF APPARATUS USED TO HOLD THE RATS' HEADS DURING IMPLANTATION OF THE CANNULA GUIDES

The corrugated tube over the animal's nose leads from a gas anaesthetic apparatus giving a mixture of O_2/N_2O and halothane. When blood pressures were recorded from pentobarbitone anaesthetised rats, a bar was placed under the incisor teeth and the tube dispensed with.

FIGURE 3 PHOTOGRAPH OF A CONSCIOUS RAT 2 WEEKS POST-CANNULATION

On the right hand side of the photograph the skull of a similar rat with cannula guide attached is shown.

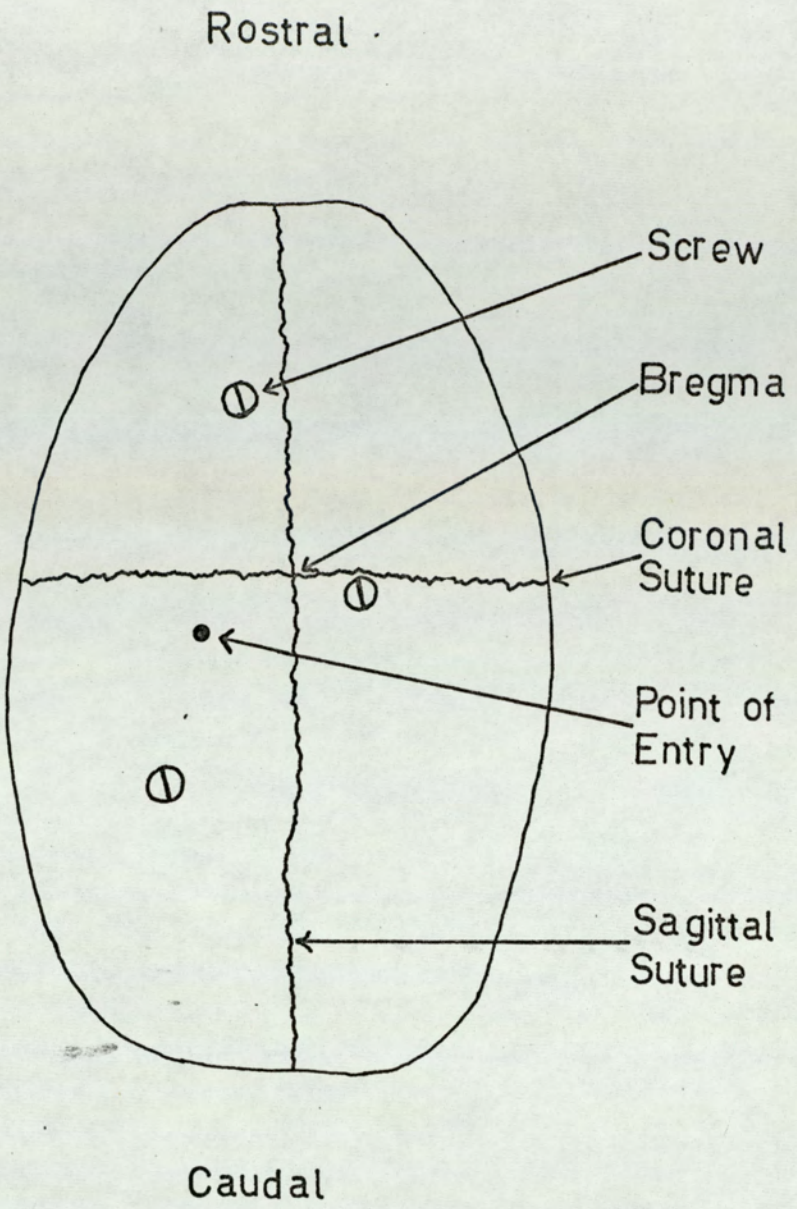

FIGURE 4 PLAN VIEW OF THE AREA OF THE SKULL EXPOSED DURING IMPLANTATION OF THE LATERAL VENTRICLE CANNULA

The diagram shows the point of entry of the cannula, and the positions of the stainless steel screws.

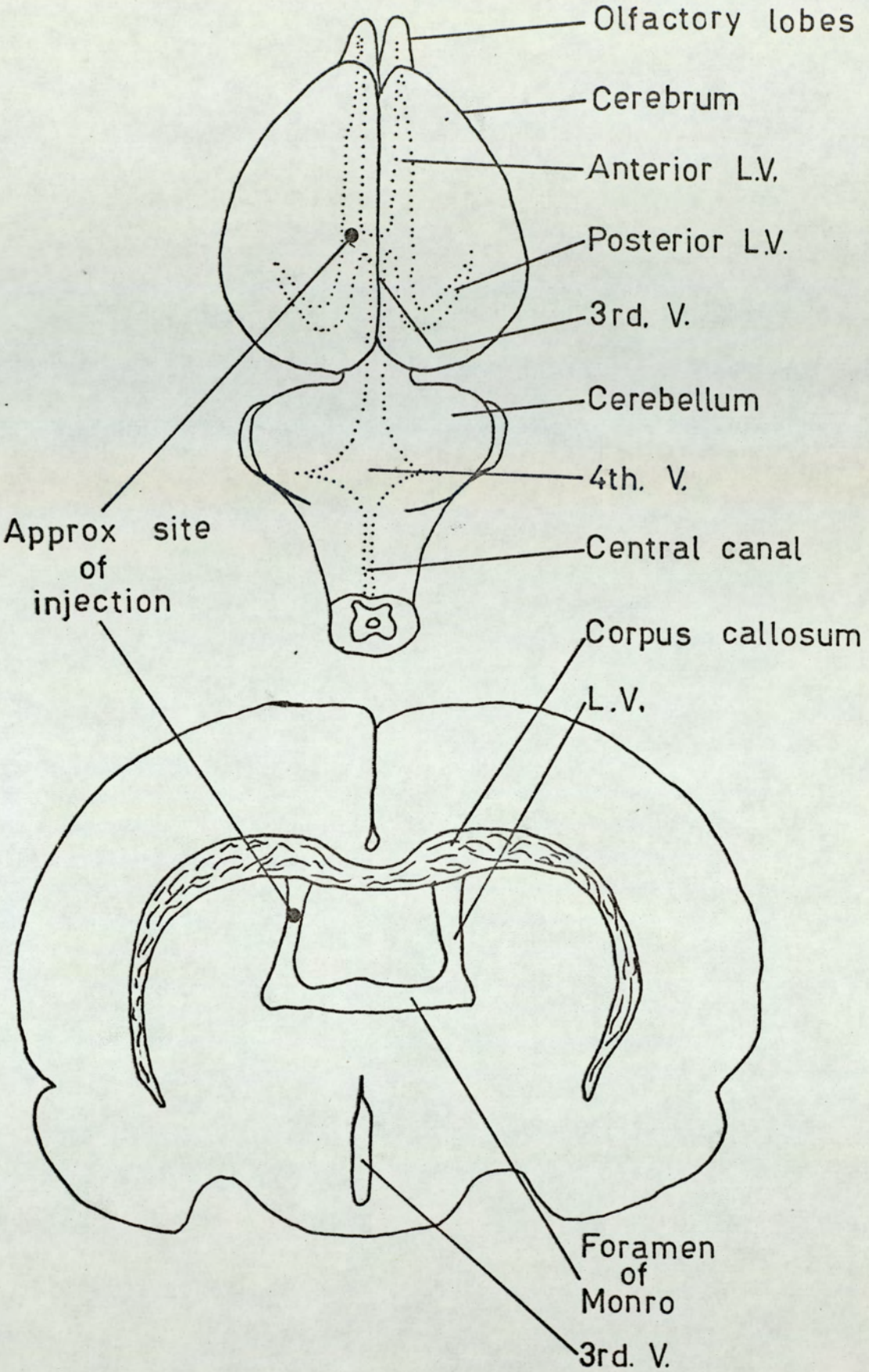

FIGURE 5 DIAGRAMS TO SHOW THE SITE OF INTRAVENTRICULAR INJECTION

Upper diagram: dorsal view of whole brain.
Lower diagram: coronal section at about A 6.2 (co-ordinate according to the de Groot (1959) stereotaxic system).

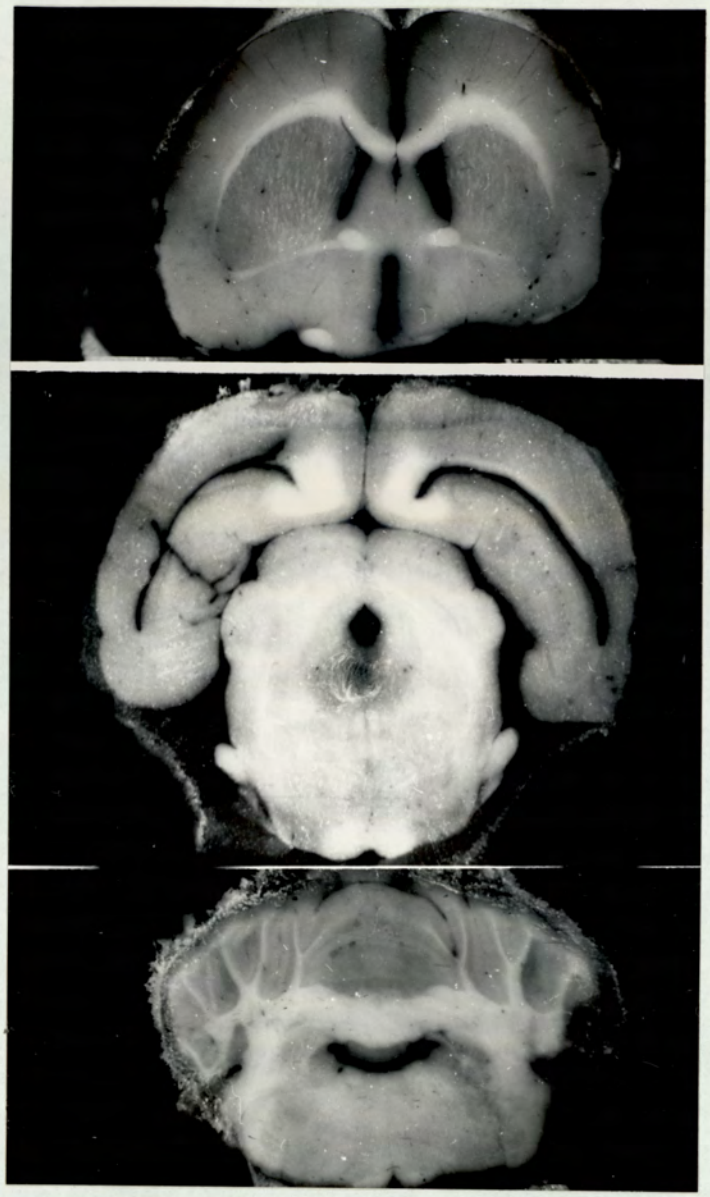

FIGURE 6 PHOTOGRAPH SHOWING THE DEGREE OF SPREAD OF PONTAMINE SKY BLUE THROUGH THE VENTRICULAR SYSTEM 30 MINUTES AFTER INTRAVENTRICULAR INJECTION

Top section shows dye in the lateral and 3rd ventricles at A 7.0 (rostral to injection site). Middle section shows dye in the lateral ventricle and in the aquaeduct at A 1.8. Lower section shows dye in the 4th ventricle at A 2.8. (Co-ordinates according to the de Groot (1959) stereotaxic system).

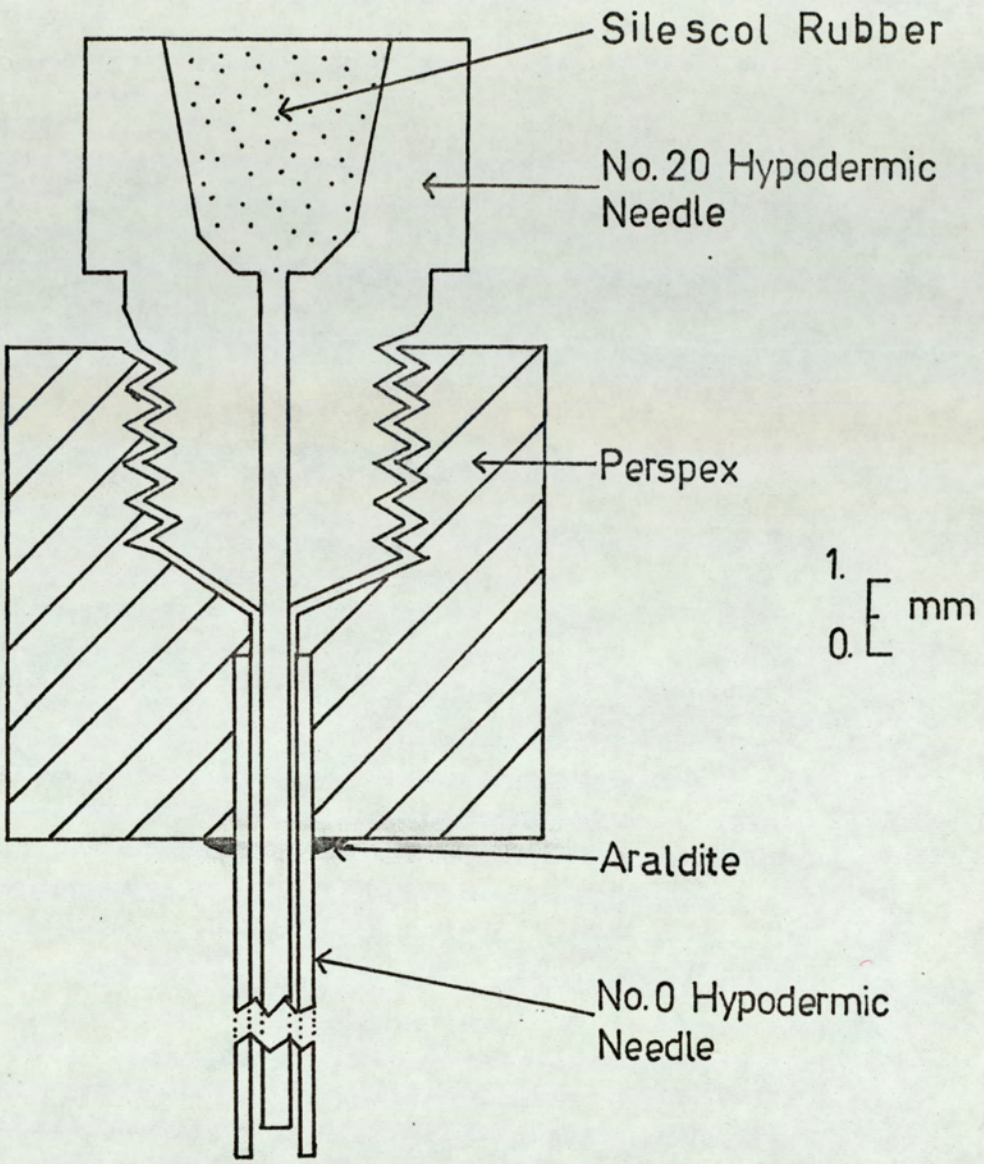

FIGURE 7 PLAN DIAGRAM OF THE CANNULA USED FOR THE IMPLANTATION OF SOLID AGENTS INTO SPECIFIC AREAS OF THE BRAIN

The length of the cannula was dependent on the location of the point to be stimulated.

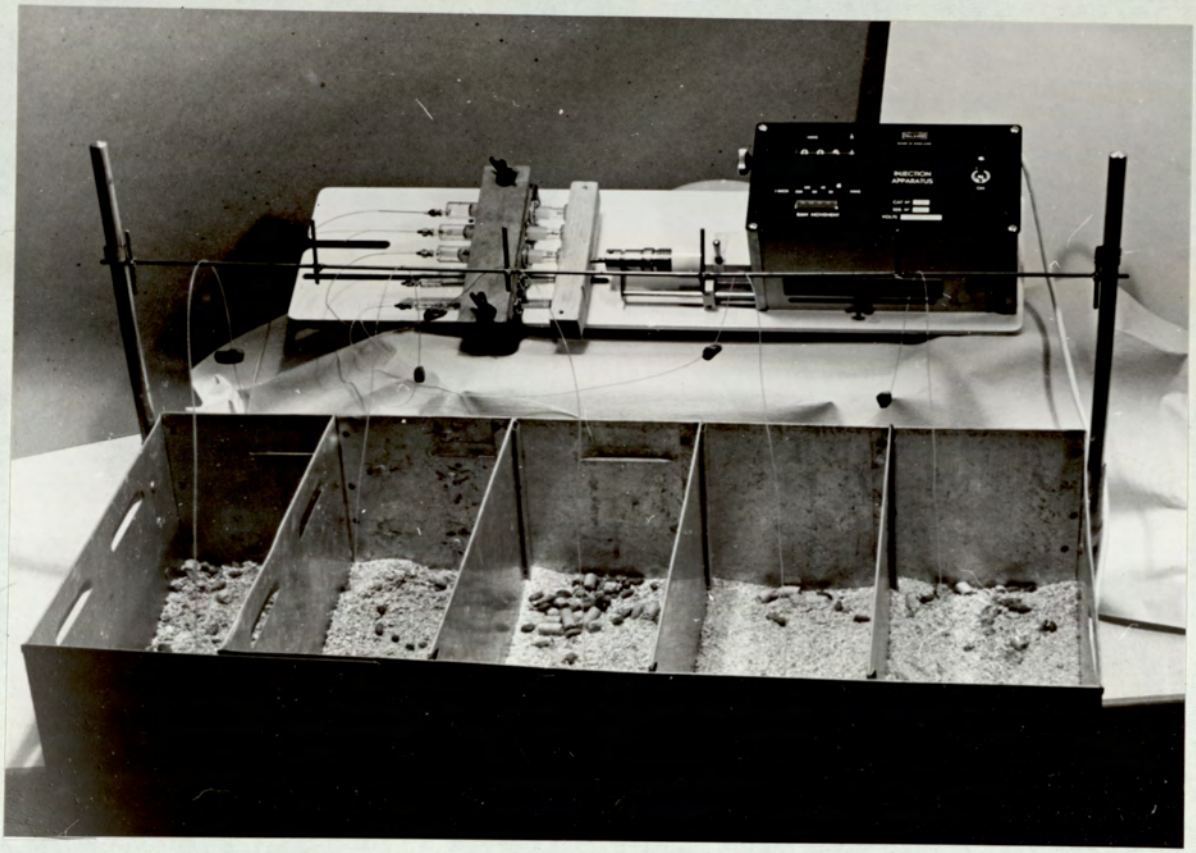

FIGURE 8 PHOTOGRAPH OF THE APPARATUS USED FOR INTRAVENTRICULAR AND INTRAVENOUS INFUSIONS

The syringes were driven by a Palmer slow injection apparatus, modified to hold a number of syringes. The box in the foreground contained one rat per compartment.

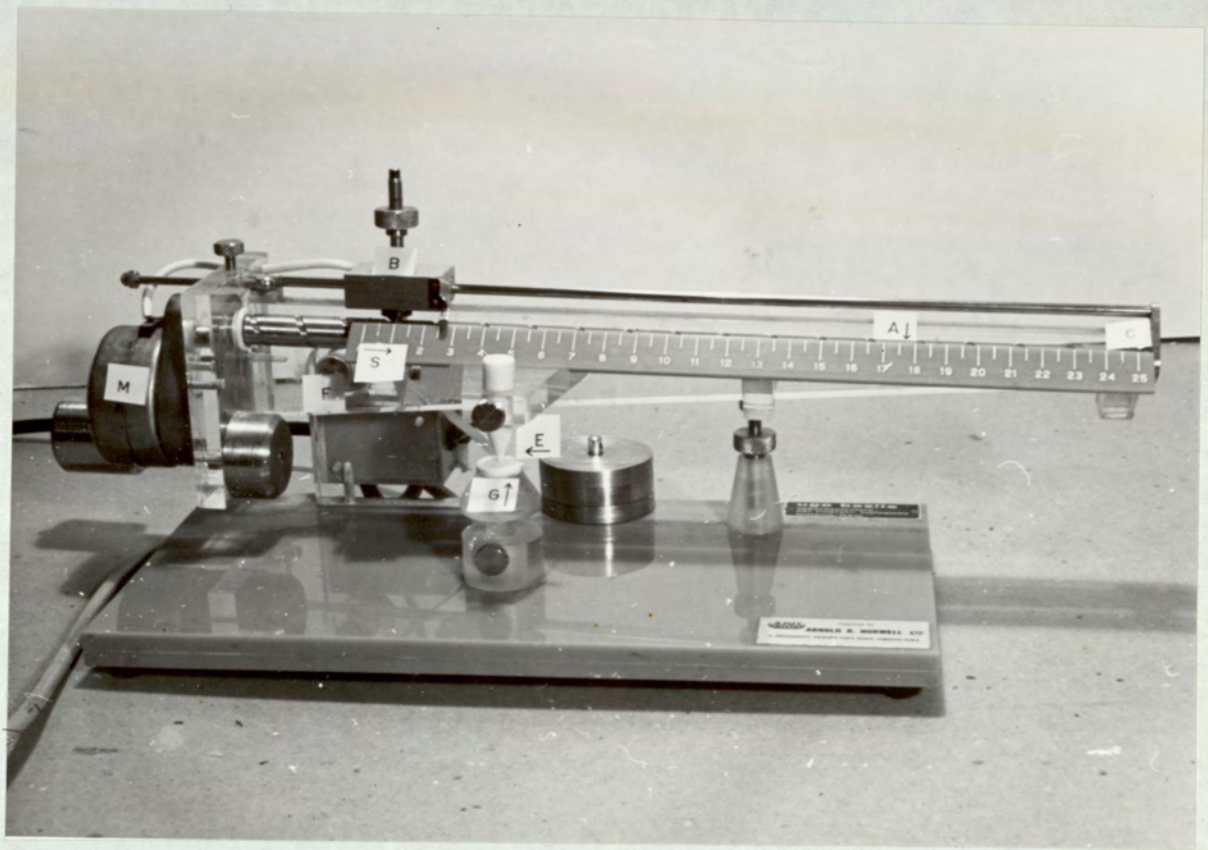

FIGURE 9 THE ANALGESY METER FOR DETERMINING THE NOCICEPTIVE THRESHOLDS OF RATS' FEET

(See text for key)

SECTION THREE

EXPERIMENTAL RESULTS

CHAPTER ONE

BEHAVIOURAL EFFECTS

BEHAVIOURAL EFFECTS

GROSS BEHAVIOUR

1. EFFECTS OF IMPLANTATION OF A CANNULA GUIDE INTO THE LATERAL VENTRICLES

For about 24 hours after implantation the animals were in a hypersensitive state and were difficult to handle. An improvement was noted about 4 days after implantation, and within 7 days the animals appeared normal to all outward appearances. They could not be distinguished from animals that had had a similar amount of handling but which had not been cannulated.

At approximately 5-6 weeks post cannulation a small proportion of animals developed strange behavioural characteristics. The syndrome manifested itself in a number of ways: some animals would sit on their hind legs with forepaws raised, rythmically waving the forepaws in front of their noses; other animals would lie in an unnatural position in the bottom of the cage, motionless and exibiting exopthalmos. A more violent manifestation was episodes of rushing round the cage with no attention paid to self-inflicted damage; in their agitation a few animals raised the lid of the cage (including a 500 ml water bottle) although escape did not appear to be their intention. Some animals would lie prone in the bottom of the cage and roll over at a great rate, usually in a direction away from the side of cannulation.

As soon as any such syndrome appeared, the animals were killed, and the brains removed for macroscopical and microscopical examination. In all cases large fluid-filled cavities were found in the brain. These hydrocephali were always associated with the ventricular system and spread both caudally and rostrally from the point where the ventricles were penetrated by the cannula. There did not appear to be any correlation between the size of the lesion and the violence or nature

of the animal's reaction.

Fig. 10 shows photographs of the sectioned brain of an animals that had started to exibit the violent syndrome. When the first symptoms appeared pontamine sky blue solution was injected into the ventricles and the animal was killed. The brain was then sectioned as described in Section 2. The hydrocephalus can be seen to spread from a point rostral to the injection site (upper photograph) and throughout the lateral ventricular system on one side of the brain (the injection side). However it is not seen in the 3rd or 4th ventricles. Fluid could be removed from these cavities if dissection had been carried out with the brain frozen. Examination of the fluid revealed a clear colourless liquid which on microscopical examination did not contain any cellular matter.

The presence of hydrocephalus was always preceded by an elevated basal core temperature of the animal. This hyperthermia always appeared prior to the components of the syndrome, though hydrocephalus could not always be seen on section of the brain of an animal - despite being sacrificed as soon as an elevated core temperature could be demonstrated.

2. INJECTION OF STERILE SOLUTIONS INTO THE CEREBRAL VENTRICLES

a) Sterile pyrogen-free normal saline

NaCl solution, 0.9% w/v was injected in volumes of 5-100 µl. Volumes of 5-50 µl had no obvious effect on the animals; they did not react to the injection any more than animals given a similar amount of handling or to having a cannula passed down the guide without injection. The rate of injection also had no effect.

When volumes of 100 µl were given the rate of injection had a slight effect. Thus, rapid delivery of 100 µl often elicited immediate vocalisation and struggling. After the injection many animals frequently shook their heads, groomed the vibrisae with the forepaws and then retired to a corner of the cage and remained there for periods of up to 2 minutes. Contrary to this, animals given small volumes always indulged in a short period of exploration on return to the cage, following the same pattern as animals that had simply been handled.

The length of the cannula guide had no effect on the behaviour. That is, when 3 mm guide tubes were tried, the penetration of 2 mm of brain tissue by the cannula had no observable effect.

Initially a stillete was not placed down the lumen of the cannula guide. In such cases, occasionally resistance could be felt on pushing the cannula down the guide. This resistance could be overcome with pressure which almost invariably induced behavioural changes in the animal. These varied from violent episodes, similar to those associated with hydrocephalus, to a very much prolonged version of the grooming of the vibrissae and period of quiet, similar to those associated with large volumes of saline. The core temperature of the animals was almost always raised after such an episode. All these effects were overcome by the use of a stilette as described in Section 2.

b) Noradrenaline

Doses of 10-80 µg of NA injected in a volume of 10 µl, produced a dose-related syndrome. The animals appeared depressed with reduced reflex and motor activity. There was also a state that gave the animal the appearance of being "hypnotised". Animals would

crouch in the cage, motionless and 'staring'. The higher doses (20-80 μg) produced vocalisation in the form of a prolonged high pitched squeak, and there was considerable muscular relaxation. The animals would lie full length in the cage with all motor and abdominal muscles relaxed. Responses were easily obtained to painful stimuli, avoidance activity being necessarily feeble, but vocalisation was considerably increased.

After 10-15 minutes, hyperphagia appeared. With high doses (20-80 μg) the animals would move slowly to any food on the bottom of the cage and eat. If there was no food on the floor they would eat faeces. With low doses (10-20 μg) animals would move to the food hopper to eat.

There was no anaesthetic-like condition as described in the cat (Feldberg & Sherwood, 1954); rather the animals were hypersensitive to painful stimuli.

Adrenaline had similar effects except that higher doses were necessary (40-100 μg) to elicit similar hypnosis, and hyperphagia was only observed in a few animals.

Subcutaneous injection or intravenous infusion of adrenaline or noradrenaline, over a period of 1 hour giving 80 and 100 μg respectively, had little or no effect on the animals except to reduce locomotor activity. Larger amounts of 300-600 μg, which were approaching lethal doses, caused hyperventilation, piloerection and an appearance of exaustion.

c) 5-Hydroxytryptamine

5-HT produced a dose-related response, in doses of 1-50 μg. A state that gave the animal the appearance of being "hypnotised"

was observed. The effect on muscular tone was the opposite of that observed with NA, high doses (15-50 μg) causing the animals to crouch in a 'tense' state. With these high doses stimulation such as pinching the tail, tapping the cage with a pencil or poking the animal caused it to rear on its hind quarters, with forepaws raised as if in defence, where it would remain for some time after removal of the stimulus. Doses very much in excess of 60 μg were toxic, causing death - apparently by respiratory depression. A few animals given these high doses would exhibit toxic effects similar to those seen in animals with hydrocephalus.

Low doses (1-10 μg) had very little effect. In a few animals slight depression was seen but the majority of animals were not significantly different from the controls.

Subcutaneous injection or intravenous infusion of 5-HT over a period of 1 hour, using doses up to 100 μg, had little effect. The highest dose (100 μg) did cause some behavioural depression when given by intravenous infusion, but it simply took the form of reduced locomotor activity.

d) Dopamine

Dopamine injected in doses up to 100 μg had no detectable effect on the behaviour of the animals.

e) Cholinergic compounds

i) Acetylcholine

Doses of Ach up to 100 μg were injected into the ventricles. Very little effect was seen except for a transitatory depression (5-10 minutes) at the 100 μg level.

ii) Carbachol

Carbachol was injected into the ventricles in doses from 0.2-3.2 μg. Doses of 0.2-1.6 μg caused the animals to become akinetic. With the low doses the animals simply remained static with muscles tense, and exhibiting slight exopthalmos. However with higher doses, a more severe manifestation of this state was seen, there being very little reaction to auditory or mechanical stimulation. If the animals were placed in abnormal positions they would take evasive action and once again become still. Occasionally slight tremor was observed at the higher doses. The nociceptive threshold was elevated at these doses, and at 1.6 μg there was no definite response to nociceptive stimuli.

Doses of 3.2 μg were toxic, often causing the death of the animals. The toxic syndrome took the form of considerable salivation, red 'tears' and episodes in which the animal appeared to attempt to burrow, or lay on its side with all limbs in violent movement.

These toxic effects were very similar to those following subcutaneous injection of carbachol using doses of 1-2 mg/kg (300-600 μg).

iii) Oxotremorine

Oxotremorine was injected into the ventricles in doses ranging from 2-80 μg. With doses from 2-20 μg a state very similar to that seen on injection of 0.2-1.6 μg of carbachol was seen. With higher doses (20-80 μg) the animals became relaxed and ataxic, there was considerable

salivation and the production of red 'tears' was more pronounced. With carbachol motor activity was produced when the animals were disturbed, but this was not seen with oxotremorine and the animals appeared depressed. Elevation of the nociceptive threshold was seen at all doses in excess of 4 µg.

Subcutaneous injection of 1-2 mg/kg (300-600 µg) of oxotremorine produced a syndrome very similar to that seen with intraventricular injection of 80 µg.

3. INFUSIONS OF SOLUTIONS INTO THE CEREBRAL VENTRICLES

Cerebrospinal fluid is manufactured at a rate of about 3 µl per minute in the rat (Cserr, 1965). Solutions were therefore infused at a rate of 2.8 µl per minute.

Saline infused at this rate for up to 8 hours had no observable effect upon the behaviour of these animals. On being placed in their boxes, after the infusion tubes had been attached the animals would carry out the normal 2-3 minute exploration and then settle down. It was found essential to accustom the animals to the box in advance otherwise the customary long spells of exploration resulted in considerable twisting of the tubes.

a) Noradrenaline

Animals were infused at dose rates of 1.0, 0.5 and 0.25 µg/rat/minute, for periods up to 3 hours.

0.25 µg/minute had the effect of causing mild hyperphagia within about 30 minutes, which continued for 1-1½ hours. There was little muscular relaxation or vocalisation. After 1-1½ hours the hyperphagia ceased and the animals became unusually restless,

wandering round and round the cage. This continued until the end of the infusions. The animals appeared normal within about 1 hour of the infusion terminating.

0.5 μg/minute caused similar effects only they were considerably more obvious. The hyperphagia started very much earlier and there was vocalisation. The obvious muscular relaxation did not inhibit the eating. The excitement phase, which again appeared after 1-1½ hours, was often so violent that the infusion had to be terminated. The activity was of an excessively restless nature, occasionally resulting in episodes of violence with the animal throwing itself around, and out of the cage. Again the animals appeared normal within 1-1½ hours of the end of the infusion.

At the 1.0 μg/minute dose level, the vocalisation and muscular relaxation were very much more intense. Hyperphagia was not so apparent as at the 0.5 μg/minute level - possibly because the animals were unable to reach the food hopper. The phase of hyperactivity came on at the same time as with the other doses, but was less violent than at 0.5 μg/minute. The activity took the form of a stereotyped exploratory behaviour, with the animals observing features of the cage in a repetitive manner, and briefly looking over the top. Loud sounds (e.g. finger clicks) or movements elicited violent responses. The animals were sedated 30 minutes after termination of the infusion and were normal within 2-3 hours.

No reduction in the nociceptive threshold was noted at any dose.

b) 5-Hydroxytryptamine

Again animals were infused at rates of 0.25, 1.05 and 1.0 μg/rat/minute, for periods up to 3 hours. 0.25 μg/minute had very

little effect except to 'tranquillize'. The animals would retire to a corner of the cage and remain there. They were quite rousable, did not appear to be in a "hypnotised" state and, although the nociceptive threshold was slightly raised for part of the infusion, there were no strong increases in the nociceptive threshold. The reaction to auditory stimuli was reduced, involving turning the head to the direction of noise or moving the pinnae, rather than the startled jump in the case of the controls.

0.5 μg/minute caused a marked sedation to appear within 5-10 minutes, the symptoms being very similar to those of the acutely injected animals. Lack of response to nociceptive stimuli appeared at about 25 minutes and got stronger until by 35-40 minutes, pinching the tail, and foot pressure caused a very much delayed response. This elevation of the nociceptive threshold faded slowly from a peak at about 1 hour until at 2-2½ hours the threshold was normal. This was accompanied by some fading of the tranquillization but at the end of the infusion the animals were still quiet.

1.0 μg/minute caused the same rearing on the hind quarters seen with the large injections. These lasted for about 35 minutes and the animals then became very difficult to arouse at all. There was little or no response to nociceptive stimuli within 30 minutes of the start of the infusion and responses only reappeared towards the end of the 3 hour infusion.

At all 3 dose levels recovery from the effects at the end of the infusion, was extremely rapid. Animals were apparently normal within 20-30 minutes of the end of the infusion.

c) Dopamine

Dopamine was infused at the rate of 1.0 μg/rat/minute for 4 hours.

Very little effect was seen though at 2-2½ hours after the start of the infusion the animals became slightly hyperactive, when compared with the controls. By about 3½ hours this hyperactivity had disappeared and the animals gave the appearance of trying to go to sleep and being unable to do so.

d) Acetylcholine

Ach was infused at rates of 0.5 and 1.0 μg/min for 3 hours.

0.5 μg/minute had very little effect and the animals did not appear to be significantly different from the saline controls, though there was a slight elevation of the nociceptive threshold.

Infusion of 1.0 μg/minute depressed the animals. They were slow to move, but did wander slowly around the cages on occasions. There was considerable elevation of the nociceptive threshold. This latter effect returned to normal with remarkable rapidity at the end of the infusion, being normal within 5-10 minutes. The recovery from depression was similarly rapid.

4. DISCUSSION

The formation of hydrocephalus in rats with permanently implanted cannulae has been reported previously (Goodrich, Greehey, Miller & Pappenheimer, 1969). Similar formations were found in this study. The hydrocephalus usually developed after about 6 weeks, except that if an animal was only cannulated (and no injections subsequently given via

the cannula) then the animals could survive very long period without such abnormalities occurring. The development of the hydrocephalus was thus associated with the repeated injections rather than implantation of the guide. Although the reasons for the phenomenon were not closely investigated they could have been due to small particles or minute hairs in the injection fluid, or blood clots formed on penetration of tissue by the cannula, blocking the normal channels of exit for the CSF. Alternatively the effect may have been due to hyperformation of CSF or to infection. However it is difficult to explain the fact that the abnormality was always confined to the cannulated lateral ventricle, since increases in pressure or infection would have been expected to affect the whole ventricular system.

Animals were normally killed either with pentobarbitone or by inhalation of chloroform and in a number of experiments morphine was administered to the animals. It was noticed that animals with hydrocephalus were unusually susceptible to the effects of these drugs. Anaesthetics have been shown to increase the toxicity of 5-HT (Correll, Lyth, Long & Vanderpool, 1952) and the effect of morphine is potentiated by 5-HT. (See Chapter 3). This is interesting in view of the finding that 5-Hydroxyindole acetic acid (5-HIAA), a catabolite of 5-HT, has been found in large quantities in hydrocephalus and brain injury in humans (Stacey, 1968).

The causes of the behavioural effects associated with hydrocephalus were not studied closely, but a number of facts could have been involved such as abnormal intracranial pressures, altered brain metabolism and the formation of lesions in nerve tracts.

Saline injected into the ventricles had very little effect except when large volumes were injected rapidly. This would indicate that the

effects were due to abnormal pressures being formed within the CNS or to the sudden displacement of CSF by saline. The volumes used subsequently in experiments did not exceed 20 µl, which was well below the lowest volume to have observable effect.

There are a number of reports on the behavioural effects of adrenaline or noradrenaline injected into the brains of animals. Though the effects seen are described in many ways (sleep, sedation, stupor, analgesia, etc.), they all are indicative of central nervous depression (Bass, 1914; Leimdorfer & Metzner, 1949; Feldberg & Sherwood, 1954; Palmer, 1959; Myers, 1964; Findlay & Robertshaw, 1967; Grossman, 1968). In the rat Marsallan and Corrado (1961) saw muscle relaxation and loss of locomotor activity similar to that now reported. More recently it has been demonstrated by Grunden (1969) that intraventricular injection of adrenaline causes hyperventilation, decreased locomotor activity, a tendency towards calming and sleep, piloerection, salivation, urination and initial eating and drinking. In the present study some of these effects were only seen after peripheral (subcutaneous) administration. On intraventricular injection, decreased locomotor activity, initial eating and in addition vocalisation were the most predominant effects seen. Grunden confirmed that intraventricular adrenaline did not cause anaesthesia, but he did find that it potentiated hexobarbital anaesthesia. On infusion of noradrenaline into the ventricles over a long period the initial depressant stage changed into an excitation stage. In studies where adrenaline or noradrenaline have been applied to various areas of the brain both the production of depression and arousal have been produced (Myers, 1964). The reason for the change during infusion may be that maintaining a relatively high concentration of the drug in the ventricles over a long period allowed either the drug or its metabolites to diffuse further through the tissues. Thus

the overall effect of intraventricular injection is depression, but infusion may allow the drug to reach such excitation sites as have been reported to be present in the dorsomedial thalamus (Myers, 1964).

Grunden (1969) also injected 5-HT into the ventricles in a dose of 0.05 μmoles (equivalent in moles to 10 μg adrenaline). He found no significant effect on locomotor activity. Feldberg and Sherwood (1954) reported that in cats, effects similar to low doses of adrenaline were elicited by 5-HT. In this study the effects seen were not very strong, but contrary to noradrenaline and adrenaline, 5-HT caused muscular 'tension'. Locomotor activity was very much reduced, the animals tending to crouch in one position for long periods. Infusing 5-HT did not appear to alter the overall behavioural effect, though increases in the nociceptive threshold were seen. Intraperitoneal administration of 5-hydroxypryptophan (5-HTP) induced a form of gnawing behaviour and steriotyped movements of the head and forelegs of rats. This gnawing can be reproduced by implanting 5-HT in the rostral part of the corpus striatum and the tremor by implanting it into the substantia nigra (Haelzovic & Ernst, 1969). That neither of these effects were seen on injection or infusion of 5-HT into the ventricles is indicative that 5-HT does not diffuse sufficiently deeply into the tissues to reach the sensitive sites.

Depression of motor activity and sedation have been reported for the peripheral administration of 5-HT using doses of 4-5 mg/kg subcutaneously and intravenously (Hoffman, 1958; Karki & Paasonen, 1959). Brunaud and Siou (1959) reported that 5-HT reduced aggressivity in rats when doses of 20 mg/kg were used and had similar effects as 5 mg/kg chlorpromazine. Peripherally administered 5-HT has also been shown to produce extinction of conditioned avoidance behaviour at doses of 10-40 mg/kg (Cook & Weidley, 1957). These effects are not necessarily centrally mediated for

at these high doses 5-HT also has marked peripheral effects. In the anaesthetised rat marked hypotension has been reported with doses as low as 0.8 μg/kg (Salmoiraghi & McCubbin, 1956). Peripherally administered 5-HT in the rat also produces a marked hypothermia in doses effective on behaviour (5 mg/kg) (Hoffman, 1958). The sedation produced by chlorpromazine and reserpine, can be prevented in mice if the animal's body temperature is prevented from falling (Lessin & Parkes, 1957), suggesting that the sedation seen in rats on peripheral injection of 5-HT may be due to the hypothermia. From the published results it would seem that hypothermia and depression following peripheral administration follow similar time courses but this was not elicited by intracerebral injection. The hypothermic effect of intraventricular 5-HT is relatively short (see Chapter 2) but the depression normally lasted for up to 2 hours following a dose of 10 μg. Further, there is no evidence that 5-HT can cross the blood-brain barrier which further indicates that the depression seen on peripheral administration is not due to central effects (see Mantegazzini, 1966).

Feldberg and Sherwood (1954) injected Ach into the cerebral ventricles of the cat and found that the animals were depressed. Similarly Dikshit (1935) found that a condition resembling sleep was produced. They used doses up to 10 μg. In the present study doses up to 100 μg were injected into the ventricles of the rat, which produced very little effect. Infusions of 1 μg per minute did produce depression in the rat, the lack of effect of the (immediate) injection possibly being due to particularly strong cholinesterase activity in this animal. Carbachol and oxotremorine on the other hand, even in quite small doses, both produced behavioural depression and an elevation of the nociceptive threshold. In the present study there was no indication that these cholinergic compounds produced drinking activity in the animals, even though water was available. However

there are many reports that cholinergic stimulation of selected areas of the limbic system produce drinking in sated animals. (Grossman, 1962; Fisher, 1964, 1969). The work of Routtenberg, (1967) suggested that the thirst stimulation may be mediated by stimulation of receptors along the walls of the ventricles. Fisher and Levitt (1967) produced evidence to show that Routtenberg was incorrect, but they did find that cholinergic substances and their blocking agents can act within or diffuse from the ventricular spaces to influence tissues involved in the mediation of drinking behaviour. These authors also saw the catatonia and tremors similar to those described in the present work, and it is possible that drinking behaviour was not seen in these experiments because it was masked by the other behavioural effects.

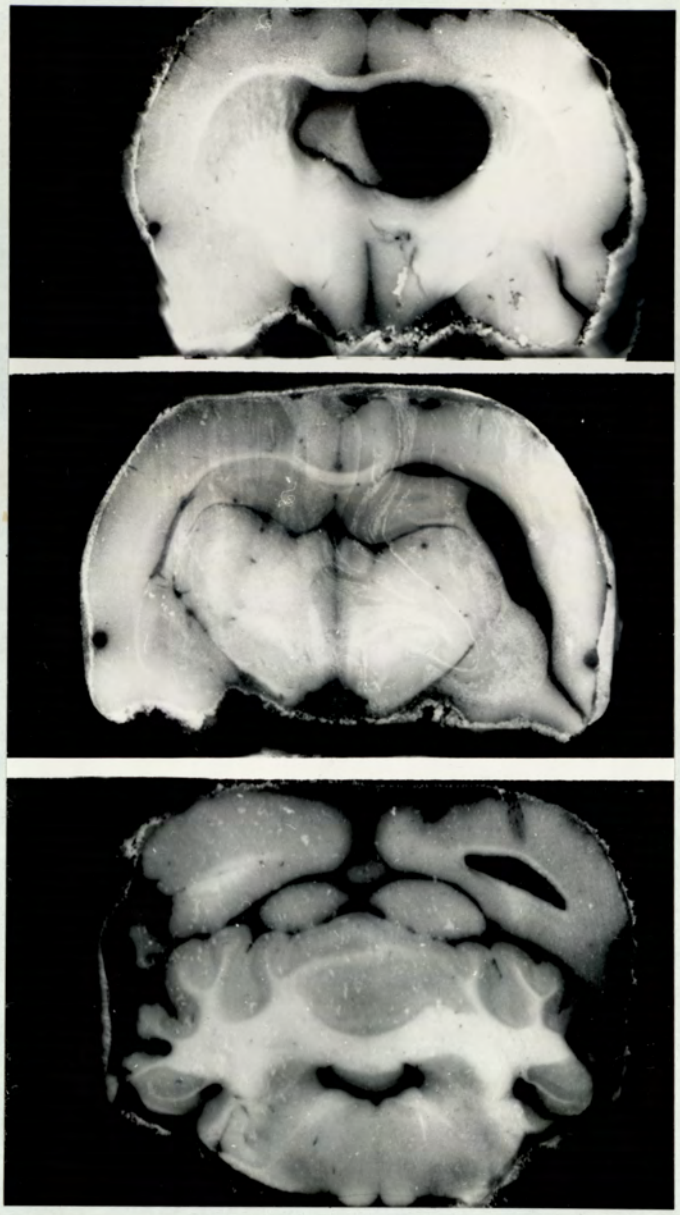

FIGURE 10 3 PHOTOGRAPHS OF THE CUT SURFACE OF A FROZEN BRAIN TAKEN TO SHOW THE SPREAD OF A HYDROCEPHALUS

The upper photograph shows a hydrocephalus in the lateral ventricle rostral to the point of injection.

The middle photograph shows the same hydrocephalus in the lateral ventricle about 3.0 mm caudal to the point of injection.

The lower photograph shows the same hydrocephalus in the lateral ventricle about 8 mm caudal to the point of injection (at the end of the lateral ventricle).

Note no swelling of the contralateral ventricle or of the 3rd and 4th ventricles.

CHAPTER TWO

BODY TEMPERATURE

CHAPTER TWO

BODY TEMPERATURE

BODY TEMPERATURE

1. DISCOVERY, SUBSEQUENT SEARCH FOR THE CAUSE, AND ELIMINATION OF AN ARTEFACT

The effects of intraventricularly administered substances on the body temperature of rats was to be investigated. Preliminary studies indicated that the body temperature was being affected by the vehicle (normal saline). The effect seen was a rise in core temperature lasting 6-9 hours. The cause of this rise was investigated before continuing the work. Possible causes were considered to be:-

a) An effect due to pyrogens in the saline.

b) An effect of the pH of the solution.

c) An effect of increasing the intraventricular pressure.

d) An effect of a contaminant in the rubber plug.

e) The effect of handling the animals or of movement of the cannula guide on insertion of the needle.

f) An effect due to the injected solution being at a lower temperature than the hypothalamic set point. The lowering of the set point causing a reflex rise in temperature.

g) An ionic effect of introducing a fluid not identical with the CSF.

h) An effect due to fluctuations in the environmental temperature.

i) An effect of cell debris, having collected in the guide, being pushed into the ventricular system.

These possibilities were studied in the following ways.

i) The cannula and syringe were carefully sterilised and commercially available pyrogen-free sterile saline for injection used (Sterivac and Steriflex, A & H).

The temperature rise was not prevented.

ii) The pH of the saline was measured and altered over the ranges 5-9.

No significant differences were observed in the hyperthermic response.

iii) The effects of the increase in pressure were investigated by injecting saline in volumes from 5-50 µl.

No significant differences were observed.

iv) To investigate the effects of the rubber plug the plug was removed prior to insertion of the cannula.

The rise was not affected.

v) Animals were handled and the exterior of the cannula guide was manipulated in order to apply a similar amount of pressure as was applied on injection.

No rise in core temperature could be detected, as shown in Fig. 11 (Table 1).

vi) Injecting saline in a syringe prewarmed to 30 or 40°C did not prevent the rise. The syringe and saline were kept at the required temperature by immersing the sterile syringe in the saline and placing the container in a water bath. Fig. 12 (Table 2) shows an identical rise in temperature for animals injected with saline at 20°C and with prewarmed to 37°C.

vii) The use of artificial CSF was not tried.

viii) Saline was injected into animals kept at environmental temperatures of 18 and 25°C. There was no significant difference in the rise due to an injection of 20 µl saline at the two temperatures.

ix) Fig. 11 (Table 1) shows that while manipulation of the cannula guide had no effect on the core temperature, simple insertion of the cannula down the guide without injection, had an effect not significantly different from the injection of 20 µl of saline. It was then shown that this increase in temperature was dependent on the length of cannula inserted. From Fig. 13 (Table 3) it can be seen that a cannula placed 1.5 mm down the guide caused a 0.7°C rise, whereas one pushed down 5.0 mm (the whole way), caused a 1.9°C rise above the controls after 4 hours. However from Fig. 14 (Table 4) it can be seen that if an injection of 20 µl saline was given, alterations in the depth of needle penetration made no significant difference to the increase in body temperature.

This, along with episodes of violence noted in Section 3, Chapter 1, (when insertion of the cannula met with resistance) indicated that some material was being pushed or washed from the guide, and subsequently causing the temperature rise.

To prevent cell matter collecting in the guide a stilette, as described in Section 2 was placed down the lumen prior to implantation. The stilette was removed

immediately before injection and ~~removed~~ replaced immediately after. This reduced the temperature rise by about 50% in the majority of cases, but a rise of 1 °C maximum was still present in a few animals. It was essential for the stilette to occupy the entire length of the guide, for the use of a 3 mm stilette did not prevent the maximum rise (see Fig. 15; Table 5).

In one experiment a rat cannulated with a 5 mm guide and no stilette was killed and the cannula guide removed. The guide was then flushed out by placing a No. 20 needle just through the rubber and 1 ml sterile pyrogen-free saline injected through the pipe. A small quantity of matter was washed out. Subsequent injection of 20 µl of the washing fluid into rats, cannulated with a 4 mm cannula guide and stilette, caused a small significant increase in temperature. In cases where sufficient matter had collected to form a plug, examination of the plug indicated the presence of dried salts that readily dissolved in water and some cellular matter.

Shortening the guide by 1-2 mm so that only 3-4 mm of tube protruded below the perspex, (thereby stopping short of the roofs of the ventricles) prevented the temperature rise in most animals. Subsequently the combination of both a 4 mm long guide and a 4mm long stilette completely abolished the rise in temperature (see Fig. 16; Table 6).

Fig. 17 (Table 7) shows that injection of volumes of saline from 10-80 µl had no significant effect on body temperature, when the shortened guide plus stilette were used.

3. DISCUSSION

The results indicate that the observed increase in temperature following a control injection of saline was due to material collecting in the cannula guide and being pushed into the ventricular system on insertion of the cannula. The use of the 4 mm cannula guide either prevented the pyrogenic material collecting in the tube or caused it to be pushed into the tissues surrounding the cannula guide, rather than into the ventricles. The use of the stilette prevented large plugs of matter collecting. However when the stilette was used in a 5mm cannula guide, a small rise of temperature was still seen. This could have been due to material getting between the walls of the guide and the stilette and subsequently being carried out by the cannula. The use of a stilette of greater diameter might have prevented this, but the stilette used was a tight fit and to use a tighter one would have been impracticable.

Having eliminated this temperature rise to 20 µl of saline, 10-80 µl were injected to see if changing the pressure within the ventricles had any effect on the temperature. No effect was observed, though Banergee, Feldberg and Lotti (1968) found that simply fixing the head of a cat into a stereotaxic frame, or implanting an intraventricular cannula, caused a rise in body temperature. Fixing the head into the frame they suggested caused a strong sensory stimulus in turn affecting the activity of monoaminergic neurones in the anterior hypothalamus. The implantation of the cannula they suggested, might have caused the release of substances into the ventricles from

damaged tissue, or caused sudden pressure changes in the ventricle. The existence of nerve endings in the ventricular system is known (Leonhardt & Lindner, 1967) and it is possible that these may have been activated, affecting hypothalamic systems. Further it has been noticed that short lasting light pressure applied to the floor of the fourth ventricle of anaesthetised cats can prevent shivering (El Hawary, Feldberg & Lotti, 1967). Again it was suggested that the reason for this was stimulation of nerve fibres terminating in the anterior hypothalamus which interfere with temperature regulating mechanisms.

In the previous chapter it was shown that large volumes of saline injected into the ventricles had minimal effects upon behaviour. In the present experiments large volumes of saline did not activate any temperature regulating mechanisms. It was therefore decided to continue the study of the effects of drugs upon temperature, using injection volumes of 10 µl.

2. THE EFFECTS OF DRUGS ON BODY TEMPERATURE

The effects on body temperature of injecting biogenic amines into the cerebral ventricles vary according to the species involved (see Introduction), Chapter 4). Thus in the rat the catecholamines and 5-HT have produced hypothermia and hyperthermia (Feldberg & Lotti, 1967; Kubikowski & Rewerski, 1969). Injection of cholinomimetics have produced hypothermia (Lomax & Kenden, 1966; Lomax, Foster & Kirkpatric, 1968). Morphine injected into the anterior hypothalamus of rats has produced a hypothermia (Lotti, Lomax & George, 1965 a & b) or a hyperthermia on injection into the cerebral ventricles of cats (Banergee, Feldberg & Lotti, 1968). Morphine has also a number of other properties which may be related; it reduces NA and adrenaline levels of the hypothalamus (Vogt, 1954; Gunne, 1959); releases 5-HT from the perfused hindquarters of rats (Battacharya & Lewis, 1956); blocks the stimulatory effects of 5-HT on the inferior mesenteric ganglion of the cat (Gyermek & Bindler, 1962); and inhibits the hydrolysis of ACh by cholinesterases (Wright & Sabine, 1943). It was therefore of interest to compare the effects of these neurotransmitters with that of morphine when injected into the cerebral ventricles of rats.

a) Noradrenaline

The effect on body temperature of NA injected into the ventricles was first studied before there were facilities available for controlling the ambient temperature. It was noted that low doses did not always produce the same effects. However once the ambient temperature was kept constant,

results were obtained. This is illustrated in Figs. 18 and 19 (Tables 8 & 9) where body temperature changes (from the saline controls) are shown for doses of 5, 10 and 20 μg of NA, injected into the ventricles of animals kept at various environmental temperatures. At a room temperature of 18°C (Fig. 18; Table 8), 20 μg of NA caused a fall in body temperature of about 2°C, followed by a significant rise of about 1.5°C. Whilst 10 μg caused a fall of about 1°C followed by a rise, 5 μg of NA only caused a rise. However when the ambient temperature was increased to 25°C (Fig. 19; Table 9), 20 μg of NA caused a significant but smaller fall, whilst both 10 and 5 μg only caused rises in temperature. The rises to 5 and 20 μg at this raised ambient temperature, were about 0.5°C larger than at 18°C, and the rises were also more prolonged. Larger doses of NA (40-80 μg) did not cause the body temperature to fall very much more than 20 μg, nor was the subsequent rise any greater - though at these higher doses the effects again were more prolonged. These results emphasise the importance of controlling and recording the ambient temperature when studying the effects of drugs on thermoregulation. As a result of these studies all future experiments were conducted in a temperature-controlled room at an even temperature of 21 ± 1°C.

It was considered possible that the hypothermia might have been due to a leakage of NA into the peripheral system. However it was found that doses of around 1 mg/kg (300 μg/rat approximately) were necessary to produce significant hyperthermia when injected subcutaneously. Intravenous infusions of NA (20 μg/hour/rat) into the jugular vein of conscious rats had no

significant effect on the body temperature. About the time these experiments were performed, Feldberg and Lotti (1967) reported that 200-500 μg NA injected intraperitoneally were required in order to produce hyperthermia in rats.

Peripheral administration of phentolamine (1 mg/kg intraperitoneally) abolished both the hypo- and hyperthermic effects of intraventricular NA (10 μg) at an ambient temperature of 18°C, as shown in Fig. 20 (Table 10). If higher doses of NA were used, the hypothermia was produced but the hyperthermia was still abolished.

b) 5-hydroxytryptamine

The effect of 5-HT on the body temperature is shown in Fig. 21 (Table 11), again expressed as the change from the saline control. The effects seen were very similar to those of NA though the compound was approximately twice as potent as NA. A temperature rise followed the fall, an observation not previously reported. It was considered possible that it might be due to the creatinine sulphate moiety, therefore creatinine sulphate in saline was injected into the ventricles but there was no significant temperature deviation from the control.

The hypothermic effect of 5-HT was prevented by the prior administration peripherally of methysergide. The effect is shown in Fig. 22 (Table 12). Pretreatment at 45 minutes with 2 mg/kg methysergide made the animals quiet and slightly depressed, with a small drop in body temperature. Both the hypo- and hyperthermic effects of 5-HT were subsequently reduced in these animals.

Upon peripheral administration of 5-HT, it was necessary to inject doses of 1,000-2,000 μg (approximately 5 mg/kg) subcutane-

ously in order to produce any temperature changes. At these doses a small hypothermia was observed, similar to the effect seen by Hoffman (1958).

c) Carbachol

Carbachol was used in this investigation following the observation that ACh lacked a definite effect; a very short hypothermia was produced with ACh but the results were not consistent. Carbachol produced a short lasting fall in body temperature, a typical response being shown in Fig. 23 (Table 13). The experiment was repeated a number of times but a fall greater than 1.5°C was not obtained. This may have been due to stimulation of motor activity at high doses; the muscular activity generating heat. However there was no subsequent rise in temperature similar to that seen with 5-HT and NA.

Fig. 25 (Table 14) shows the inhibition of the hypothermic effect of carbachol by pretreatment of the animals with hyoscine at 30 minutes. Although 0.9 μg of carbachol still showed a small hypothermic effect in the presence of hyoscine the response was significantly reduced.

Peripheral administration of carbachol produced hypothermia in relatively small doses of around 150-200 μg (approximately 0.5 mg/kg). The hypothermic action of systemically administered cholinergic compounds has been shown to be due to a central action by Spencer (1965) and Lomax and Jenden (1966) using tremorine and oxotremorine. The need for a lower dose of carbachol to have an effect when administered peripherally, when compared with the effects of NA and 5-HT, is due possibly to the ease with which

this compound passes the blood-brain barrier, compared to the relative impermeability of the barrier for the other two compounds. Also carbachol is probably more resistant to cholinesterases than are 5-HT and NA to uptake and degredation enzymes (monoamine oxidase etc.).

d) Morphine

The effect of morphine injected into the ventricles is shown in Fig. 25 (Tables 15 and 16). Small doses (10 and 20 μg) caused an increase in temperature of about 2.5°C which returned to normal within 6 hours. Higher doses (40-160 μg) caused a fall in temperature followed by a rise. The increase in the hypothermic response with increase in dose was not as pronounced as with NA, 5-HT and carbachol (flatter dose response curve).

Because of the similarity between the effects of intraventricular morphine and intraventricular NA, the previously mentioned association of morphine with NA and also the finding that ergotamine abolishes the hyperthermic effect of intraventricular morphine in cats, (Banergee, Feldberg & Lotti, 1968), the effect of pretreatment with phentolamine (1 mg/kg intraperitoneally) on the temperature responses to morphine was investigated. The results are illustrated in Fig. 26 (Table 17). The hypothermia due to morphine was not altered. However the secondary temperature increase was usually significantly reduced (as shown) and sometimes almost totally abolished - although this effect was variable. Since 5-HT had both a hypo- and hyperthermic, and carbachol had a hypothermic effect (all of which could be blocked), the effects of pretreatment with methysergide and hyoscine were also tested on the effects of intraventricular morphine.

Hyoscine (1 mg/kg) given 30 minutes prior to the intraventricular morphine reduced and almost totally abolished the hypothermia caused by morphine, but had no significant effect on the hyperthermia. From Fig. 27 (Table 18) it can be seen that the hypothermia was in fact reversed to a hyperthermia, giving the graph an appearance very similar to that obtained with lower doses of morphine.

Pretreatment with methysergide (2.0 mg/kg intraperitoneally 30 minutes previously) had no significant effect on either the hyper- or hypothermia caused by intraventricular injection of 80 µg morphine.

3. DISCUSSION

a) Naturally occurring substances

Hypothermia followed by hyperthermia caused by the intraventricular injection of NA in the rat has been reported previously by Feldberg and Lotti (1967). Kubikowski and Rewerski (1969) found that higher doses (50 µg) caused a fall in body temperature, but that small doses of 0.5 µg - which they considered more physiological - caused a hyperthermia. The present finding - that low doses of NA caused either hypo- or hyperthermia, depending on the ambient temperature - indicates that the thermoregulatory centre had been put out of action, rather than a reduction in the set point of the central "thermostat". The ability of the alpha-adrenergic blocking drug phentolamine to prevent the fall and rise in body temperature indicates that the effect of the intraventricularly administered amine was mediated through an alpha-adrenergic receptor system (or something

similar) in or near the ventricular system. A most likely site is the temperature-control centre in the hypothalamus. Feldberg and Lotti (1967) confirmed our control results, showing that very much larger doses of NA were necessary to produce temperature changes when given peripherally.

Similar conclusions were drawn from the experiments involving the injection of 5-HT into the ventricles. However the similarity between NA and 5-HT reported here has not been reported previously. Feldberg and Lotti (1967), although using very similar doses of 5-HT creatinine sulphate, only recorded a fall in body temperature. Kubikowski and Rewerski (1969) found that the response varied from rat to rat, but that the predominant effect was one of hyperthermia, though small hypothermic effects were sometimes observed. Sheard and Agajanian (1967) noted that neural release of 5-HT was elicited by stimulation of the mid-brain raphé region (below the aquaeduct in the central grey matter) and they noted a concomitant rise in body temperature. They suggested that the temperature rise was mediated via the released 5-HT. Our finding that the effects of intraventricular 5-HT could be blocked by methysergide would again indicate the presence of receptors sensitive to 5-HT.

The hypothermic effect of muscarinic agents administered systematically and intracerebrally to rats has also been reported previously (Lomax & Jenden, 1966; Kirkpatric, Jenden & Lomax, 1967). They found that the implantation of oxotremorine and carbachol into the rostral hypothalamus produced a profound decrease in body temperature that could be prevented by pretreatment with atropine. In the present experiments, the effects of intraventricular injection were similar, except that the fall in

body temperature was not so great as that recorded by these previous authors. The erratic effect of even larger doses of ACh could be explained by its rapid breakdown by cholinesterases.

Feldberg and Myers (1964), on the basis of experiments carried out on cats, have postulated that regulation of body temperature depends on a balance between the release of NA and 5-HT in the rostral hypothalamus. There is considerable variation in the response to these agents; those causing a fall in body temperature in one species may well cause a rise in another (see Introduction, Chapter 4). However there is reasonable evidence that central control of temperature may be at least partly cholinergic in nature in the rat, since cholinergic compounds cause hypothermia on intracerebral and systemic administration. It has been suggested that catecholamines may modulate cholinergic transmission in the rat since injection of NA into the thermoregulatory centres blocks the hypothermia caused by a previous systemic dose of pilocarpine (Lomax, Foster & Kirkpatric, 1968). The dose of NA used was sufficiently small that it would not itself have caused hypothermia on injection.

In the monkey, NA injected into the anterior hypothalamus caused a fall in body temperature and both 5-HT and carbachol cause a rise. From this a cholinergic heat production pathway, activated by 5-HT, has been postulated (Myers & Yaksh, 1969), in which a sustained release of 5-HT may be necessary to maintain normal body-heat production in this species. The release of NA could block the hyperthermic effect of 5-HT either by inhibiting the latter's release or by competing for receptor sites post-synaptically. A second alternative is that NA

directly blocks a cholinergic pathway delegated to heat production.

It is not unlikely that similar mechanisms are present in the rat, though in this case the cholinergically mediated pathway would stimulate heat-loss. We have shown that 5-HT caused a profound and rapid fall in temperature in releatively small doses. This could be due to potentiation or stimulation of a cholinergic heat-loss mechanism, or 5-HT itself may mediate a heat-loss mechanism. The rise in temperature seen after small doses of NA could be due to inhibition of the 5-HT. This is supported by the finding that NA does interfere with the contractile action of 5-HT on smooth muscle (Armitage & Vane, 1964). An alternative explanation is that NA blocks the cholinergic pathway, as has been shown to occur in cholinergically-mediated pathways in the superior cervical ganglion of the cat (De Groot & Volle, 1966). The involvement of 5-HT in stimulating heat-loss in rats is supported by a number of related observations: exposing animals to heat accelerates 5-HT synthesis in the brain, whereas cold-stress has no effect (Reid, Volicer, Smookler, Beaven & Brodie, 1968; Corrodi, Fuxe & Hokfelt, 1967); the hypothermia of systemically-administered oxotremorine is accompanied by increased brain 5-HT levels (Cox & Potkonjak, 1967); and the release of 5-HT from the perfused 3rd ventricles of cats caused by addition of tranylcypromine to the perfusion fluid, is associated with a rise in body temperature and shivering (El Hawary, Feldberg & Lotti, 1967). The rise in temperature following intraventricular 5-HT could be due to the displacement of NA ~~from~~ from storage sites by 5-HT (Reid et al, 1968), and this could also be the reason for the increase in temperature seen during the electrical stimulation of the mid-brain raphé ~~region~~ region.

Since the rat normally lives at an ambient temperature well below its body temperature, it is unlikely that NA only increases body temperature by inhibiting heat-loss mechanisms. Also, if it is to fit into the scheme as a promotor of heat gain, the heat loss seen on larger injections seems paradoxical. Exposure of rats to both heat and cold stress increases the turnover rate of NA in the brain (Reid, Volicer, Smookler, Beaven & Brodie, 1968). They suggested that the increase in turnover on cold-exposure stimulates heat production mechanisms in the hypothalamus in an attempt to keep the body temperature normal. They further suggested that the increase in NA production in heat-stress is due to circulatory responses and that in this situation the neurons which elicit heat production are selectively blocked. This may be the kind of response seen on injection of large doses of NA into the brain, the high concentrations actively inhibiting heat production, and lowering body temperature. The small doses would have an effect similar to exposure to cold-stress - the heat production mechanisms being stimulated. Alternatively, it has been reported in Chapter 1 (Section 3) that large intraventricular doses of NA cause CNS depression, which in turn causes a reduction in motor activity. Since muscular activity is a major source of heat production the hypothermia may simply be due to a reduction in the natural production of heat.

b) Morphine

Peripheral administration of morphine produces both hypo- and hyperthermia, there being a threshold dose above which temperature decreases and below which it increases (Gunne, 1960). Lotti, Lomax and George, (1965a & c) found that micro-injection of 50 µg morphine sulphate into the anterior hypothalamus caused a profound

hypothermia. Peripheral administration of nalorphine during this hypothermia or before the morphine injection, reversed the effect, causing a hyperthermia (Lotti, Lomax & George, 1965b). Also, acute tolerance to this hypothermic effect was seen upon repeated intracerebral and intravenous administration or morphine. After the production of this tolerance, intracerebral injection of morphine caused hyper- (and not hypothermia) (Lotti, Lomax & George, 1966b). These authors suggested that the hyperthermic effects in the tolerant animals and in the nalorphine-treated animals are due to the unmasking of a direct stimulating effect of morphine on the thermoregulatory centres.

In our results the blocking effect of phentolamine on the hyperthermia caused by intracerebral morphine would indicate that the hyperthermia was due to an alpha-adrenergic effect. Similarly the hyperthermia due to intraventricular morphine has been shown to be inhibited by pretreatment with ergotamine in cats (Banergee, Feldberg & Lotti, 1968). However in this species NA is reported to decrease body temperature only, and 5-HT to elevate it (Feldberg & Myers, 1964). It should be pointed out that in their experiments the cats were anaesthetised with pentobarbitone, and the effect of morphine was to arrest and reverse the hypothermia of anaesthesia, this being following by a large hyperthermia.

Morphine has been shown to deplete the hypothalamus of NA. (Vogt, 1954; Gunne, 1960) and in view of the similarity between the hyperthermia due to morphine and NA, and the inhibition of this effect by phentolamine, it would seem likely that the hyperthermic effects of morphine are due to the release of NA in the hypothalamus. In the rabbit, intraventricular injection of morphine also

caused a rise in temperature, which was preceded by a small fall when large doses were used (Bahergee, Burks, Feldberg & Goodrich, 1968). In this species, NA injected into the ventricles caused a rise in body temperature, 5-HT having little effect (Cooper, Cranston & Honour, 1965). The reason for the lack of a hyperthermia following the hypothermia in the experiments of Lotti et al is not known. The dose of 50 µg morphine sulphate injected directly into the hypothalamus caused a very much longer lasting hypothermia than the doses we have used. Reid, Volicer, Smookler, Beaven & Brodie (1968) reported that after long exposure to cold the increased NA turnover in the hypothalamus returned to normal possibly through an adaption mechanism. It may be that a similar effect was seen in the experiments of Lotti et al.

The hypothermic effect of morphine was not significantly affected by pretreatment with phentolamine or methysergide. This indicates that morphine's hypothermic effect is not mediated by NA or 5-HT, since the hypothermic effects of these amines are blocked by phentolamine and methysergide respectively. The fact that the hypothermia was blocked by hyoscine is indicative that the effect was mediated by a cholinergic mechanism. It is possible that in high dosage morphine released sufficient ACh to cause a hypothermia. It should be pointed out that Lotti (1967) found that the hypothermic effect of morphine (20 mg/kg intravenously) was not blocked by atropine. However the effects of peripherally administered morphine are not necessarily the same as those of the agent injected centrally and also 20 mg/kg intravenously is a very large dose. Wright and Sabine (1943) showed that morphine had anticholinesterase activity and this could explain the hypothermic

effect of morphine - the anticholinesterase effect causing a build-up of ACh to occur in the hypothalamus. However it has since been shown that nalorphine has a similar effect on ACh levels (Foldes, Erdos, Baart, & Zwartz, 1959), which would not explain the reversal of morphine hypothermia by nalorphine. Schaumann (1957) has shown that morphine and nalorphine interfere with the release of ACh from the guinea-pig ileum on electrical stimulation of the preparation. Also Schuberth and Sundwall (1967) have shown that morphine is a competitive inhibitor of the uptake of ACh by cortex slices of mouse brain.

It is therefore possible that high doses of morphine cause ACh levels to build up in the anterior hypothalamus, causing a drop in body temperature. Nalorphine on the other hand may have the same action as it possesses in the guinea-pig ileum, preventing the release of ACh.

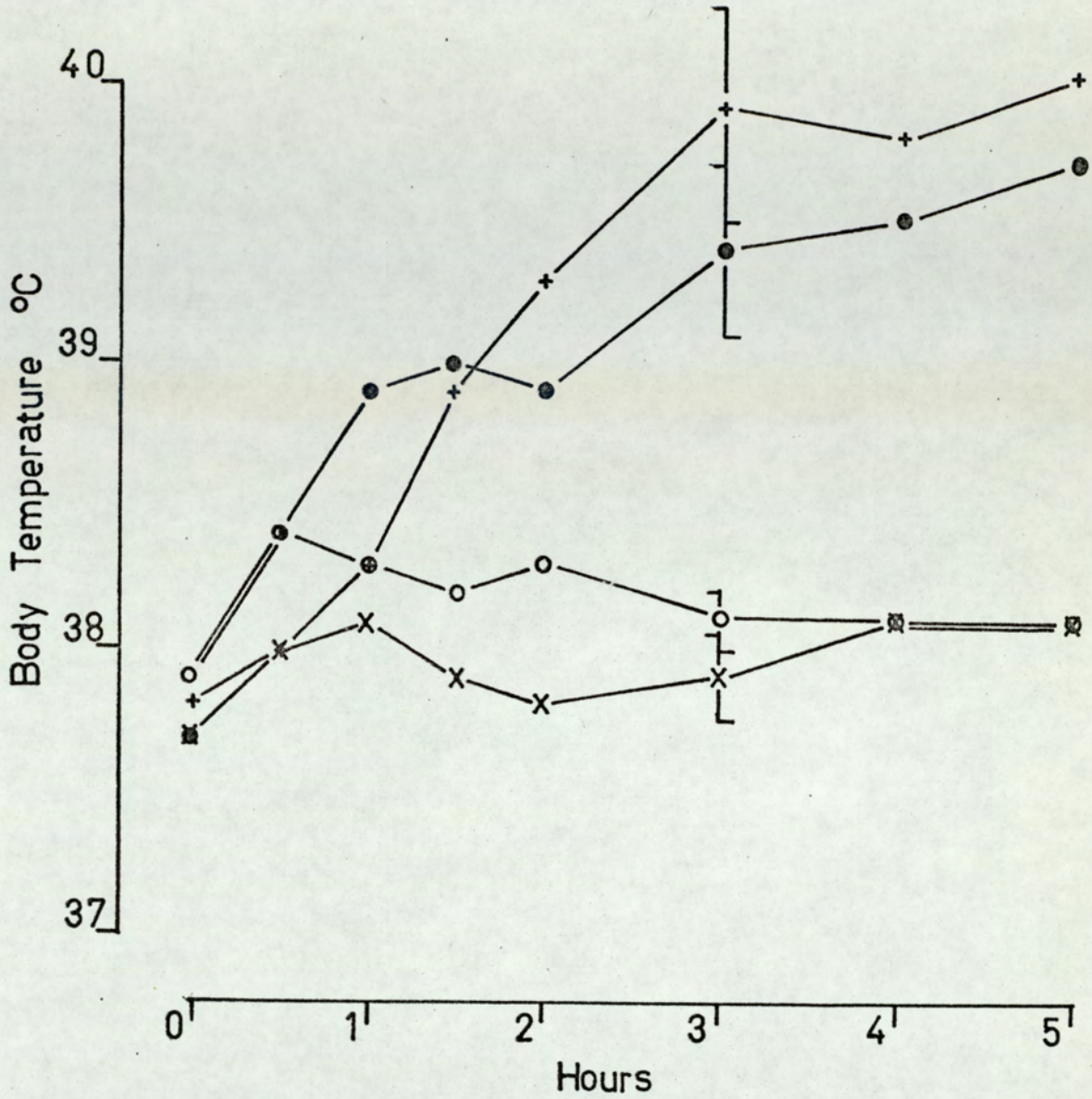

FIGURE 11 THE EFFECT ON BODY TEMPERATURE OF MANIPULATIONS OF THE CANNULA GUIDE

Each animal was fitted with a 5 mm cannula guide without a stilette, and a 5 mm cannula was used. 4 groups of 5 animals were used. In one group the cannula was inserted into the guide and no injection was made (—+—); in another 20 µl saline was injected after insertion of the cannula (—●—); in another the cannula guide was held in forceps and shaken (—o—) and in another the animals were only handled, the cannula being left untouched (—✗—).

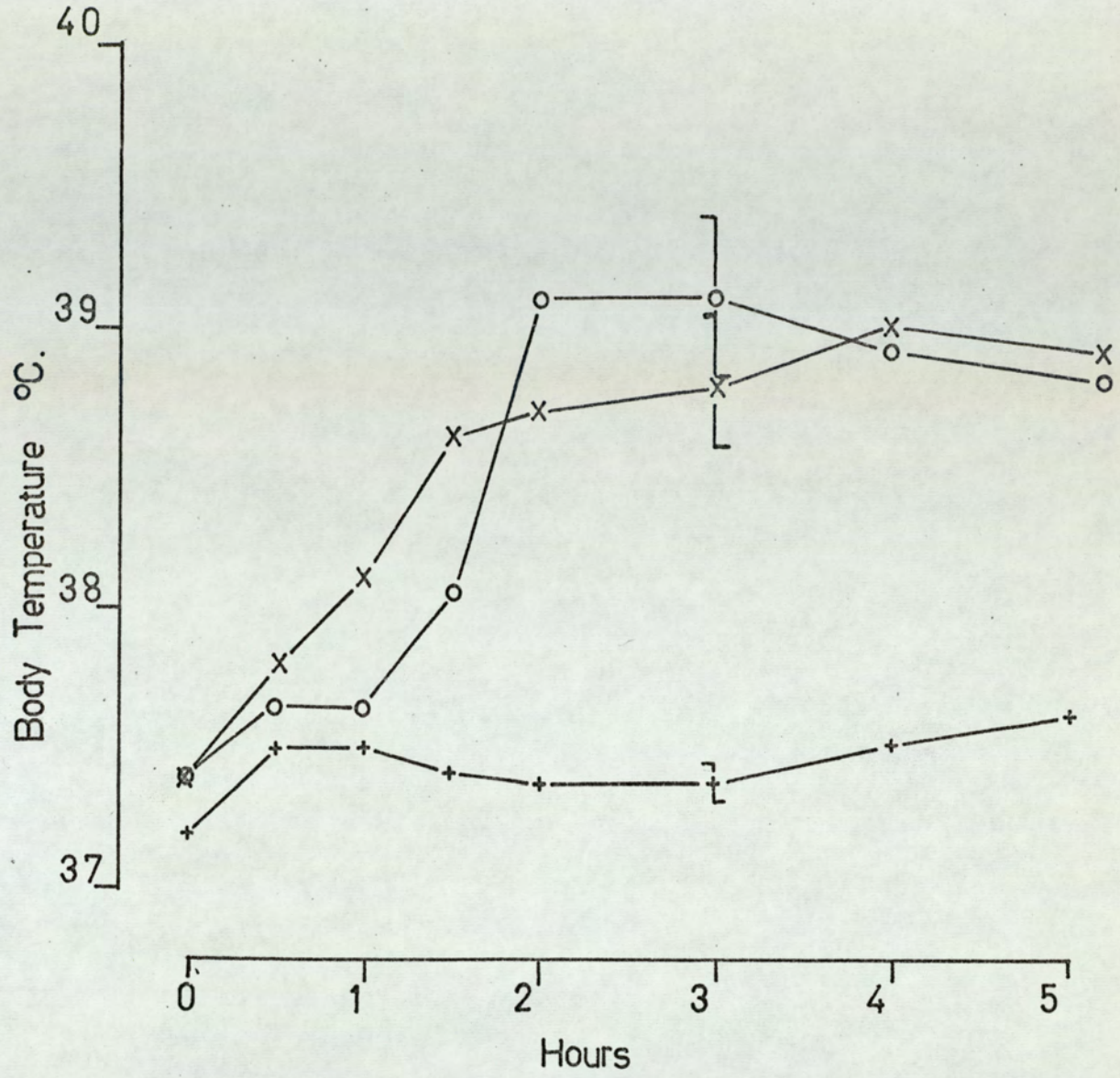

FIGURE 12 THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF SALINE AT 21°C AND 37°C

Animals were fitted with a 5 mm guide and a 5 mm cannula was used. 3 groups of 5 animals were used. One group received 20 µl saline at 37°C (—X—); one group 20 µl saline at 21°C (— o —) and one received no treatment (— + —).

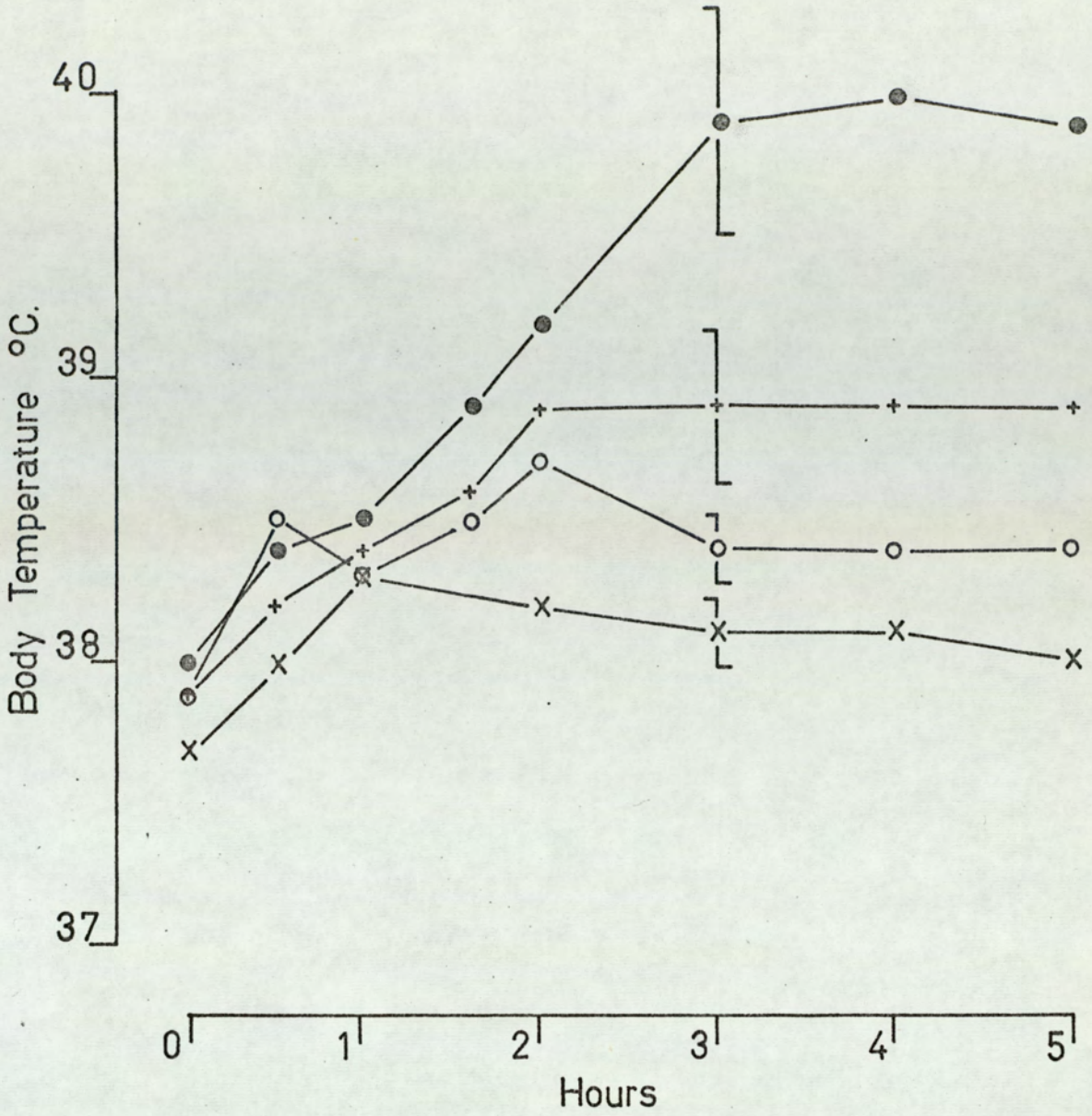

FIGURE 13 THE EFFECTS ON BODY TEMPERATURE OF THE USE OF VARIOUS LENGTHS OF CANNULA WITH A 5 mm CANNULA GUIDE

4 groups of 5 animals were used. Each animal was fitted with a 5 mm cannula guide without stilette. The following lengths of cannula were then inserted without an injection being made:

5 mm (—●—); 3.5 mm (—+—);

1.5 mm (—o—) and no insertion (—x—).

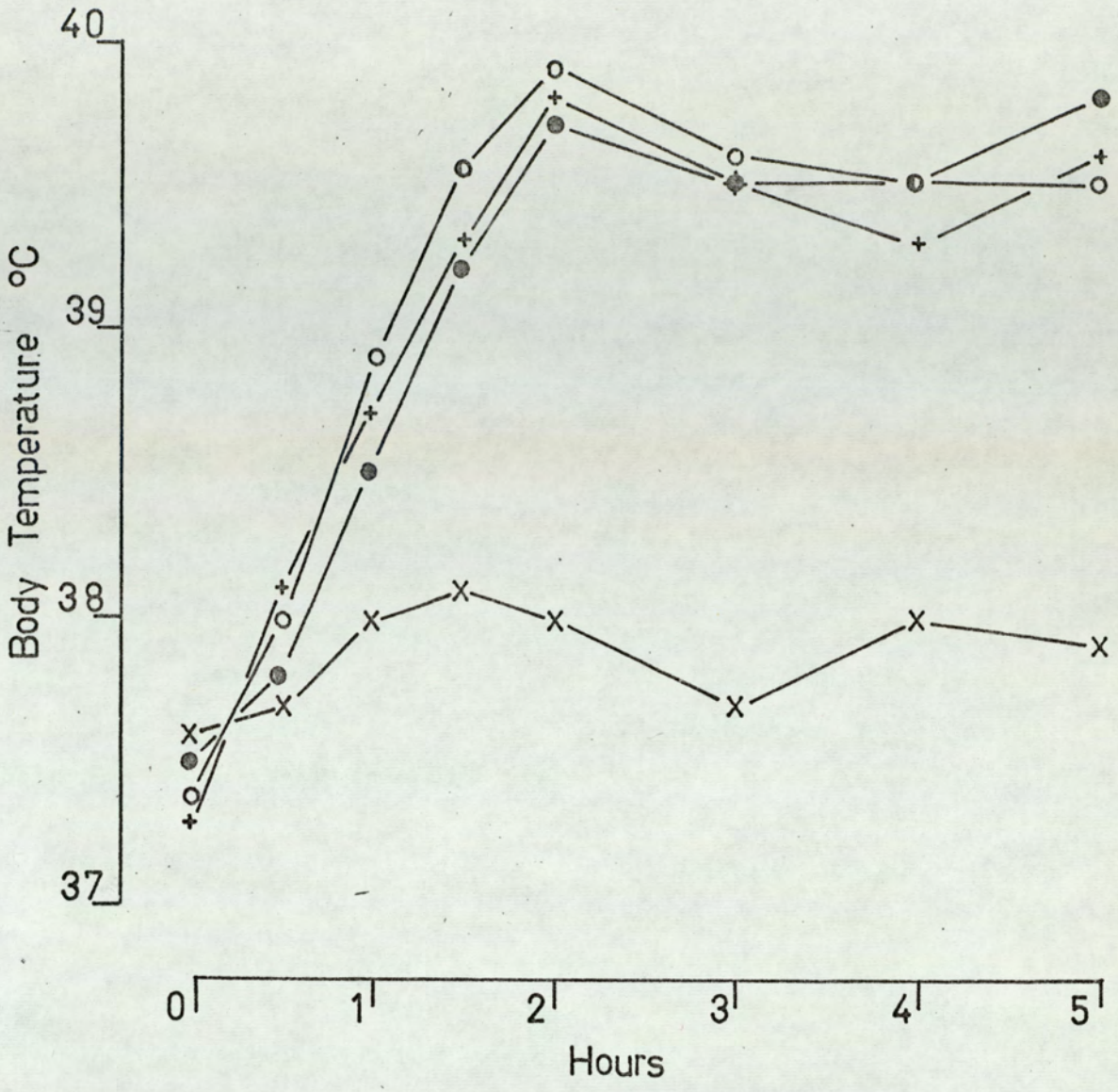

FIGURE 14 THE EFFECTS ON BODY TEMPERATURE OF 20 µl SALINE INJECTED VIA DIFFERENT LENGTHS OF CANNULA

4 groups of 5 animals were used. Each animal was fitted with a 5 mm cannula guide. 20 µl saline was then injected intraventricularly using the following lengths of cannula:

5.0 mm (—●—); 3.5 mm (— + —);

1.5 mm (— o —) and no treatment (—X—).

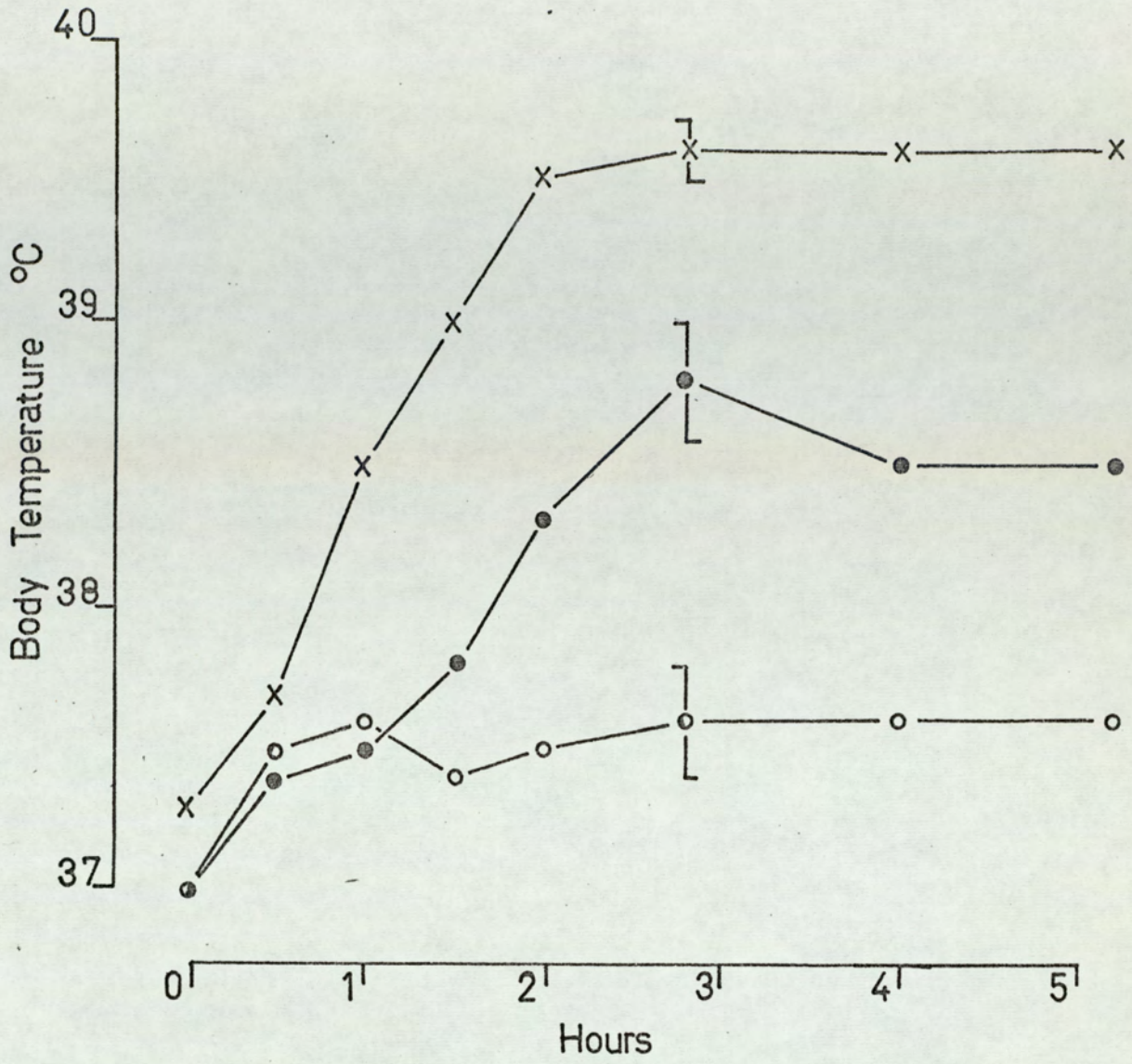

FIGURE 15 THE EFFECT ON BODY TEMPERATURE OF DIFFERENT LENGTHS OF STILETTE IN A 5 mm CANNULA GUIDE

3 groups of 5 animals were used. Each animal was fitted with a 5 mm cannula guide. Intraventricular injections of 20 µl saline were made using a 5 mm cannula. One group was previously fitted with a 3 mm stilette (—X—) and one group with a 5 mm stilette (—●—). The third group received no treatment (—o—).

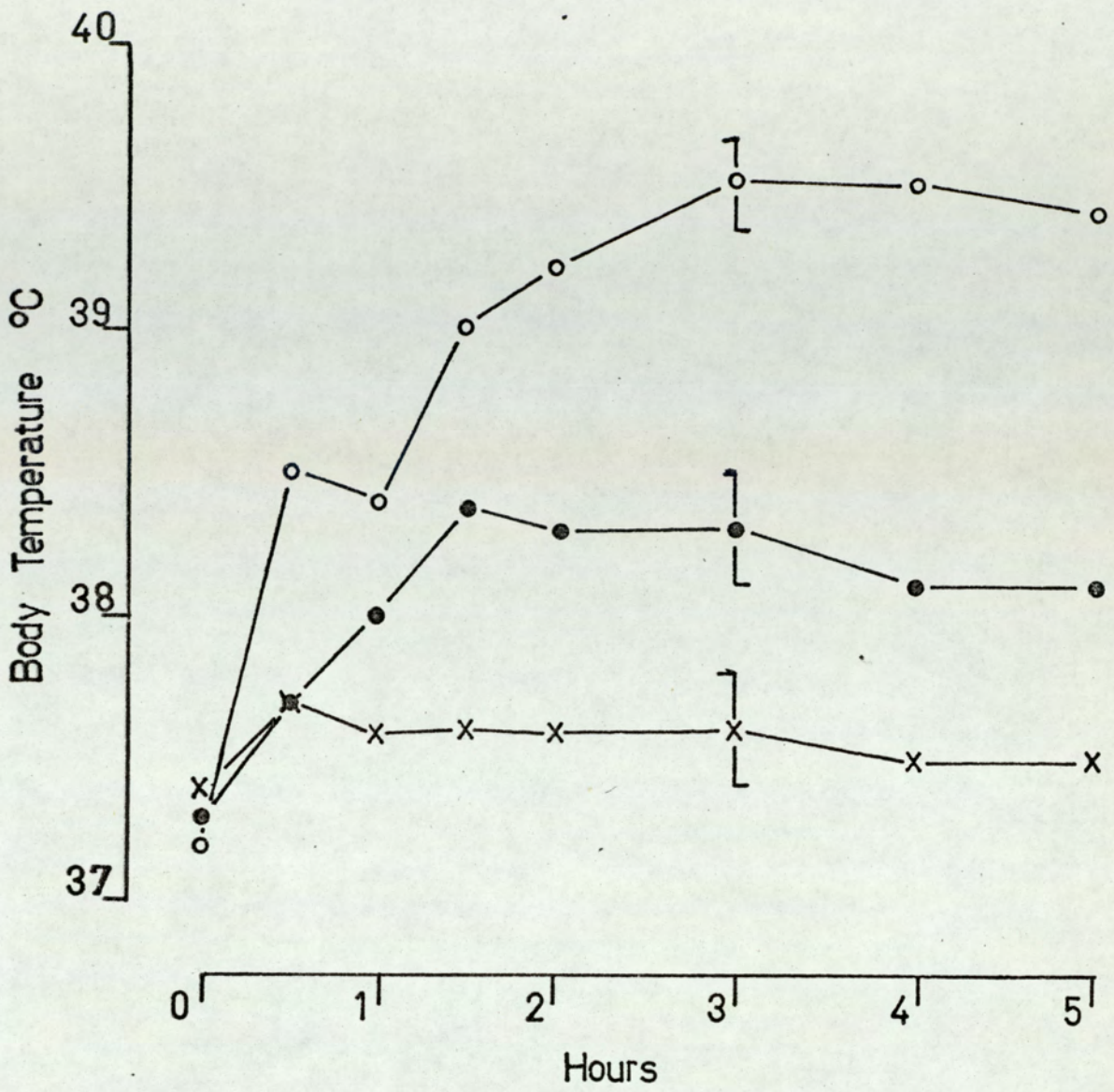

FIGURE 16 THE EFFECTS ON BODY TEMPERATURE OF DIFFERENT LENGTHS OF CANNULA GUIDE AND STILETTE

3 groups of 5 animals were used. All groups received a 20 µl injection of saline intraventricularly via a 5 mm cannula. One group was fitted with a 5 mm cannula and a 5 mm stilette (—●—); one group with a 5 mm guide and no stilette (—o—) and one group with a 4 mm guide and a 4 mm stilette (—x—).

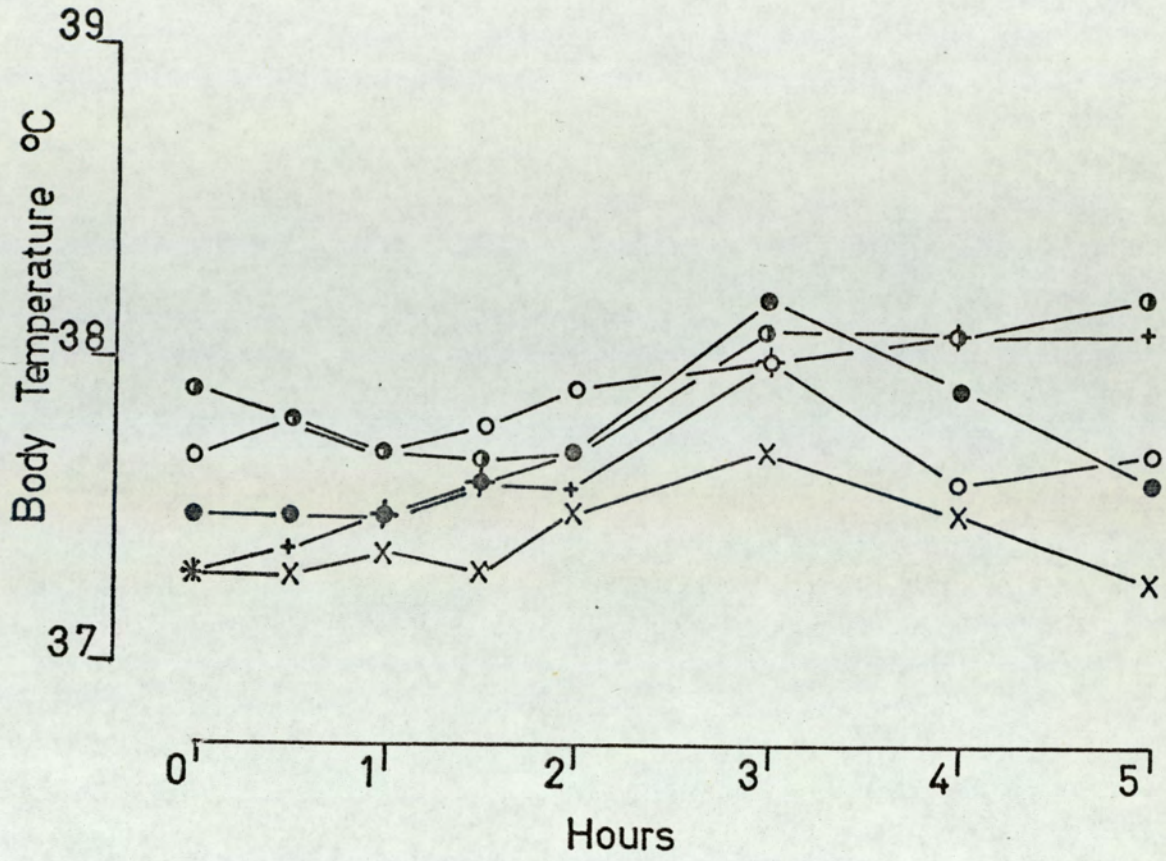

FIGURE 17 THE EFFECT ON BODY TEMPERATURE OF VARIOUS VOLUMES OF SALINE INJECTED INTRAVENTRICULARLY USING A 4 mm CANNULA AND STILETTE

5 groups of 5 animals were used. Each animal was fitted with a 4 mm cannula guide and a 4 mm stilette and injections were made using a 5 mm cannula. The following volumes of saline were injected intraventricularly:

10 µl (—o—); 20 µl (—●—); 40 µl (—◐—);

80 µl (— + —) and one group only had the cannula inserted without an injection being made (— X—).

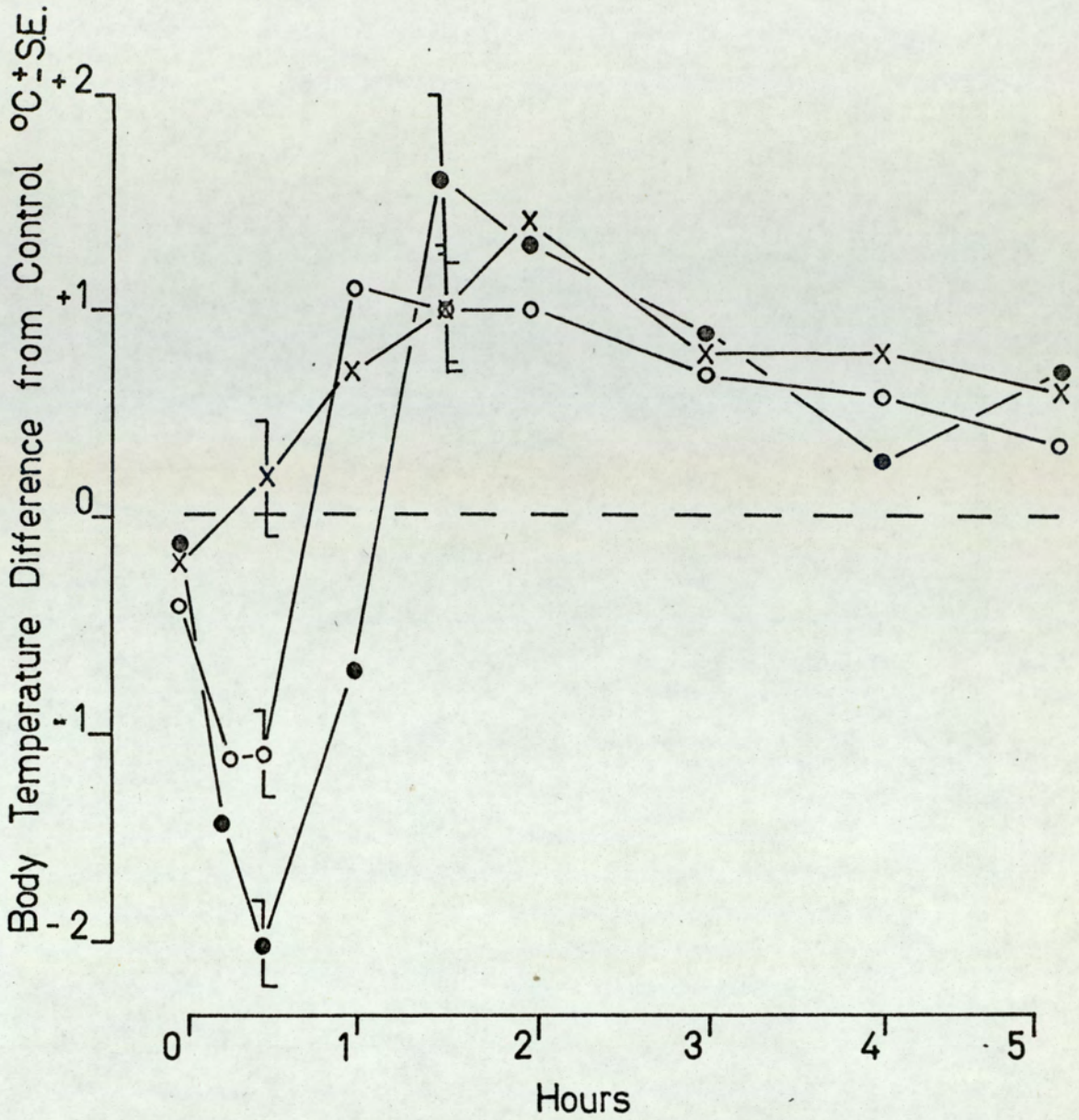

FIGURE 18 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF NA AT AN AMBIENT TEMPERATURE OF 18°C

4 groups of 5 animals were used. One group received 5 μg NA intraventricularly (—X—); one group 10 μg NA (—o—); and one group 20 μg NA (—●—). The results are expressed as difference from the control group, which received 10 μl saline intraventricularly.

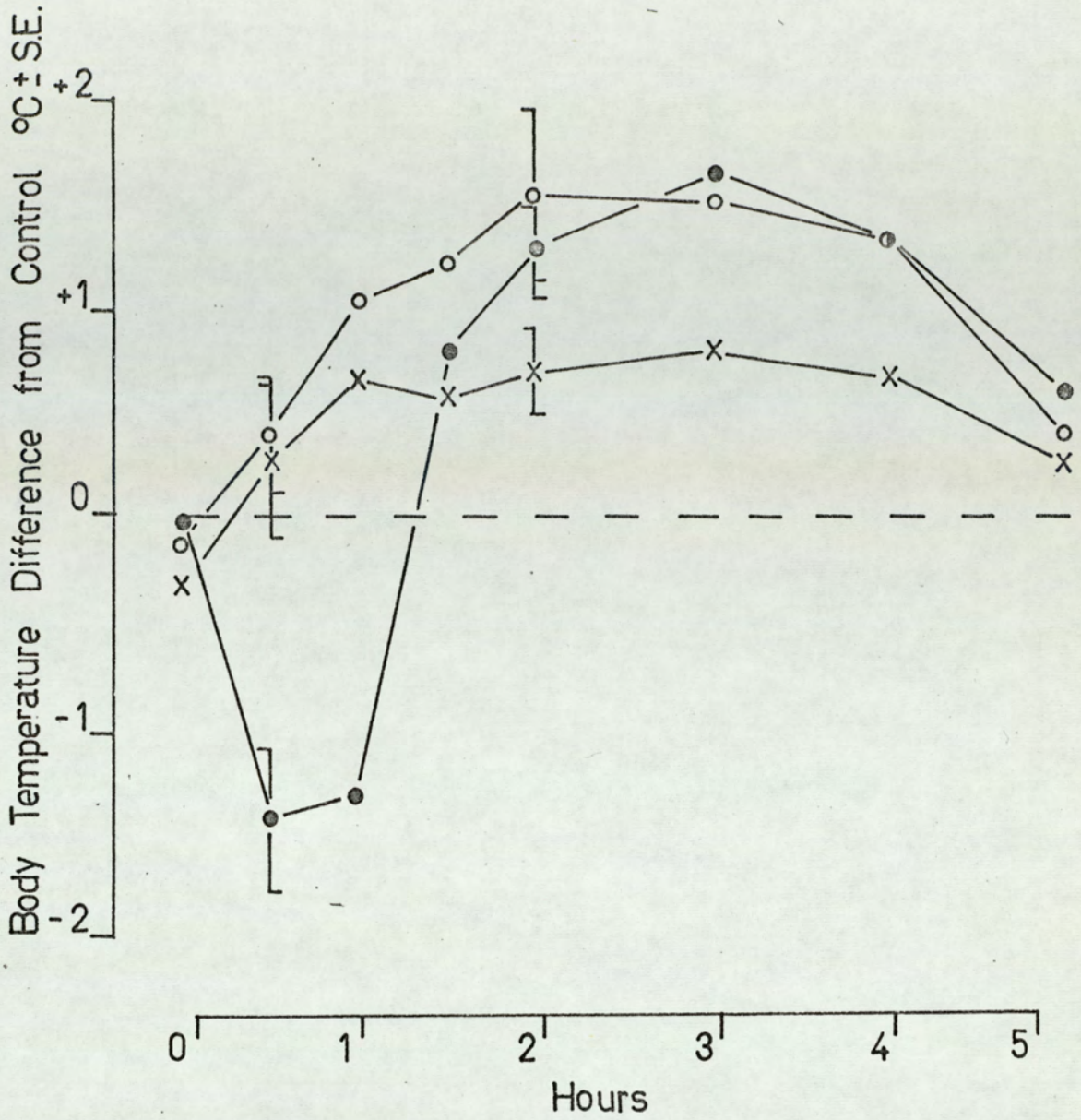

FIGURE 19 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF NA AT AN AMBIENT TEMPERATURE OF 25°C

4 groups of 5 animals were used. One group received 5 µg NA intraventricularly (—X—); one group 10 µg (—o—) and one group 20 µg (—●—). The results are expressed as difference from the control group which received 10 µl saline intraventricularly.

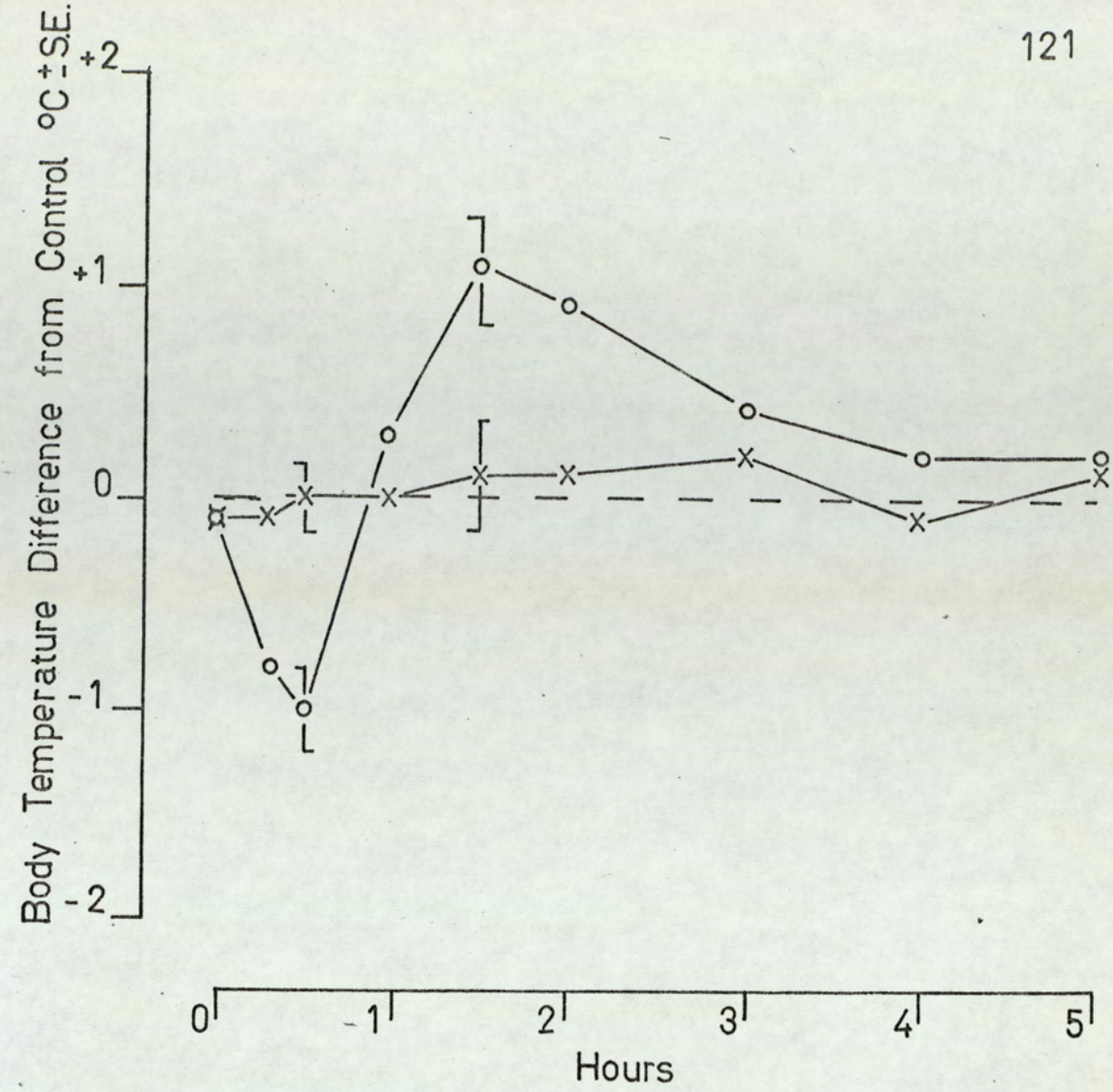

FIGURE 20 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF NA FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED PHENTOLAMINE

3 groups of 5 animals were used. One group received 1 mg/kg phentolamine i.p. at -30 minutes (—X—) and one 1.0 ml/kg saline (—o—). At time 0 both groups received 10 μg NA intraventricularly. The results are expressed as difference from the control group, which received phentolamine i.p. and saline intraventricularly.

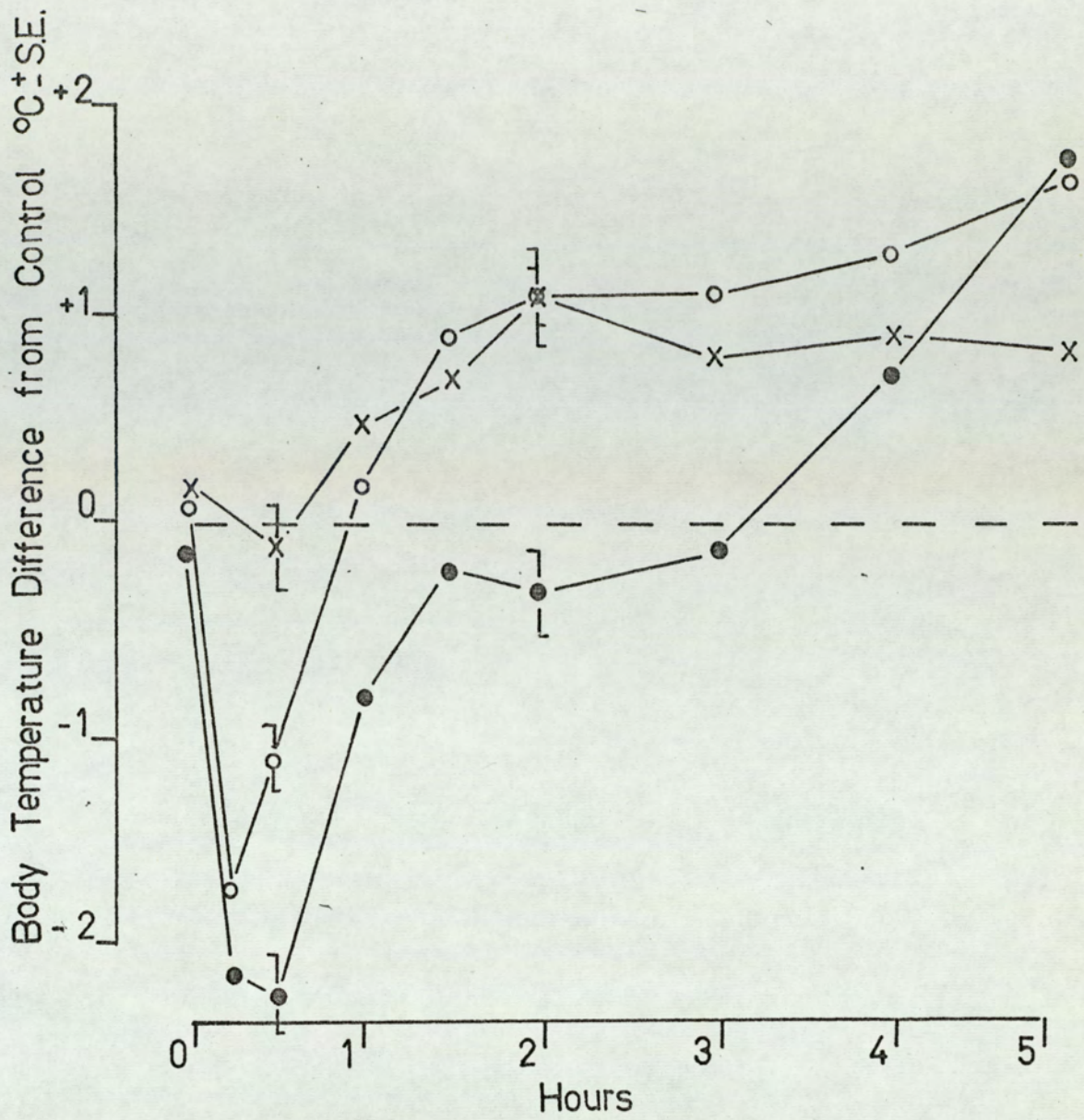

FIGURE 21 THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF 5-HT

4 groups of 5 animals were used. One group received 2.5 μg 5-HT intraventricularly (—X—); one group 5.0 μg (—o—); and one group 10 μg (—●—). The results are expressed as difference from the control group which received intraventricular saline (10 μl).

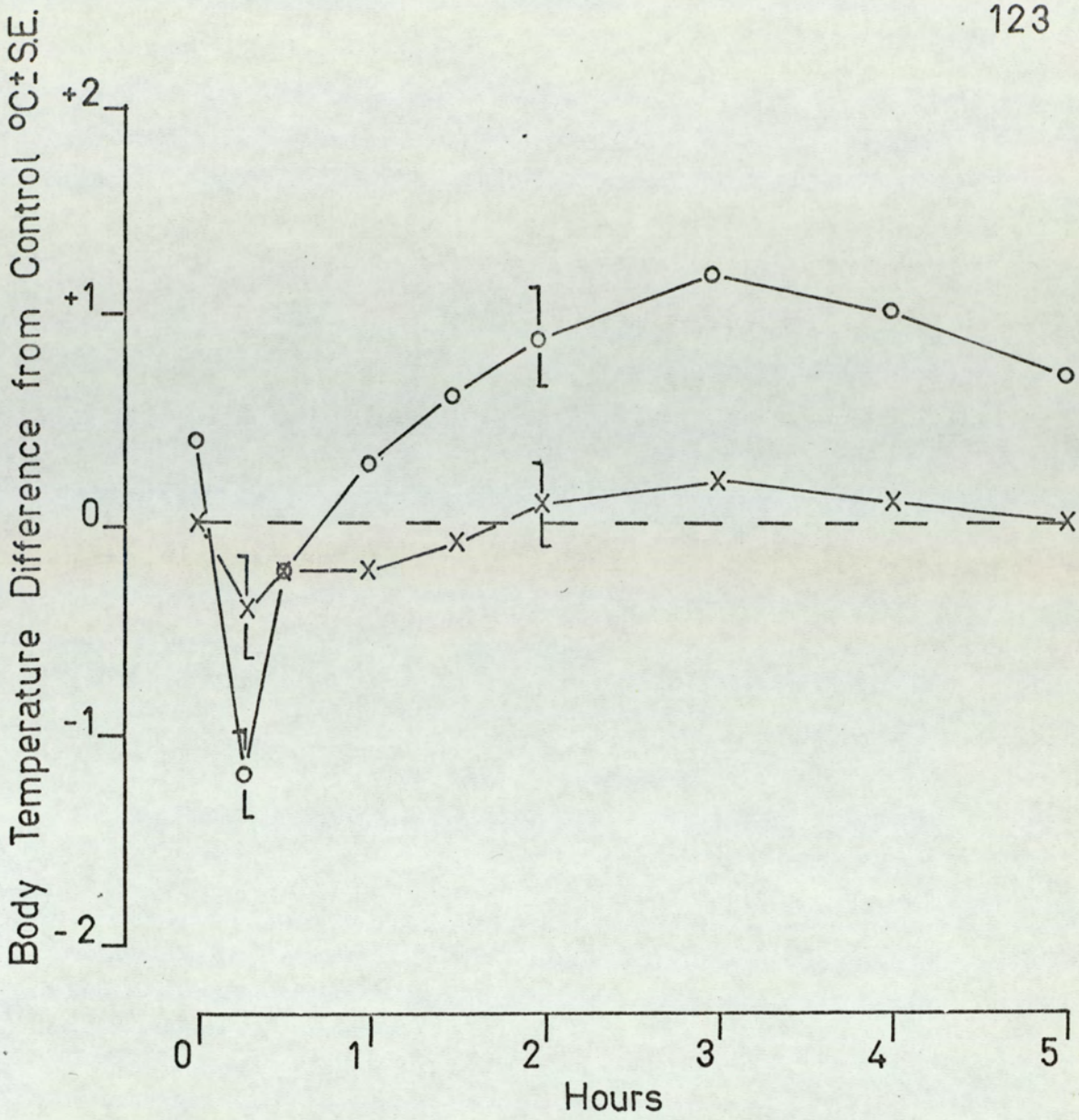

FIGURE 22 THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF 5-HT FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED METHYSERGIDE

3 groups of 5 animals were used. One group received 2 mg/kg methysergide i.p. at -45 minutes (—X—); one group received 1 mg/kg saline i.p. (— o —). At time 0 both groups received 5 μg 5-HT intraventricularly. The results are expressed as difference from the control group which received methysergide i.p. and saline intraventricularly.

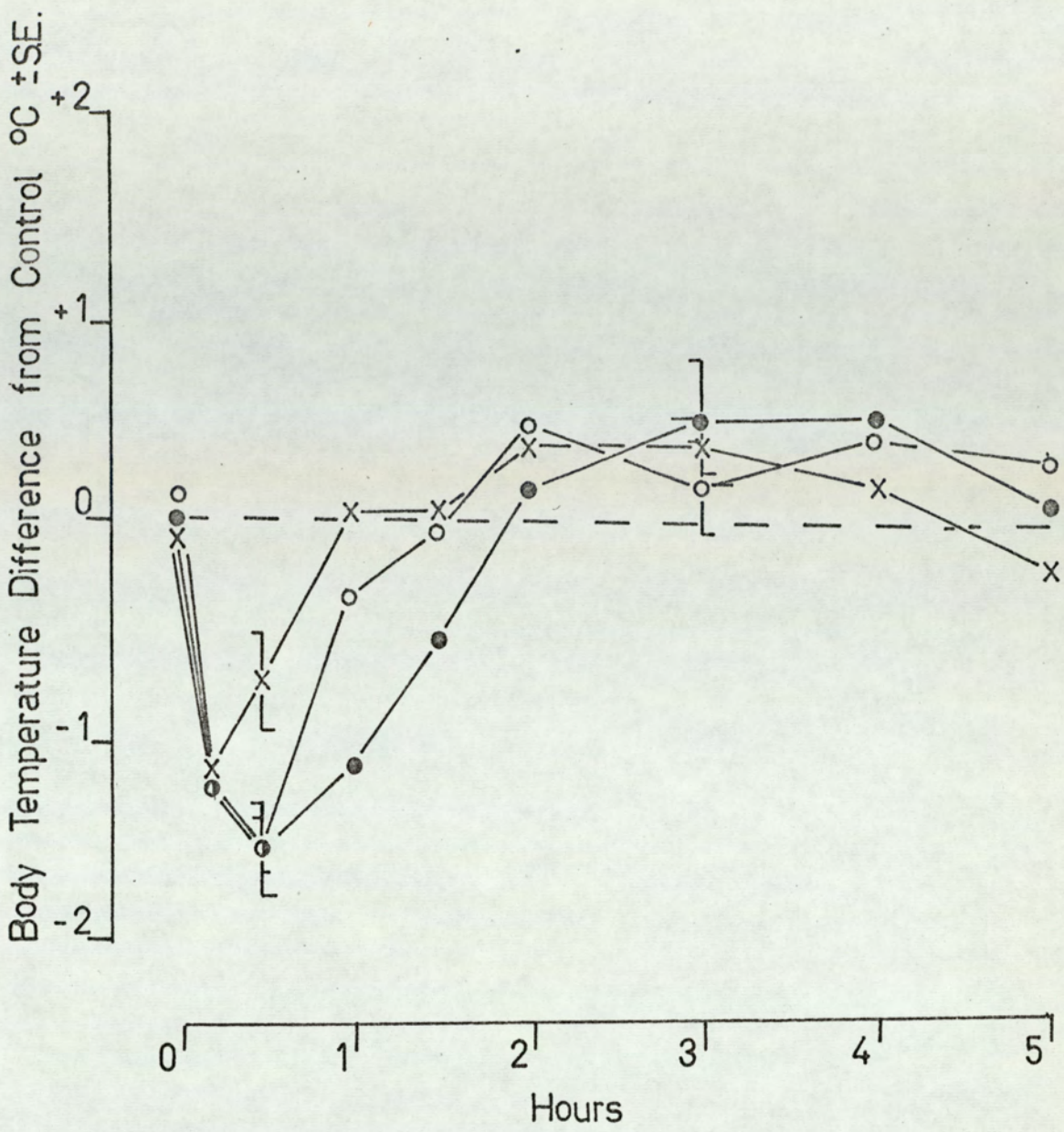

FIGURE 23 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF CARBACHOL

4 groups of 5 animals were used. One group received 0.9 µg carbachol intraventricularly (—X—); one group 1.8 µg (—o—) and one group 3.6 µg (—●—). The results are expressed as difference from the control group which received saline (10 µl).

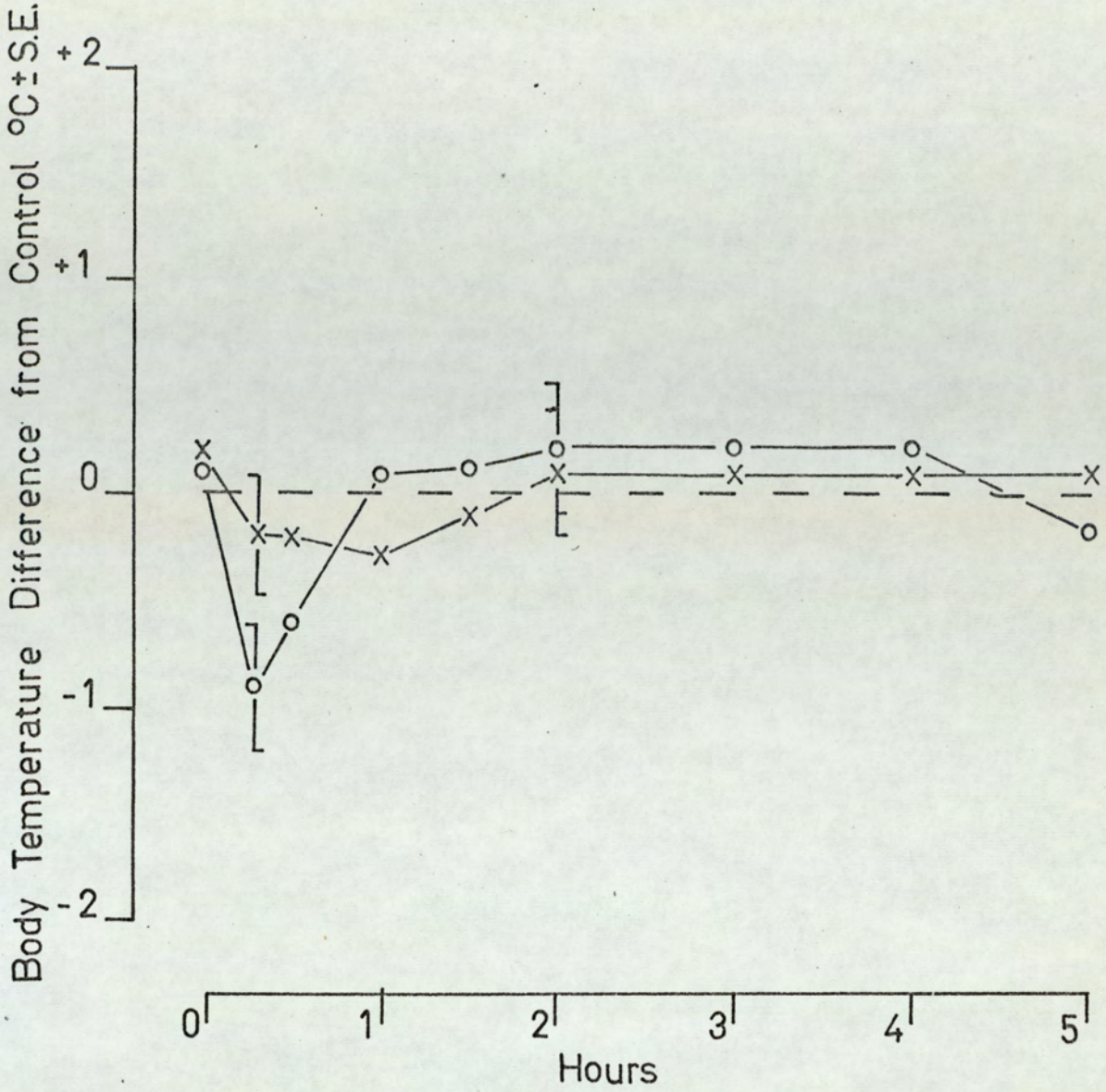

FIGURE 24 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF CARBACHOL FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED HYOSCINE

3 groups of 5 animals were used. One group received hyoscine 1 mg/kg i.p. at -30 minutes (—X—) and one 1 ml/kg saline (—o—). At time 0 both groups received 0.9 μg carbachol intraventricularly. The results are expressed as difference from the control group which received hyoscine i.p. and saline intraventricularly.

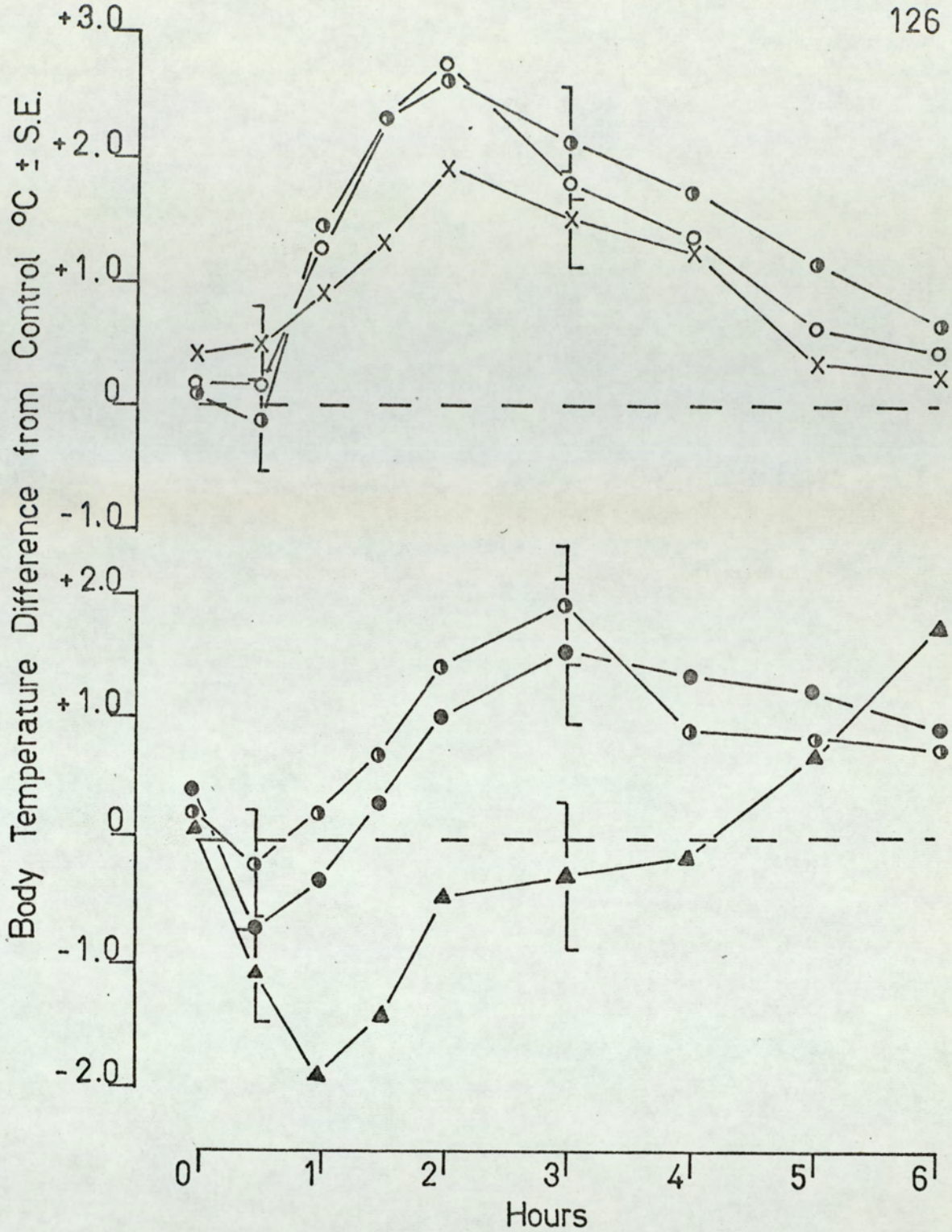

FIGURE 25 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF MORPHINE

The figure shows the results from 2 experiments. 8 groups of 5 animals were used. The following doses of morphine were injected intraventricularly:

10 μg (—X—); 20 μg (—o—); 40 μg (—◑—); 80 μg (—●—); 160 μg (—▲—).

The results are expressed as differences from the control groups of each experiment, which received saline (10 μl).

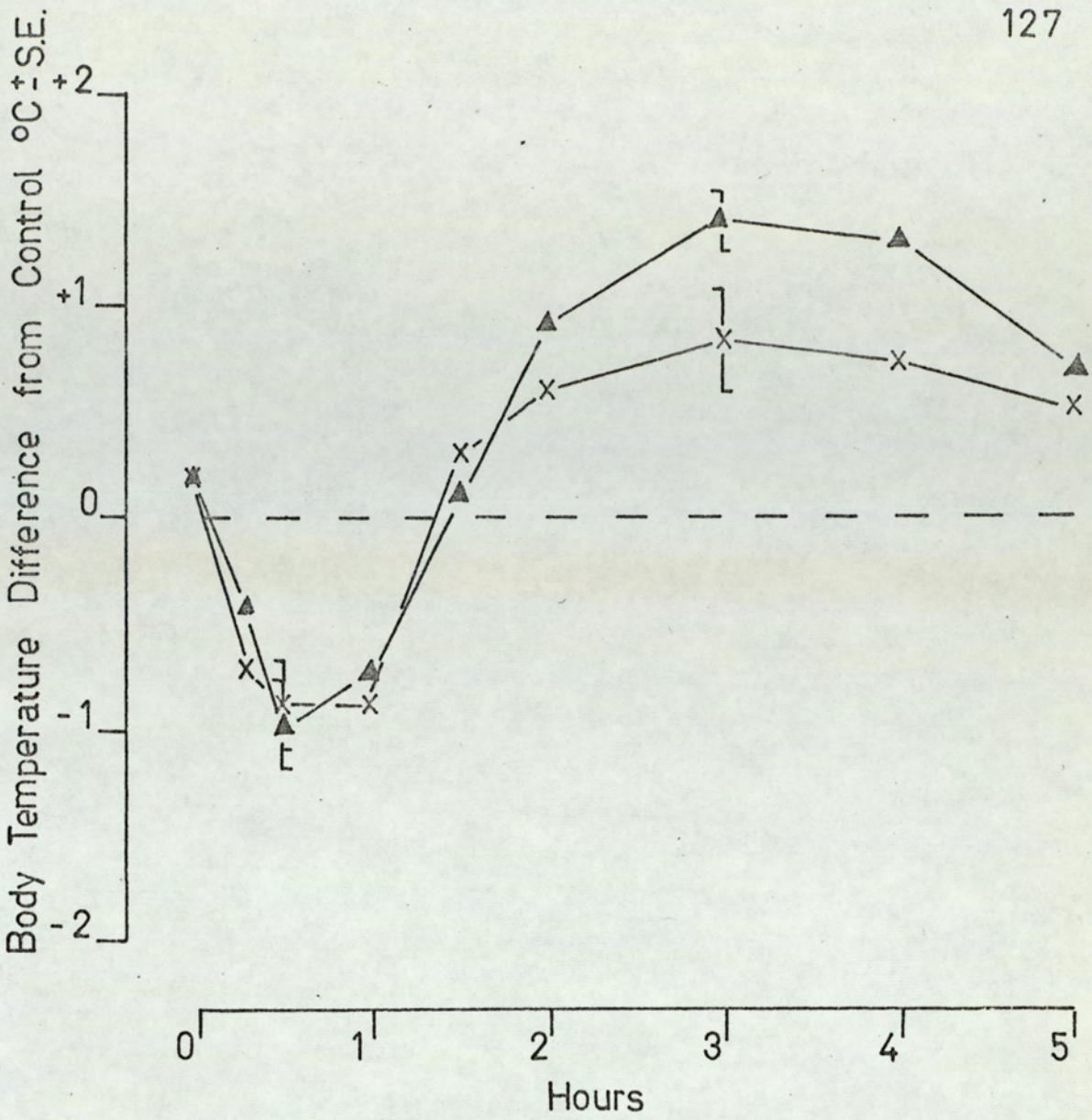

FIGURE 26 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF MORPHINE FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED PHENTOLAMINE

3 groups of 5 animals were used. One group received 1 mg/kg phentolamine i.p. at -30 minutes (—X—) and one 1 ml/kg saline (—▲—). At time 0 both groups received 80 μg morphine intraventricularly. The results are expressed as difference from the control group, which received phentolamine i.p. and saline intraventricularly.

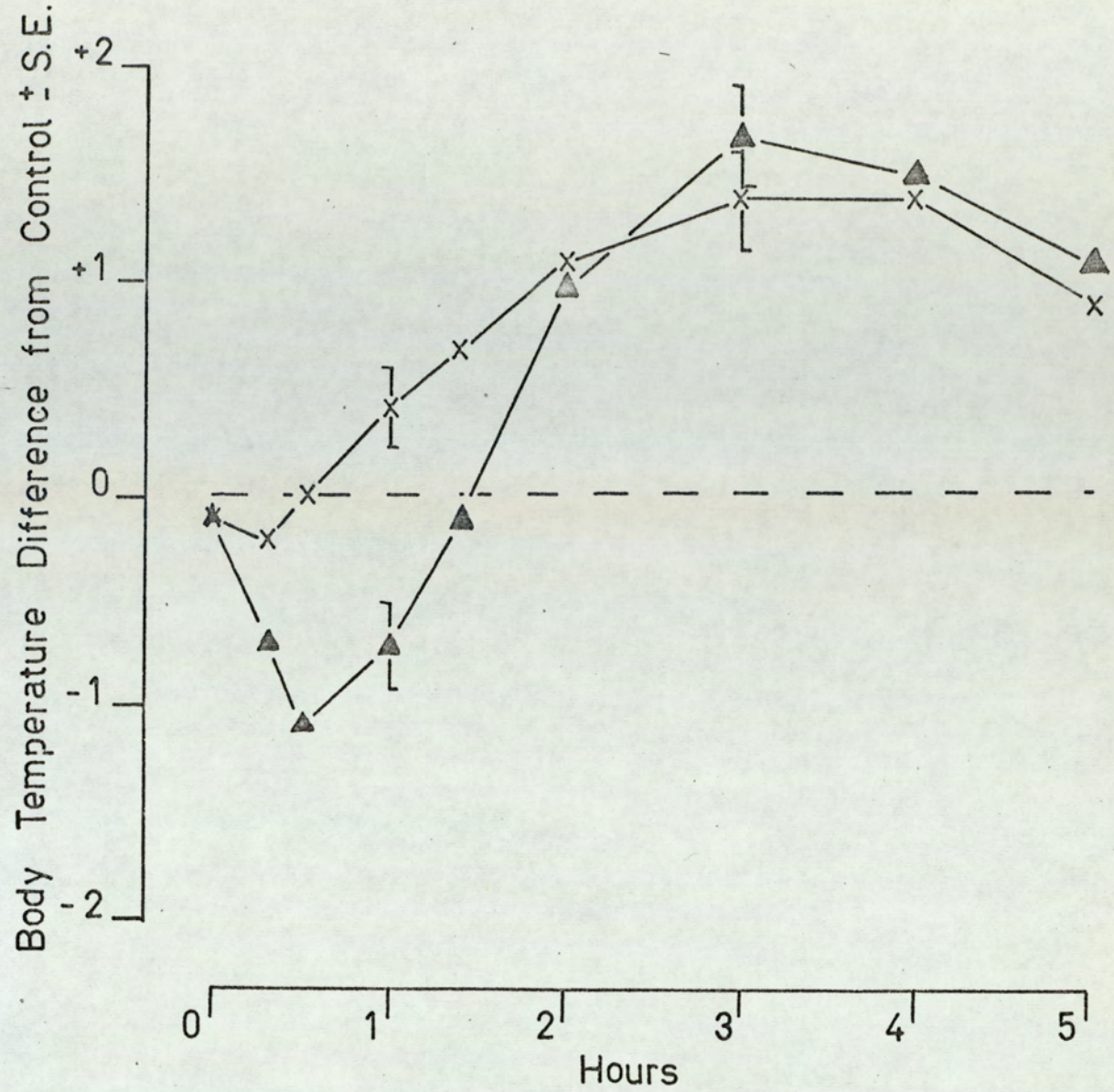

FIGURE 27 THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF MORPHINE FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED HYOSCINE

3 groups of 5 animals were used. One group received 1 ml/kg saline i.p. at -30 minutes (—▲—) and one group hyoscine 1 mg/kg i.p. (—X—). At time 0 both groups received an intraventricular injection of 80 μg morphine. The results are expressed as difference from the control group, which received hyoscine i.p. and saline intraventricularly.

TABLE 1

THE EFFECT ON BODY TEMPERATURE OF MANIPULATIONS OF THE CANNULA GUIDE

(see Fig. 11)

	Body temperature $^{o}C \pm$ Standard Error							
Time in hours	0	0.5	1.0	1.5	2	3	4	4
Handling only	37.7 ± 0.15	28.0 ± 0.20	38.1 ± 0.20	37.9 ± 0.12	37.8 ± 0.20	37.9 ± 0.14	38.1 ± 0.12	38.1 ± 0.15
Cannula guide manipulated	37.9 ± 0.13	38.4 ± 0.19	38.3 ± 0.24	38.2 ± 0.20	38.3 ± 0.15	38.1 ± 0.07	38.1 ± 0.18	38.1 ± 0.24
Cannula inserted (no injection)	37.8 ± 0.09	38.0 ± 0.16	38.3 ± 0.19	38.9 ± 0.05	39.3 ± 0.33	39.9 ± 0.46	39.8 ± 0.36	40.0 ± 0.21
20 µl saline injected	37.7 ± 0.20	38.4 ± 0.40	38.9 ± 0.56	39.0 ± 0.39	38.9 ± 0.30	39.4 ± 0.32	39.5 ± 0.29	39.7 ± 0.30

TABLE 2

THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF SALINE AT 21°C AND 37°C

(see Fig. 12)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Handling only	37.2 ± 0.03	37.5 ± 0.07	37.5 ± 0.05	37.4 ± 0.08	37.4 ± 0.10	37.4 ± 0.04	37.5 ± 0.08	37.6 ± 0.05
5 mm guide : 5 mm cannula No stilette 20 µl saline at 21°C	37.4 ± 0.24	37.8 ± 0.34	38.1 ± 0.34	38.6 ± 0.35	38.7 ± 0.39	38.8 ± 0.37	39.0 ± 0.31	38.9 ± 0.30
5 mm guide : 5 mm cannula No stilette 20 µl saline at 37°C	37.4 ± 0.17	37.9 ± 0.17	37.9 ± 0.20	38.3 ± 0.35	39.1 ± 0.35	38.1 ± 0.24	38.9 ± 0.18	38.8 ± 0.16

TABLE 3

THE EFFECTS ON BODY TEMPERATURE OF THE USE OF VARIOUS LENGRHS OF CANNULA WITH A 5 mm CANNULA GUIDE

(see Fig. 13)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Handling only	37.7 ± 0.12	38.0 ± 0.16	38.3 ± 0.14	38.1 ± 0.19	38.2 ± 0.13	38.2 ± 0.14	38.1 ± 0.13	38.0 ± 0.17
5 mm guide : 1.5 mm cannula No stilette 20 µl saline	37.9 ± 0.15	38.5 ± 0.19	38.3 ± 0.27	38.5 ± 0.26	38.7 ± 0.38	38.4 ± 0.16	38.4 ± 0.18	38.4 ± 0.21
5 mm guide : 3.5 mm cannula No stilette 20 µl saline	37.9 ± 0.13	38.2 ± 0.13	38.4 ± 0.16	38.6 ± 0.14	38.7 ± 0.25	38.9 ± 0.35	38.9 ± 0.35	38.9 ± 0.37
5 mm guide : 5 mm cannula No stilette 20 µl saline	38.0 ± 0.14	38.4 ± 0.19	38.5 ± 0.27	38.9 ± 0.27	39.2 ± 0.29	39.9 ± 0.44	40.0 ± 0.38	39.9 ± 0.42

TABLE 4

THE EFFECTS ON BODY TEMPERATURE OF 20 µl SALINE INJECTED VIA DIFFERENT LENGTHS OF CANNULA

(see Fig. 14)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2	3	4	5
Handling only	37.6 ± 0.12	37.7 ± 0.22	38.0 ± 0.29	38.1 ± 0.31	38.0 ± 0.22	37.7 ± 0.16	38.0 ± 0.06	37.9 ± 0.10
1.5 mm cannula 20 µl saline	37.4 ± 0.06	38.0 ± 0.16	38.9 ± 0.13	39.3 ± 0.14	39.9 ± 0.11	39.6 ± 0.08	39.5 ± 0.08	39.5 ± 0.09
3.5 mm cannula 20 µl saline	37.4 ± 0.14	38.1 ± 0.21	38.7 ± 0.21	39.3 ± 0.21	39.8 ± 0.19	39.5 ± 0.21	39.4 ± 0.20	39.6 ± 0.21
5.0 mm cannula 20 µl saline	37.5 ± 0.10	37.8 ± 0.15	38.5 ± 0.15	39.2 ± 0.20	39.7 ± 0.16	39.5 ± 0.17	39.5 ± 0.15	39.8 ± 0.16

TABLE 5

THE EFFECT ON BODY TEMPERATURE OF DIFFERENT LENGTHS OF STILETTE IN A 5 mm CANNULA GUIDE

(see Fig. 15)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Handling only	37.0 ± 0.4	37.5 ± 0.10	37.6 ± 0.16	37.4 ± 0.15	37.5 ± 0.11	37.6 ± 0.10	37.6 ± 0.12	37.6 ± 0.15
5 mm guide : 5 mm cannula 3 mm stilette 20 µl saline.	37.3 ± 0.05	37.7 ± 0.14	38.5 ± 0.10	29.0 ± 0.19	38.5 ± 0.09	39.6 ± 0.06	39.8 ± 0.10	39.8 ± 0.13
5 mm guide : 5 mm cannula 5 mm stilette 20 µl saline	37.0 ± 0.05	37.4 ± 0.12	37.5 ± 0.07	37.8 ± 0.15	38.3 ± 0.13	38.8 ± 0.11	38.5 ± 0.18	38.5 ± 0.15

TABLE 6

THE EFFECTS ON BODY TEMPERATURE OF DIFFERENT LENGTHS OF CANNULA GUIDE AND STILETTE

(see Fig. 16)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2	3	4	5
5 mm guide : 5 mm cannula No stilette 20 µl saline	37.2 ± 0.09	38.5 ± 0.10	38.4 ± 0.16	39.0 ± 0.20	39.2 ± 0.25	39.5 ± 0.16	38.5 ± 0.10	39.4 ± 0.12
5 mm guide : 5 mm cannula 5 mm stilette 20 µl saline	37.3 ± 0.05	37.7 ± 0.10	38.0 ± 0.15	38.4 ± 0.16	38.3 ± 0.18	38.3 ± 0.20	38.1 ± 0.22	38.1 ± 0.21
4 mm guide : 5 mm cannula 4 mm stilette 20 µl saline	37.4 ± 0.10	37.7 ± 0.20	37.6 ± 0.16	37.6 ± 0.12	37.6 ± 0.10	37.6 ± 0.22	37.5 ± 0.17	37.5 ± 0.09

TABLE 7

THE EFFECT ON BODY TEMPERATURE OF VARIOUS VOLUMES OF SALINE INJECTED INTRAVENTRICULARLY USING A 4 mm CANNULA AND STILETTE

(see Fig. 17)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Cannula only (no injection)	37.3 ± 0.10	37.3 ± 0.18	37.4 ± 0.18	37.3 ± 0.19	37.5 ± 0.18	37.7 ± 0.20	37.5 ± 0.21	37.3 ± 0.19
10 µl saline	37.7 ± 0.18	37.8 ± 0.18	37.7 ± 0.17	37.7 ± 0.20	37.8 ± 0.19	38.0 ± 0.18	37.6 ± 0.19	37.7 ± 0.18
20 µl saline	37.5 ± 0.12	37.5 ± 0.10	37.5 ± 0.05	37.6 ± 0.07	37.7 ± 0.08	38.2 ± 0.15	37.9 ± 0.16	37.6 ± 0.19
40 µl saline	37.9 ± 0.05	37.8 ± 0.02	37.7 ± 0.10	37.7 ± 0.15	37.7 ± 0.10	38.1 ± 0.07	38.1 ± 0.09	38.2 ± 0.14
80 µl saline	37.3 ± 0.19	37.4 ± 0.17	37.5 ± 0.14	37.6 ± 0.20	37.6 ± 0.24	38.0 ± 0.24	38.1 ± 0.24	38.1 ± 0.20

TABLE 8

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF NA AT AN AMBIENT TEMPERATURE OF 18°C

(see Fig. 18)

	Body temperature °C ± Standard Error								
Time in hours	0	0.25	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Saline 10 µl	37.4 ± 0.08	37.3 ± 0.10	37.5 ± 0.08	37.7 ± 0.15	37.8 ± 0.19	37.9 ± 0.15	38.6 ± 0.18	38.8 ± 0.20	38.5 ± 0.15
NA 5 µg	37.2 ± 0.10	36.8 ± 0.23	37.7 ± 0.25	38.4 ± 0.26	38.8 ± 0.25	39.3 ± 0.30	39.4 ± 0.29	39.6 ± 0.26	39.1 ± 0.25
NA 10 µg	37.0 ± 0.09	36.2 ± 0.18	36.4 ± 0.17	38.8 ± 0.19	38.8 ± 0.20	38.9 ± 0.19	39.3 ± 0.30	39.4 ± 0.31	38.9 ± 0.25
NA 20 µg	37.3 ± 0.10	35.9 ± 0.12	35.3 ± 0.12	37.0 ± 0.15	39.4 ± 0.32	39.2 ± 0.30	39.5 ± 0.31	39.1 ± 0.29	38.8 ± 0.25

TABLE 9

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF NA AT AN AMBIENT TEMPERATURE OF 25°C

(see Fig. 19)

	Body temperature °C ± Standard Error							
Time in hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Saline 10 µl	38.1 ± 0.09	38.2 ± 0.20	38.3 ± 0.18	38.4 ± 0.20	38.5 ± 0.16	38.3 ± 0.16	38.5 ± 0.19	38.8 ± 0.14
NA 5 µg	37.8 ± 0.17	38.5 ± 0.17	39.0 ± 0.16	39.0 ± 0.21	39.1 ± 0.26	39.1 ± 0.19	39.2 ± 0.20	39.1 ± 0.21
NA 10 µg	38.0 ± 0.22	38.6 ± 0.30	39.3 ± 0.30	39.5 ± 0.22	39.9 ± 0.34	39.8 ± 0.35	39.7 ± 0.36	39.2 ± 0.34
NA 20 µg	38.1 ± 0.13	36.8 ± 0.25	37.8 ± 0.36	39.1 ± 0.20	39.7 ± 0.12	39.9 ± 0.12	39.8 ± 0.18	39.6 ± 0.20

TABLE 10

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR (i.c.) INJECTIONS OF NA FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED PHENTOLAMINE

(see Fig. 20)

	Body temperature °C ± Standard Error								
Time (hours)	0	0.25	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Phentolamine 1 ml/kg i.p. Saline 10 µl i.c	37.4 ± 0.05	37.4 ± 0.10	37.5 ± 0.14	37.6 ± 0.08	37.6 ± 0.15	37.5 ± 0.09	37.6 ± 0.06	37.4 ± 0.07	37.3 ± 0.12
Phentolamine 1 mg/kg i.p. NA 10 µg i.c.	37.3 ± 0.12	37.3 ± 0.13	37.5 ± 0.10	37.6 ± 0.09	37.7 ± 0.18	37.6 ± 0.15	37.8 ± 0.17	37.3 ± 0.07	37.4 ± 0.09
Saline 1 ml/kg i.p. NA 10 µg i.c.	37.3 ± 0.10	36.6 ± 0.07	36.5 ± 0.16	37.9 ± 0.15	38.7 ± 0.27	38.4 ± 0.11	38.0 ± 0.14	37.6 ± 0.05	37.5 ± 0.11

TABLE 11

THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF 5-HT

(see (Fig. 21)

	Body temperature °C ± Standard Error								
Time in hours	0	0.25	0.5	1.0	1.5	2	3	4	5
Saline 10 µl	37.1 ± 0.12	37.7 ± 0.12	37.8 ± 0.07	37.7 ± 0.07	37.7 ± 0.15	37.8 ± 0.10	38.0 ± 0.06	37.9 ± 0.04	37.7 ± 0.05
5-HT 2.5 µg	37.3 ± 0.08	37.4 ± 0.34	37.7 ± 0.29	38.2 ± 0.20	38.4 ± 0.20	38.9 ± 0.27	38.8 ± 0.30	38.8 ± 0.22	38.5 ± 0.21
5-HT 5.0 µg	37.2 ± 0.18	36.0 ± 0.13	36.7 ± 0.22	37.9 ± 0.14	38.6 ± 0.17	38.9 ± 0.13	39.1 ± 0.22	39.2 ± 0.19	39.3 ± 0.17
5-HT 10.0 µg	37.0 ± 0.06	35.6 ± 0.22	35.6 ± 0.24	36.9 ± 0.15	37.5 ± 0.33	37.5 ± 0.27	37.9 ± 0.16	38.6 ± 0.14	39.4 ± 0.09

TABLE 12

THE EFFECTS ON BODY TEMPERATURE OF INTRAVENTRICULAR (i.c.) INJECTIONS OF 5-HT FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED METHYSERGIDE

(see Fig. 22)

	Body temperature °C ± Standard Error								
Time (hours)	0	0.25	0.5	1.0	1.5	2	3	4	5
Methysergide 2 mg/kg i.p. Saline 10 µl i.c.	36.9 ± 0.14	37.0 ± 0.18	36.8 ± 0.12	36.9 ± 0.10	37.0 ± 0.14	37.1 ± 0.12	37.1 ± 0.16	37.2 ± 0.16	37.2 ± 0.09
Saline 1.0 ml/kg i.p. 5-HT 5 µg i.c.	37.3 ± 0.10	35.8 ± 0.10	36.6 ± 0.24	37.2 ± 0.25	37.6 ± 0.26	38.0 ± 0.20	38.3 ± 0.24	38.2 ± 0.20	37.9 ± 0.19
Methysergide 2 mg/kg i.p. 5-HT 5 µg i.c.	36.9 ± 0.15	36.6 ± 0.20	36.6 ± 0.22	36.7 ± 0.21	36.9 ± 0.23	37.2 ± 0.21	37.3 ± 0.18	37.3 ± 0.16	37.2 ± 0.20

TABLE 13

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF CARBACHOL

(see Fig. 23)

	Body temperature °C ± Standard Error								
Time in hours	0	0.25	0.5	1	1.5	2	3	4	5
Saline 10 µl	37.3 ± 0.09	37.5 ± 0.08	37.4 ± 0.09	37.5 ± 0.19	37.8 ± 0.16	37.7 ± 0.15	37.8 ± 0.20	38.1 ± 0.16	37.8 ± 0.20
Carbachol 0.9 µg	37.3 ± 0.12	36.4 ± 0.23	36.7 ± 0.26	37.6 ± 0.14	37.9 ± 0.19	38.1 ± 0.16	38.2 ± 0.12	38.3 ± 0.12	37.5 ± 0.10
Carbachol 1.8 µg	37.5 ± 0.08	36.3 ± 0.17	35.9 ± 0.22	37.2 ± 0.3	37.8 ± 0.29	38.2 ± 0.25	38.0 ± 0.16	38.5 ± 0.14	38.1 ± 0.09
Carbachol 3.6 µg	37.4 ± 0.11	36.3 ± 0.10	35.9 ± 0.12	36.4 ± 0.32	37.3 ± 0.30	37.9 ± 0.30	38.3 ± 0.24	38.7 ± 0.09	38.9 ± 0.10

TABLE 14

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR (i.c.) INJECTIONS OF CARBACHOL FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED HYOSCINE

(see Fig. 24)

	Body temperature °C ± Standard Error								
Time (hours)	0	0.25	0.5	1.0	1.5	2	3	4	5
Hyoscine 1 mg/kg i.p. Saline 10 µl i.c.	37.1 ± 0.10	37.3 ± 0.16	37.2 ± 0.08	37.3 ± 0.12	37.4 ± 0.16	37.4 ± 0.18	37.3 ± 0.12	37.2 ± 0.13	37.2 ± 0.09
Saline 1 ml/kg i.p. Carbachol 0.9 µg i.c.	37.2 ± 0.09	35.4 ± 0.20	36.6 ± 0.24	37.4 ± 0.20	37.5 ± 0.22	37.6 ± 0.21	37.5 ± 0.22	37.4 ± 0.19	37.0 ± 0.21
Hyoscine 1 mg/kg i.p. Carbachol 0.9 µg i.c.	37.3 ± 0.21	37.1 ± 0.20	37.0 ± 0.21	37.0 ± 0.22	37.3 ± 0.24	37.5 ± 0.20	37.4 ± 0.19	37.3 ± 0.15	37.3 ± 0.14

TABLE 15

.THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF MORPHINE (LOW DOSES)

(see Fig. 25)

	Body Temperature °C ± Standard Error								
Time (Hours	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
Saline 10 µl i.c.	36.7 ± 0.25	37.2 ± 0.15	37.1 ± 0.17	37.0 ± 0.15	37.0 ± 0.16	37.6 ± 0.19	37.6 ± 0.33	37.8 ± 0.31	37.8 ± 0.20
Morphine 10 µg i.c.	37.1 ± 0.31	37.7 ± 0.29	38.0 ± 0.39	38.3 ± 0.42	38.9 ± 0.53	39.2 ± 0.14	38.8 ± 0.21	38.1 ± 0.32	38.0 ± 0.24
Morphine 20 µg i.c.	36.9 ± 0.21	37.4 ± 0.21	38.4 ± 0.19	39.3 ± 0.12	39.8 ± 0.15	39.4 ± 0.14	38.9 ± 0.13	38.4 ± 0.12	38.2 ± 0.16
Morphine 40 µg i.c.	36.8 ± 0.13	37.1 ± 0.24	38.5 ± 0.36	39.3 ± 0.43	39.6 ± 0.24	39.7 ± 0.26	39.3 ± 0.14	38.9 ± 0.24	38.4 ± 0.42

TABLE 16

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR INJECTIONS OF MORPHINE (HIGH DOSES)

(see Fig. 25)

	Body Temperature °C ± Standard Error								
Time (hours)	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
Saline 10 µl i.c.	37.3 ± 0.14	37.5 ± 0.13	37.4 ± 0.14	37.5 ± 0.17	37.5 ± 0.30	37.4 ± 0.26	37.8 ± 0.18	37.7 ± 0.14	37.7 ± 0.10
Morphine 40 µg i.c.	37.5 ± 0.09	37.3 ± 0.17	37.6 ± 0.27	38.2 ± 0.16	38.9 ± 0.14	39.3 ± 0.19	38.7 ± 0.05	38.5 ± 0.14	38.4 ± 0.27
Morphine 80 µg i.c.	37.7 ± 0.14	36.8 ± 0.27	37.1 ± 0.32	37.8 ± 0.39	38.5 ± 0.44	38.9 ± 0.32	39.1 ± 0.10	38.9 ± 0.16	38.6 ± 0.17
Morphine 160 µg i.c.	37.4 ± 0.114	36.4 ± 0.26	35.5 ± 0.26	36.1 ± 0.30	37.0 ± 0.42	37.1 ± 0.34	37.6 ± 0.16	38.4 ± 0.19	39.4 ± 0.27

TABLE 17

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR (i.c.) INJECTION OF MORPHINE FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED PHENTOLAMINE

(see Fig.26)

	Body Temperature °C ± Standard Error								
Time (hours)	0	0.25	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Phentolamine 1.0 ml/kg i.p. Saline 10 µl i.c.	37.3 ± 0.09	37.3 ± 0.15	37.4 ± 0.14	37.4 ± 0.18	37.4 ± 0.17	37.5 ± 0.05	37.5 ± 0.11	37.5 ± 0.16	37.5 ± 0.14
Phentolamine 1.0 mg/kg i.p. Morphine 80 µg i.c.	37.5 ± 0.08	36.6 ± 0.14	36.5 ± 0.15	36.5 ± 0.16	37.7 ± 0.16	38.1 ± 0.22	38.0 ± 0.08	37.9 ± 0.05	37.7 ± 0.09
Saline 1.0 ml/kg i.p. Morphine 80 µg i.c.	37.5 ± 0.13	36.9 ± 0.12	36.4 ± 0.13	36.7 ± 0.10	37.5 ± 0.05	38.4 ± 0.19	38.9 ± 0.21	38.8 ± 0.10	38.2 ± 0.23

TABLE 18

THE EFFECT ON BODY TEMPERATURE OF INTRAVENTRICULAR (i.c.) INJECTIONS OF MORPHINE FOLLOWING PRETREATMENT WITH INTRAPERITONEALLY (i.p.) ADMINISTERED HYOSCINE

(see Fig. 27)

	Body Temperature °C ± Standard Error								
Time (hours)	0	0.25	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Hyoscine 1.0 mg/kg i.p. Saline 10 µl i.c.	37.5 ± 0.08	37.4 ± 0.12	37.4 ± 0.18	37.6 ± 0.12	37.5 ± 0.11	37.5 ± 0.12	37.4 ± 0.11	37.5 ± 0.13	37.6 ± 0.09
Hyoscine 1.0 mg/kg i.p. Morphine 80 µg i.c	37.4 ± 0.10	37.2 ± 0.14	37.4 ± 0.16	38.0 ± 0.12	38.2 ± 0.18	38.6 ± 0.12	38.8 ± 0.13	38.9 ± 0.20	38.5 ± 0.10
Saline 1.0 ml/kg i.p.	37.4 ± 0.12	36.7 ± 0.10	36.3 ± 0.15	36.9 ± 0.14	37.6 ± 0.14	38.5 ± 0.15	39.1 ± 0.16	39.0 ± 0.12	38.7 ± 0.10

CHAPTER THREE

MORPHINE, BIOGENIC AMINES AND NOCICEPTION

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MORPHINE, BIOGENIC AMINES AND NOCICEPTION

1. INTRODUCTION

The mechanism of the analgesic action of morphine at the cellular level is still unknown. Several theories have been proposed involving interactions with neurotransmitters in the brain. Some authors have proposed that the analgesic action is mediated by acetylcholine (Slaughter & Munsell, 1939; Slaughter, 1950; Szerb, 1957; Saxena, 1958; Gordonoff, 1959), some propose noradrenaline (Schaumann, 1958; Verri, Graeff & Corrado, 1968; Colville & Chaplin, 1964; Leimdorfer & Metzner, 1949; Raduco-Thomas, 1957) and some propose 5-HT (Medakovic & Banic, 1964; Tenen, 1968). It has also been suggested that neither acetylcholine nor the amines are involved (Rudzik & Mennear, 1965). However it is generally agreed that compounds that deplete central stores of NA and 5-HT or block the action of ACh, inhibit the action of morphine.

In view of these reports and the results in the previous chapter it became of interest to investigate morphine-neurotransmitter interactions further.

The word analgesia is derived from the Greek, meaning without pain. Thus in order to talk about analgesia one should be able to define pain. However pain is such a subjective experience that although most people know of it from personal experience, it is very difficult to define. This aspect of analgesia can not be considered in depth here, and has been thoughtfully reviewed by Winter (1965).

There is also a fundamental lack of knowledge about the physiology of pain. There are at present 3 main theories covering the nature of the perception of pain (i) the specificity theory (ii) the pattern theory and (iii) the gate-control theory. The specificity theory proposes that a mosaic of specific pain receptors in the body tissues project to a pain centre in the brain. It maintains that free nerve endings are the pain receptors and they generate pain impulses that are carried via the spinal cord to a pain centre in the thalamus. The pattern theory proposes that all fibre endings (apart from those which innervate hair cells) are alike, so that the pattern for pain, is produced by intense stimulation of non-specific receptors. This theory is not entirely satisfactory as it ignors the facts of physiological specialisation in each receptor-fibre unit. (see Melzack & Wall, 1965). Thirdly the gate control theory suggests that the system responsible for pain perception and response is triggered after the sensory input has been modulated by both sensory feedback mechanisms and influences of the CNS. This then takes into account that pain perception is not simply a function of the amount of damage alone, but is determined by past experience, attention and expectation, anxiety and the meaning of the situation in which injury occurs (Melzack & Wall, 1965 & 1968).

The complexity of the nature of pain makes it difficult to conduct laboratory experiments to predict the analgesic activity of drugs. Animals are unable to report if pain is felt, so observable responses must be used to indicate whether a noxious stimulus has taken effect. In such cases it is difficult to determine whether an animal has 'felt' pain or whether the reaction is a simple escape reflex. Thus it may be better to use the word nociceptive, rather than pain, to describe the stimulus and the response (Winter, 1965; Collier, 1964). A very large number of different methods have been used to apply nociceptive stimuli

and these can be classified into 5 main categories: thermal, electrical, mechanical, chemical, and behavioural techniques. These tests measure the power of a drug to increase the smallest stimulus needed to elicit either pain in man or a given nociceptive response in man or animals, most of the tests being based on noxious stimulation of the skin (Collier, 1964). A number of problems are encountered in the use of these tests, for drugs thought not to have analgesic action in man may appear to have analgesic action in animals, and secondly drugs known to be active in man frequently have no such effect in animals (Winter, 1967). False positive reactions can be due to a number of factors, one of the more important is that many of the analgesic tests require a response from the animals that is a co-ordinate movement. Thus drugs that dis-co-ordinate the animals will appear to be analgesic. In order to expose such effects, co-ordination tests can be run. One such test is to place animals on a rotating drum, those animals that fall off being taken as dis-co-ordinated (Collier, 1964).

The majority of narcotic analgesics are active in man and animals. Unlike the narcotics, the antipyretic analgesics and narcotic-antagonists are inactive in the majority of animal tests. Yet the narcotic antagonists such as nalorphine and the antipyretic analgesics such as aspirin have been shown to inhibit pain in man. Thus when screening for analgesics the choice of test is not easy. However in the situation presented here, it was intended to use narcotic analgesics only (though later a non-narcotic analgesic was used in one test) and in such cases tests depending on superficial stimulation are suitable (Collier, 1964). In 1957 Randall and Selitto designed a test which they claimed was suitable for both narcotic and antipyretic analgesics. One foot of a rat is subjected to steadily increasing pressure until the

animals makes a characteristic withdrawal response. If the foot has previously been treated with an inflammatory agent (such as a subcutaneous injection of live yeast) the test is suitable for antipyretic analgesics, although in the form used here the test would not be sensitive to reasonable doses of the antipyretic analgesics.

2. THE EFFECTS OF PERIPHERALLY ADMINISTERED MORPHINE ON THE NOCICEPTIVE THRESHOLD IN RATS

a) ED_{50} (Foot pressure method)

Preliminary experiment indicated that the time of peak effect for sub-maximal injections of morphine given subcutaneously was between 30 and 60 minutes. Groups of 5 rats received injections of morphine in doses of 4, 8, or 12 mg/kg subcutaneously at the back of the neck. Nociceptive thresholds were recorded immediately before the injection, and 60 minutes later.

The results are shown in Fig. 28 (Table 19). The ED_{50}* was found to be 6.6 mg/kg and the ED_{75}* 9.8 mg/kg. 8.0 mg/kg was chosen as a dose which should have given a response greater than 50% of the maximum, but less than 75%.

The efficacy of this dose increased with time until after several months it consistently produced an analgesic index of 1.0 in 3 or more of the animals in a group. The dose-effect relationship was again investigated. The 3 dose levels used were 2.0, 4.0 and 8.0 mg/kg. The result of this experiment is also shown in Fig. 28. The ED_{50} was then found to be 3.5 mg/kg and the ED_{75}, about 5.4 mg/kg. A dose of 4.0 mg/kg was then used to produce an analgesic index between 0.5 and 0.75.

* 50% & 75% increases in A.I., respectively.

The effect of morphine was also found to vary by small amounts from day to day. A given dose of morphine was found to be more effective in a group of individually caged animals than in a group of animals all in the same cage. Therefore, unless otherwise stated, all results are for groups of individually caged animals.

b) Tolerance to a single dose of morphine

Cannulated rats were used repeatedly for up to 5 weeks after cannulation. Usually 2 days were left between each experiment so that the animals could recover completely from the effects of drugs given previously. However, repeated doses of morphine are known to produce tolerance. Consequently, as a preliminary it was necessary to determine the minimum time interval between experiments such that a single 8.0 mg/kg dose of morphine would not affect a subsequent dose.

6 groups of 5 animals were used. On day 0, each group received a dose of 8 mg/kg morphine subcutaneously, and nociceptive thresholds were estimated 1 hour later. Subsequently each group was again tested 1 hour following a dose of 8.0 mg/kg morphine such that the interval between the two doses was 1, 2, 4, 8 and 16 days respectively for each group.

The results are shown in Fig. 29 (Table 20). 1 day after the initial dose there was a significant reduction in the response (of about 30%) which continued up to day 4. By day 8 this had reduced to about 15%, and by day 16 the response had returned to normal.

It has been noted previously that there was occasionally considerable variation in the response to a dose of 8.0 mg/kg

morphine. To show that any effect seen was not due to this, a group of animals treated with 1.0 ml/kg saline on day 0 was injected with each test group. The responses of these groups were not significantly different from the responses of the treated groups on day 0. (Controls in Fig. 29).

In a subsequent experiment, a group of 10 animals was injected with morphine on day 0. 12 days later the procedure was repeated. The anti-nociceptive effect on the two occasions was not significantly different.

3. THE EFFECTS OF INTRAVENTRICULAR INJECTIONS OF 5-HT AND NA ON THE ANTI-NOCICEPTIVE EFFECT ON A SINGLE DOSE OF MORPHINE

a) 5-HT

Groups of 5 animals were used. At time 0 the nociceptive threshold was measured and the animals given 8.0 mg/kg morphine subcutaneously. 30 minutes later the pain threshold was again measured, followed by an intraventricular injection of 5 µg of 5-HT in 10 µl saline. Nociceptive thresholds were measured at regular intervals thereafter.

The results are shown in Fig. 30 (Table 21). The normal response to morphine disappeared between 2.5 and 3.0 hours. Injection of 5-HT into the ventricles did not significantly prolong the anti-nociceptive activity, but caused a marked potentiation of the effect. A smaller but still significant response to 2.5 µg 5-HT is shown in Fig. 31 (Table 20).

b) NA

The experiments described in the above paragraph were repeated except that instead of 5-HT being injected, NA was used.

The results are shown in Fig. 32 (Table 21). The usual anti-nociceptive effect of morphine, 8.0 mg/kg, is promptly and completely curtailed by 20 µg of NA.

In other experiments, where a dose of 10 µg NA was used, there was initial abolition of the anti-nociceptive effect lasting for about 45 minutes; after this the nociceptive threshold rose to approach the level of the group injected intra-ventricularly with saline (Fig. 33; Table 22).

4. THE EFFECTS OF INTRAVENTRICULAR INJECTIONS OF PHENTOLAMINE AND LSD-25 ON THE ANTI-NOCICEPTIVE ACTION OF A SINGLE DOSE OF MORPHINE

a) Phentolamine

The experimental design was again identical to that previously described in paragraph 3 a). At 30 minutes 10 µg of phentolamine (in 10 µl saline) was injected into the ventricles.

The results are shown in Fig. 34 (Table 23). The usual response to morphine was shorter in this case, lasting only 1.5 - 2.0 hours. However a significant potentiation of the response was seen. At 45, 60 and 75 minutes 3 or more animals had an analgesic index of 1.0. There was no increase in the duration of action of morphine.

b) LSD-25

The experimental design was again identical to that described in paragraph 3 a). At 30 minutes, 1.0 µg of LSD-25 was injected into the ventricles in a volume of 10 µl.

The results are shown in Fig. 35 (Table 23). The anti-nociceptive effect of morphine alone was shorter than expected. However this response to morphine was significantly inhibited by the LSD-25 and there was no sign of there being any recovery from this inhibition during the period of observation ($3\frac{1}{2}$ hr).

5. THE EFFECTS OF INTRAVENTRICULAR INJECTIONS OF 5-HT, NA, PHENTOLAMINE AND LSD-25 ON THE NOCICEPTIVE THRESHOLDS IN RATS

In order to control for any inherent effects the intracerebrally administered compounds themselves may have had, they were all administered in the absence of morphine.

The experimental design was again identical to that described in previous sections, except that saline 1.0 ml/kg was injected subcutaneously at time 0 instead of morphine. NA and 5-HT were also administered in doses 2 and 3 times larger than those given previously in the presence of morphine.

Alone, 5-60 μg of 5-HT had no significant effect upon the nociceptive threshold, when injected intraventricularly. Further, 5-80 μg of NA did not increase the nociceptive threshold, although 40 and 80 μg gave the appearance of the animals being in a hyperalgesic state; the animals vocalised very much more readily on pinching the tail or prodding them, though a quantitative measure of this hyperalgesia could not be demonstrated by this test.

Phentolamine and LSD-25 were only given at the same dose levels previously used. No alteration of the nociceptive threshold could be detected.

6. THE EFFECTS OF PERIPHERAL ADMINISTRATION OF 5-HT, NA, PHENTOLAMINE AND LSD-25 ON THE ANTI-NOCICEPTIVE EFFECT OF A SINGLE DOSE OF MORPHINE

As with the temperature experiments, it was possible that the effects seen were due to actions of the amines at peripheral sites following leakage from the ventricles. To rule-out this possibility the amines were administered peripherally both by subcutaneous injection and by slow intravenous infusion over a period of 1-2 hours and their effects on the nociceptive threshold, in the presence or absence of morphine, determined.

a) Injections and infusions of 5-HT

Subcutaneous injections of small doses of 5-HT (5-20 μg) had no effect on the nociceptive threshold, nor on the anti-nociceptive action of morphine. Similarly, intravenous infusions of these doses, over periods of 1-2 hours, had no effect. Larger doses (1-2 mg/kg or 300-600 μg/rat) had depressant effects on the animals, but no significant effects on the nociceptive thresholds. These larger doses caused small apparent increases in the anti-nociceptive action of morphine, presumably because of the additive effects of the depression caused by the two drugs.

b) Injections and infusions of NA

Subcutaneous injections of small doses of NA (20-80 μg) had no effect on the nociceptive threshold and had no significant effect on the anti-nociceptive effect of morphine. Similar results were obtained when these doses of NA were infused intravenously over a period of 1 hour. Subcutaneous injections of 1-2 mg/kg caused marked behavioural effects in the animals, including muscular weakness which reduced their ability to respond to the

nociceptive stimulus. The animals usually died from the effects of such high doses, but unless the animal was very close to death the nociceptive threshold was not significantly altered. However the high doses did slightly increase the anti-nociceptive effect of morphine, and the morphine considerably reduced the toxic effects of the NA. This increase in the anti-nociceptive effect of morphine appeared to be due to a combination of the depressant effects of the two drugs.

c) Injections of phentolamine and LSD-25

Phentolamine was injected both subcutaneously and intravenously (via the tail vein) at a dose identical to that given intraventricularly (10 μg). There were no detectable effects on the nociceptive threshold and the anti-nociceptive effect of morphine was unaltered.

Similarly LSD-25 was given by the subcutaneous and intravenous routes at the same dose as that given previously by intraventricular injection (1.0 μg); again there was no detectable effect on either the nociceptive threshold nor on the anti-nociceptive effect of morphine.

7. THE EFFECT OF NALORPHINE, PENTAZOCINE AND NALOXONE ON THE NOCICEPTIVE THRESHOLD. INTERACTIONS WITH INTRAVENTRICULAR 5-HT

A number of narcotic antagonists not only antagonise the effects of morphine, but in man also show anti-nociceptive activity of their own. Most of these compounds do not have anti-nociceptive activity in the majority of animal tests (see Blumberg, Wolf & Dayton, 1965). Since intraventricular 5-HT produced an increase in the nociceptive threshold in reserpinised animals treated with morphine, some of these compounds

were given to rats and followed by 5-HT intraventricularly.

Subcutaneous injections of nalorphine hydrobromide (up to 20 mg/kg), pentazocine lactate (up to 30 mg/kg) and naloxone HCl (up to 50 mg/kg) had no detectable effects on the nociceptive thresholds. Nalorphine and pentazocine, at their highest doses, caused the animals to become excited, but naloxone had no effect.

In further experiments nalorphine hydrobromide (15 mg/kg) pentazocine lactate, (15 mg/kg) and naloxone HCl (50 mg/kg) were injected subcutaneously, followed 30 minutes later by 5 μg 5-HT intraventricularly. In the case of pentazocine and naloxone, intraventricular 5-HT had no detectable effect, but with nalorphine a short but significant anti-nociceptive effect was produced.

20 μg of NA injected into the ventricles at this time had no significant effect on the nociceptive threshold (see Fig. 36; Table 24).

8. DISCUSSION

Throughout the period of experimental work involving the anti-nociceptive action of morphine, a considerable variation in the response to a given dose was found from day to day. Also it was found that the sensitivity of animals to morphine appeared to change progressively with time. This latter change may have been due to practice on the part of the operator. In each test the animal under investigation was held by hand and a change in the attitude of the operator towards the animals (e.g. increasing confidence) may have affected their response. The change from day to day could not easily be explained on this basis since the differences were quite marked. However the levels of 5-HT in the brain are subject to circadian rythms (Quay, 1968), as are the levels of NA in the hypothalamus (Manshardt & Wurtman, 1968). A daily

susceptibility rythm to morphine analgesia has been shown in mice (Morris & Lutsch, 1969), the morphine analgesia being at a peak when motor and neural activity as well as catecholamine levels were maximal. The majority of the experiments were conducted at the same time of day (2-8 p.m.) but such variations could explain the differences since the intensity of the analgesic action of morphine appears to be dependent on the levels of 5-HT in the brain (see this chapter).

Single dose tolerance to morphine has been shown previously (Kornetsky & Bain, 1968). They found that there was no tolerance at 24 hours, but the effect increased up to 180 days. However the test used was of the behavioural type (foot-shock attentuation in which the rat could adjust the shock intensity) and it may be that different mechanisms are involved in this type of test than in the foot pressure test.

Many times it has been suggested that 5-HT plays a mediator role in morphine analgesia (Medakovic & Banic, 1964; Nicak, 1965; Schaumann, 1958; Tenen, 1968; Verri, Graeff & Corrado, 1968), and the results have been variable. Medakovic and Banic (1964) suggested that 5-HT antagonises morphine analgesia; Herold and Cahn (1968) and Saarnivaara (1969a) suggested that 5-HT enhances the sensation of pain and is the mediator of pain sensation and would therefore antagonise morphine. Contrary to this, other authors have suggested that increases in 5-HT levels potentiate the analgesic effects of morphine (Sigg, Caprio & Schneider, 1958; Nicak, 1965; Tenen, 1968). The results presented here would support the hypothesis that increases in 5-HT levels in the brain do not have an anti-nociceptive effect themselves, but do potentiate the anti-nociceptive action of morphine.

The finding that higher doses of 5-HT given peripherally potentiated the anti-nociceptive effect of morphine has been seen in rabbits by Saarnivaara (1969a). However since no detectable increases in brain levels of 5-HT have been observed after large doses (60 mg/kg) given peripherally (Udenfriend, Weissbach & Bogdanski, 1957) it was concluded that this effect was due to peripheral actions. Saarnivaara (1969a) also found that this peripheral analgesic effect could be blocked by a peripheral antagonist of 5-HT (xylamidine). It was concluded that the increase in morphine analgesia on peripheral administration of 5-HT could have been due to either constriction of cerebral vessels, stimulation of sympathetic ganglia or by an indirect central effect of reflex origin.

The sympathomimetic amines have frequently been implicated in the mechanism of morphine analgesia, and again the results have been variable. Various authors have reported that NA and related substances have analgesic activity of their own (Colville & Chaplin, 1964; Leimdorfer & Metzner, 1949; Radouco-Thomas, Radouco-Thomas & Le Breton, 1957; Rothballer, 1959; Saarnivaara, 1969b; Handley & Spencer, 1969). They have been reported also to have no effect of their own (Milosevic, 1955; Tsou & Jang, 1964); finally, they have been found to potentiate the anti-nicoceptive effects of morphine (Sigg, Caprio & Schneider, 1958; Contreras & Tamayo, 1966; Nott, 1968, Verri, Graeff & Corrado, 1968; Saarnivaara, 1969b). Milosevic (1955) reported that peripherally-administered NA reduced the anti-nociceptive activity of morphine mice. Our results show that increasing the ventricular brain levels of NA abolishes the anti-nociceptive effect of morphine and that high doses injected peripherally have only very slight potentiating effects. Since there is an active barrier against NA entering the CNS, it would seem likely that the potentiating effects seen after peripheral admin-

istration were due to peripheral actions, possibly similar to those suggested for 5-HT. Thus our results would indicate that the analgesic effect of morphine is at least modulated by the levels of 5-HT and NA in the brain, 5-HT potentiating and NA attentuating the action. These conclusions are supported by our finding that the alpha-adrenergic blocking agent phentolamine significantly potentiated the anti-nociceptive effect of morphine without having an effect of its own, and that the 5-HT antagonist LSD-25 inhibited this action.

The ability of intraventricular 5-HT to produce an elevation of the nociceptive threshold in animals treated with nalorphine is difficult to explain. It was thought possible that these narcotic antagonists were without effects in animals due to their inability to adequately alter 5-HT levels in the brain. The result for nalorphine seemed to support this. However pentazocine, a narcotic antagonist that is a potent analgesic in man was without effect in our test, as was naloxone (also a potent morphine antagonist) and no elevation in the threshold was seen for either of these agents in the presence of 5-HT. However it has been reported that physostigmine produces an elevation of the nociceptive threshold in mice, and that if threshold doses of physostigmine are given along with narcotic-antagonist analgesics then the anti-nociceptive activity of these compounds is uncovered. Further, naloxone - which has little or no analgesic activity itself - inhibited the physostigmine-induced inhibition of the tail-flick reflex in mice (Dewey & Harris, 1967; Harris, Dewey & Howes, 1968).

However there has been considerable speculation concerning the roles played by brain amines in the mediation of morphine's anti-nociceptive action. Some workers have implicated NA (Schaumann, 1958; Verri, Graeff & Corrado, 1968; Heller, Saavedra & Fischer,

1968), and some 5-HT (Medakovic & Banic, 1964). Yet others have considered either both (Tagaki, Takashima & Kimura, 1964), or neither (Rudzik & Mennear, 1965), to be involved. A number of these results have been obtained using substances that alter the levels of these amines in the brain. Consequently it was decided to investigate further the effects of NA and 5-HT on the anti-nociceptive effect of morphine, using some of these compounds - see Chapter 4.

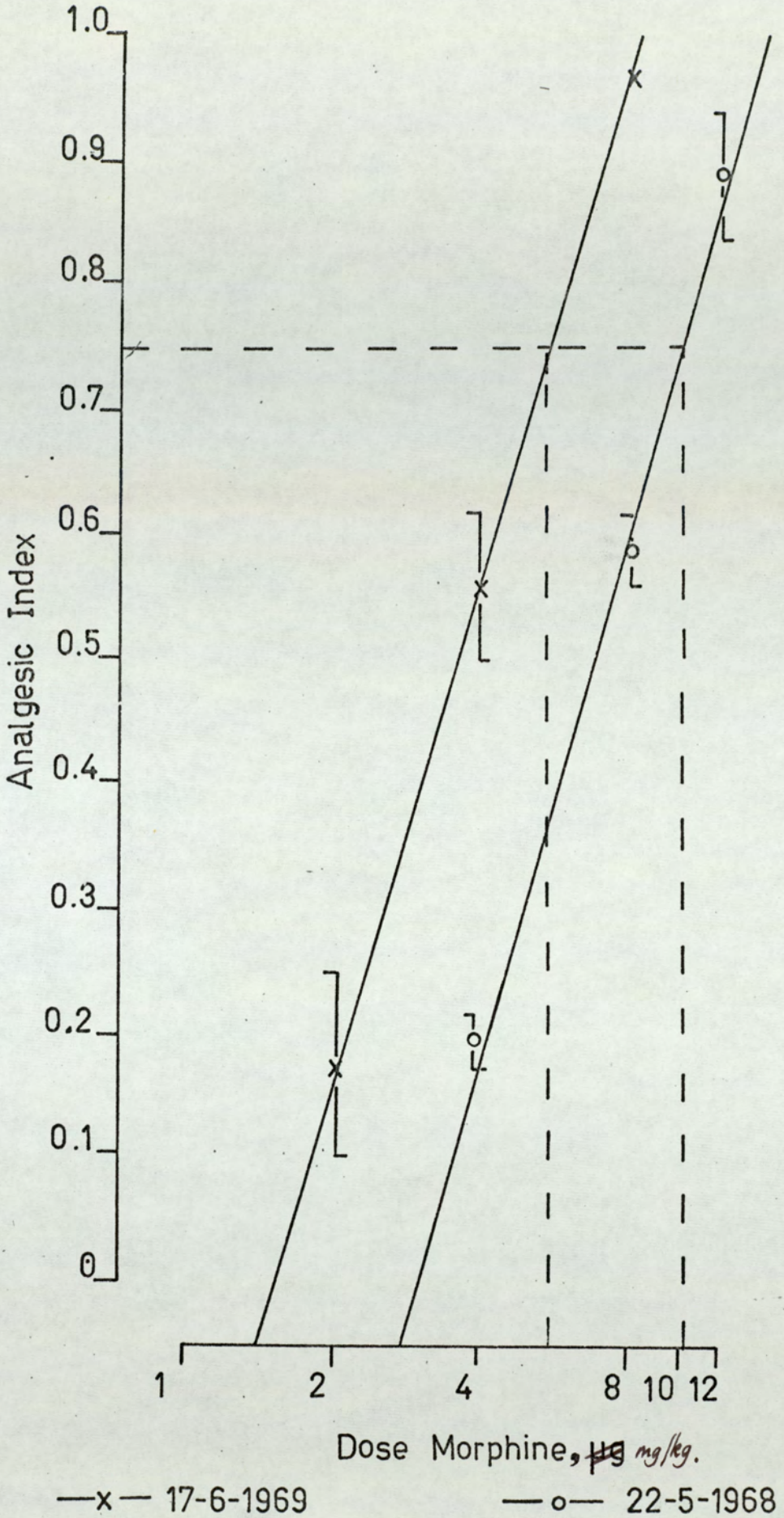

FIGURE 28 ED_{50} MORPHINE (FOOT PRESSURE METHOD)

To show the apparent change in sensitivity over a period of 1 year. (See Table 19).

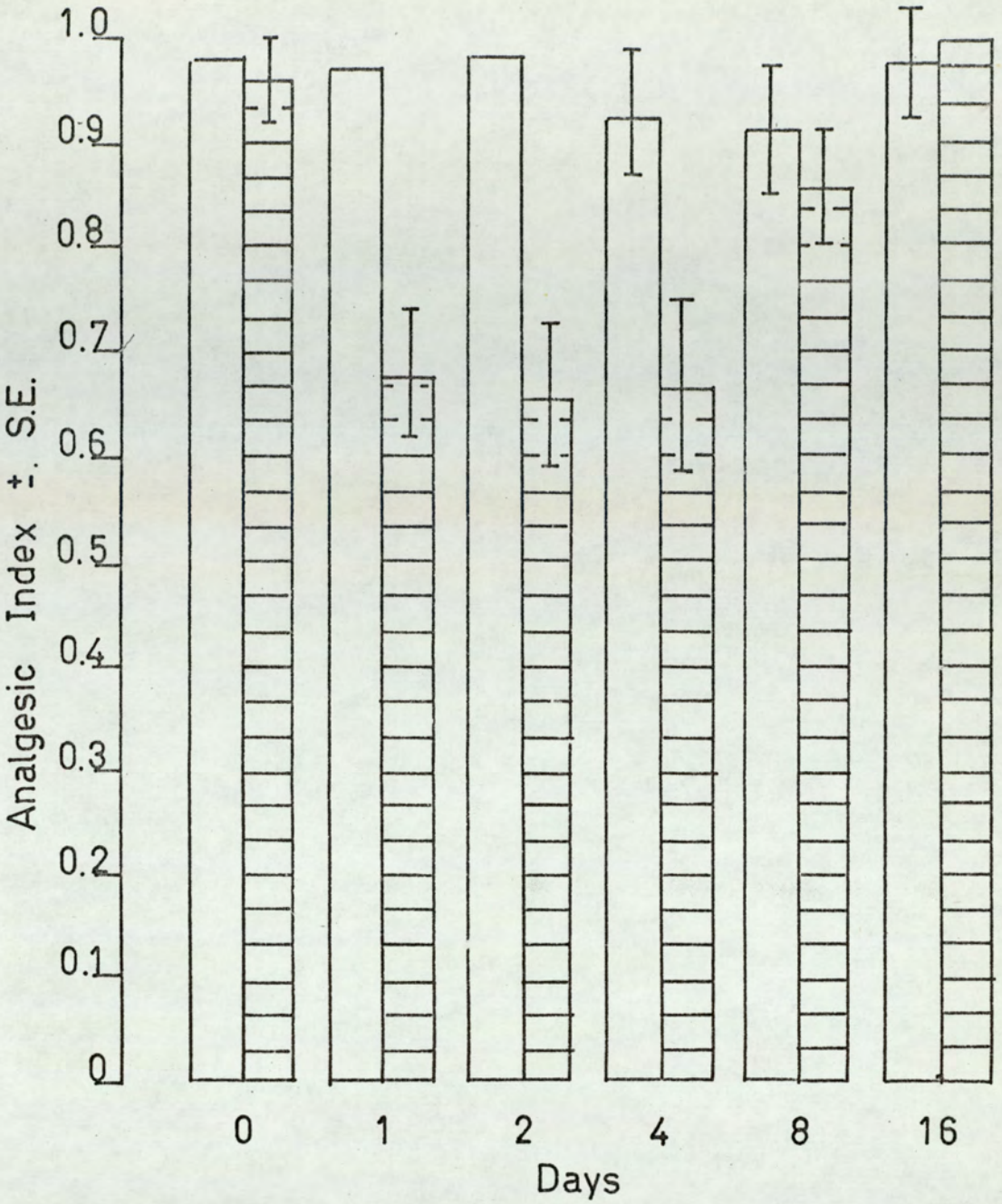

FIGURE 29 TOLERANCE TO A SINGLE DOSE OF MORPHINE

6 groups of 5 animals were used. On day 0, all animals received a single dose of 8.0 mg/kg morphine. At intervals of either 1, 2, 4, 8, or 16 days the animals received a 2nd s.c. injection of 8.0 mg/kg morphine. Changes in the nociceptive threshold were recorded 1 hour after the morphine injection. (Control animals open columns; morphine pretreated animals shaded columns). (See Table 20).

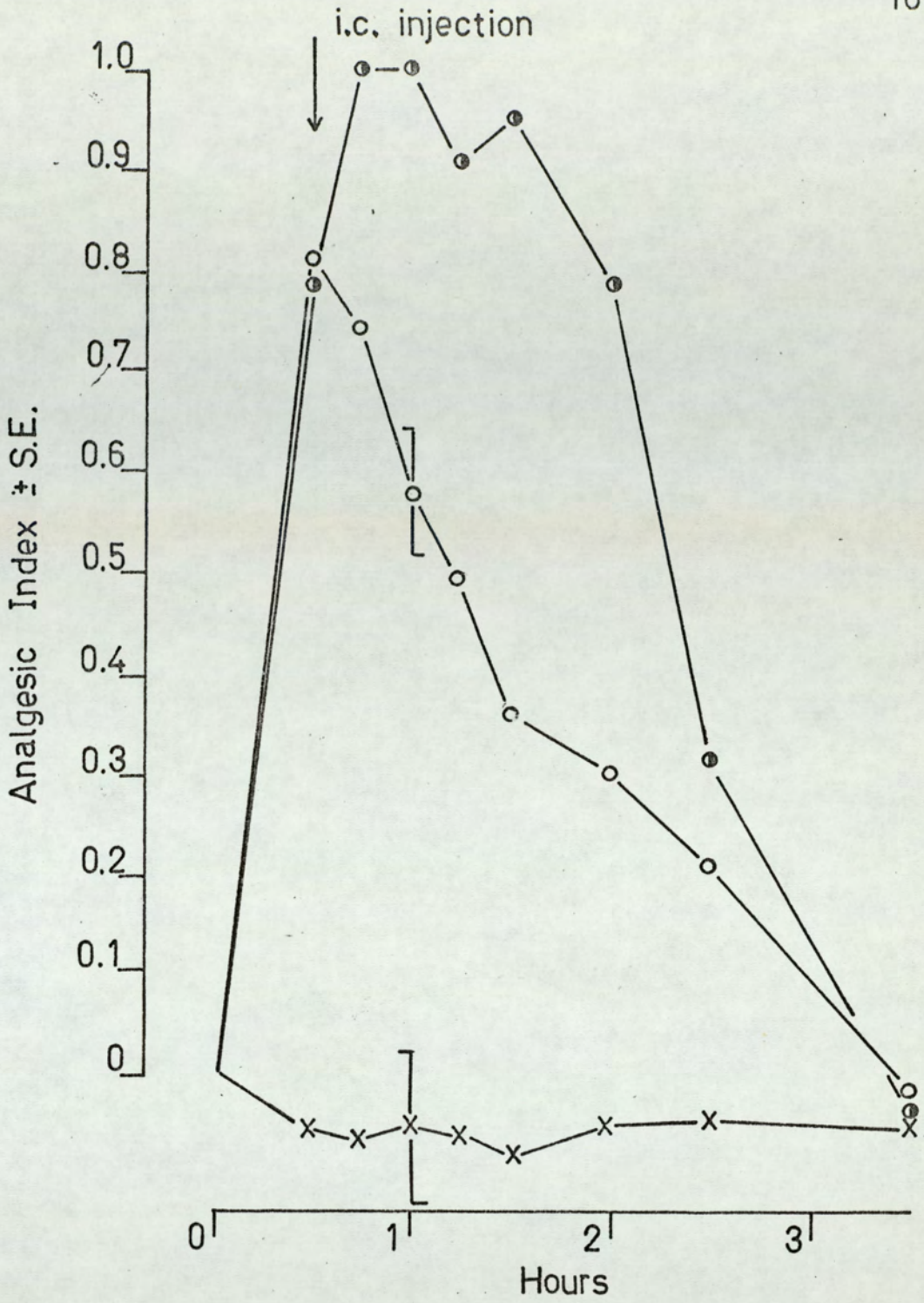

FIGURE 30 EFFECT OF INTRAVENTRICULAR 5-HT (HIGH DOSES) ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. and one group received 1.0 ml/kg saline s.c. 30 minutes later (at arrow) 5.0 µg 5-HT was injected into the cerebral ventricles (i.c.) of one treated group (—●—). 10 µl saline was injected i.c. into the other morphine treated group, (— o —) and the saline treated group (—X—). Changes in the nociceptive threshold were recorded at the times indicated. . (See Table 21).

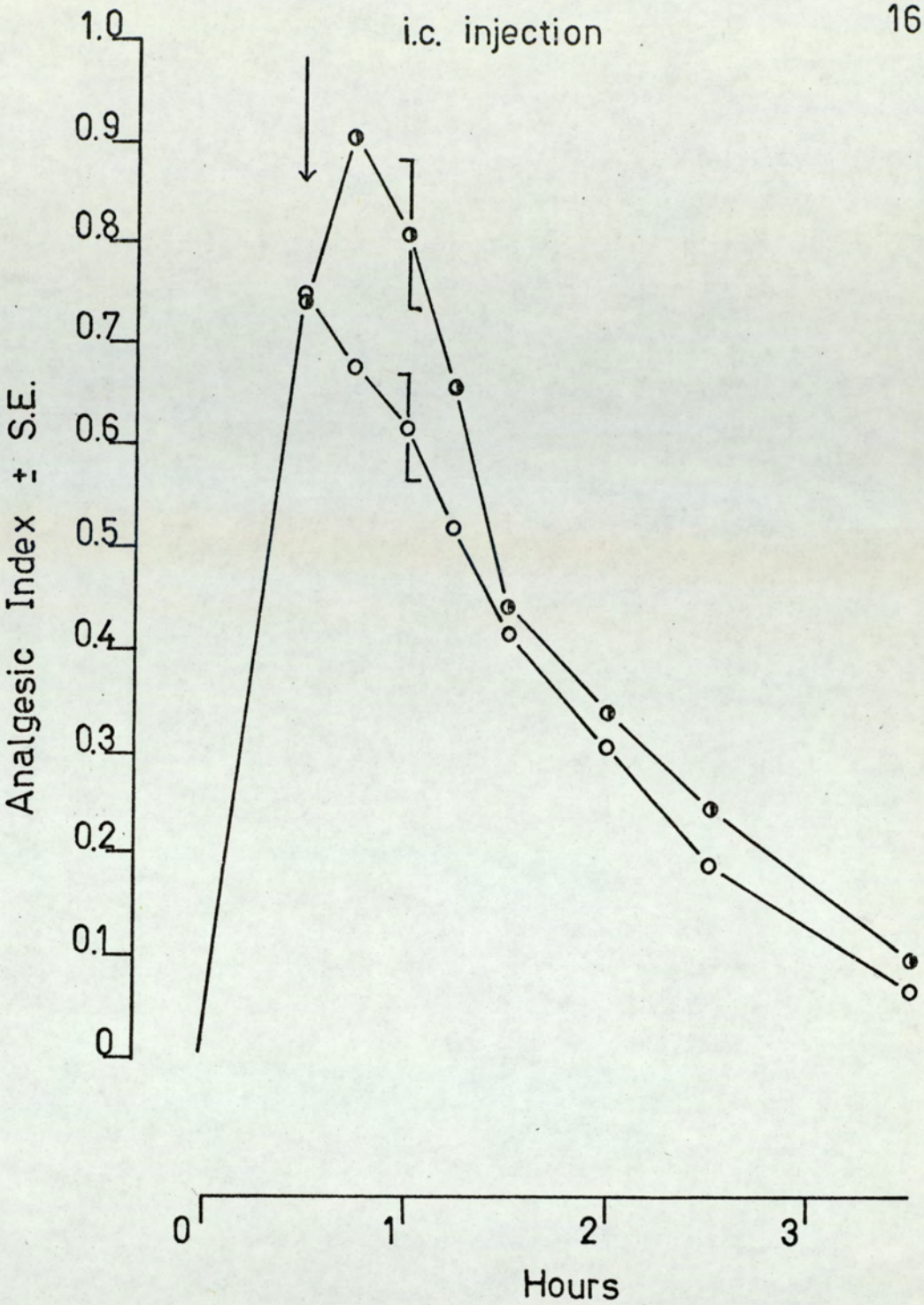

FIGURE 31 EFFECT OF INTRAVENTRICULAR 5-HT (LOW DOSES) ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. 30 minutes later (at arrow) 2.5 µg of 5-HT was injected into the cerebral ventricles (i.c.) of one group (—●—) and 10 µl saline injected i.c. into the other (—○—). Changes in the nociceptive threshold were recorded at times indicated. (See Table 22).

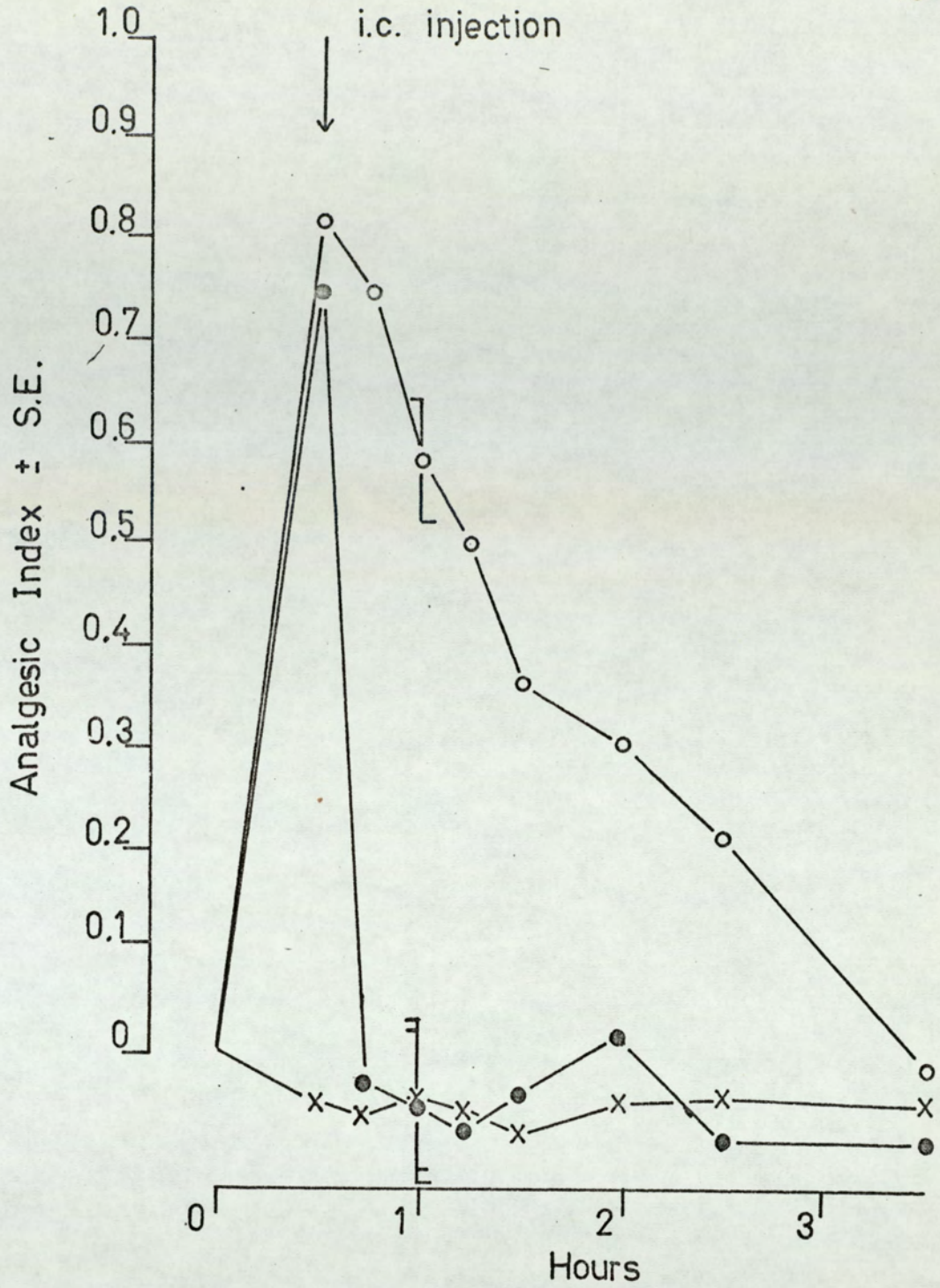

FIGURE 32 EFFECT OF INTRAVENTRICULAR NA (HIGH DOSES) ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. and one group received 1.0 ml/kg saline s.c. 30 minutes later (at arrow) 20 µg NA was injected into the cerebral ventricles (i.c.) of one morphine treated group (—●—). 10 µl saline was injected i.c. into the other morphine treated group (—o—) and the saline treated group (—x—). Changes in the nociceptive threshold were recorded at the times indicated. (See Table 21).

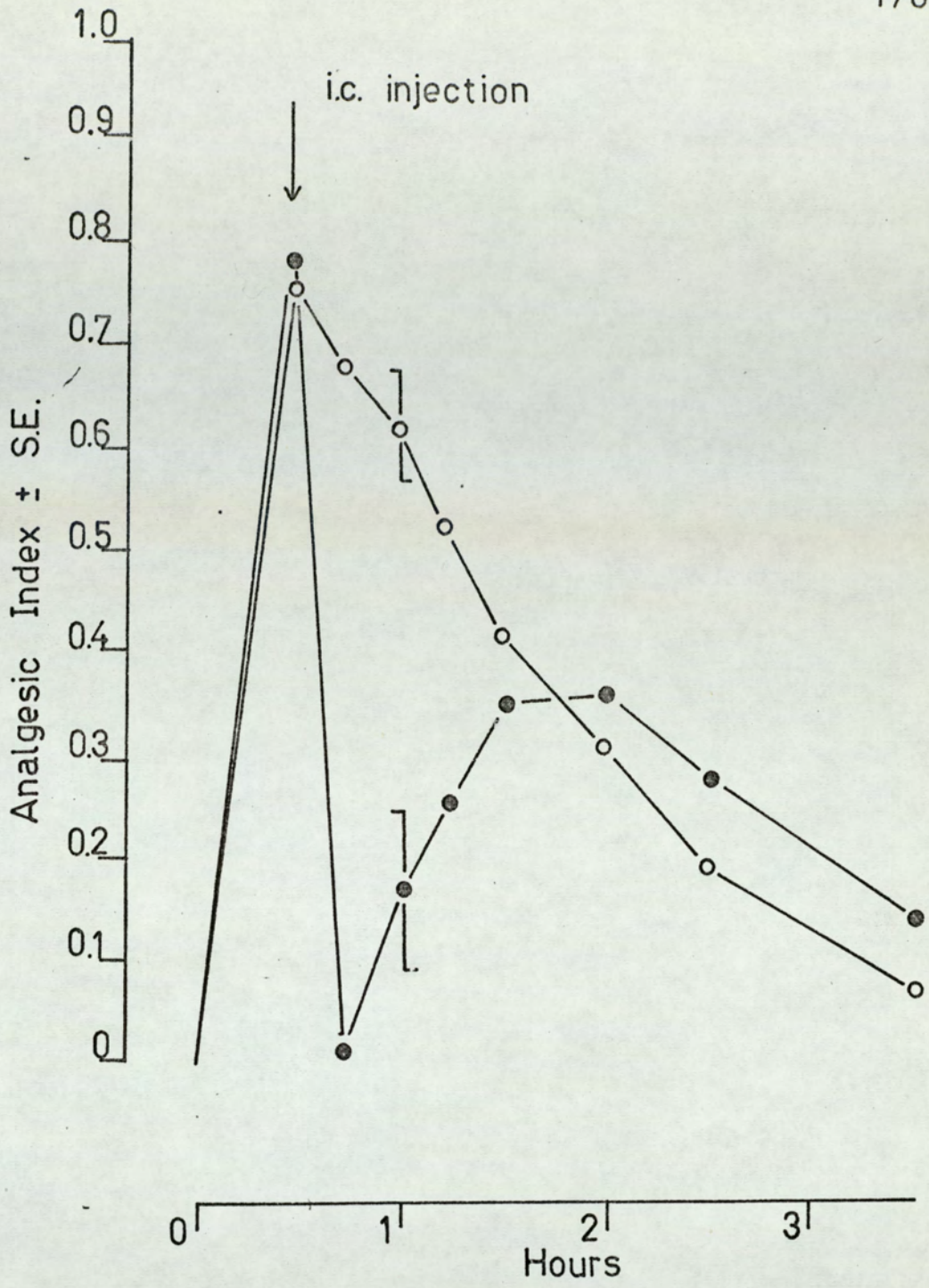

FIGURE 33 EFFECT OF INTRAVENTRICULAR NA (LOW DOSE) ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. 30 minutes later (at arrow) 10 μg NA was injected into the cerebral ventricles (i.c.) of one group (—●—) and 10 μl saline injected i.c. into the other (—o—). Changes in the nociceptive threshold were recorded at the times indicated. (See Table 22).

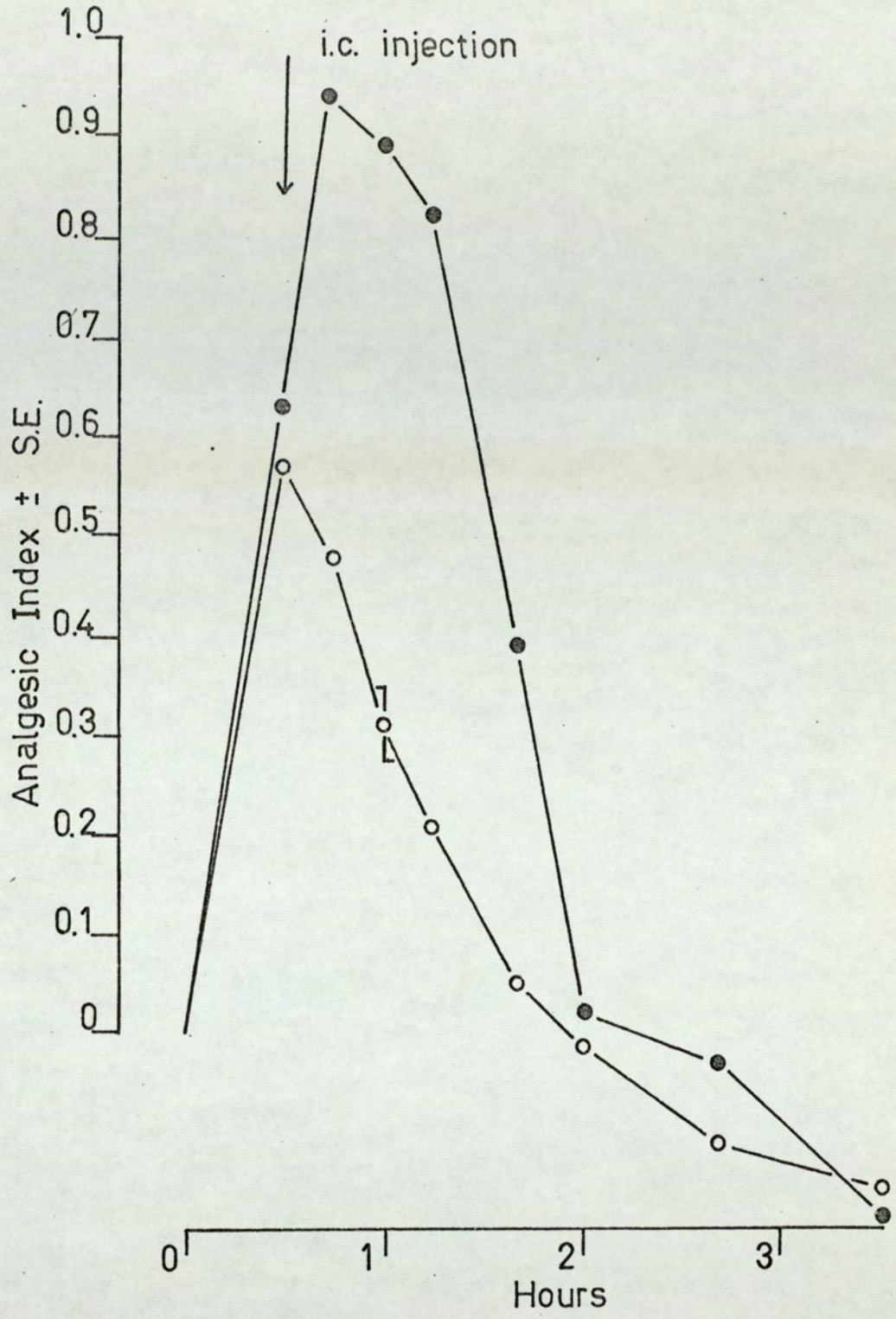

FIGURE 34 EFFECT OF INTRAVENTRICULAR PHENTOLAMINE ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. 30 minutes later (at arrow) 10 µg phentolamine was injected into the cerebral ventricles (i.c.) of one group (—●—) and 10 µl of saline injected i.c. into the other group (—o—). Changes in the nociceptive threshold were recorded at the times indicated. (See Table 23).

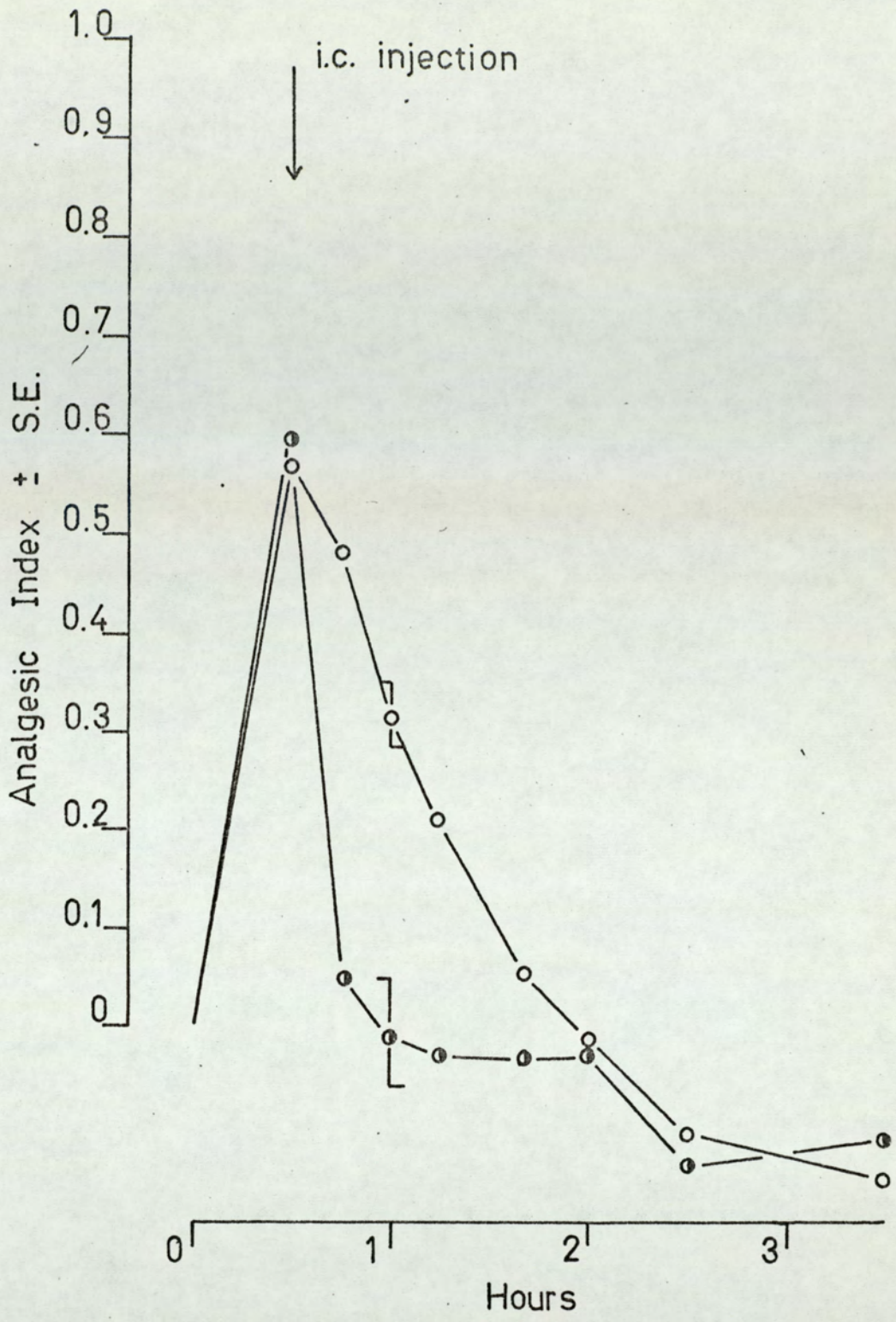

FIGURE 35 EFFECT OF INTRAVENTRICULAR LSD-25 ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

Groups of 5 animals were used. At time 0, 2 groups received 8.0 mg/kg morphine s.c. 30 minutes later (at arrow) 1.0 µg of LSD was injected into the cerebral ventricles (i.c.) of one group (—●—) and 10 µl of saline injected i.c. into the other group (—o—). Changes in the nociceptive threshold were recorded at the times indicated. (See Table 23).

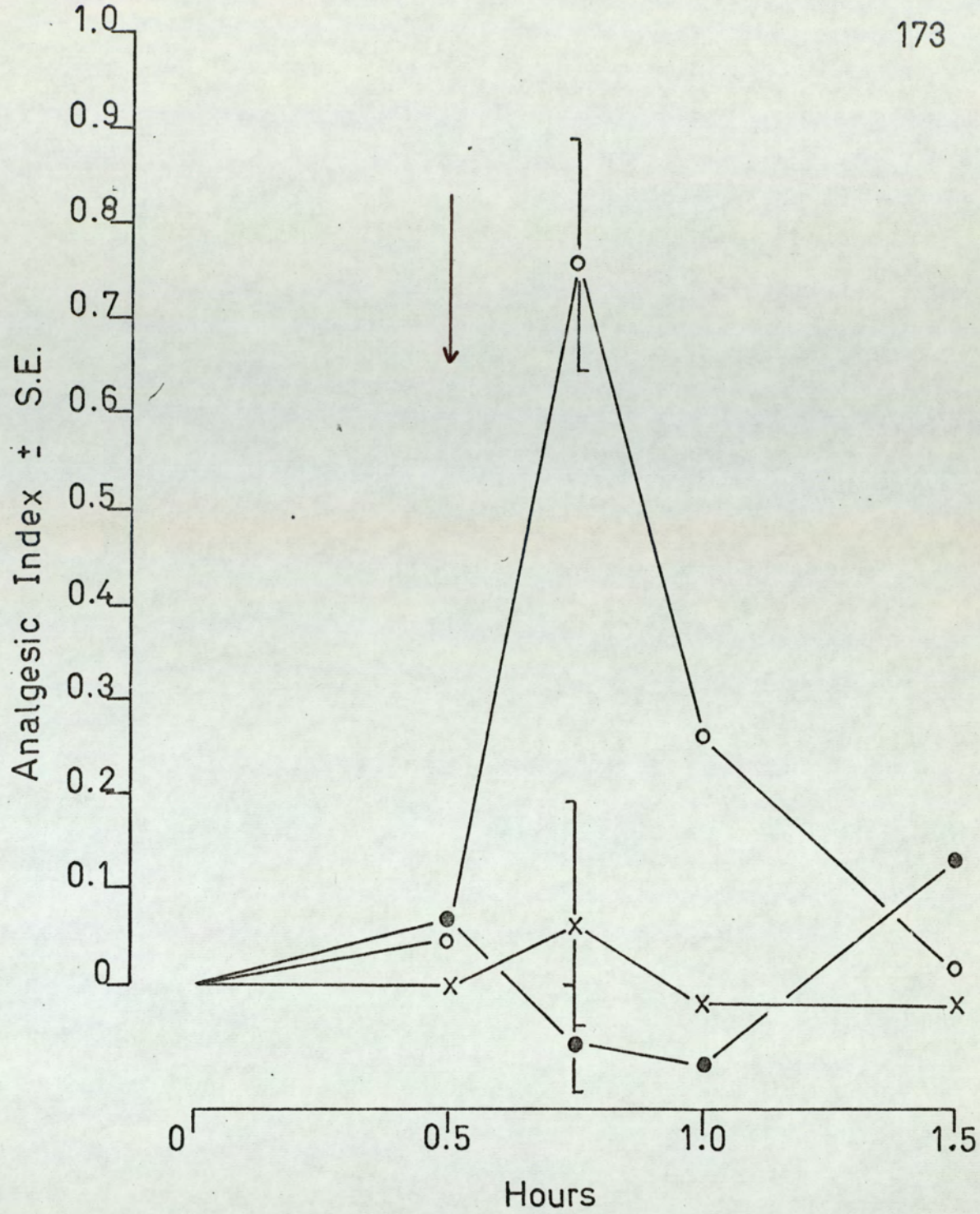

FIGURE 36 THE EFFECT OF INTRAVENTRICULAR INJECTION OF 5-HT INTO RATS PREVIOUSLY GIVEN SUBCUTANEOUS INJECTIONS OF NALORPHINE

Groups of 5 animals were used. At time 0, 3 groups were injected with nalorphine 10 mg/kg s.c. 30 minutes later (at arrow) one group received an intraventricular injection of 10 µl saline (—X—) one 5 µg 5-HT (—o—) and one 20 µg NA (—●—). Changes in the nociceptive threshold were recorded at times indicated. (See Table 24).

TABLE 19

ED_{50} MORPHINE (FOOT PRESSURE METHOD)

To show the apparent change in sensitivity over a period of one year. 3 groups of 5 rats were used. The nociceptive threshold for each animals was recorded, followed by a subcutaneous injection of morphine. 1 hour later the nociceptive threshold was again determined and the analgesic index (AI) calculated. (See Fig. 28).

Morphine mg/kg	2	4	8	12
AI 17.6.69.	0.172 ± 0.07	0.554 ± 0.06	0.967 -	- -
AI 22.5.68.	- -	0.194 ± 0.02	0.593 ± 0.03	0.890 ± 0.05

TABLE 20

TOLERANCE TO A SINGLE DOSE OF MORPHINE

(see Fig. 29)

	Analgesic index ± Standard Error (1 hour after s.c. morphine)					
Time (Days)	0	1	2	4	8	16
Control Saline 1 ml/kg s.c. day 0 8.0 mg/kg M s.c. test day	0.982 -	0.972 -	0.984 -	0.920 ± 0.062	0.910 ± 0.058	0.980 ± 0.046
8.0 mg/kg M s.c. day 0 8.0 mg/kg M s.c. test day	0.964 ± 0.040	0.686 ± 0.070	0.674 ± 0.072	0.678 ± 0.086	0.873 ± 0.062	1.000 -

M = Morphine

TABLE 21

MODIFICATION OF THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE
BY THE INTRAVENTRICULAR INJECTION OF NA AND 5-HT (HIGH DOSE)

(see Figs. 30 and 32)

	Analgesic index ± Standard Error							
Time (Hours)	0.5	0.75	1.0	1.25	1.5	2.0	2.5	3.5
Saline 1.0 ml/kg s.c. Saline 10.0 µl i.c.	0.052 ± 0.025	0.054 ± 0.086	0.049 ± 0.082	0.057 ± 0.069	0.075 ± 0.060	0.052 ± 0.029	0.045 ± 0.060	0.046 ± 0.039
M 8 mg/kg s.c. NA 20 µg i.c.	0.736 ± 0.088	0.132 ± 0.093	0.055 ± 0.100	0.078 ± 0.098	0.041 ± 0.082	0.012 ± 0.082	0.086 ± 0.092	0.087 ± 0.069
M 8 mg/kg s.c. Saline 10 µl i.c.	0.811 ± 0.081	0.744 ± 0.110	0.575 ± 0.077	0.499 ± 0.070	0.361 ± 0.110	0.300 ± 0.092	0.206 ± 0.120	0.019 ± 0.090
M 8 mg/kg s.c. 5-HT 5 µg i.c.	0.790 ± 0.130	1.000 -	1.000 -	0.910 -	0.955 -	0.794 -	0.314 ± 0.119	0.021 ± 0.072

M = Morphine

TABLE 22

MODIFICATION OF THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE BY THE INTRAVENTRICULAR INJECTION OF NA AND 5-HT (LOW DOSE)

(see Figs. 31 and 33)

	Analgesic index ± Standard Error							
Time (Hours)	0.5	0.75	1.0	1.25	1.5	2.0	2.5	3.5
M 8.0 mg/kg s.c. 5-HT 2.5 µg i.c.	0.740 ± 0.082	0.908 ± 0.079	0.804 ± 0.079	0.580 ± 0.072	0.434 ± 0.062	0.341 ± 0.081	0.250 ± 0.072	0.108 ± 0.082
M 8.0 mg/kg s.c. Saline 10 µl i.c.	0.742 ± 0.081	0.682 ± 0.072	0.640 ± 0.072	0.542 ± 0.063	0.446 ± 0.091	0.321 ± 0.084	0.198 ± 0.081	0.076 ± 0.077
M 8.0 mg/kg s.c. NA 10 µg i.c.	0.781 ± 0.072	0.015 ± 0.071	0.176 ± 0.088	0.257 ± 0.088	0.351 ± 0.088	0.366 ± 0.072	0.277 ± 0.076	0.137 ± 0.074

M = Morphine

TABLE 23

MODIFICATION OF THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE BY INTRAVENTRICULAR INJECTIONS OF PHENTOLAMINE AND LSD

(see Figs. 34 and 35)

	Analgesic index ± Standard Error							
Time (Hours)	0.05	0.75	1.0	1.25	1.5	2.0	2.5	3.5
M 8.0 mg/kg s.c. Phentolamine 10 µg i.c.	0.638 ± 0.083	0.946 -	0.895 -	0.823 -	0.395 ± 0.118	0.028 ± 0.140	0.026 ± 0.140	0.184 ± 0.020
M 8.0 mg/kg s.c. Saline 10 µl i.c.	0.575 ± 0.027	0.480 ± 0.030	0.305 ± 0.037	0.210 ± 0.042	0.069 ± 0.042	0.080 ± 0.028	0.103 ± 0.030	0.157 ± 0.042
M 8.0 mg/kg s.c. LSD 1 µg i.c.	0.591 ± 0.132	0.051 ± 0.091	- 0.005 ± 0.056	0.031 ± 0.049	- 0.027 ± 0.058	0.025 ± 0.030	- 0.144 ± 0.029	- 0.109 ± 0.036

M = Morphine

TABLE 24

THE EFFECT OF INTRAVENTRICULAR INJECTION OF 5-HT INTO RATS PREVIOUSLY GIVEN SUBCUTANEOUS INJECTIONS OF NALORPHINE

(see Fig. 36)

	Analgesic index ± Standard Error			
Time (Hours)	0.5	0.45	1.0	1.5
Nalorphine 10 mg/kg s.c. Saline 10 µl i.c.	0.000 ± 0.014	0.016 ± 0.140	- 0.025 ± 0.023	0.118 ± 0.097
Nalorphine 10 mg/kg s.c. 5-HT 5 µg i.c.	0.052 ± 0.050	0.752 ± 0.131	0.262 ± 0.110	0.026 ± 0.030
Nalorphine 10 mg/kg s.c. NA 10 µg i.c.	0.001 ± 0.045	- 0.166 ± 0.051	- 0.082 ± 0.053	0.142 ± 0.067

CHAPTER FOUR

THE EFFECTS OF DRUGS THAT ALTER CENTRAL AMINE LEVELS ON THE ANTI-NOCICEPTIVE ACTION OF MORPHINE

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Reserpine depletes tissues of both catecholamines and 5-HT, and has been shown to antagonise the anti-nociceptive effect of morphine in rats and mice (Schneider, 1954; Schaumann, 1958; Sigg, Caprio & Schneider, 1958; Medakovic & Banic, 1964; Takagi, Takashima & Dimura, 1964; Rudzik & Mennear, 1965; Contreras & Tamayo, 1967; Ross & Ashford, 1967; Verri, Graeff & Corrado, 1967). However, contrary to this, potentiation of morphine has been reported in mice (Tripod & Gross, 1957; Garcia Leme & Roche E Silva, 1961). Attentuation of the anti-nociceptive effect of morphine has also been reported for tetrabenazine pretreatment (Takagi, Takashima & Kimura, 1964). A number of other workers have used the precursors of NA and 5-HT, which were administered peripherally to normal and reserpinised animals, in order to alter the brain levels of these amines in the presence and absence of morphine. However such experiments have also yielded inconsistent results (Sigg, Caprio & Schneider, 1958; Colville & Chaplin, 1964; Contreras & Tamayo, 1966). In a similar way confusing results have been obtained using specific NA depletors such as α-methyldopa and α-methyl-m-tyrosine (Medakovic & Banic, 1964; Contreras, Tamayo & Quijada, 1969), and to confuse the issue further there appear to be differences between results obtained with rats, mice and rabbits in these experiments.

In this chapter the effect of reserpine pretreatment on the anti-nociceptive effect of morphine in the rat will again be described, and also modifications of this effect of reserpine by intraventricularly

administered amines.

1. THE EFFECT OF PRETREATMENT WITH RESERPINE

Medakovic and Banic (1964) reported that the anti-nociceptive effect of morphine in rats could be abolished by pretreatment with reserpine (1 mg/kg) given 3 hours before the morphine (4 mg/kg). Preliminary studies conducted here indicated that at 3 hours pre-treatment with 5 mg/kg reserpine there was an increase in the activity of morphine. Consequently a chronic study to determine the time course of the effect of reserpine on morphine analgesia was conducted.

10 groups of 5 animals were injected with 5 mg/kg reserpine intraperitoneally at time 0. At 3, 6, 9, 12, 15, 18, 21, 30, 60 and 90 hours later the nociceptive threshold of one group was determined, the animals were injected with morphine 8 mg/kg subcutaneously, and the nociceptive threshold tested again 1 hour later.

The results are shown in Fig. 37. (Table 25). There was a suggestion of potentiation of the morphine effect at 3 hours, followed by a progressive reduction in effect up to a maximum at 15 hours. This complete inhibition remained for about 6 hours and then there was a slow recovery until by 5 days after reserpine, the effect of morphine was restored.

All the following experiments were therefore carried out 16-20 hours after the administration of reserpine.

2. THE EFFECT OF INTRAVENTRICULAR 5-HT IN RESERPINISED ANIMALS

Groups of 5 animals were pretreated with reserpine, 5 mg/kg i.p. at -16 hours. This was followed by 8 mg/kg morphine subcutaneously at

0 hours after measurement of the basal nociceptive threshold. At 30 minutes the nociceptive threshold was again determined and the animals given intraventricular injections of either 5 µg of 5-HT in 10 µl saline, or 10 µl saline. Nociceptive thresholds were then determined at regular intervals.

The results are shown in Fig. 38 (Table 26). On the left hand side the normal response to morphine is seen and its inhibition by reserpine pretreatment. On the right hand side the effect of 5-HT given to reserpinised animals in the presence and absence of morphine is seen. In the absence of morphine the 5-HT had no effect on the nociceptive threshold, but in the presence of morphine a significant anti-nociceptive effect was apparent.

3. THE EFFECT OF INTRAVENTRICULAR 5-HT IN TETRABENAZINE-PRETREATED ANIMALS

Tetrabenazine has been reported to antagonise the anti-nociceptive effect of morphine in mice at a dose of 20-40 mg/kg, when given 2-4 hours in advance of the morphine (Takagi, Takashima & Kimura, 1964).

Groups of 5 animals were treated with 30 mg/kg tetrabenazine intraperitoneally. 4 hours later the nociceptive thresholds were determined, and 8 mg/kg morphine injected subcutaneously. 30 minutes later one group received saline (10 µl) and the other group 5 µg of 5-HT in 10 µl of saline, injected into the ventricles.

The results are shown in Fig. 39 (Table 27). The anti-nociceptive effect of morphine was totally inhibited. Injection of 5-HT into the ventricles produced a brief anti-nociceptive effect, in the presence of morphine, but was without effect in the absence of morphine. These results were similar to those previously seen with reserpine.

4. THE EFFECT OF INTRAVENTRICULAR NA IN RESERPINISED ANIMALS

Since a number of authors believe that NA plays an important role in the mechanism of anti-nociceptive effect of morphine, the experiment described in paragraph 2 above was repeated, using 20 μg of NA instead of 5-HT.

The results are shown in Fig. 40 (Table 28). On the left of the figure the normal effect of morphine is again seen and its inhibition by reserpine. On the right it can be seen that NA had no significant effects on the nociceptive thresholds of these animals in the presence nor in the absence of morphine. This is in contrast to the effects seen when 5-HT was injected intraventricularly.

5. THE EFFECT OF PERIPHERALLY ADMINISTERED DOPA

Radouco-Thomas, Singh, Garcin and Radouco-Thomas (1967) have shown that DOPA given peripherally to guinea-pigs increases the nociceptive threshold. This effect is only observed in rabbits if pyrogallol (a catechol-o-methyltransferase inhibitor) is present (Munoz & Paeile, 1967). Peripheral administration of DOPA causes increases in brain levels of NA. Thus from the results in Chapter 3 it would be expected that subcutaneous injection of DOPA in the rat should inhibit the anti-nociceptive effect of morphine, whilst having no effect of its own on the nociceptive threshold.

The results of such an experiment are shown in Fig. 41 (Table 29). Four groups of 5 rats were used: 2 groups received 300 mg/kg. DOPA intraperitoneally and the other 2 groups 5 ml/kg saline. Forty-five minutes later the groups were treated with morphine (8 mg/kg subcutaneously) or with saline. A normal response to morphine was seen in the animals pretreated with saline. There was a significant elevation

of the nociceptive threshold in the animals treated with DOPA. However the administration of DOPA before morphine significantly reduced the morphine response to a level that was not significantly different from that of the DOPA alone. To see if this effect of DOPA on the nociceptive threshold was a central or a peripheral effect, it was next injected directly into the cerebral ventricles (see below).

6. THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF DOPA

Preliminary experiments indicated that DOPA (5-40 μg) injected into the ventricles of conscious rats had no effect on the nociceptive threshold. When DOPA was injected into the ventricles 30 minutes after the morphine (as was the case for NA) little if any effect was seen. However if the DOPA was injected at the same time as the morphine, a significant inhibition of the anti-nociceptive effect of morphine was seen. This is shown in Fig. 42 (Table 30) where both the morphine (8 mg/kg subcutaneously) and the DOPA (40 μg intraventricularly) were given at time 0. Thus although DOPA does inhibit morphine when injected into the cerebral ventricles the effect does not appear as readily as with NA. This dose of DOPA did not have any significant anti-nociceptive effect of its own.

Since DOPA is the precursor of dopamine which is then converted to NA, these effects could have been due to the DOPA, dopamine or NA alone or in combination. Consequently dopamine was then injected into the cerebral ventricles. (see below)

7. THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF DOPAMINE

Preliminary experiments indicated that dopamine 5-40 μg had no detectable effect on the nociceptive threshold.

However in similar experiments to those described above for DOPA, it was found that 20 μg injected into the ventricles at the same time as a subcutaneous injection of 8 mg/kg of morphine significantly inhibited the anti-nociceptive effect of morphine. (see Table 31) Again there was little or no effect when it was injected 30 minutes after the morphine - i.e. there was no immediate effect.

8. DISCUSSION

The difference between the results obtained here, and those of Medakovic and Banic (1964), concerning the reserpine pretreatment time necessary to inhibit the action of morphine, is difficult to explain. They reported that reserpine was effective if given 3 hours before the morphine, but we found that a minimum of 16 hours was required. The dosage of reserpine used may have been responsible for they used 1 mg/kg and we used 5 mg/kg. Differences in the duration of reserpine's action according to the type of tests used in rats has been described (Faith, Young, Grabaritis & Harvey, 1968). Five classes of recovery time from the effects of reserpine were found. The recovery from inhibition of the anti-nociceptive effect of morphine, in our results, fell into their class 4 (slow recovery: 72-98 hours). However Medakovic and Banic used a heat stimulus applied to the tail to obtain a nociceptive response, rather than a pressure method, and it may be that recovery from such a stimulus falls into class 1 (rapid recovery: 24-26 hours), with a consequent earlier onset of action. Potentiation of the anti-nociceptive effect of morphine by reserpine has been reported in mice (Tripod & Gross, 1957; Garcia Leme & Roche E Silva, 1961) and it is possible that the observed difference was due to species, the test used or the dose of reserpine employed. Ross and Ashford (1967) have noted that in mice, there is agreement among workers on the interaction of reserpine and

morphine in the tail-clip test, but results differ when methods based on heat are used. However using mice and the tail-flick test for measuring the nociceptive threshold, Dewey, Harris, Howes and Nuite (1968) found that administration of reserpine at the same time as morphine moved the dose response curve for morphine to the left (potentiation) and when given 16 hours previously it was moved to the right (inhibition). This essentially agrees with our results. A possible explanation is that the potentiation was due to an initial release of catecholamines by reserpine, whereas the inhibition 16 hours later was due to depletion of these amines from central stores.

Reserpine prevents the storage of NA, dopamine and 5-HT into neurons, leading to a disappearance of these amines from both central and peripheral tissues (Anden, 1968). Our results showing that intracerebral 5-HT restored anti-nociceptive activity to morphine after its inhibition by reserpine, would indicate that the anti-morphine effect of reserpine was mediated by its ability to deplete central stores of 5-HT rather than NA.

Tetrabenazine depletes NA and 5-HT from the brain, having little effect peripherally (Quinn, Schore & Brodie, 1959). From our results, the mechanism of action of tetrabenazine in inhibiting morphine would appear to be similar to that of reserpine, since a raised nociceptive threshold was restored by intraventricular 5-HT. Also, because tetrabenazine's action is primarily central, our results tend to confirm that the action of 5-HT in this situation is central too. Our results are supported by the findings that tetrabenazine also antagonises the effects of morphine in mice. (Takagi, Takashima & Kimura, 1964; Takagi & Nakama, 1966; 1968). However Takagi & Nakama, (1966 & 1968) also found that the dopamine content of mouse brain is reduced by tetrabenazine, and they suggested that the antagonism was

mediated through this mechanism.

Reserpine and tetrabenazine deplete both NA and 5-HT but various agents that deplete either one or the other have also been used. Para-chlorophenylalanine (pCPA) has been reported to reduce central levels of 5-HT without affecting the NA or dopamine levels (Koe & Weissman, 1966). Tenen (1967) found that this drug caused rats to appear more sensitive to nociceptive stimuli. This increase in sensitivity has been returned to normal by treating the rats with the 5-HT precursor 5-hydroxytrytophan (5-HTP) (Harvey, Lints & Grabaritis, 1968). Tenen (1968) also found that pretreatment with pCPA reduced the anti-nociceptive action of morphine. From this it was suggested that the morphine was antagonised because of a deficiency of central 5-HT. However in rabbits pretreatment with pCPA increased the anti-nociceptive effect of morphine as did pretreatment with the 5-HT antagonist cyproheptadine (Saarnivaara, 1969a), again indicating a species difference. Reserpine, guanethidine and tolazoline each have been found to inhibit the anti-nociceptive action of morphine (Contreras & Tamayo, 1967). These authors found that administration of 5-HTP could reverse this effect of reserpine and guanethidine and they concluded that the action of these drugs may have been due to actions on central 5-HT levels. Tolazoline was unaffected by 5-HTP and its action may have been due to other effects.

Our results, showing that intraventricular NA had no effect on the reserpine-induced inhibition of morphine, support the hypothesis that depletion of NA from central sites is not the cause of reserpine's inhibition. α-methyl-m-tyrosine and α-methyl-DOPA are selective catecholamine depletors (Hess, Connamacher, Osaki & Udenfriend, 1961). In rats pretreatment with α-methyl-m-tyrosine causes potentiation of morphine's anti-nociceptive effect (Medakovic & Banic, 1964) and a

similar effect has been seen using α-methyl-DOPA (Contreras, Tamayo & Quijada, 1969; Vedernikov & Afrikanov, 1969). It is possible that the perception of nociceptive stimuli in rats is dependent on a dynamic balance between NA and 5-HT in the brain. If this is the case, then the above mentioned results would support our findings, since selectively decreasing the NA levels would remove the inhibiting effect of NA on morphine, causing an increased effect. However the authors of the papers themselves interpreted their results differently, suggesting that the effect of morphine is mediated via catecholamines. Nevertheless the finding that the antagnosim of morphine by reserpine was prevented by α-methyl-DOPA (Contreras & Tamayo, 1966) is confusing. Again contrasting results have been obtained in mice. Medakovic and Banic (1964) and Verri, Graeff and Corrado (1968) found that α-methyl-m-tyrosine antagonised morphine, whilst Contreras, Tamayo and Quijada (1969) found that α-methyl-DOPA caused a potentiation, and further, Ross and Ashford (1967) found that alpha-methyl-DOPA had no effect on the anti-nociceptive effect of morphine. In the rabbit α-methyl-m-tyrosine has been shown to decrease the anti-nociceptive effect of morphine. (Saarnivaara, 1969b).

The replacement of amines depleted by reserpine, using precursors of NA and 5-HT, has also been carried out. Radouco-Thomas, Singh, Garcin and Radouco-Thomas (1967) found that both 5-HTP and DOPA had their own anti-nociceptive effects following peripheral administration. These compounds also showed synergistic effects with pethidine and to some extent reversed the inhibitory effect of reserpine on pethidine. However the report that DOPA had an anti-nociceptive action of its own was contrary to our results where NA had no effect when given either peripherally or centrally. Nevertheless we also found that 300 mg/kg

DOPA given intraperitoneally to rats, significantly increased the nociceptive threshold, but that this dose also reduced the normal morphine response to a value similar to that obtained with DOPA alone. In the body DOPA is converted to dopamine, and then to NA, so that the effects observed could have been due to an effect of DOPA itself, or to increases in dopamine or NA levels, either centrally or peripherally. It would seem likely that the analgesic action of DOPA was due to some peripheral mechanism that was not affected by increased central levels of NA. The possibility that the reduction in the anti-nociceptive response to morphine could have been due to increases in the central levels of dopamine or DOPA was tested by injecting dopamine or DOPA directly into the cerebral ventricles. The finding that there was inhibition of morphine, but that this was subject to delay indicates that these agents were acting indirectly, either by the release of another substance or by an action through one of their metabolites. In view of the previous results, the most likely explanation is that they were converted into NA in the brain tissue and it was this NA that was inhibiting the morphine. This is supported by the findings of Vedernikov and Afrikanov (1969) who reported that the β-oxidase inhibitor disulfiram (prevents the conversion of dopamine to noradrenaline), weakened the anti-nociceptive effect of morphine in rats. Alternatively our results may have been due to differences in the dose-response characteristics of DOPA, dopamine and NA.

In summary, the present findings, like those of the previous chapter, suggest that in the rat 5-HT and NA - although having no effects of their own on the nociceptive threshold of the rat - can modulate the effect of morphine. It is possible that morphine exerts its anti-nociceptive action by interfering with the levels of these amines in the brain.

Alterations in the levels of ACh in the CNS are known to have effects on the nociceptive threshold (see Section 3), Chapter 3,

paragraph 1). Also there is a strong possibility that ACh is a central transmitter (see Section 1, Chapter 1). Consequently it was decided to investigate next the involvement of cholinergic mechanisms in anti-nociception (see Chapter 5).

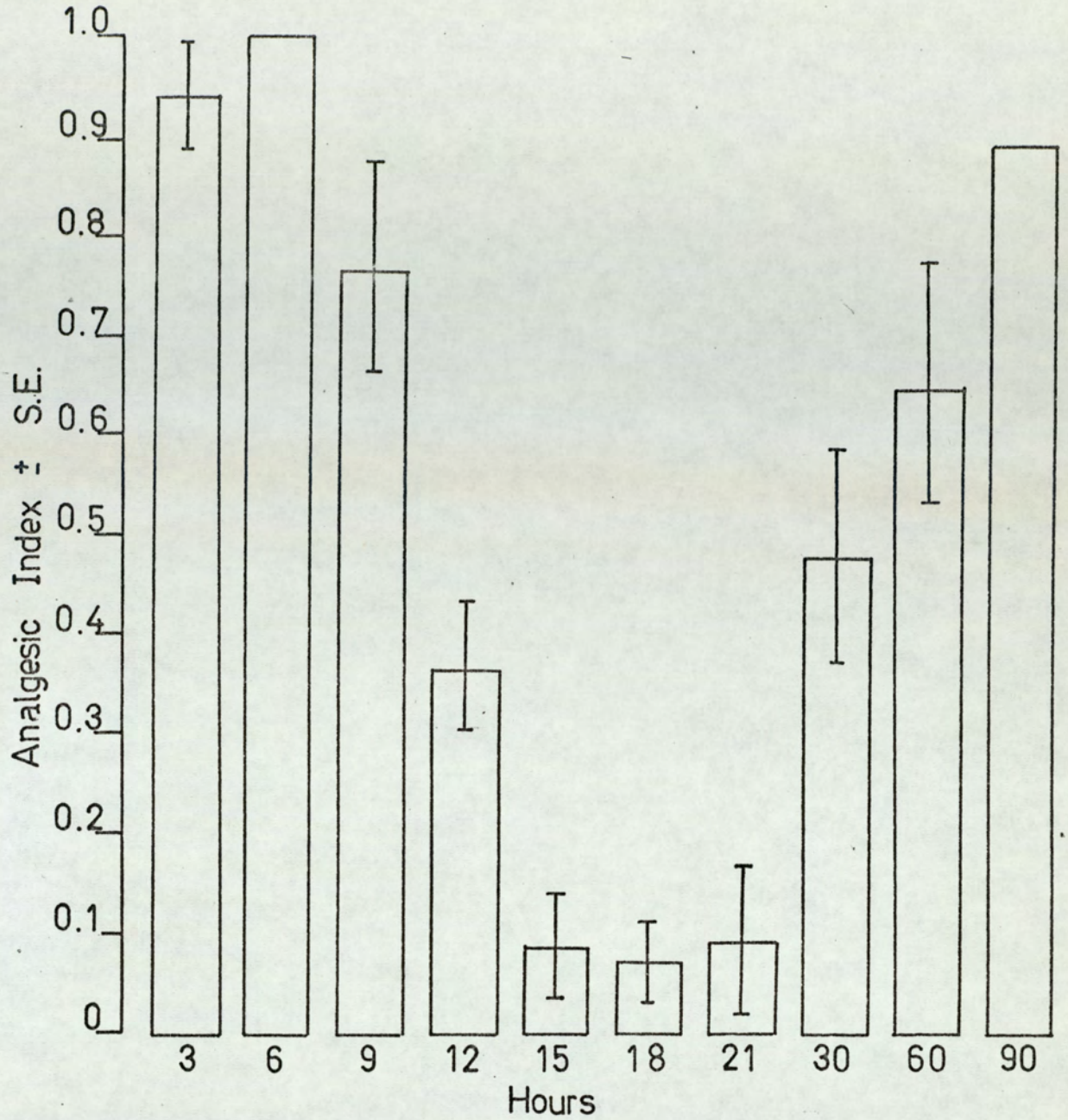

FIGURE 37 THE EFFECT OF PRETREATMENT WITH RESERPINE, ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

10 groups of 5 animals were used. Each animal received 5.0 mg/kg reserpine i.p. at time 0. 1 hour before the time indicated the nociceptive threshold was determined for 1 group, and the group then received 8.0 mg/kg morphine s.c. The nociceptive threshold was again determined 1 hour later. (see Table 25).

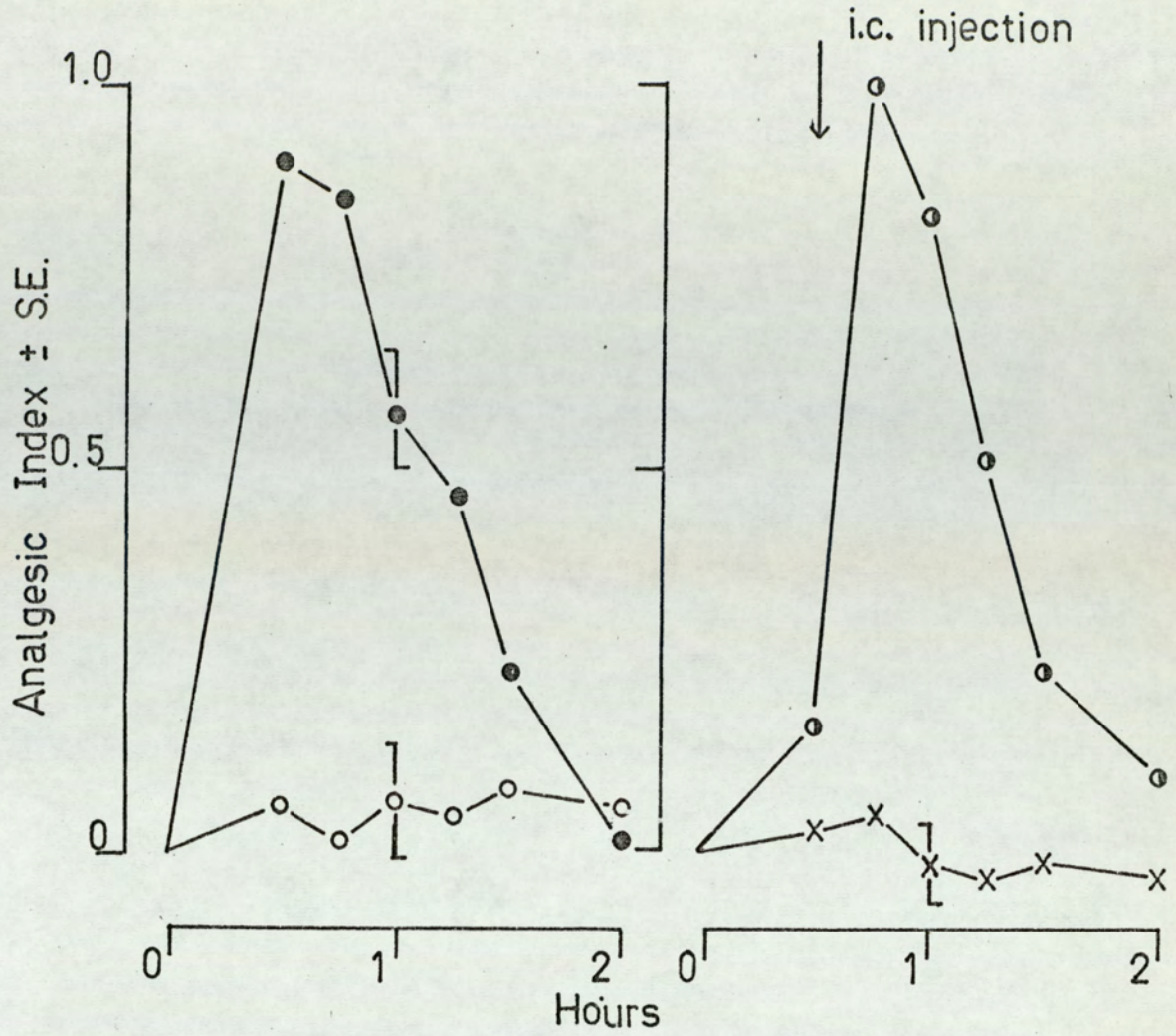

FIGURE 38 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN RESERPINISED ANIMALS

4 groups of 5 animals were used. 3 groups were pre-treated with 5.0 mg/kg reserpine i.p. at 16 hours. On the left hand side the analgesic effect of 8.0 mg/kg morphine given s.c. at time 0 is seen, in non-pretreated (—●—) and reserpine-pretreated (—o—) rats. The right hand side shows the effects of intraventricular (i.c.) injections of 5-HT (at arrow) given to pretreated animals in the presence (—●—) and absence (—X—) of morphine. (see Table 26).

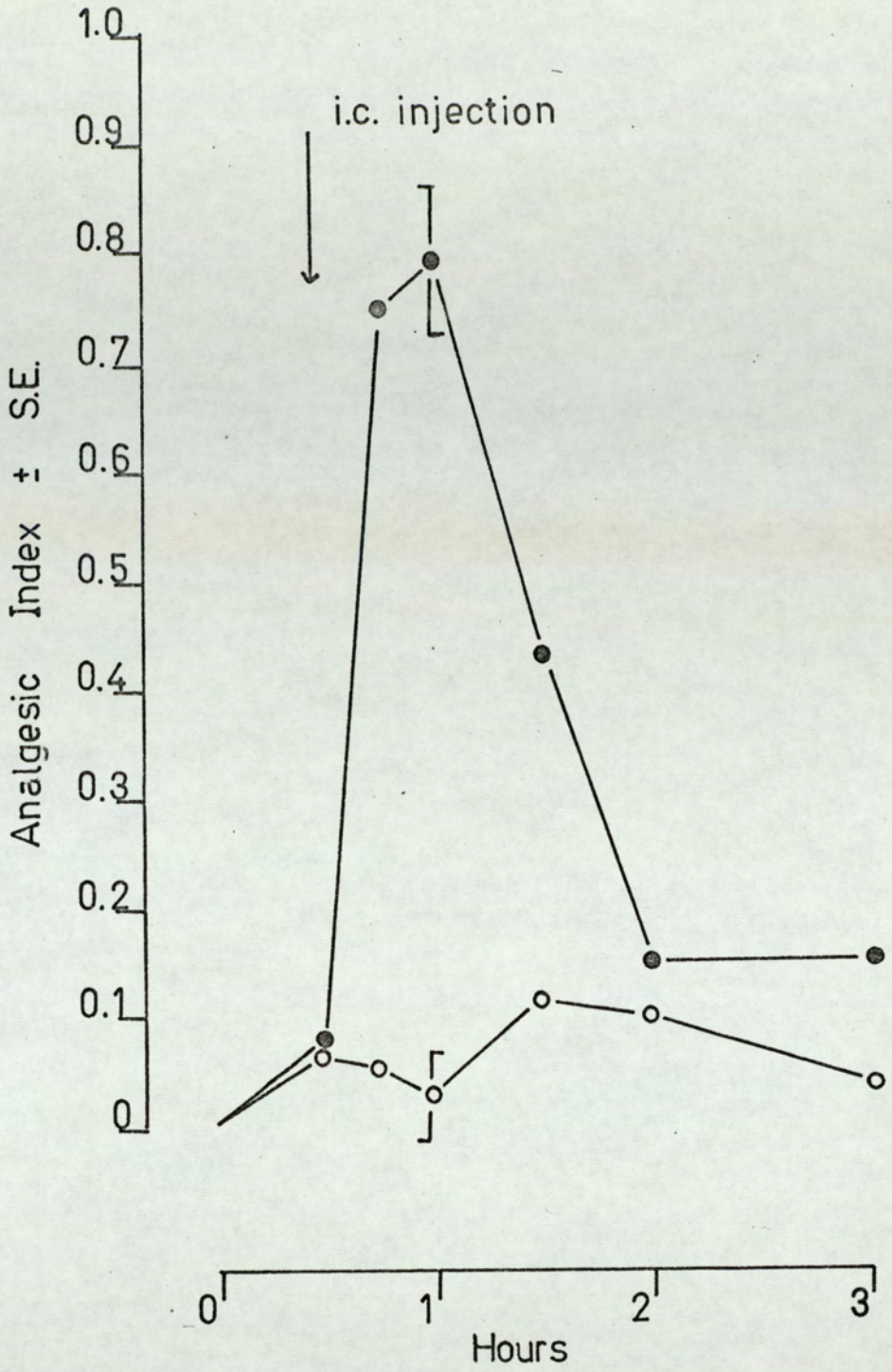

FIGURE 39 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN TETRABENAZINE-PRETREATED ANIMALS

2 groups of 5 animals were used. Both groups were pretreated with tetrabenazine 30 mg/kg at 4 hours. At time 0 nociceptive thresholds were determined and 8.0 mg/kg morphine injected s.c. 30 minutes later (at arrow) 5.0 µg 5-HT was injected into the cerebral ventricles of one group (—●—) and 10 µl saline injected into the other group (—o—). (see Table 27).

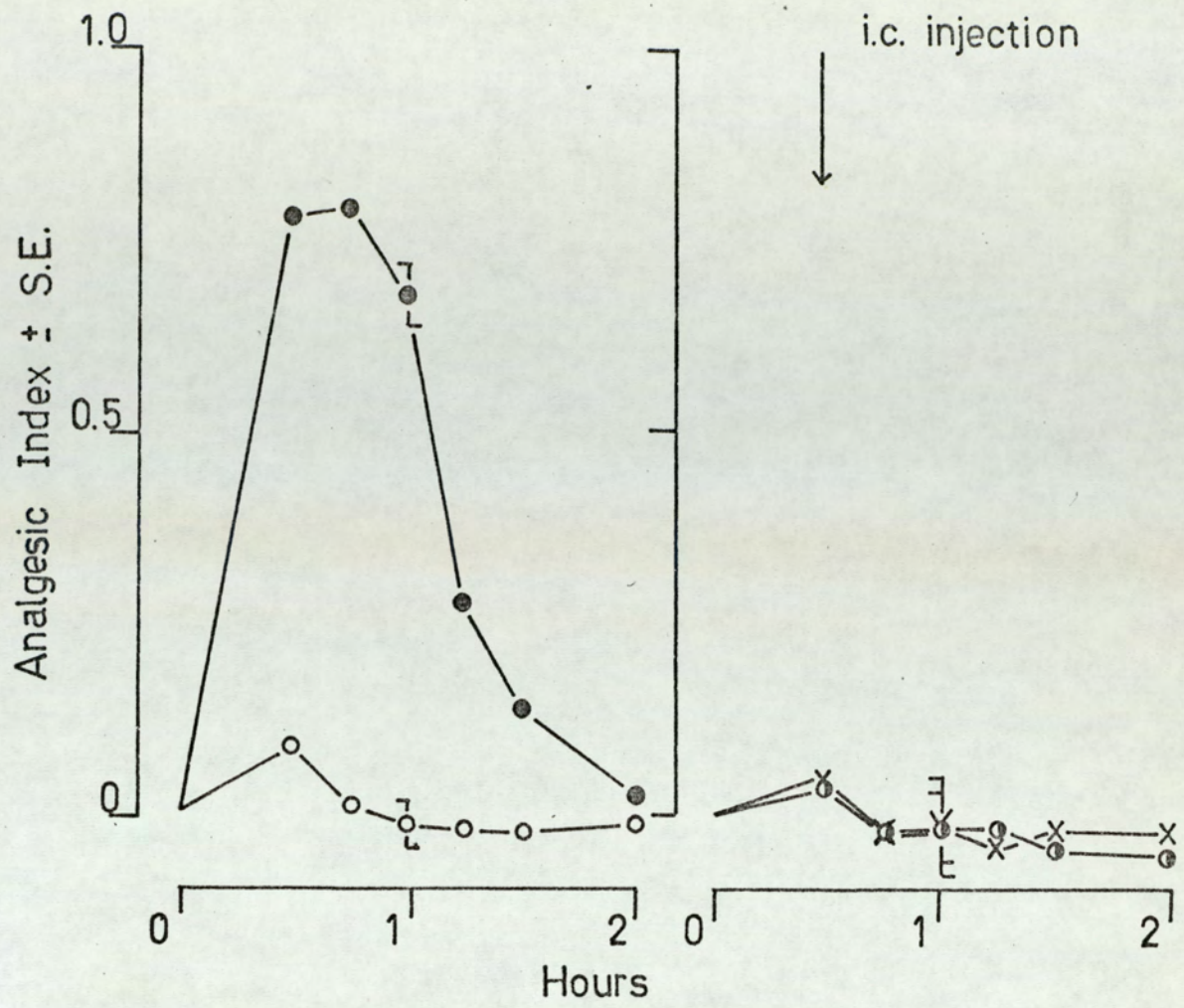

FIGURE 40 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF NA ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN RESERPINISED ANIMALS

4 groups of 5 animals were used. 3 groups were pretreated with 5.0 mg/kg reserpine i.p. at 16 hours. On the left hand side the analgesic effect of 8.0 mg/kg ~~reserpine~~ morphine given s.c. at time 0 is seen, in non-pretreated (—●—) and in reserpine-pretreated rats (—o—). The right hand side shows the effects of intraventricular (i.c.) injections of NA given to pretreated animals in the presence (—o—) and absence (—X—) of morphine. (see Table 28).

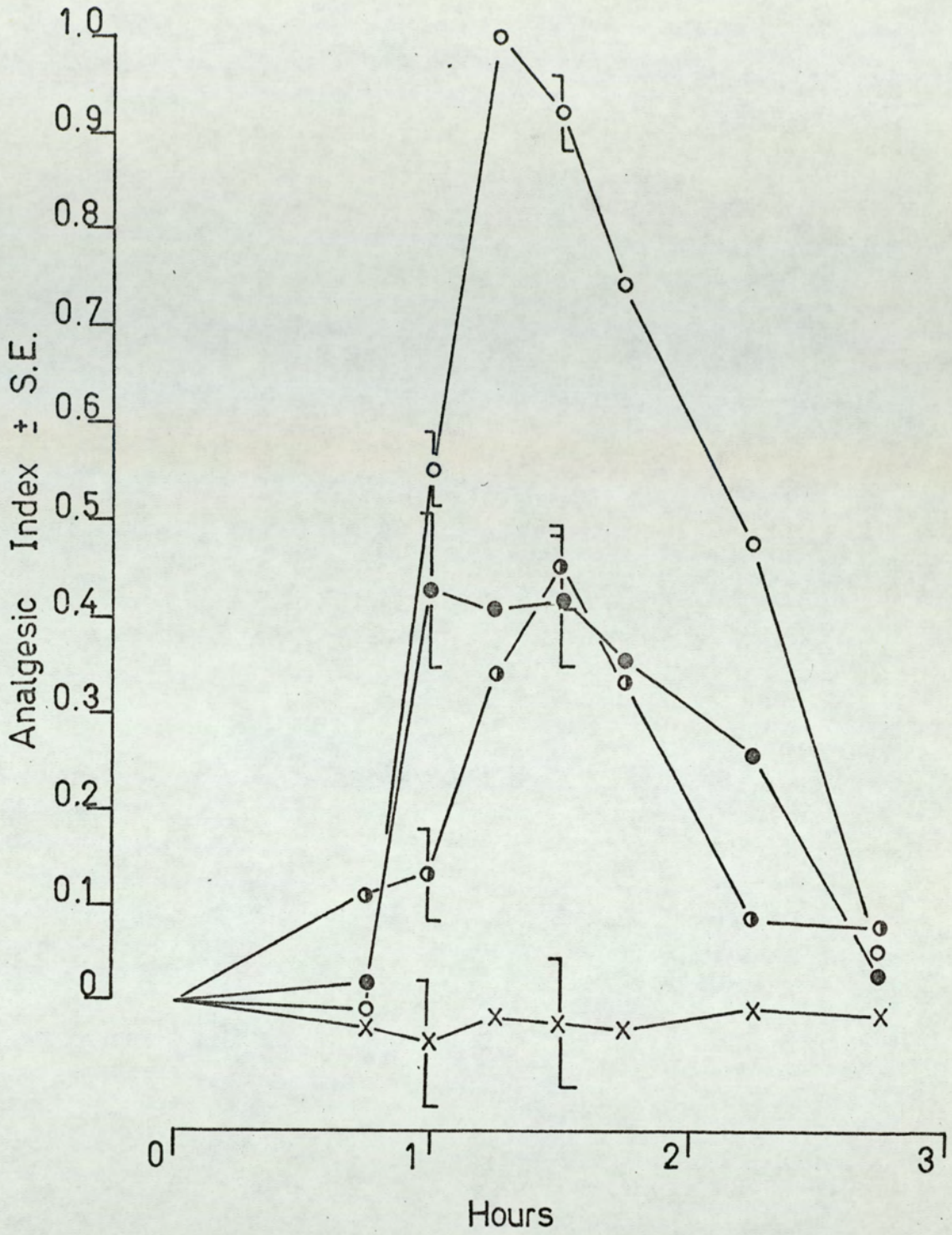

FIGURE 41 THE EFFECT OF PERIPHERALLY ADMINISTERED DOPA ON THE NOCICEPTIVE THRESHOLD AND ON THE ANTI-NOCICEPTIVE ACTION OF MORPHINE

4 groups of 5 animals were used. 2 groups were pretreated at 2 hours with 300 mg/kg DOPA i.p. The figure shows the effect on the nociceptive threshold of:

1) Morphine 8.0 mg/kg s.c. (time 0) (—o—)
2) Morphine 8.0 mg/kg s.c. (time 0) in DOPA pre-treated animals (—●—)
3) DOPA pretreatment alone (—●—)
4) Saline controls (—x—)

(see Table 29)

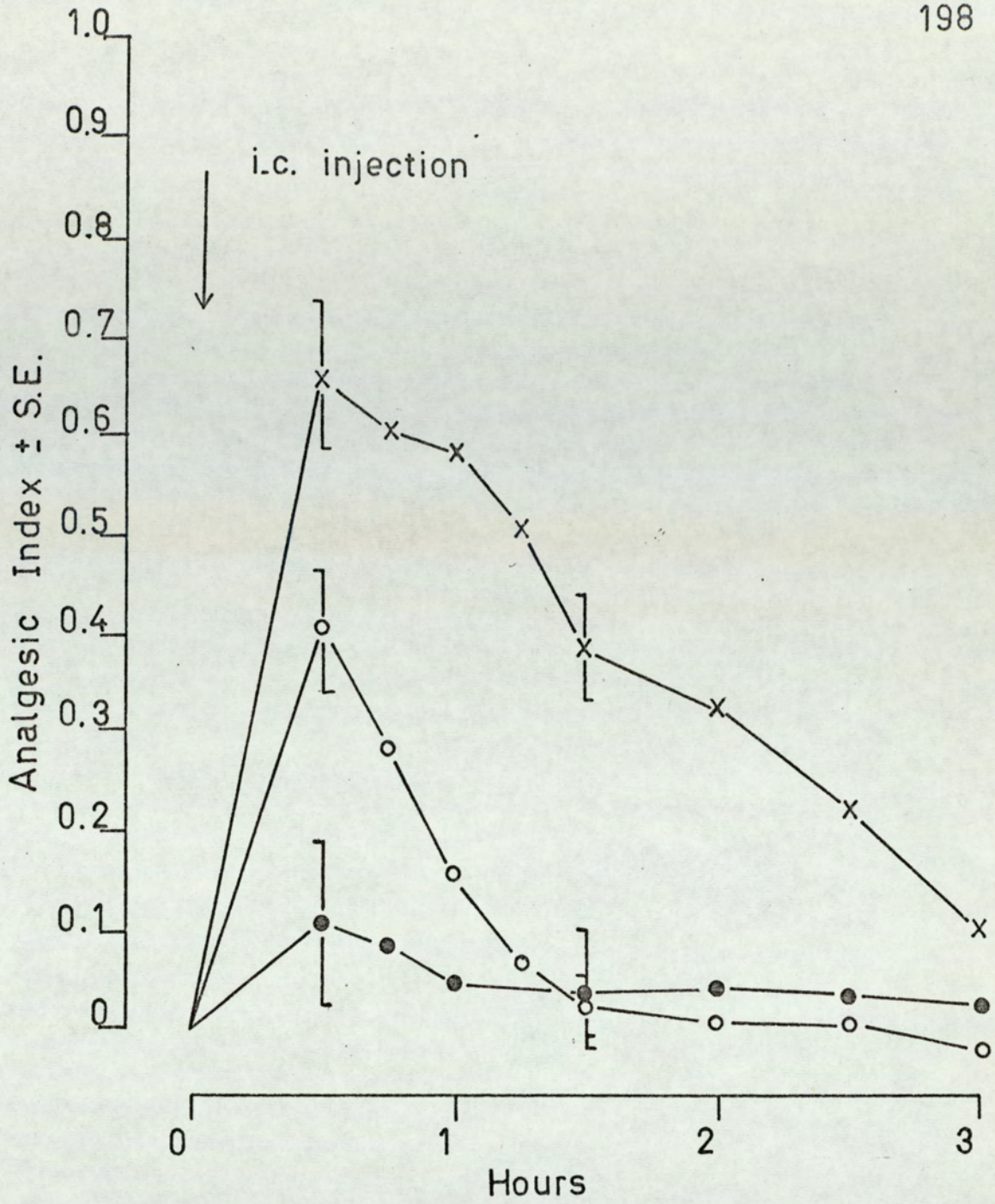

FIGURE 42 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF DOPA ON THE NOCICEPTIVE THRESHOLD AND ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

3 groups of 5 animals were used. 1 group received morphine 8.0 mg/kg s.c. at time 0 (—X—); 1 group received morphine 8.0 mg/kg s.c. and DOPA 40 µg i.c. at time 0 (—o—); 1 group received 40 µg DOPA i.c. at time 0 (—●—). Changes in the nociceptive threshold were recorded at times indicated. (see Table 30).

TABLE 25

THE EFFECT OF PRETREATMENT WITH RESERPINE, ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

(see Fig. 37)

	Analgesic index ± Standard Error									
Time (Hours)	3	6	9	12	15	18	21	30	60	90
Control (No reserpine pretreatment)	0.981 ± 0.042	0.960 ± 0.056	0.942 ± 0.063	0.964 ± 0.073	0.970 -	0.942	0.956 ± 0.072	0.944 ± 0.076	0.984 -	0.992 -
5.0 mg/kg reserpine i.p. (+ at time 0)	0.941 ± 0.062	1.000 -	0.777 ± 0.110	0.376 ± 0.070	0.092 ± 0.061	0.084 ± 0.052	0.096 ± 0.082	0.489 ± 0.162	0.651 ± 0.130	0.890 -

Each group received 8.0 mg/kg morphine s.c., 1 hour before determination of the nociceptive threshold.

TABLE 26

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN RESERPINISED ANIMALS

(see Fig. 38)

	Analgesic index ± Standard Error					
Time (Hours)	0.5	0.75	1.0	1.25	1.5	2.0
Reserpine 5 mg/kg i.p. Morphine 8 mg/kg s.c. Saline 10 µl i.c.	0.057 ± 0.040	0.0133 ± 0.032	0.073 ± 0.080	0.049 ± 0.012	0.088 ± 0.22	0.062 ± 0.046
Reserpine 5 mg/kg i.p. Morphine 8 mg/kg s.c. 5-HT 5 µg i.c.	0.166 ± 0.020	1.000 -	0.829 -	0.514 ± 0.142	0.239 ± 0.092	0.100 ± 0.086
Reserpine 5 mg/kg i.p. Saline 1 ml/kg i.p. 5-HT 5 µg i.c.	0.030 ± 0.062	0.050 ± 0.074	- 0.010 ± 0.050	- 0.026 ± 0.040	- 0.010 ± 0.042	- 0.026 ± 0.051
Saline 2 ml/kg i.p. Morphine 8 mg/kg s.c. Saline 10 µl i.c.	0.895 -	0.846 -	0.575 ± 0.080	0.461 ± 0.042	0.229 ± 0.110	0.001 ± 0.092

TABLE 27

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN TETRABENAZINE-PRETREATED ANIMALS

(see Fig. 39)

	Analgesic index ± Standard Error					
Time (Hours)	0.5	0.75	1.0	1.5	2.0	3.0
Tetrabenazine 30.0 mg/kg i.p. Morphine 8.0 mg/kg s.c. 5-HT 5.0 µg i.c.	0.088 ±0.037	0.752 ±0.140	0.797 ±0.074	0.425 ±0.101	0.152 ±0.046	0.152 ±0.042
Tetrabenazine 30.0 mg/kg i.p. Morphine 8.0 mg/kg s.c. Saline 10 µl i.c.	0.100 ±0.027	0.089 ±0.034	0.067 ±0.041	0.113 ±0.032	0.102 ±0.030	0.039 ±0.041

TABLE 28

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF NA ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE IN RESERPINISED ANIMALS

(see Fig. 40)

	Analgesic index ± Standard Error					
Time (Hours)	0.5	0.75	1.0	1.25	1.5	2.0
Reserpine 5 mg/kg i.p. Morphine 8 mg/kg s.c. Saline 10 µl i.c.	0.094 ± 0.056	0.001 ± 0.083	- 0.012 ± 0.029	- 0.016 ± 0.014	- 0.017 ± 0.022	- 0.011 ± 0.017
Reserpine 5 mg/kg i.p. Morphine 8 mg/kg s.c. NA 20 µg i.c.	0.037 ± 0.057	- 0.019 ± 0.062	- 0.014 ± 0.057	- 0.011 ± 0.054	- 0.040 ± 0.026	- 0.047 ± 0.039
Reserpine 5 mg/kg i.p. Saline 1 ml/kg s.c. NA 20 µg i.c.	0.048 ± 0.035	- 0.018 ± 0.037	0.008 ± 0.047	- 0.038 ± 0.029	- 0.013 ± 0.032	- 0.018 ± 0.032
Saline 1 ml/kg i.p. Morphine 8 mg/kg s.c. Saline 10 µl i.c.	0.788 ± 0.110	0.798 ± 0.087	0.679 ± 0.043	0.288 ± 0.089	0.135 ± 0.068	0.029 ± 0.029

TABLE 29

THE EFFECT OF PERIPHERALLY ADMINISTERED DOPA ON THE NOCICEPTIVE THRESHOLD AND ON THE ANTI-NOCICEPTIVE ACTION OF MORPHINE

(see Fig. 41)

	Analgesic index ± Standard Error						
Time (Hours)	0.75	1.0	1.25	1.5	1.75	2.25	2.75
DOPA 300 mg/kg i.p. Morphine 8 mg/kg s.c.	0.020 ± 0.062	0.427 ± 0.078	0.405 ± 0.092	0.416 ± 0.048	0.354 ± 0.079	0.266 ± 0.073	0.025 ± 0.060
DOPA 300 mg/kg i.p. Saline 1 ml/kg s.c.	0.114 ± 0.058	0.134 ± 0.047	0.343 ± 0.070	0.456 ± 0.063	0.336 ± 0.052	0.099 ± 0.035	0.089 ± 0.025
Saline 5 ml/kg i.p. Morphine 8 mg/kg s.c.	- 0.011 ± 0.046	0.555 ± 0.039	1.000 -	0.915 ± 0.042	0.737 ± 0.060	0.472 ± 0.091	0.070 ± 0.045
Saline 5 ml/kg i.p. Saline 8 ml/kg s.c.	- 0.220 ± 0.072	- 0.042 ± 0.068	- 0.011 ± 0.070	- 0.021 ± 0.079	- 0.032 ± 0.025	- 0.005 ± 0.052	- 0.012 ± 0.076

TABLE 30

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF DOPA ON THE NOCICEPTIVE THRESHOLD AND ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

(see Fig. 42)

	Analgesic index ± Standard Error							
Time (Hours)	0.5	0.75	1.0	1.25	1.5	2.0	2.5	3.0
Saline 10 µl i.c. Morphine 8 mg/kg s.c.	0.665 ± 0.113	0.605 ± 0.106	0.596 ± 0.104	0.506 ± 0.062	0.383 ± 0.071	0.334 ± 0.044	0.237 ± 0.059	0.109 ± 0.023
DOPA 40 µg i.c. Morphine 8 mg/kg s.c.	0.409 ± 0.098	0.286 ± 0.059	0.167 ± 0.022	0.078 ± 0.017	0.003 ± 0.034	0.005 ± 0.019	0.005 ± 0.014	- 0.018 ± 0.023
DOPA 40 µg i.c. Saline 8 mg/kg s.c.	0.112 ± 0.092	0.096 ± 0.048	0.052 ± 0.017	0.050 ± 0.034	0.042 ± 0.071	0.050 ± 0.062	0.041 ± 0.059	0.032 ± 0.042

TABLE 31

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF DOPAMINE ON THE NOCICEPTIVE THRESHOLD AND ON THE ANTI-NOCICEPTIVE ACTION OF MORPHINE

	Analgesic index ± Standard Error							
Time (Hours)	0.5	0.75	1.0	1.15	1.5	2.0	2.5	3.0
Saline 10 µl i.c. Morphine 8 mg/kg s.c.	0.720 ± 0.121	0.694 ± 0.094	0.554 ± 0.086	0.512 ± 0.071	0.410 ± 0.044	0.321 ± 0.036	0.242 ± 0.019	0.094 ± 0.063
Dopamine 20 µg i.c. Morphine 8 mg/kg s.c.	0.324 ± 0.091	0.210 ± 0.086	0.092 ± 0.042	0.014 ± 0.051	- 0.001 ± 0.043	0.032 ± 0.062	0.021 ± 0.011	0.001 ± 0.012
Dopamine 20 µg i.c. Saline 8 mg/kg s.c.	0.112 ± 0.021	0.012 ± 0.011	0.034 ± 0.020	0.022 ± 0.026	0.010 ± 0.042	- 0.021 ± 0.054	- 0.034 ± 0.021	0.012 ± 0.026

CHAPTER FIVE

THE EFFECTS OF CHOLINERGIC COMPOUNDS ON THE NOCICEPTIVE THRESHOLD OF RATS

CHAPTER FIVE

THE EFFECTS OF CHOLINERGIC COMPOUNDS ON THE NOCICEPTIVE THRESHOLD OF RATS

THE EFFECTS OF CHOLINERGIC COMPOUNDS ON THE NOCICEPTIVE THRESHOLD OF RATS

Cholinergic mechanisms in the brain have been implicated frequently in the mechanism of action of morphine (see Martin, 1963; Winter, 1965; Maynert, 1967). The evidence includes demonstrations that several compounds with cholinergic activity elevate the nociceptive threshold in mice (Chen, 1958; Harris, Dewey & Howes, 1968; Handley & Spencer, 1969). Physostigmine has been reported to potentiate the anti-nociceptive action of morphine in rats (Saxena, 1958), in the cat (Slaughter & Munsell, 1940) and in man in both experimental (Slaughter, 1950) and clinical (Hand & Audin, 1944) situations. Also, administration of morphine increases brain ACh concentrations in rats (Maynert, 1967).

In this chapter the effects on the nociceptive threshold of some drugs that have cholinergic activity are described, and these effects are compared with those of morphine.

1. THE EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF ACh AND CARBACHOL

ACh was injected into the ventricles of conscious rats in doses from 1-100 μg. A small, very short and somewhat erratic elevation of the nociceptive threshold was seen at the higher doses. However since there was some indication of an anti-nociceptive response to ACh, it was then infused into the ventricles at 0.5 and 1.0 μg/minute. The effect of this infusion on the nociceptive threshold is shown in Fig. 43 (Table 32). Both rates of administration produced a significant elevation of the nociceptive threshold. The recovery from the effects of the infusion was extremely rapid. In view of the generally

unsatisfactory nature of the response to ACh, carbachol was used subsequently.

Fig. 44 (Table 33) shows the effects on the nociceptive threshold of 0.2 - 1.6 µg carbachol injected into the cerebral ventricles of conscious rats. The response/time curves were very similar in shape to that of morphine, the difference being that the peak effect was at about 15 minutes (45-60 minutes for morphine) and the effect was very much shorter. The prolonged effect at 1.6 µg may have been due to overloading of the cholinesterase systems, or to other unspecific effects, for animals given a dose of 3.2 µg of carbachol usually exhibited toxic symptoms.

2. THE EFFECTS OF INTRAVENTRICULARLY ADMINISTERED OXOTREMORINE (OTMN)

OTMN was injected into the ventricles of conscious rats in doses of 2.0 - 16.0 µg. The effects of these doses are shown in Fig. 45 (Table 34). The elevation of the nociceptive threshold was markedly similar to that obtained for carbachol, though the potency of carbachol was almost exactly 10 times that of OTMN. As noted in Section 3, Chapter 1, the OTMN was very much less toxic than carbachol. Thus the dose of OTMN that gave a 95% anti-nociceptive response, was not prolonged.

300-600 µg of OTMN or of carbachol (i.e. 10-100 times intraventricular doses) produced a significant elevation of the nociceptive threshold (approximately 45% when injected subcutaneously).

3. COMPARISON OF THE DOSE RESPONSE CURVES FOR OTMN CARBACHOL AND MORPHINE

Fig. 46 shows the log. dose response curves for carbachol and OTMN. The effects 15 minutes after the injection, shown in Tables 33

and 34 were compared. The deviance from parallelism was not significant according to the method of Litchfield and Wilcoxon (1949).

Fig. 47 shows the log. dose response curves for carbachol and morphine. The values used were those obtained at 15 minutes after the intraventricular injection of carbachol (given in Table 34) and at 60 minutes after a subcutaneous injection of morphine (given in Table 19: 17.6.69). Again, the difference from parallelism was not significant according to the method of Litchfield and Wilcoxon (1949).

4. THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF INTRAVENTRICULAR CARBACHOL AND OTMN

Rats were treated with 1.0 mg/kg hyoscine or 1.0 ml/kg saline given intraperitoneally. 30 minutes later 0.8 μg carbachol was injected into the ventricles. Fig. 48 (Table 35) shows a complete inhibition of the usual increase in nociceptive threshold produced by carbachol.

Very similar results were obtained with OTMN (8.0 μg intraventricularly) when the animals were pretreated with hyoscine (Table 36).

5. THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

The experimental procedure was similar to that used in paragraph 4. Hyoscine 1.0 mg/kg was given intraperitoneally and morphine (6.0 mg/kg subcutaneously), given after 30 minutes (at time 0). The effects are shown in Fig. 49 (Table 37). This dose of hyoscine significantly inhibited the anti-nociceptive action of morphine, though complete abolition of the effect was not seen. Hyoscine alone had no significant effect on the nociceptive threshold.

6. THE EFFECT OF INTRAVENTRICULAR CARBACHOL AND OF INTRAVENTRICULAR CARBACHOL PLUS 5-HT, IN RESERPINISED ANIMALS

Dewey, Harris, Howes and Nuite (1968) reported that reserpine antagonised OTMN anti-nociceptive activity in the tail flick test in the mouse. It was therefore decided to examine whether or not this effect was also seen with carbachol in the rat.

The previous procedure for inhibiting morphine's action with reserpine was followed (see Section 3, Chapter 4). Rats were pre-treated with 5.0 mg/kg reserpine. 16 hours later carbachol (0.8 µg) was injected into the ventricles. As can be seen from Fig. 50 (Table 38) the anti-nociceptive action of carbachol was completely abolished.

In view of the restorative effect of intraventricular 5-HT on the anti-nociceptive effect of morphine in this situation, 0.8 µg of carbachol and 5.0 µg of 5-HT were mixed in a volume of 10 µl and injected into the ventricles of reserpinised rats. The effect is also shown in Fig. 50 (Table 38). Carbachol and 5-HT together produced a significantly larger and longer lasting elevation of the nociceptive threshold in reserpinised rats, than carbachol alone in untreated rats. Again, as was shown in Section 3, Chapter 4, intra-ventricular injections of 5-HT into reserpinised rats did not affect the nociceptive threshold.

7. DISCUSSION

The observation that intraventricular injections of carbachol into rats elevate the nociceptive threshold has been recorded previously. Metys, Wagner, Metysova and Herz (1969) immobilised rats with

succinylcholine and stereotaxically injected carbachol into the ventricles in doses of 0.315, 1.25 and 5 µg, and noted a significant increase in the nociceptive threshold above that of the controls. They used an electrical nociceptive stimulus and in this test the controls (saline injected) also showed an elevated threshold. They also noted that in this test OTMN and arecoline produced an elevated threshold on peripheral administration while carbachol had no such effect. Contrary to this, in the present experiments, doses of 1-2 mg/kg OTMN or carbachol produced significant elevation of the nociceptive threshold when injected subcutaneously.

It has been reported that tremorine has an anti-nociceptive action in mice (Chen, 1958; Keranen, Zaratzian & Coleman, 1961). Harris, Dewey, Howes, Kennedy and Pars (1969) found that both OTMN and physostigmine were active in raising the nociceptive threshold when given peripherally to mice and this is also true of OTMN given intracerebrally to mice (Handley & Spencer, 1969). Harris et al (1969) also found OTMN to be 250 times more potent than morphine when given peripherally, and again the same effect was seen by Handley & Spencer (1969) following intracerebral OTMN. In our results no valid comparison of potency can be given since the two drugs were given by different routes. However we did find that 300-600 µg (1-2 mg/kg) of OTMN were necessary to produce a significant elevation of the nociceptive threshold when given subcutaneously, though a dose response curve for this effect was not obtained.

Harris, Dewey, Howes, Kennedy and Pars (1969) found that the dose response curves for OTMN and physostigmine were parallel to each other and to that of morphine. We found that the dose response curves for oxotremorine and carbachol given intraventricularly were parallel to each other and to that of subcutaneously administered morphine,

indicating that these agents probably have a similar site of action.

Pretreatment of rats with hyoscine inhibited the anti-nociceptive action of both intraventricularly administered oxotremorine and carbachol. Pretreatment with hyoscine in the same dose that abolished the activity of the cholinergic compounds, reduced the efficacy of morphine by some 50%. Higher doses of hyoscine were not tried, though if they had been it is likely that complete inhibition would have been obtained. Inhibition of morphine by atropine has been recorded previously (Slaughter & Munsell, 1940), although other authors have failed to find this effect (Levy & Edwards, 1956; Saxena, 1968).

The inhibition of the anti-nociceptive effect of carbachol by reserpine provides a further similarity between cholinergic agents and morphine. The effect was previously reported for OTMN by Dewey, Howes and Nuite (1968). Again similarity is seen in the inhibition of the anti-nociceptive effects of both morphine and carbachol by reserpine and the reversal of this inhibition by intraventricular 5-HT (this chapter and Section 3, Chapter 3).

Acute administration of morphine in rats causes an increase in the concentration of brain ACh which reaches a peak in about 30 minutes and returns to normal 4-6 hours later, and it has been suggested that these increases could be brought about in 3 ways: (i) by inhibition of cholinesterase (ii) by an increase in synthesis of ACh or (iii) by inhibition of the release of transmitter from nerve endings. (Bernheim & Bernheim, 1936; Maynert, 1967). It is unlikely that morphine increases synthesis of ACh (Maynert, 1967) though it has various effects on the uptake and release of ACh in vitro. It inhibits the release of ACh from the

electrically stimulated intestine (Schaumann, 1957; Paton, 1957; Cox & Weinstock, 1966), inhibits the uptake of ACh by mouse brain cortex slices (Schuberth & Sundwall, 1967), and also inhibits release of ACh from rat brain cortex slices (Sharkawi & Schulman, 1969). However considerable doubt has been placed on the theory that morphine mediates its effects by increasing brain levels of ACh (Szerb, 1957; Saxena, 1958; Maynert, 1967). Since nalorphine antagonises the anti-nociceptive effect of morphine it would be expected that nalorphine would inhibit the effect of morphine on brain ACh levels if the theory were correct. Maynert (1967) showed that nalorphine given to rats in doses sufficient to inhibit the anti-nociceptive and depressant effects of morphine, did not inhibit the increased brain levels of ACh. Contrary to this, Howes, Harris, Dewey and Voyda (1969), while investigating the tail-flick reflex in mice, found that the increased brain ACh caused by morphine was reversed by nalorphine, even though nalorphine alone itself caused increased brain ACh. This latter observation was further supported by Cox and Weinstock (1966) who noted that low doses of nalorphine, (that were without effect themselves) antagonised the inhibitory effect that morphine and related drugs have on the release of ACh from the electrically stimulated guinea-pig intestine. There are indications that morphine may have similar effects in inhibiting the release of ACh from the brain. Morphine added to the fluid used to perfuse the cerebral subarachnoid space (Belesin, Polak & Sproull, 1965) or the cerebral ventricles (Belesin & Polak, 1965) of cats, inhibits the release of ACh into perfusion fluid. From this evidence it seems possible that morphine exerts its action by preventing the release of ACh from specific sites thus causing a build-up of brain ACh levels. In contrast, the intraventricular injections of cholinergic compounds would ~~have a similar effect~~ serve to increase the amount of extraneuronal ACh.

Further evidence for the involvement of cholinergic mechanisms in the action of narcotic analgesics is provided by the work of Harris, Dewey, Howes, Kennedy and Pars (1969). They demonstrated that physostigmine was active in inhibiting the tail-flick reflex in mice. They then noted that a number of narcotic antagonists (nalorphine, pentazocine, cyclazocine, and cyclorphan) which had no activity themselves in this test, gave highly significant increases in the reaction time of mice in the presence of a nearly inactive dose of physostigmine. All these compounds have been shown to have analgesic activity in man. They also noted that naloxone (a compound with nearly pure narcotic antagonist activity) antagonised the inhibition of the tail-flick response caused by physostigmine - and naloxone does not produce analgesia in man.

The results presented in this and previous chapters suggest that in the rat at least, cholinergic, adrenergic and tryptaminergic systems are involved in the mediation of the anti-nociceptive effects of morphine. However it should be pointed out that Herz (1968) reported that peripheral administration of cholinergic stimulants (tremorine and arecoline) to rats inhibit well established conditioned responses in rats. Also Grossman (1964) found similar effects on application of cholinomimetics to various brain structures. Herz (1968) also found that inhibition of conditioned responses runs parallel to inhibition in the reaction to nociceptive stimuli, indicating that central cholinergic stimulation may result in decreased responsiveness in a general sense.

In Section 3, Chapter 2, it was postulated that the effects of morphine on body temperature may be mediated by ACh and modulated by 5-HT and NA. From the results obtained in the study of changes in

the nociceptive threshold it would seem that similar interactions might be involved. The suggestions found in the literature, that one such transmitter substance is involved to the exclusion of the others, are not easy to accept. In fact, it is postulated that morphine may mediate its effect via ACh and this effect can be modulated by NA and 5-HT levels in the brain. In view of the fact that morphine still increases brain ~~release~~ ACh in tolerant animals (Maynert, 1968) it is possible that changes in NA or 5-HT levels are responsible for the phenomena of tolerance and dependence, and this is briefly examined in the following chapter.

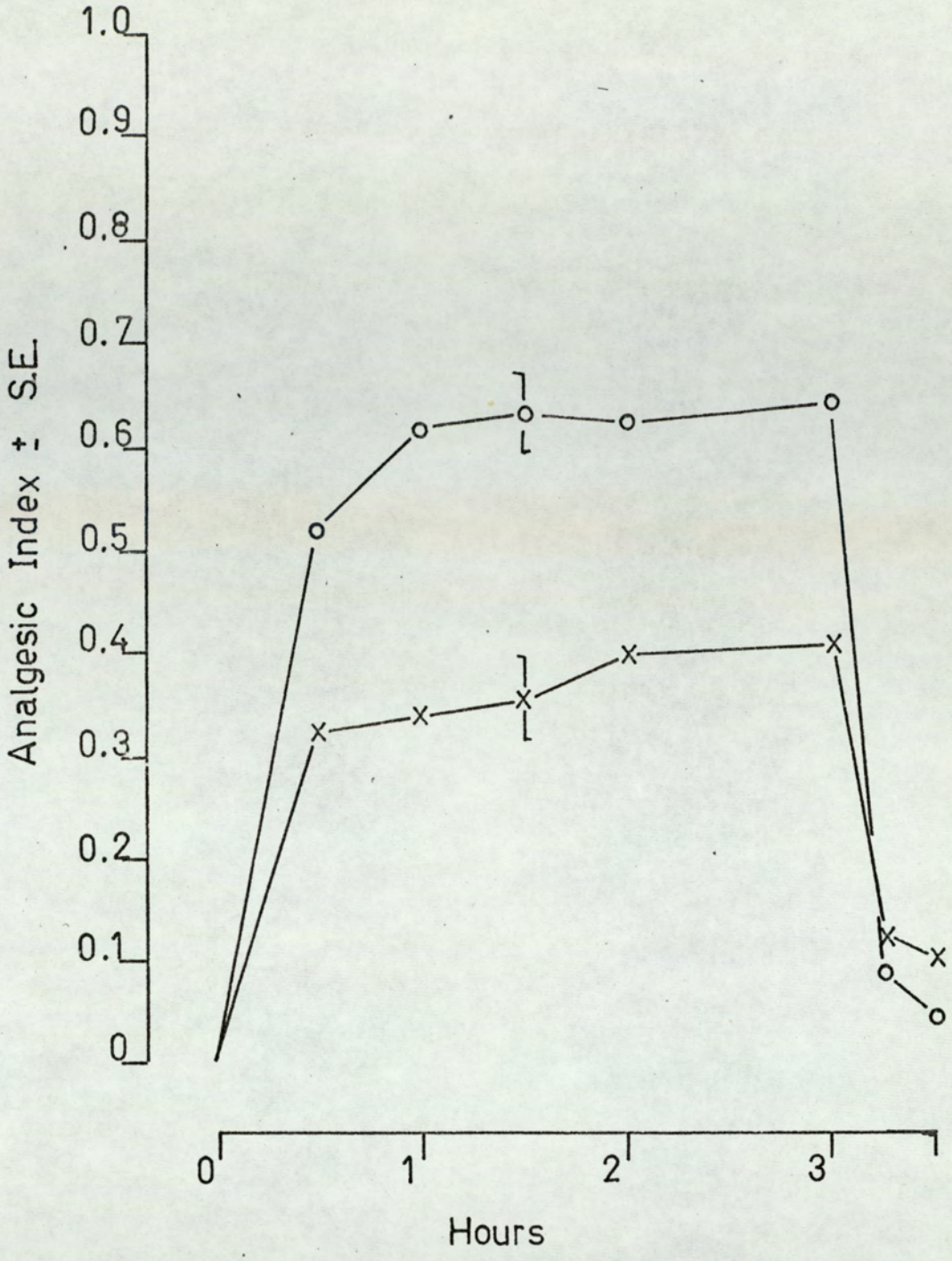

FIGURE 43 THE EFFECT OF INTRAVENTRICULAR INFUSION OF ACh ON THE NOCICEPTIVE THRESHOLD OF RATS

2 groups of 5 animals were used. The infusions were not carried out simultaneously. ACh was infused into the ventricles at 0.5 µg/minute (—X—), and 1.0 µg/minute (—o—) for 3.5 hours. Changes in the nociceptive threshold were recorded at the times indicated. (see Table 32).

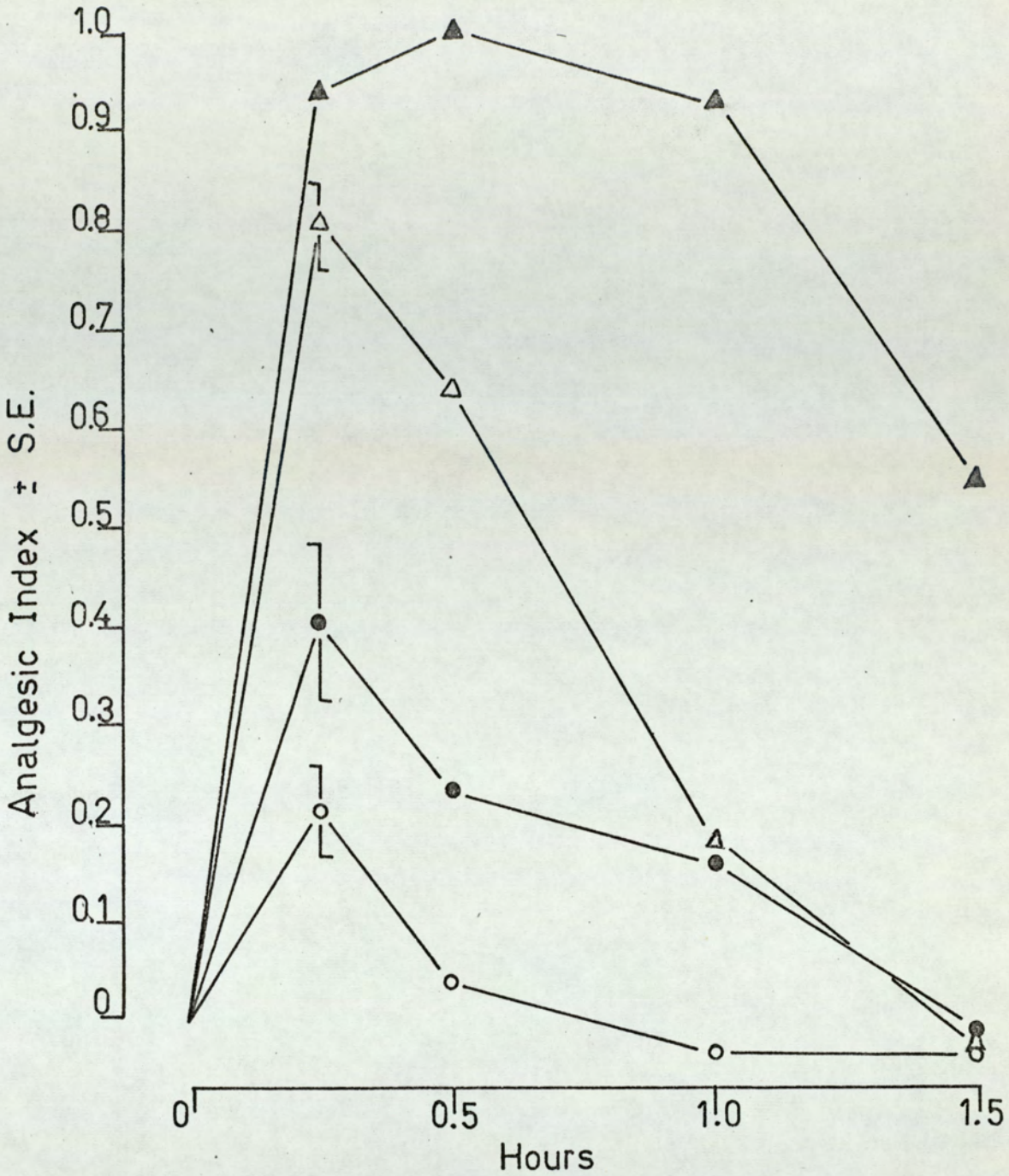

FIGURE 44 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF CARBACHOL ON THE NOCICEPTIVE THRESHOLD IN RATS

4 groups of 5 animals were used. At time 0 the following doses of carbachol were injected into the cerebral ventricles:
0.2 µg (—o—); 0.4 µg (—●—);
0.8 µg (—Δ—); 1.6 µg (—▲—).
Changes in the nociceptive threshold were recorded at the times indicated.
(see Table 33).

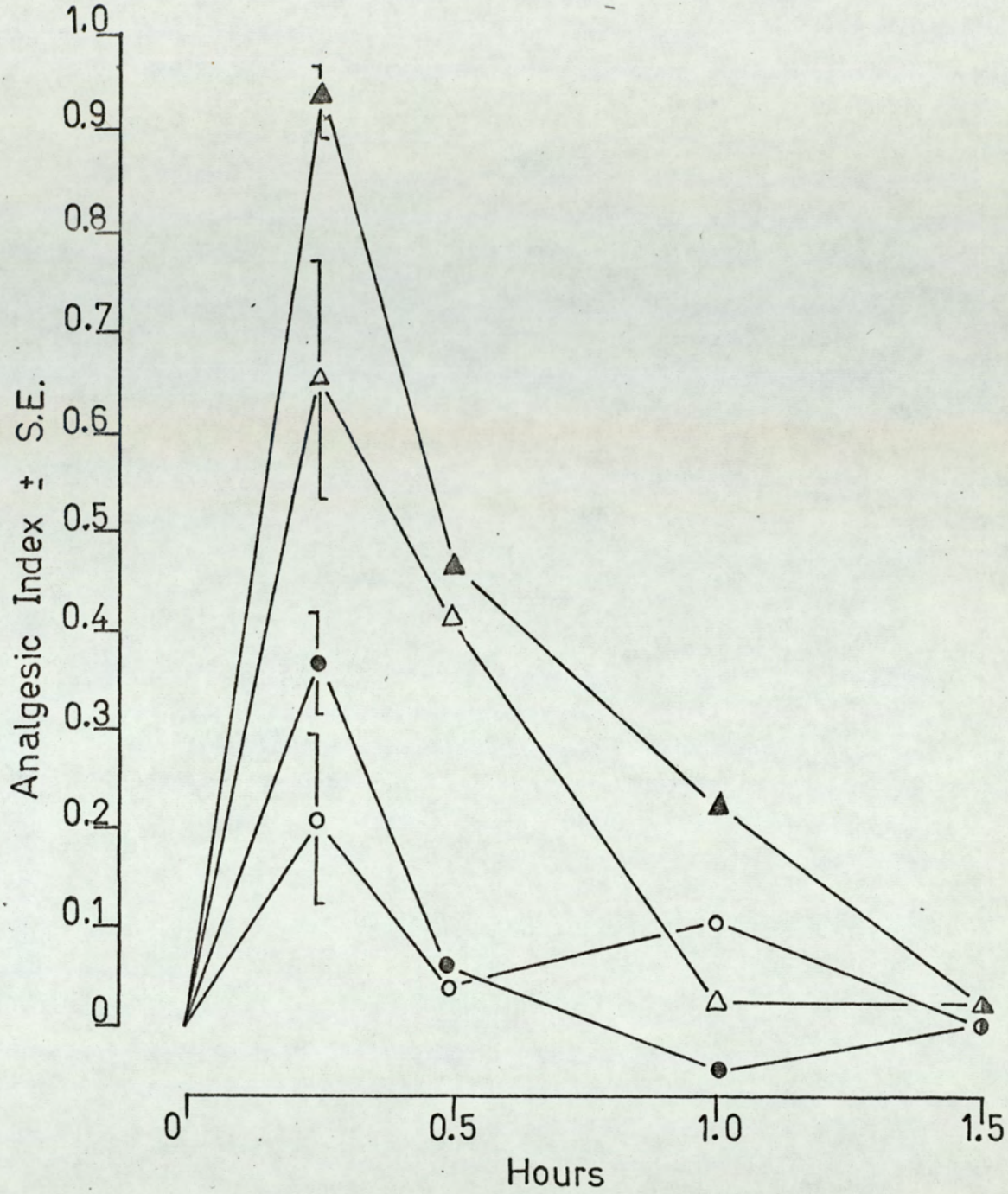

FIGURE.45 THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF OTMN ON THE NOCICEPTIVE THRESHOLD OF RATS

4 groups of 5 animals were used. At time 0 the following doses of OTMN were injected into the cerebral ventricles:
2.0 µg (—o—); 4.0 µg (—●—);
8.0 µg (—△—); 1.6 µg (—▲—).
Changes in the nociceptive threshold were recorded as indicated.
(see Table 34).

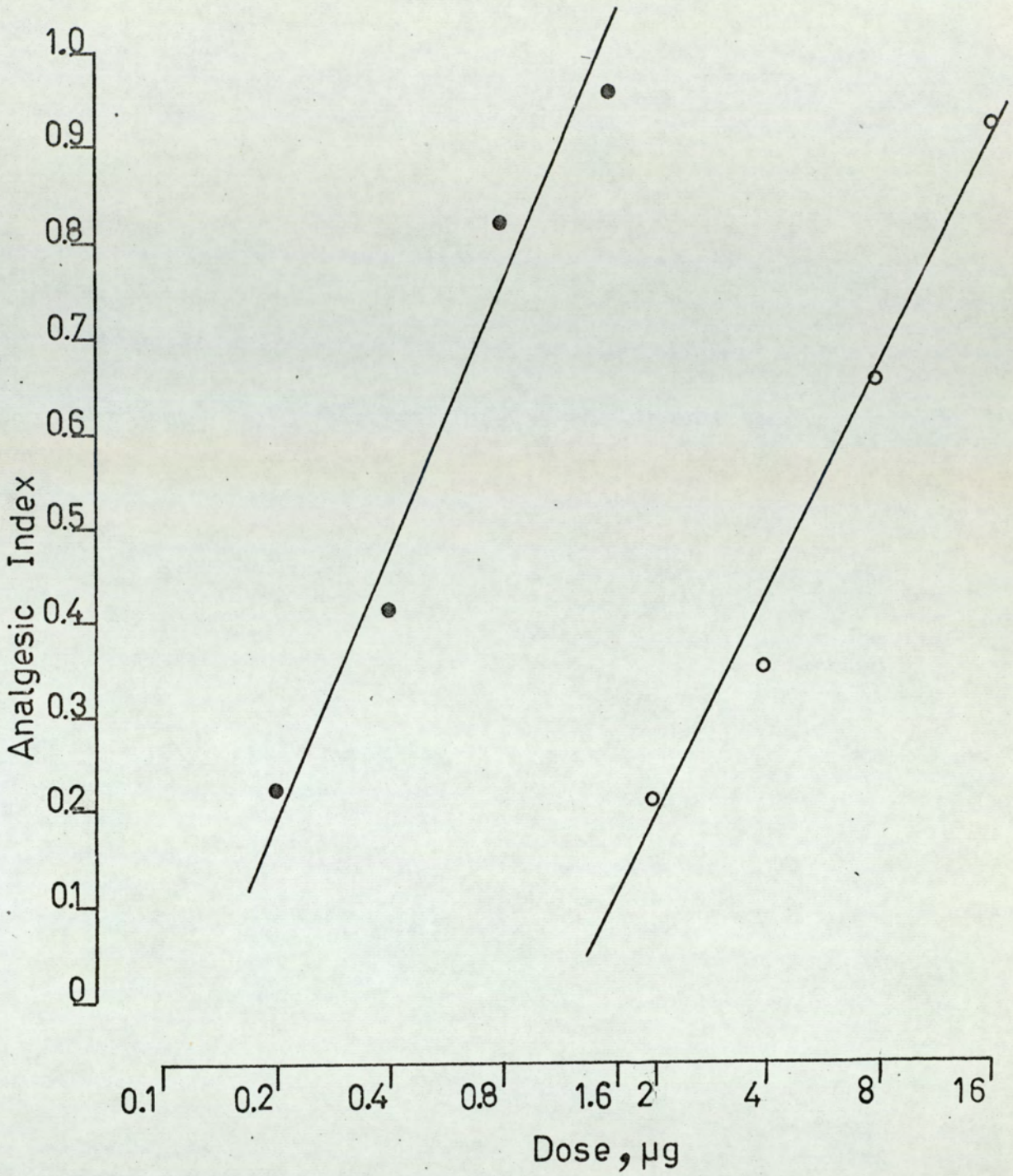

FIGURE 46 COMPARISON OF THE DOSE-RESPONSE CURVES FOR THE ANTI-NOCICEPTIVE EFFECTS OF CARBACHOL AND OTMN

The analgesic indices shown 15 minutes after intraventricular injection of carbachol (Table 34; (—●—)) and OTMN (Table 35; (—o—)) are compared. The difference from parallelism is not significant.

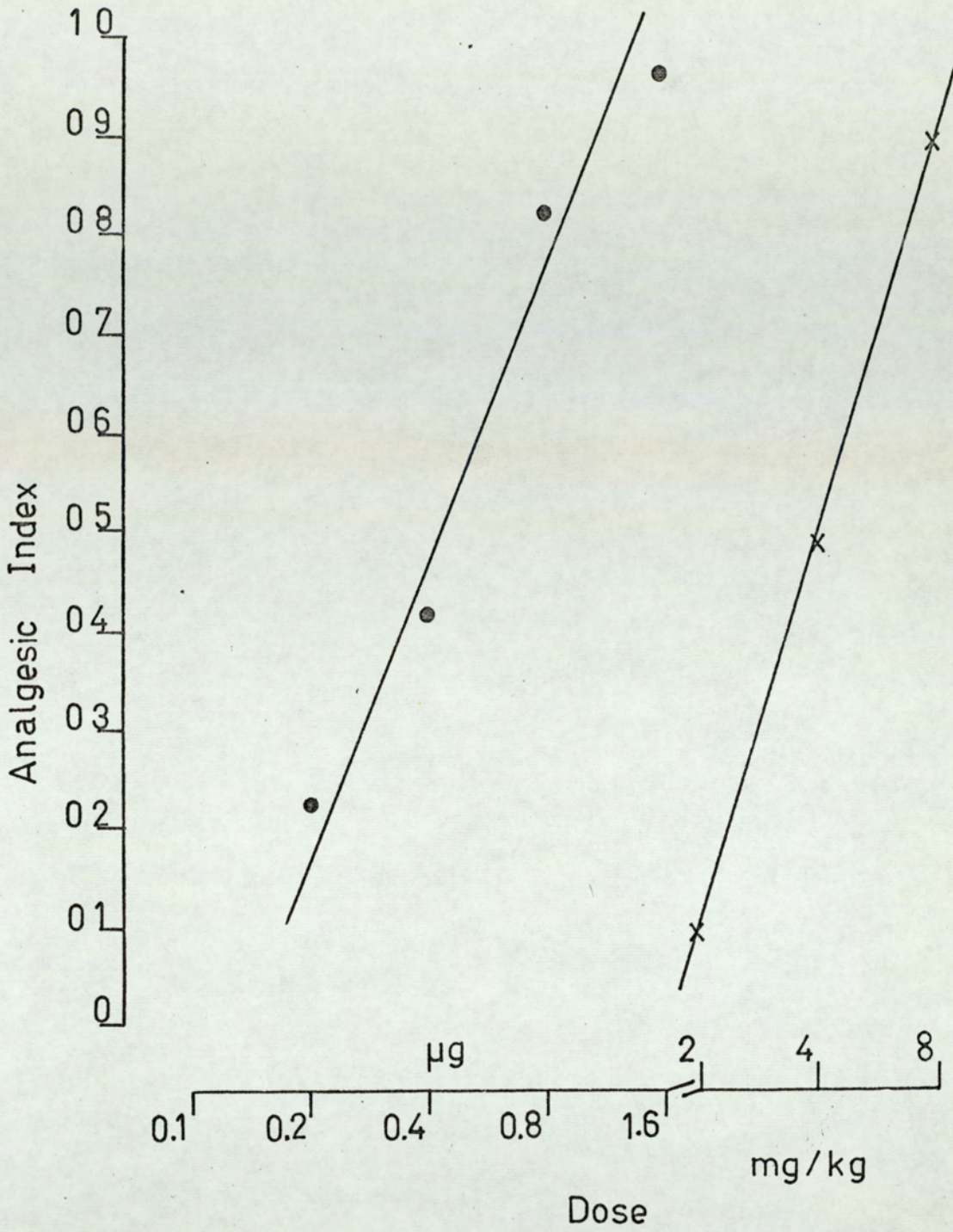

FIGURE 47 COMPARISON OF THE DOSE-RESPONSE CURVES FOR THE ANTI-NOCICEPTIVE EFFECTS OF MORPHINE AND CARBACHOL

The analgesic indices shown 15 minutes after intraventricular injection of carbachol (Table 34; (—●—)) and 60 minutes after the s.c. injection of morphine (Table 19; (—X—)). The deviation from parallelism is not significant.

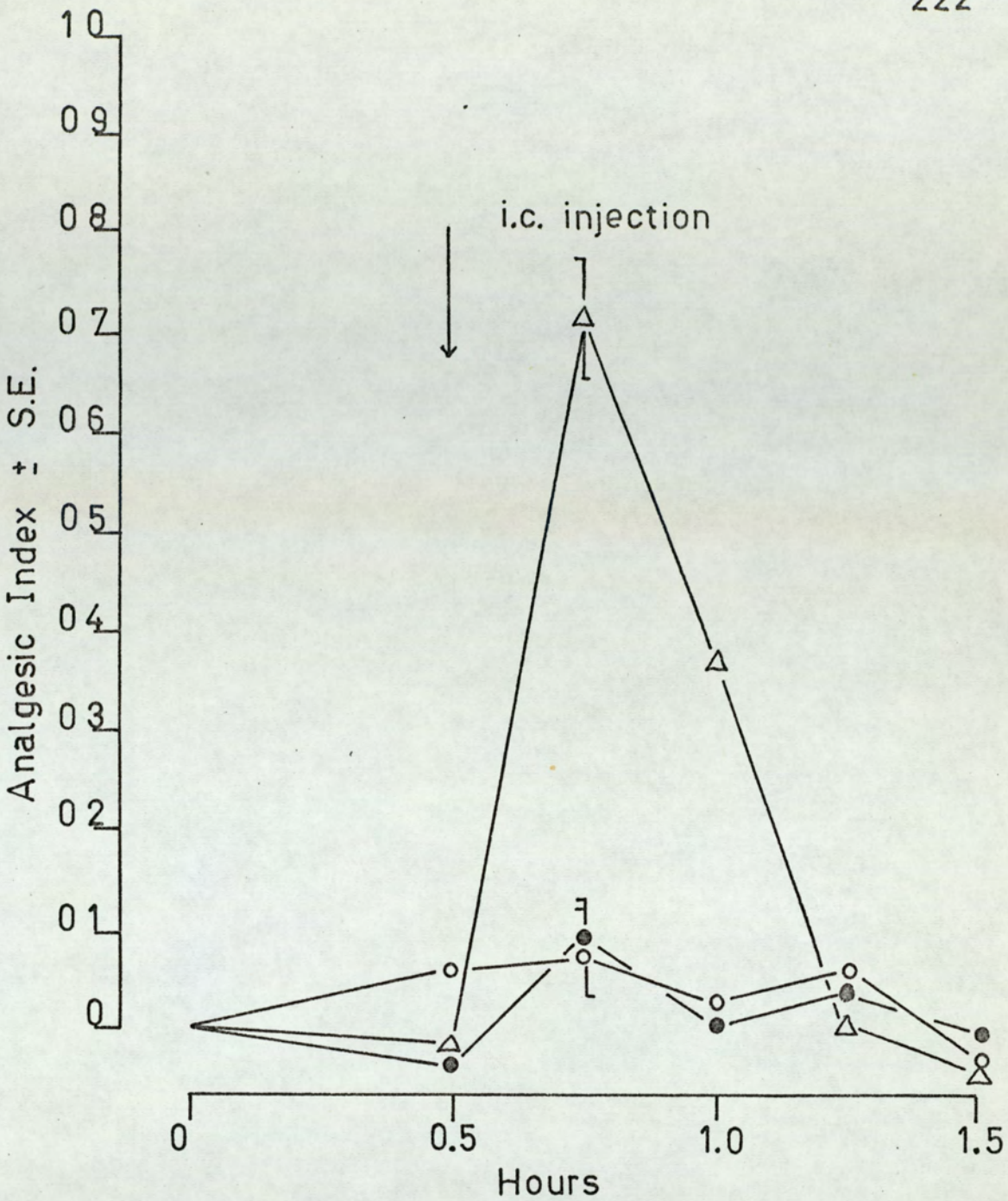

FIGURE 48 THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF CARBACHOL

3 groups of 5 animals were used. 2 groups received 1.0 mg/kg hyoscine i.p. and one received 1.0 ml/kg saline, at time 0. 30 minutes later (at arrow), one hyoscine treated group received an intraventricular (i.c.) injection of saline (—o—). The saline pre-treated group also received 0.8 µg carbachol i.c. at 30 minutes (—●—). Changes in the nociceptive threshold were recorded as indicated. (see Table 35).

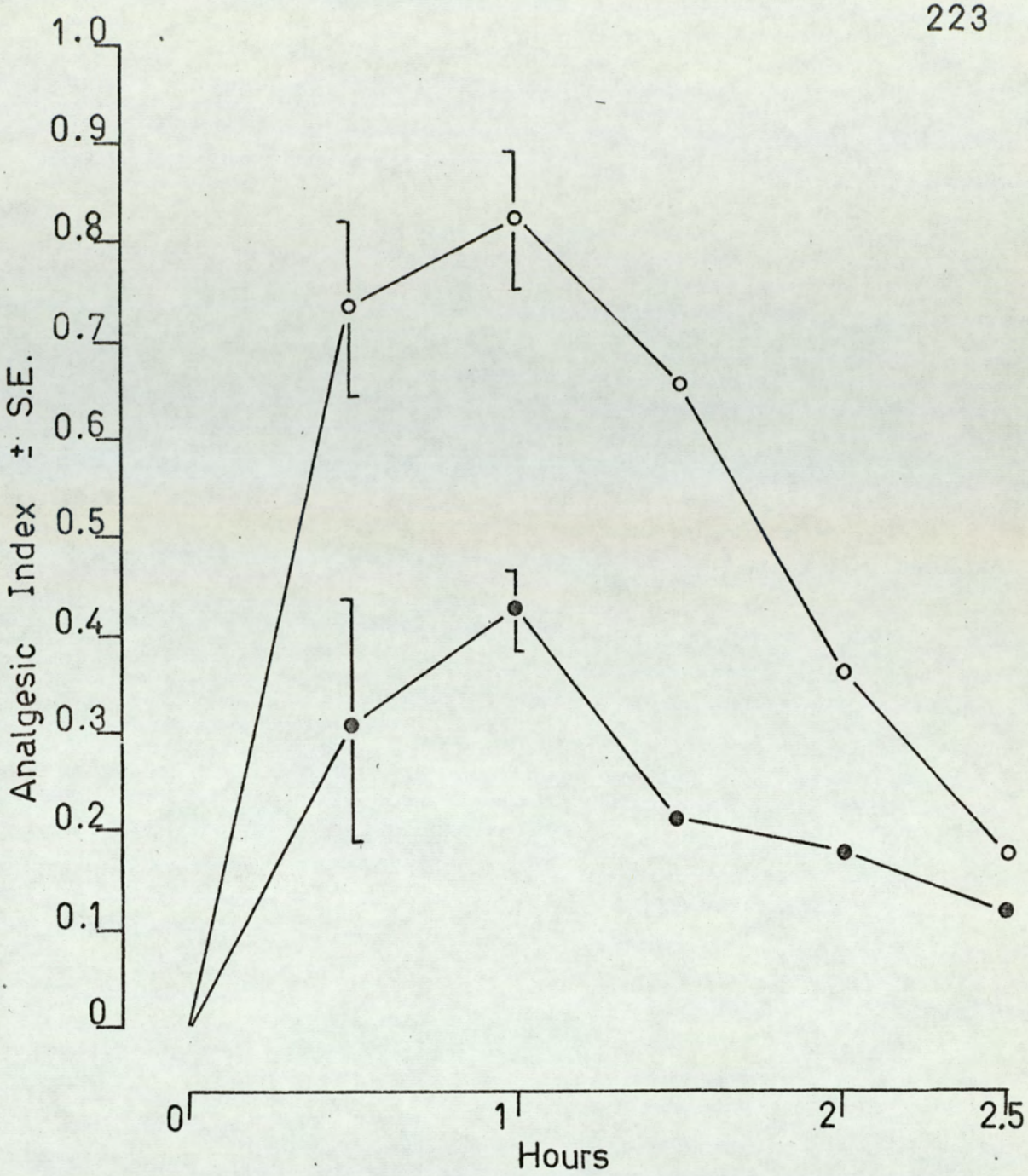

FIGURE 49 THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

2 groups of 5 animals were used. One group of animals was pretreated with hyoscine 1.0 mg/kg, i.p. at 30 minutes (—●—) and one with saline 1.0 ml/kg (—o—). At time 0 both groups received 6.0 mg/kg morphine s.c. Changes in the nociceptive threshold were recorded at the times indicated.
(see Table 37).

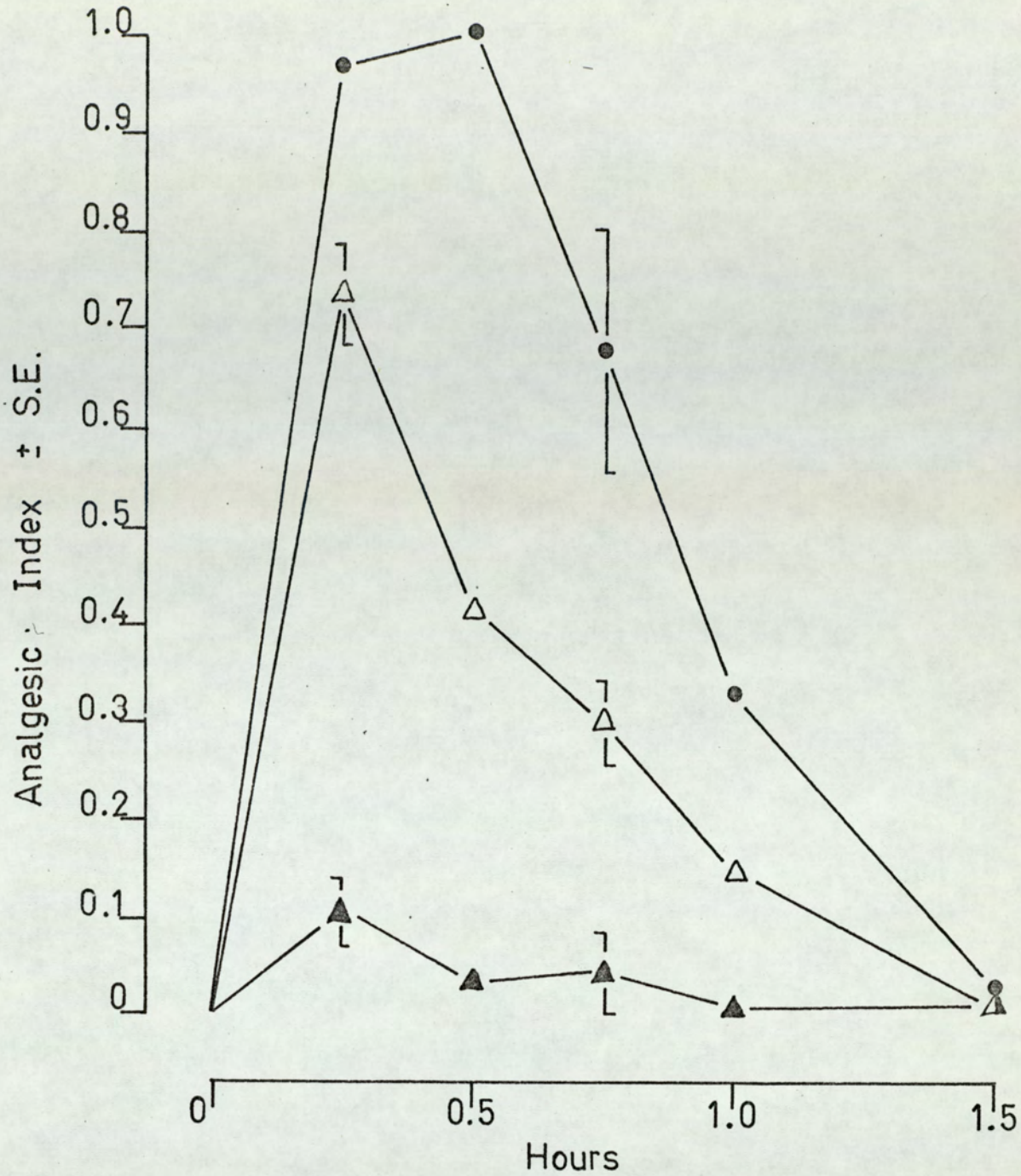

FIGURE 50 THE EFFECT OF INTRAVENTRICULAR CARBACHOL AND OF INTRAVENTRICULAR CARBACHOL PLUS 5-HT, ON THE NOCICEPTIVE THRESHOLD OF RESERPINISED ANIMALS

3 groups of 5 animals were used. 2 groups were pretreated with reserpine 5.0 mg/kg i.p. at 16 hours. At time 0 one of the reserpine groups received an intraventricular (i.c.) injection of 0.8 µg carbachol (—▲—), and the other received an i.c. injection of 0.8 µg carbachol mixed with 5.0 µg 5-HT (—●—). The third group (not reserpine pretreated) received 0.8 µg carbachol at time 0. Changes in the nociceptive threshold were recorded at the times indicated.
(see Table 38).

TABLE 32

THE EFFECT OF INTRAVENTRICULAR INFUSION OF ACh ON THE NOCICEPTIVE THRESHOLD OF RATS

(see Fig. 43).

	Analgesic index ± Standard Error						
Time (Hours)	0.5	1.0	1.5	2.0	3.0	3.25	3.5
ACh 0.5 µg/minute	0.324 ± 0.094	0.346 ± 0.062	0.372 ± 0.042	0.400 ± 0.051	0.412 ± 0.020	0.120 ± 0.021	0.110 ± 0.020
ACh 1.0 µg/minute	0.524 ± 0.121	0.621 ± 0.090	0.630 ± 0.024	0.624 ± 0.032	0.654 ± 0.046	0.096 ± 0.024	0.054 ± 0.046

TABLE 33

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF CARBACHOL ON THE NOCICEPTIVE THRESHOLD IN RATS

(see Fig. 44)

	Analgesic index ± Standard Error			
Time (Hours)	0.25	0.5	1.0	1.5
Carbachol 0.2 µg	0.216 ± 0.050	0.058 ± 0.040	- 0.045 ± 0.030	- 0.046 ± 0.030
Carbachol 0.4 µg	0.411 ± 0.120	0.253 ± 0.160	0.139 ± 0.097	- 0.019 ± 0.032
Carbachol 0.8 µg	0.819 ± 0.053	0.662 ± 0.160	0.173 ± 0.150	- 0.022 ± 0.053
Carbachol 1.6 µg	0.950 -	1.000 -	0.940 -	0.559 ± 0.190

TABLE 34

THE EFFECT OF INTRAVENTRICULAR INJECTIONS OF OTMN ON THE NOCICEPTIVE THRESHOLD OF RATS

(see Fig. 45)

	Analgesic index ± Standard Error			
Time (Hours)	0.25	0.5	1.0	1.5
Oxotremorine 2.0 µg	0.208 ± 0.100	0.045 ± 0.020	0.102 ± 0.089	0.009 ± 0.029
Oxotremorine 4.0 µg	0.370 ± 0.070	0.056 ± 0.032	- 0.051 ± 0.028	0.006 ± 0.039
Oxotremorine 8.0 µg	0.662 ± 0.150	0.419 ± 0.088	0.044 ± 0.027	0.019 ± 0.010
Oxotremorine 16.0 µg	0.941 ± 0.028	0.456 ± 0.070	0.226 ± 0.070	- 0.019 ± 0.055

TABLE 35

THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF CARBACHOL

(see Fig. 48)

	Analgesic index ± Standard Error				
Time (Hours)	0.5	0.75	1.0	1.25	1.5
Hyoscine 1.0 mg/kg i.p. Saline 10 µl i.c.	0.058 ± 0.04	0.075 ± 0.06	0.039 ± 0.057	0.059 ± 0.050	- 0.008 ± 0.062
Saline 1.0 ml/kg i.p. Carbachol 0.8 µg i.c.	- 0.023 ± 0.045	0.713 ± 0.070	0.379 ± 0.092	0.000 ± 0.064	- 0.072 ± 0.052
Hyoscine 1.0 mg/kg i.p. Carbachol 0.8 µg i.c.	- 0.040 ± 0.024	0.098 ± 0.042	0.003 ± 0.041	0.052 ± 0.50	0.003 ± 0.050

TABLE 36

THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF OTMN

	Analgesic index ± Standard Error				
Time (Hours)	0.5	0.75	1.0	1.25	1.5
Hyoscine 1.0 mg/kg i.p. Saline 10.0 µl i.c.	0.077 ± 0.042	0.028 ± 0.033	- 0.047 ± 0.040	0.105 ± 0.067	0.047 ± 0.045
Saline 1.0 mg/kg i.p. OTMN 8.0 µg i.c.	- 0.045 ± 0.053	0.950 -	0.686 ± 0.120	0.471 ± 0.160	- 0.025 ± 0.030
Hyoscine 1.0 mg/kg i.p. OTMN 8.0 µg i.c.	- 0.029 ± 0.031	0.153 ± 0.080	0.068 ± 0.036	0.066 ± 0.040	0.088 ± 0.035

TABLE 37

THE EFFECT OF HYOSCINE PRETREATMENT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

(see Fig. 49)

	Analgesic index ± Standard Error				
Time (Hours)	0.5	1.0	1.5	2.0	2.5
Saline 1.0 ml/kg i.p. Morphine 6.0 mg/kg s.c.	0.740 ± 0.94	0.836 ± 0.074	0.657 ± 0.015	0.354 ± 0.075	0.183 ± 0.077
Hyoscine 1.0 mg/kg i.p. Morphine 6.0 mg/kg s.c.	0.306 ± 0.130	0.427 ± 0.030	0.215 ± 0.060	0.185 ± 0.021	0.113 ± 0.034

TABLE 38

THE EFFECT OF INTRAVENTRICULAR CARBACHOL AND OF INTRAVENTRICULAR CARBACHOL PLUS 5-HT, ON THE NOCICEPTIVE THRESHOLD OF RESERPINISED ANIMALS

(see Fig. 50)

	Analgesic index ± Standard Error				
Time (Hours)	0.25	0.5	0.75	1.0	1.5
Carbachol 0.8 µg i.c. Saline 1.0 ml/kg i.p.	0.741 ± 0.049	0.415 ± 0.059	0.307 ± 0.045	0.157 ± 0.072	0.018 ± 0.079
Reserpine 5.0 mg/kg i.p. Carbachol 0.8 µg i.c.	0.107 ± 0.032	0.044 ± 0.032	0.049 ± 0.042	0.006 ± 0.019	0.016 ± 0.012
Reserpine 5.0 mg/kg i.p. (Carbachol 0.8 µg) (5-HT 5.0 µg) i.c.	0.963 -	1.000 -	0.688 ± 0.144	0.347 ± 0.170	0.021 ± 0.010

CHAPTER SIX

ACUTE TOLERANCE

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The demonstration that morphine causes a release of catecholamines from the brain stem (Vogt, 1954) has aroused considerable interest in their possible importance in the morphine abstinence syndrome. Maggiolo and Huidobro (1962) conducted a number of studies in mice and concluded that the intensity of the abstinence syndrome was not directly related to cencentrations of NA or 5-HT in nervous structures. However they did suggest that these amines are involved in some way in the production of the syndrome (Huidobro & Maggiolo, 1961; Maggiolo & Huidobro, 1962; Huidobro, Contreras & Croxatto, 1963). Repeated chronic dosing of rats with 200 mg/kg of morphine has been shown to increase NA levels in the brain stem (Klingman & Maynert, 1962), whilst an increase in the whole brain NA occurred in rats treated with either 30 mg/kg or 120 mg/kg of morphine for several weeks (Akera & Brody, 1968). However, morphine causes a reduction in the NA content of peripheral tissues (Klingman & Maynert, 1962; Sloan, Brooks, Eisenman & Martin, 1962). A reduction in the granular vesicle population that contains NA in rat arteriolar smooth muscle has also been induced by the production of morphine tolerance (Graham, Lever & Spriggs, 1969). In 1963 Paton suggested that the abstinence syndrome is due to the excessive release of ACh that has been dammed up in cholinergic axons by morphine-like drugs. Thus amines and ACh in both the central and peripheral nervous systems have been implicated in the abstinence syndrome. However few definite conclusions can be drawn from the literature because widely differing doses and species of animals have been used (Gunne, 1963; Vogt, 1964).

Cox, Ginsburg and Osman (1968) have demonstrated a method of inducing acute tolerance in rats by slow intravenous infusion of morphine. In this chapter we describe the effects of intraventricularly administered amines on tolerance induced in this way.

1. THE PRODUCTION OF ACUTE TOLERANCE IN RATS

Cox, Ginsburg and Osman (1968) reported the effects of infusions with 5.0, 7.5 and 10 mg/kg/hour of morphine, intravenously, into rats. They found that a dose of 7.5 - 10 mg/kg/hour produced a maximum anti-nociceptive effect (analgesic index of 1.0) at 2-3 hours after the start of infusion, and that the response had declined to an analgesic index of about 0.2, within 7-8 hours.

Our results are very similar, as can be seen in Fig. 51 (Table 39). An infusion of morphine at a rate of 5 mg/kg/hour (as base) produced maximum increase in the nociceptive threshold at around 3 hours, and the analgesic index had declined to values approaching those of the controls within 7 hours. As the anti-nociceptive effects declined, so did the behavioural depression associated with morphine. Saline infused intravenously at a rate of 1.0 ml/hour had no detectable effect on the nociceptive threshold.

2. THE EFFECT OF INTRAVENTRICULAR INFUSION OF NA ON ACUTE TOLERANCE TO MORPHINE

In this experiment, the mean for groups of only two animals are given: because of the smallness of the group no standard error is shown. The reason for the small group is that it was found difficult to supervise these double infusions on more than 4 animals (2 test, 2 control) at any one time.

The results are shown on Fig. 52 (Table 40). Animals were infused intravenously with 5 mg/kg/hour of morphine, and intraventricular infusions of either normal (physiological) saline (0.3 µl/minute) or NA (0.5 µg/minute) started one hour later. The effect of the NA was to inhibit the anti-nociceptive effect of morphine and it produced either a 'premature tolerance' or a prolonged antagonism of morphine. Both the elevation of the nociceptive threshold and the depression due to morphine were reduced by the NA, the NA causing a certain amount of hyperexcitability in the animals.

NA infused alone in doses of 0.25, 0.5 and 1.0 µg/minute caused excitement in the rats, but no elevation of the nociceptive threshold (see Section 3, Chapter 1).

3. EFFECT OF INTRAVENTRICULAR INFUSION OF 5-HT ON ACUTE TOLERANCE TO MORPHINE

From the results presented in Section 3, Chapter 3, it might have been expected that 5-HT would inhibit the tolerance, or at least postpone its appearance, when infused into the cerebral ventricles of the rat.

The effects of starting an intraventricular infusion of 5-HT (0.5 µg/minute), 1 hour after the start of an intravenous infusion of morphine (5 mg/kg/hour), are shown in Fig. 53 (Table 41). Again there were 2 animals per group. 5-HT had no significant influence on the effect of morphine. In other similar experiments a potentiation of the anti-nociceptive effect of morphine was sometimes seen at 1.5 and 2.0 hours.

4. THE EFFECT OF INTRAVENTRICULAR INFUSIONS OF 5-HT ON THE NOCICEPTIVE THRESHOLD

As reported in Section 3, Chapter 1, infusion of 0.25, 0.5 and 1.0 µg/minute of 5-HT elevated the nociceptive threshold in rats. In

Fig. 54 (Table 42) the effect of 0.5 μg/minute, infused into the ventricles over a period of 6 hours is shown. A significant increase in the nociceptive threshold was apparent within ½ hour, which increased to a maximum at 1 hour, and had faded by 4 hours.

Thus tolerance had been demonstrated to both intravenous morphine and intraventricular 5-HT, when they were infused over long periods. In the experiment described in paragraph 3, where both agents were infused simultaneously, tolerance to each of them would have appeared at about the same time. It was therefore decided to investigate the effect of injections of 5-HT into animals previously made tolerant to morphine.

5. EFFECT OF INTRAVENTRICULAR INJECTIONS OF 5-HT IN RATS PREVIOUSLY MADE TOLERANT TO MORPHINE

4 rats were infused intravenously with 5 mg/kg/hour of morphine until they were tolerant (7 hours). 8 hours after the start of this infusion, 5 μg of 5-HT was injected into the ventricles of 2 of these animals. The results are shown in Fig. 55 (Table 43). Injections of 5-HT into the tolerant animals caused a significant return of the anti-nociceptive effect. This response of 5-HT is not seen in animals not treated with morphine (see Section 3, Chapter 3). The intensity of the effect was variable in different experiments but there was always an elevation to give an analgesic index of 0.5 or more, in response to the 5.0 μg dose of 5-HT.

6. DISCUSSION

The acute tolerance to morphine infusions produced in the present experiments was very similar to that produced by Cox, Ginsburg and Osman (1968), although we used a slightly smaller dose of morphine to produce a maximal increase in the nociceptive threshold. In view of the

results obtained in Section 3, Chapter 3, the inhibition of the anti-nociceptive effects of morphine by infusion of NA into the ventricles was to be expected.

In view of the effects of infusion of NA, it was tempting to propose that a tolerance developed to morphine due to increased central levels of NA during the infusion. Since assays on the central NA levels were not carried out, we have been unable to correlate directly central NA levels with the observed decreasing effect of morphine, but various reports by other authors on the levels of NA after morphine have been published.

Urinary catecholamine metabolites increase in relation to the morphine dose in chronic treatment and during abstinence manifestations (Gunne, 1961; Akera & Brody, 1968). Maynert and Klingman, (1962) found catecholamine depletion to take place in the brains of rats at nearly convulsive doses of morphine. Smaller doses caused marked behavioural effects without detectable effect on brain NA levels in rats (Gunne, 1963). However prior to this Gunne (1959) had reported that brain catecholamine levels of rats decreased after withdrawal of morphine following chronic treatment. Later he decided that this was only seen in a specific strain of rats (Gunne, 1962). Acute morphine administration has been found to increase activity in ascending dopamine neurons in rats. This effect disappeared on chronic administration, whilst the withdrawal of morphine after chronic administration reduced the activity of brain dopamine neurons (Gunne, Jonsson & Fuxe, 1969). Nalorphine-induced abstinence caused increases in NA neuron activity, which the authors suggested may have been due to excitement. No change in NA neurons following withdrawal of morphine could be demonstrated though in this case the symptoms of withdrawal were less profound than

with nalorphine, and the peak effects scattered with respect to time. Further involvement of catecholamines in the abstinence syndrome has been shown by Takagi et al; 1968; 1969). Takagi and Nakama (1968) reported that tetrabenazine reduced brain levels of both NA and dopamine and that DOPA administration restored dopamine levels and partly restored NA levels. Takagi and Kuriki (1969) then found that tetrabenazine given daily to mice, before a daily morphine injection, reduced the development of tolerance to morphine. Further this suppressive effect could be reduced by repeated administration of DOPA. Thus the evidence of these authors suggest that a reduction in central levels of dopamine and NA inhibits the development of tolerance. (c.f. our finding that increased brain NA levels create a situation similar to tolerance).

Central 5-HT has also been implicated in the development of tolerance (Shen, Loh & Leong Way, 1968). It was found that an accelerated rate of 5-HT synthesis accompanied the development of tolerance to, and physical dependence on, morphine. It was also observed that inhibition of 5-HT synthesis with p-CPA, prior to morphine administration, greatly reduced the development of tolerance to morphine. Loh, Shen and Leong Way (1969) demonstrated inhbition of morphine tolerance using the protein synthesis inhibitor cyclohexamide in chronically treated mice. Similarly, Cox, Ginsburg and Osman (1968) inhibited acute morphine tolerance using actinomycin D in rats infused intravenously with morphine (though they could not inhibit 'established tolerance' in animals that had been chronically treated with morphine). Loh et al (1969) reported that the administration of cyclohexamide also blocked the increase in 5-HT synthesis that they had previously reported to accompany the development of morphine tolerance (Shen, Loh & Leong Way, 1968). This and their results with p-CPA induced them to suggest that increased synthesis of 5-HT may play an important part in

tolerance and physical dependence mechanisms. Our results also suggest that 5-HT is involved in the production of tolerance. Again it is tempting to speculate on the role of 5-HT in morphine tolerance. From these results it would seem possible that increased levels of 5-HT were necessary for the production of the anti-nociceptive effect of morphine. As tolerance develops to the effects of 5-HT the rate of synthesis increases accordingly. Finally the increase in turnover is overcome by the tolerance and the anti-nociceptive effect of morphine diminishes. Alternatively the anti-nociceptive effect of morphine may be mediated via increased turnover or increased brain levels of 5-HT; tolerance to the 5-HT may then appear as seen in the intraventricular infusion.

However cholinergic systems have also been implicated in the development of tolerance (Paton, 1963). We did not investigate the possibility of ACh's involvement and more work is needed in this direction.

It seems most likely however that all 3 systems (cholinergic, adrenergic and tryptaminergic) are involved in the production of tolerance, although with the information available at present it is difficult to see how they may be inter-related. It is equally possible that none of these systems are involved and that the phenomenon of tolerance is caused by other factors such as silent receptors or drug-induced changes in the numbers of receptors, as postulated by Collier (1965), and the effects observed in this work are secondary to this tolerance.

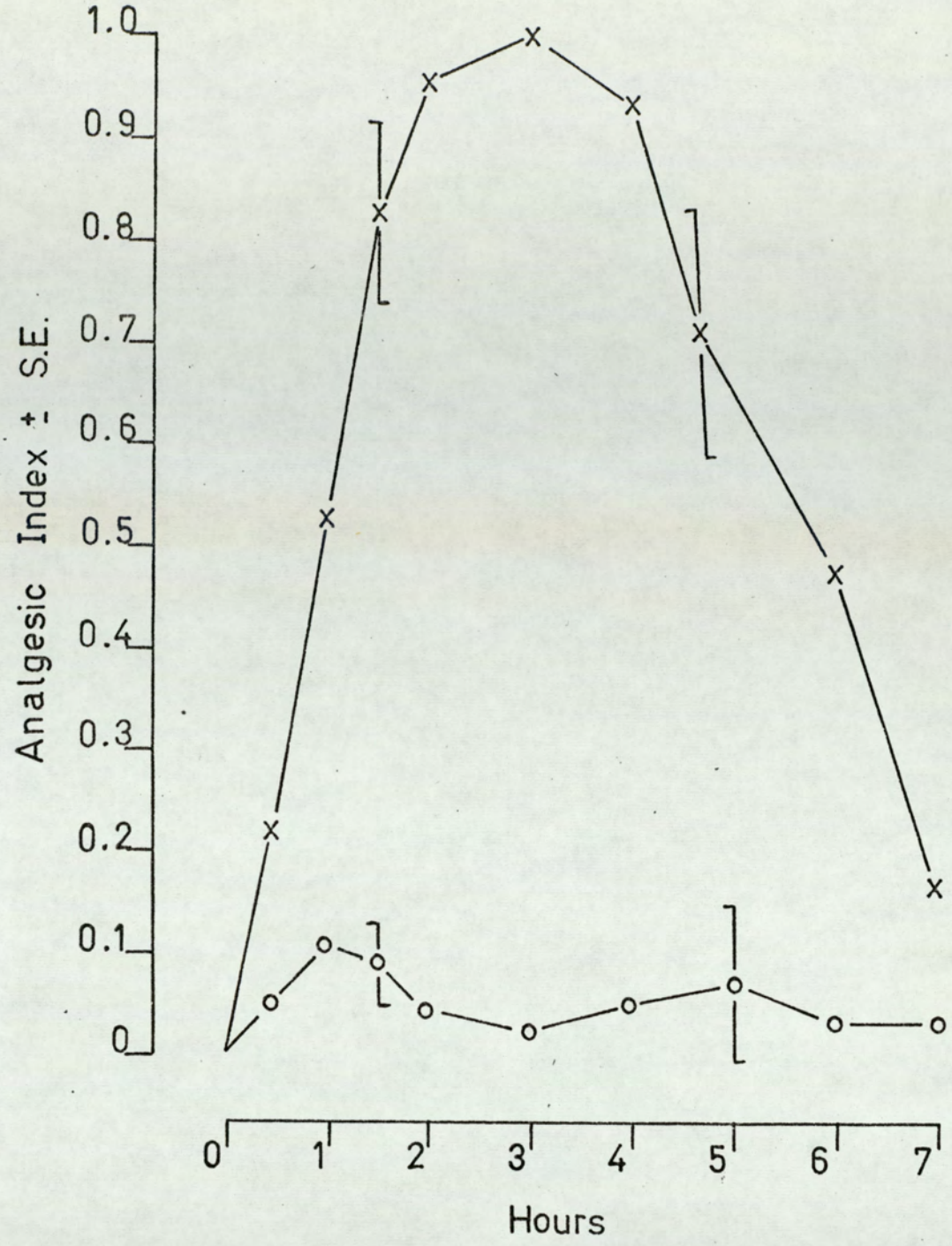

FIGURE 51 THE PRODUCTION OF ACUTE TOLERANCE TO MORPHINE

2 groups of 5 animals were used. One group received an i.v. infusion of 1.0 ml/hour saline (—o—), and the other group received an i.v. infusion of 5.0 mg/kg/hour morphine (—X—). Infusions were maintained for 7 hours, and were not given simultaneously. Changes in the nociceptive threshold were recorded at the times indicated.
(see Table 39).

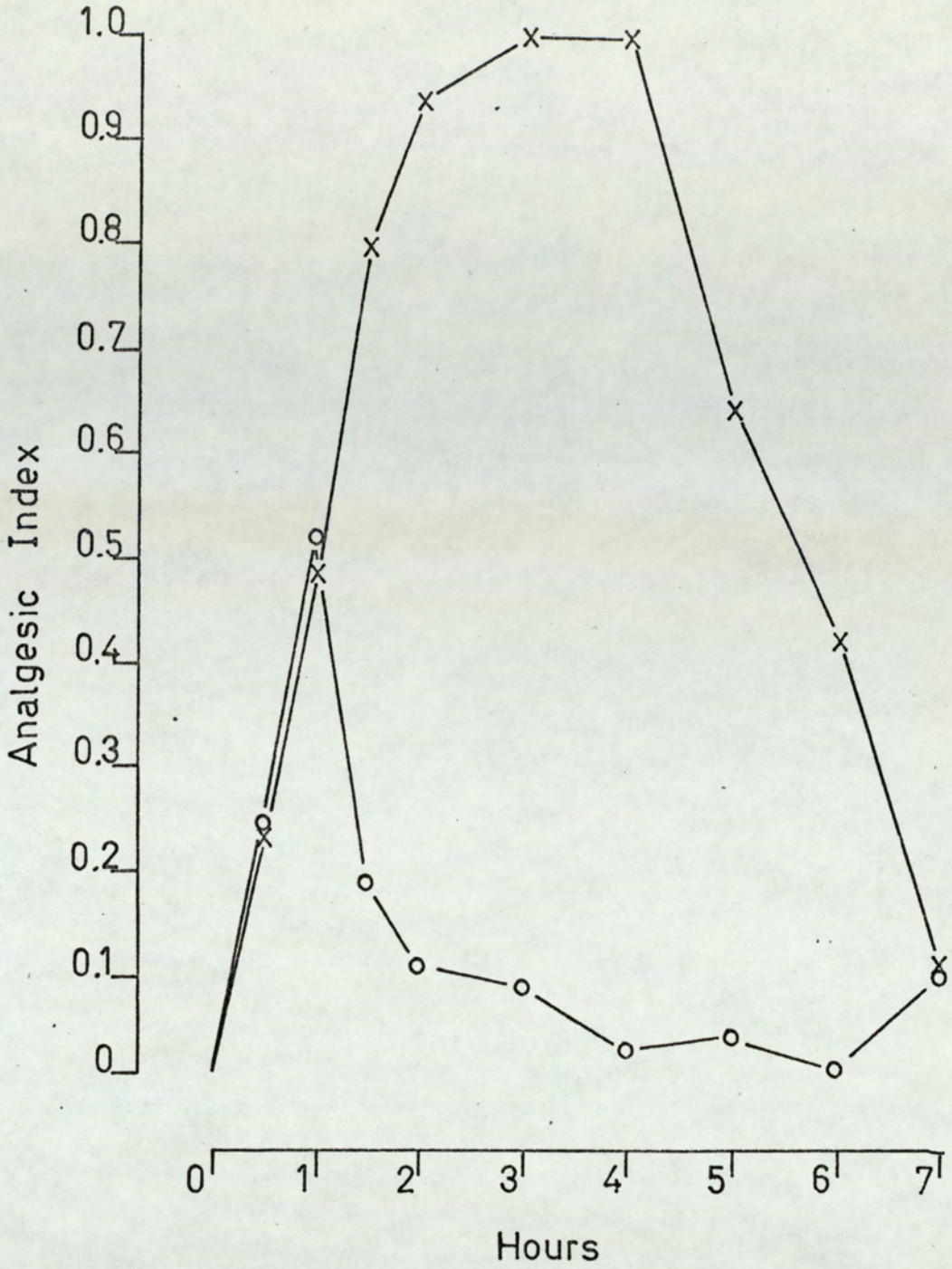

FIGURE 52 EFFECT OF INTRAVENTRICULAR INFUSIONS OF NA ON ACUTE TOLERANCE TO MORPHINE

2 groups of 2 animals were used. Both groups received i.c. infusions of 5.0 mg/kg morphine, maintained for 7 hours. One hour after the start of the i.v. infusion, intraventricular infusion of 0.5 µg/min NA was started in one group (—o—) and of 0.3 µl/min saline started in the other group (—X—). These intraventricular infusions were maintained for 6 hours. Changes in the nociceptive threshold were recorded at the time indicated. (see Table 40).

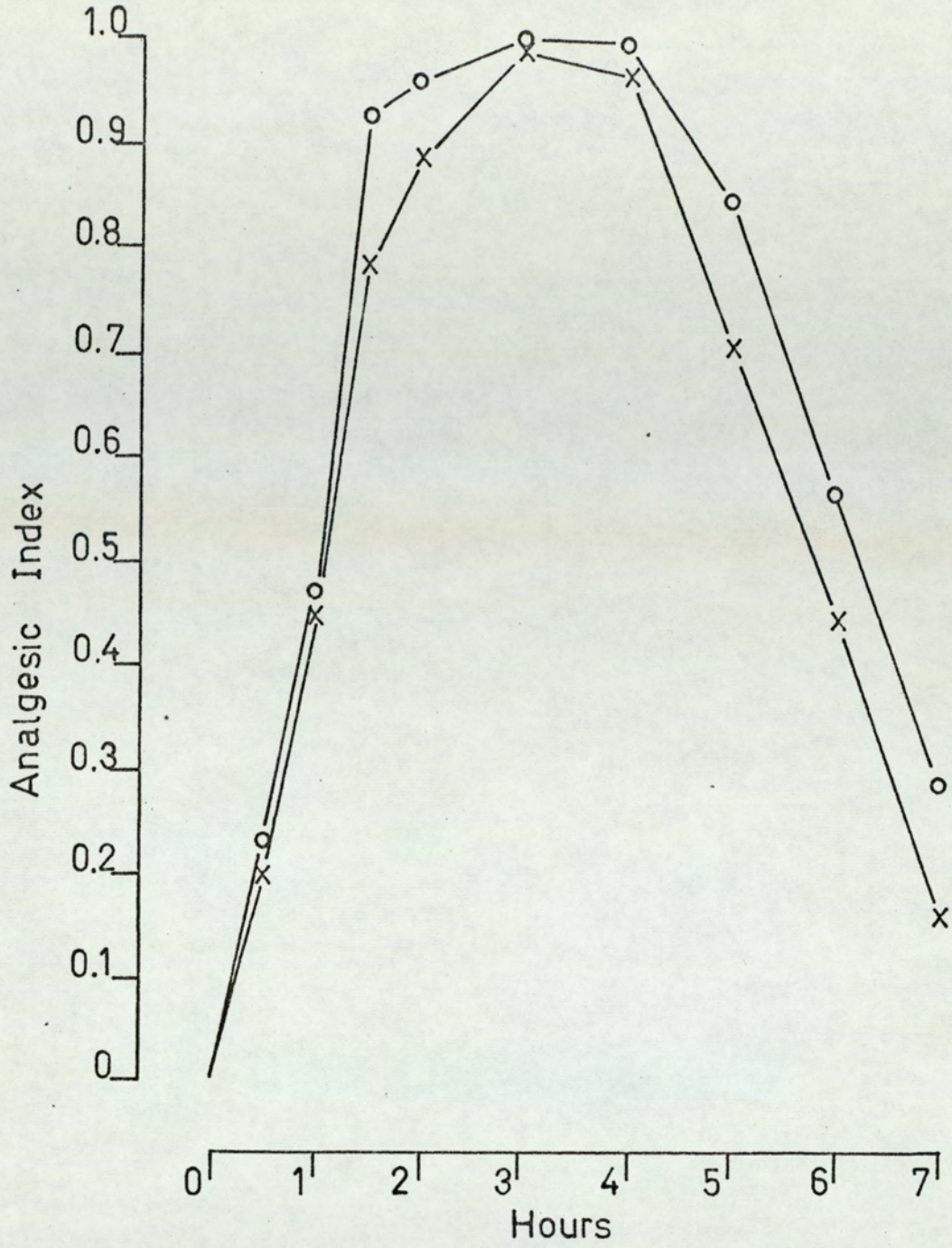

FIGURE 53 EFFECT OF INTRAVENTRICULAR INFUSIONS OF 5-HT ON ACUTE TOLERANCE TO MORPHINE

2 groups of 2 animals were used. Both groups received i.v. infusions of 5.0 mg/kg morphine, maintained for 7 hours. One hour after the start of the i.v. infusion, intraventricular infusion of 0.5 µg/minute 5-HT was started in one group (—o—) and of 0.3 µl/minute saline started in the other group (—x—). These intraventricular infusions were maintained for 6 hours. Changes in the nociceptive threshold were recorded at the times indicated.
(see Table 41).

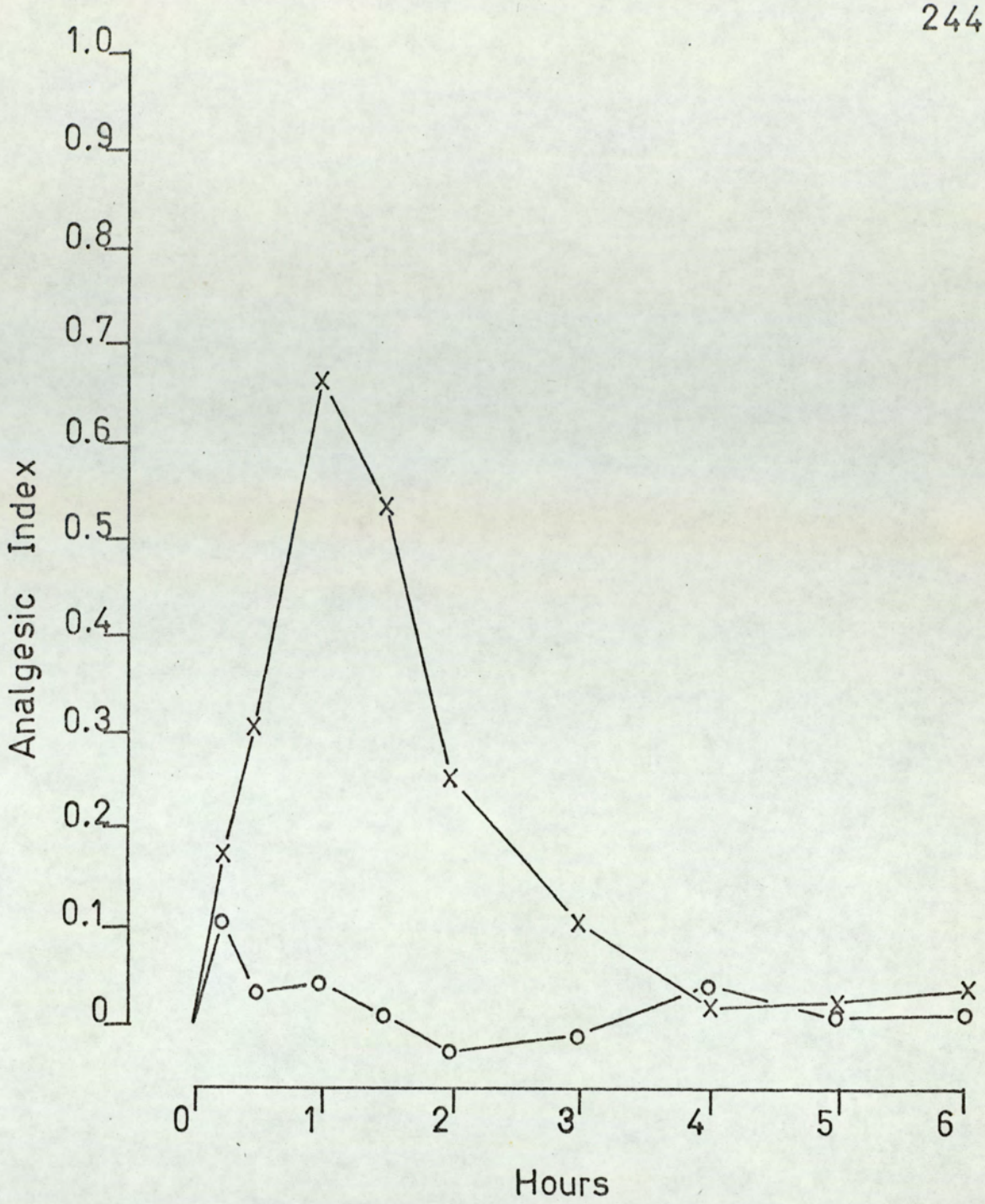

FIGURE 54 EFFECT ON THE NOCICEPTIVE THRESHOLD OF INTRAVENTRICULAR INFUSIONS OF 5-HT

2 groups of 2 animals were used. One group received an intraventricular infusion of saline 3.0 µl/min saline (—o—) and the other group received an infusion of 0.5 µg/minute 5-HT (—X—). Changes in the nociceptive threshold were measured at the times shown. (see Table 42).

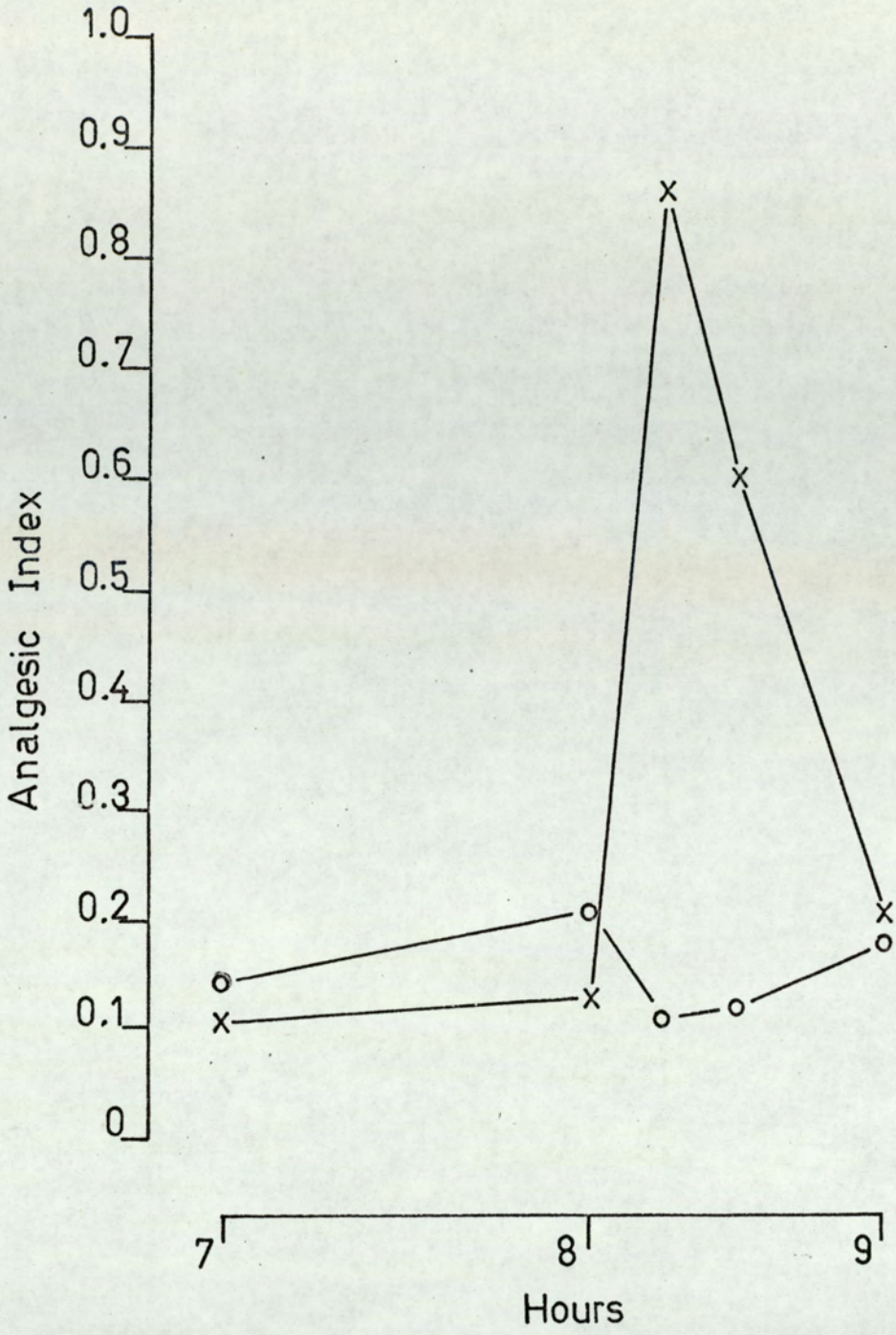

FIGURE 55 EFFECT OF INTRAVENTRICULAR INJECTION OF 5-HT INTO RATS PREVIOUSLY MADE TOLERANT TO MORPHINE

2 groups of 2 animals were used. Both groups were infused i.v. with 5.0 mg/kg morphine for 7 hours (which made the animals tolerant). At the 8th hour after the start of infusion, 5.0 µg of 5-HT was injected into the cerebral ventricles of one group (—X—) and 10 µl saline injected into the cerebral ventricles of the other (—O—). Changes in the nociceptive threshold were recorded at the times shown.
(see Table 43).

TABLE 39

THE PRODUCTION OF ACUTE TOLERANCE TO MORPHINE

(see Fig. 51)

	Analgesic index ± Standard Error								
Time (Hours)	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
Saline infusion	0.056 ± 0.095	0.112 ± 0.052	0.093 ± 0.042	0.052 ± 0.050	0.041 ± 0.061	0.072 ± 0.094	0.084 ± 0.071	0.041 ± 0.082	0.042 ± 0.071
Morphine 5 mg/kg/hour	0.266 ± 0.087	0.537 ± 0.159	0.832 ± 0.095	0.956 -	0.1000 -	0.947 -	0.711 ± 0.127	0.488 ± 0.127	0.168 ± 0.097

TABLE 40

EFFECT OF INTRAVENTRICULAR INFUSIONS OF NA ON ACUTE TOLERANCE TO MORPHINE

(see Fig. 52)

	Analgesic index								
Time (Hours	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
Morphine 5 mg/kg/hour Saline 3 µl/minute	0.232	0.496	0.084	0.942	0.1000	0.1000	0.649	0.432	0.145
Morphine 5 mg/kg/hour NA 0.5 µg/minute	0.241	0.522	0.182	0.110	0.092	0.041	0.062	0.014	0.011

TABLE 41

EFFECT OF INTRAVENTRICULAR INFUSIONS OF 5-HT ON ACUTE TOLERANCE TO MORPHINE

(see Fig. 53)

	Analgesic index								
Time (Hours	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
Morphine 5 mg/kg/hour Saline 3 µl/hour	0.201	0.445	0.792	0.892	0.994	0.972	0.702	0.451	0.162
Morphine 5 mg/kg/hour 5-HT 0.5 µg/minute	0.242	0.461	0.942	0.981	0.1000	0.1000	0.854	0.561	0.284

TABLE 42

EFFECT ON THE NOCICEPTIVE THRESHOLD OF INTRAVENTRICULAR INFUSIONS OF 5-HT

(see Fig. 54)

	Analgesic index								
Time (Hours)	0.25	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
5-HT 0.5 µg/minute	0.167 ± 0.046	0.301 ± 0.021	0.679 ± 0.091	0.542 ± 0.084	0.261 ± 0.042	0.102 ± 0.061	0.022 ± 0.041	0.034 ± 0.022	0.042 ± 0.026
Saline 3 µl/minute	0.102 ± 0.021	0.045 ± 0.042	0.056 ± 0.021	0.011 ± 0.046	- 0.041 ± 0.039	- 0.022 ± 0.028	0.041 ± 0.036	0.021 ± 0.041	0.016 ± 0.044

TABLE 43

EFFECT ON INTRAVENTRICULAR INJECTION OF 5-HT INTO RATS PREVIOUSLY MADE TOLERANT TO MORPHINE

(see Fig. 55)

	Analgesic index				
Time (Hours)	7.0	8.0	8.25	8.5	9.0
Morphine 5.0 mg/kg/hour i.v. Saline 10 µl i.c.c.	0.102	0.146	0.864	0.601	0.212
Morphine 5.0 mg/kg/hour i.v. 5-HT 5.0 µg i.c.c.	0.146	0.214	0.102	0.122	0.168

CHAPTER SEVEN

INTERACTIONS OF INTRAVENTRICULAR NA AND 5-HT WITH A NON-NARCOTIC ANALGESIC

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INTERACTIONS OF INTRAVENTRICULAR NA AND 5-HT WITH A NON-NARCOTIC ANALGESIC

In previous chapters only narcotic agents have been discussed. However there are other classes of compounds that have analgesic properties. The anti-inflammatory agents such as aspirin have their primary action at the site of initiation of the nociceptive impulse, though part of their anti-nociceptive and anti-pyretic action may spread into the synapses of the spinal cord, mid-brain and thalamic areas. The muscle relaxant type of anti-nociceptive agent such as carisoprodol blocks pain impulses primarily in the spinal cord and mid-brain areas, and the tranquillizers block at higher synaptic sites - the reticular formation, mid-brain and thalamic areas (Randall, 1963).

This chapter reports the effects of acetylsalicylic acid (aspirin) on the elevation of the nociceptive threshold of the inflamed rat's paw, and also reports the effects of intraventricular NA and 5-HT on this elevation.

1. PRODUCTION OF INFLAMMATION IN THE RAT PAW

Inflammation was produced according to the method of Randall and Selitto (1957). A 7% w/v suspension of dried yeast in water (equivalent to a 20% solution of baker's yeast) was prepared immediately before use. The nociceptive threshold of the rats was recorded. They were then lightly anaesthetised with a gaseous mixture of oxygen, nitrous oxide and halothane and 0.1 ml of the yeast suspension injected under the skin on the plantar surface of one hind foot. This significantly increased the sensitivity of the paws to pressure. Fig. 56

(Table 44) shows a comparison of the analgesic indices for the yeast injected foot and the contralateral untreated foot in a group of 5 rats. Maximum sensitivity was reached 2 hours after the yeast injection. The sensitivity then slowly returned to normal and within 24 hours there was no significant difference between the response of the two feet to the nociceptive stimulus.

2. THE EFFECT OF ASPIRIN ON THE NOCICEPTIVE RESPONSE TO THE INFLAMED FOOT OF THE CONSCIOUS RAT

The effect of aspirin was tested 2 hours after the injection of the yeast and 1 hour after the administration of aspirin. The nociceptive threshold of 4 groups of 5 rats was recorded. The animals were then lightly anaesthetised as previously described, and 0.1 ml of a 7% suspension of dried yeast injected into the plantar surface of one hind foot. The animals were then allowed to recover and suspensions of aspirin were prepared, using a small quantity of gum acacia. An hour after the yeast treatment the appropriate dose of aspirin was injected subcutaneously. One hour later the nociceptive thresholds were again determined. The results are shown in Fig. 57 (Table 45). The yeast increased the sensitivity of the foot to pressure. Aspirin in doses of 25, 50 and 100 mg/kg elevated the nociceptive threshold in the inflamed foot but had no detectable effect on the untreated foot. It should be noted that the aspirin not only reduced the increased sensitivity to nociceptive stimuli caused by yeast, but it also increased the nociceptive threshold above that in the untreated foot.

3. THE EFFECT OF INTRAVENTRICULAR NA AND 5-HT ON THE ELEVATION OF PAIN THRESHOLD PRODUCED BY ASPIRIN IN THE INFLAMED FOOT

The experiment described in paragraph 2 was repeated using a dose of 50 mg/kg aspirin, with the exception that 1 hour 45 minutes after the

injection of yeast into the foot (45 minutes after subcutaneous injection of the aspirin), either 5 μg 5-HT or 20 μg NA was injected into the cerebral ventricles. The results are presented in Fig. 58 (Table 46). 50 mg/kg of aspirin produced a significant elevation ($p < 0.005$) of the nociceptive threshold in the inflamed foot. Intraventricular injection of 5-HT had no significant effect on the increased nociceptive threshold produced by aspirin. However intraventricular NA (20 μg) caused a significant ($0.05 > p > 0.01$) decrease in aspirin's effect.

From Fig. 58 (Table 46) it can be seen that neither amine had any significant effect on the inflamed foot of animals treated with saline instead of aspirin.

4. DISCUSSION

Randall and Selitto (1957) found that the nociceptive threshold in the rat foot decreased to a basal level 1 hour after the injection of yeast, and did not return to normal for 48 hours. The difference between this and the results reported here may be due to differences in the type of yeast used. Randall and Selitto used a 20% suspension of brewer's yeast and we used dried baker's yeast that was made up to produce a suspension equivalent to a 20% suspension of normal baker's yeast. However a satisfactory inflammation was produced by the dried yeast, and this caused an increase in the sensitivity of the rat foot to the nociceptive stimulus. Randall and Selitto (1957) found that sodium salicylate increased the nociceptive threshold of the inflamed foot without having any significant effect on the uninflamed foot and the results presented here are in good agreement with this. Randall (1963) demonstrated that the decreased threshold of the inflamed foot to nociceptive stimuli was accompanied by oedema and an increase in the

temperature of the foot. Sodium salicylate reduced both of these effects at the same time that it reduced the nociceptive threshold. It is likely that these anti-pyretic and anti-oedema effects play a major role in the anti-nociceptive effects of aspirin in this particular experimental situation.

Injection of substances into the cerebral ventricles therefore was not expected to have any major effect on the anti-nociceptive effect of aspirin. This was found to be the case with 5-HT which did not significantly alter the nociceptive threshold in the presence or absence of aspirin. However 20 µg of NA was found to produce a significant inhibition of the anti-nociceptive effects of aspirin. It is unlikely that this could have been due to leakage into the peripheral circulation, for adrenergic compounds (e.g. amphetamine) have been found in contrast to be anti-nociceptive when administered peripherally to rats with inflamed feet (Randall, 1963) - possibly due to their vasoconstrictive properties. NA injected intraventricularly in the absence of aspirin did not significantly increase or decrease the sensitivity of the inflamed foot (as shown in previous chapters, this is also the case in the normal foot). However intraventricular NA does give the animals the appearance of being more sensitive to mild noxious stimuli (see Section 3, Chapter 1), though any direct increase in sensitivity was not picked up by this test when NA alone was injected in the absence of aspirin.

Adrenergic mechanisms have been implicated in the mechanism of action of anti-pyretic analgesics by other authors. As previously reported (Randall, 1963) found that various adrenergic compounds administered peripherally had anti-nociceptive effects in rats with inflamed feet. Contreras, Tamayo, Pincheira and Quijada (1969) found that peripherally administered adrenergic blocking agents such as dibenamine, phenoxybenzamine and tolazoline antagonised the anti-nociceptive effect of sodium salicylate and mefenamic acid in a test involving electrical

stimulation of rats. They also found that amine-depleting agents, such as reserpine, given peripherally antagonised the effects of sodium salicylate and mefenamic acid. Treatment with nialamide (a monoamine oxidase inhibitor) increased the effect of mefenamic acid but decreased the effect of sodium salicylate. From their results they concluded that there may well be an adrenergic component involved in the anti-nociceptive action of non-narcotic drugs, but they did not suggest whether they considered this to be central or peripheral.

Since intraventricular NA inhibited the anti-nociceptive effects of both morphine and aspirin, two agents unrelated both chemically and in their mechanism of action, it is possible that the mechanism of action of NA is to increase the sensitivity of central receptor mechanisms rather than block the activity of the analgesic drugs directly. On the other hand, although anti-inflammatory mechanisms seem to be the major mechanism of aspirin's action, it was noted both here and by Randall and Selitto (1957) that salicylates not only abolish the increased sensitivity to nociceptive stimuli brought about by inflammation, but they also increase the threshold above that seen in the non-inflamed foot. This indicates that effects upon inflammation may not be the complete explanation for the anti-nociceptive effects of the salicylates, and it is possible that there is a central mechanism common to these two anti-nociceptive agents upon which NA may exert its effects. If NA mediates its effect in this system by increasing the sensitivity of central receptor mechanisms (rather than directly antagonising the effects of morphine), then the suggestions put forward in previous chapters would need considerable revision, especially those in the previous chapter concerning tolerance. However before any major conclusions based on the work of the present chapter can be made,

further work is necessary. So far, the increased sensitivity to nociceptive stimuli following intraventricular NA has only been observed and not recorded, since the test used here was not capable of detecting it quantitatively. If other tests (such as the tail-flick in response to a heat stimulus) were to be used, results may be obtained to clarify the picture.

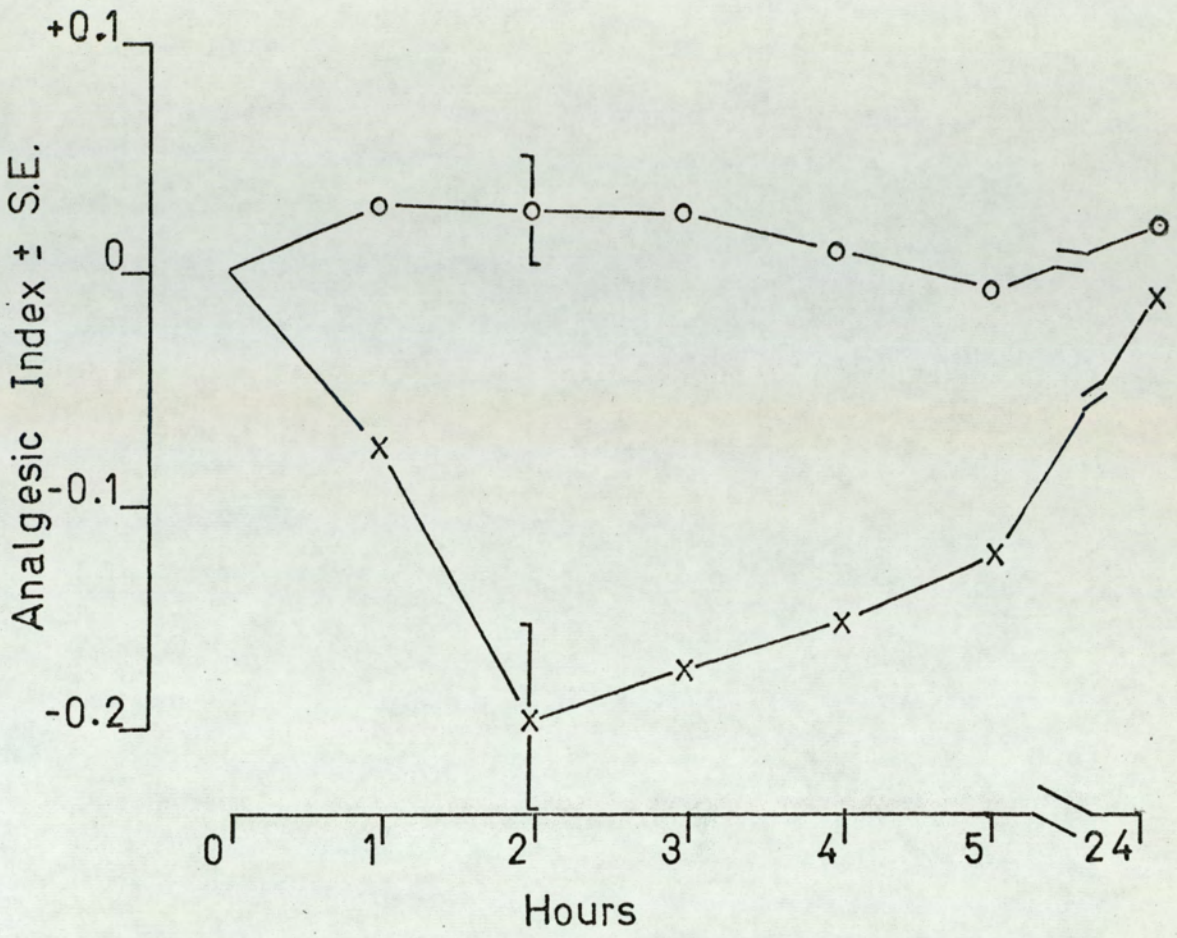

FIGURE 56 THE EFFECT OF INFLAMMATION ON THE NOCICEPTIVE SENSITIVITY OF THE RAT PAW

2 groups of 5 rats were used. One group was lightly anaesthetised and 0.1 ml of a 7% suspension of dried yeast in water injected under the skin on the plantar surface of one hind foot (—X—). The other group was anaesthetised but no injections were made (—o—). The nociceptive thresholds were recorded at the times indicated.
(see Table 44).

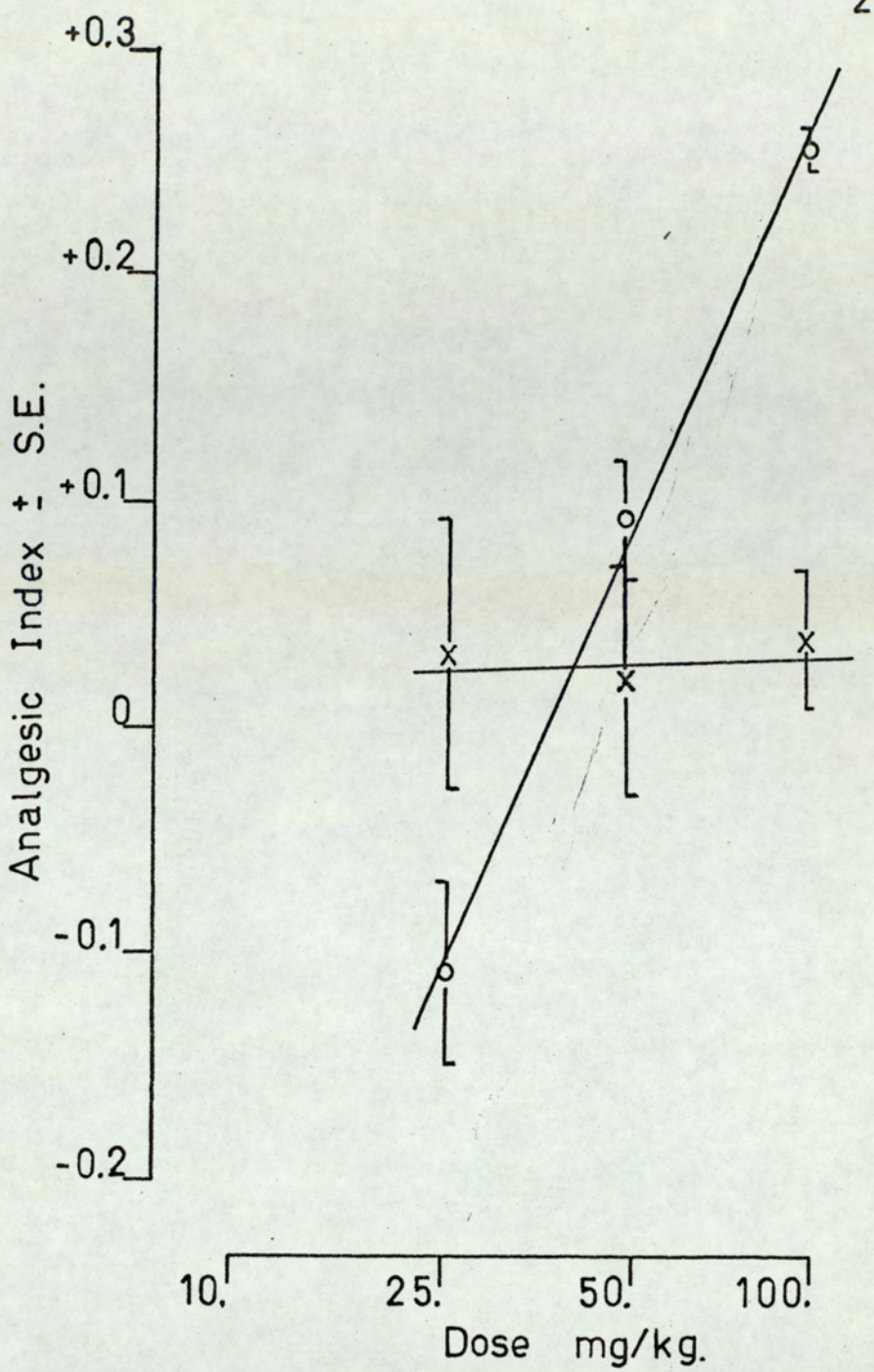

FIGURE 57 THE EFFECT OF ASPIRIN ON THE NOCICEPTIVE RESPONSE OF THE INFLAMED FOOT OF THE RAT

4 groups of 5 rats were used. The nociceptive threshold of both hind feet of the animals was recorded. Animals were then lightly anaesthetised and 0.1 ml of a 7% suspension of dried yeast in was was injected under the skin on the plantar surface of one hind foot. One hour later one group received 5.0 ml/kg saline s.c. and the other 3 groups received either 25, 50, or 100 mg/kg aspirin s.c. One hour after the aspirin the nociceptive threshold of both the inflamed (—o—) and the non-inflamed (—x—) hind feet was recorded. (see Table 45).

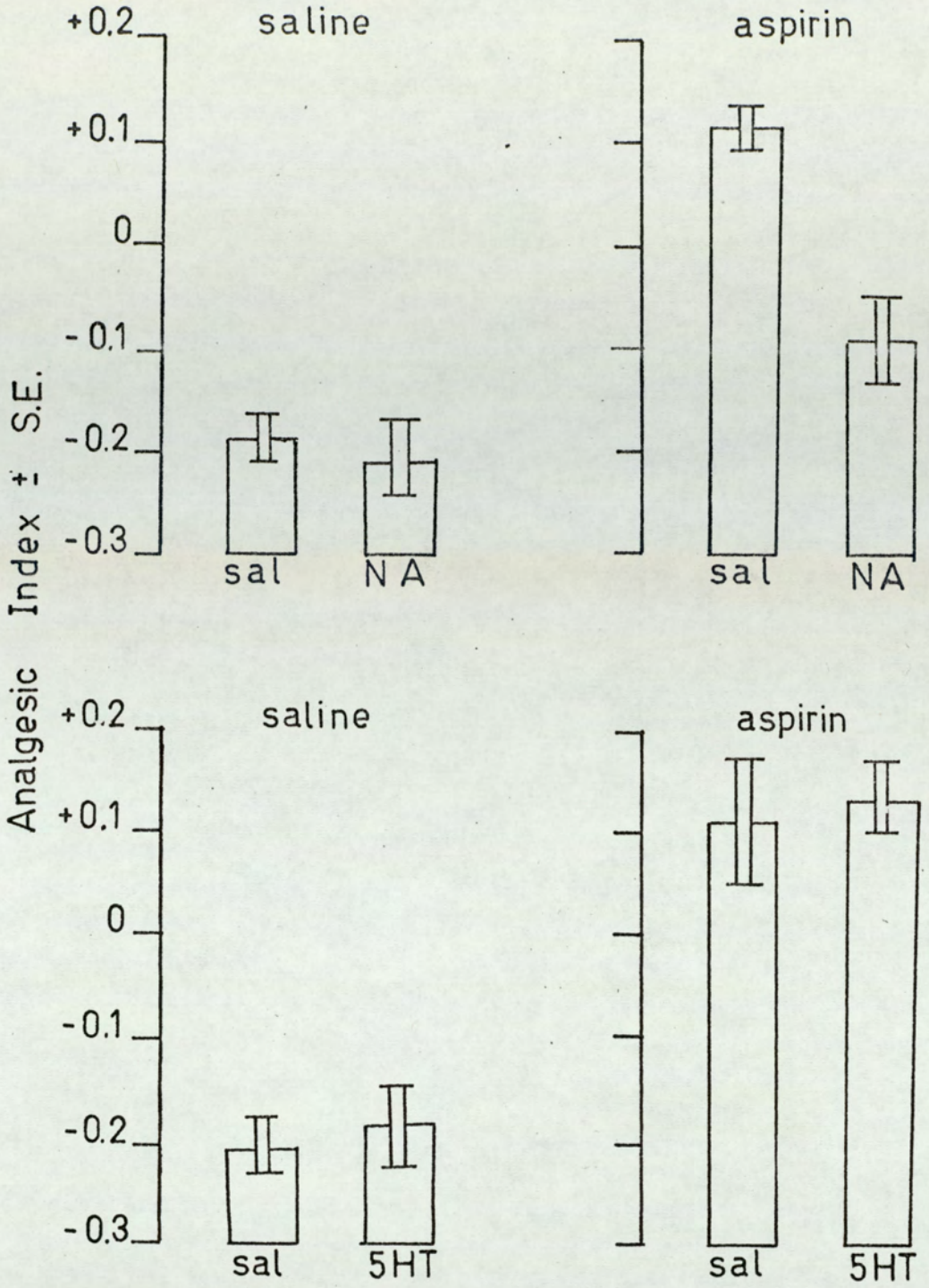

FIGURE 58 THE EFFECTS OF INTRAVENTRICULARLY ADMINISTERED NA AND 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF ASPIRIN ON THE INFLAMED RAT PAW

8 groups of 5 rats were used. 2 ml/kg saline or 50 mg/kg aspirin were injected subcutaneously 1 hour after a yeast suspension had been injected into the rats' paws. 45 minutes after the aspirin injection either NA or 5-HT was administered intraventricularly and the nociceptive threshold determined 15 minutes later. The significance levels between groups are given in Table 46.

TABLE 44

THE EFFECT OF INFLAMMATION ON THE NOCICEPTIVE SENSITIVITY OF THE RAT PAW

(see Fig. 56)

	Analgesic index ± Standard Error					
Time (Hours)	1.0	2.0	3.0	4.0	5.0	24
Inflamed foot	- 0.077 ± 0.119	- 0.192 ± 0.045	- 0.170 ± 0.040	- 0.153 ± 0.042	- 0.119 ± 0.044	0.004 ± 0.042
Untreated foot	0.034 ± 0.007	0.031 ± 0.019	0.031 ± 0.019	0.010 ± 0.028	- 0.006 ± 0.028	0.017 ± 0.031

TABLE 45

THE EFFECT OF ASPIRIN ON THE NOCICEPTIVE RESPONSE OF THE INFLAMED FOOT OF THE RAT

(see Fig. 57)

	Analgesic index ± Standard Error	
	Inflamed foot	Untreated foot
Saline 2 ml/kg s.c.	- 0.192 ± 0.045	0.031 ± 0.019
Aspirin 25 mg/kg s.c.	- 0.108 ± 0.040	0.037 ± 0.063
Aspirin 50 mg/kg s.c.	0.092 ± 0.024	0.024 ± 0.051
Aspirin 100 mg/kg s.c.	0.251 ± 0.004	0.049 ± 0.034

TABLE 46

THE EFFECTS OF INTRAVENTRICULARLY ADMINISTERED NA AND 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF ASPIRIN ON THE INFLAMED RAT PAW

The levels of significance between groups are:-

a; $0.005 > P^*$ c; $0.8 > P > 0.6$

b; $0.7 > P > 0.6$ d; $0.05 > P > 0.01^*$

* = Difference significant

(see Fig. 58)

Treatment	Analgesic index ± Standard Error	
Saline 2.0 ml/kg s.c. Saline 10 µl i.c.	- 0.201 ± 0.032	a c
Saline 2.0 ml/kg s.c. 5-HT 5 µg i.c.	- 0.186 ± 0.040	c
Aspirin 50 mg/kg s.c. Saline 10 µl i.c.	0.102 ± 0.061	a b
Aspirin 50 mg/kg s.c. 5-HT 5 µg i.c.	0.136 ± 0.041	b

Treatment	Analgesic index ± Standard Error	
Saline 2.0 ml/kg s.c. Saline 10 µl i.c.	- 0.187 ± 0.021	a c
Saline 2.0 ml/kg s.c. NA 20 µg i.c.	- 0.212 ± 0.042	c
Aspirin 50 g/kg s.c. Saline 10 µl i.c.	0.116 ± 0.046	a d
Saline 2.0 ml/kg s.c. NA 20 µg i.c.	- 0.097 ± 0.061	d

CHAPTER EIGHT

PLACEMENT OF AGENTS IN DISCRETE AREAS OF THE BRAIN

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PLACEMENT OF AGENTS IN DISCRETE AREAS OF THE BRAIN

The exact site and mode of action of morphine still remains poorly understood. In previous chapters interactions between peripherally administered morphine and intraventricularly administered amines has been described. However apart from localising an agent to the brain, intraventricular administration of a compound gives little indication of its site of action within the brain since an injected agent may spread rapidly from its site of injection throughout the ventricular system (see Section 2, Fig. 6). Histamine or bromophenol blue infused into the ventricles of cats has been shown to penetrate 2-2.5 mm into the tissues surrounding the ventricles (Feldberg, 1963); sodium fluorescein perfused into the cerebral ventricles of the rat is taken up in different amounts by different areas of the hypothalamus (Scremin, 1967). Such studies demonstrate the impossibility of predicting the exact site of action of an agent administered by simple intraventricular injection.

This chapter reports an attempt to localise the site at which previously observed interactions with morphine were taking place.

Cannulae designed for the implantation of substances into specific brain areas were fitted to 20 rats. The co-ordinates were according to the de Groot system (1959). One tamp of the crystalline material was given on each occasion, producing a dose of compound of approximately 50 μg. The amount of drug taken into the cannula was not measured on each occasion, but in preliminary experiments (using a balance accurate up to $\pm$ 1.0 μg) it was found that between 40 and 70 μg of drug was taken up on one tamping.

Since the concentration of the chemical at the tip of the cannula was always maximal, regardless of dosage, variations of the quantity inside the cannula can only extend the effective spread or prolong the duration of action of the implanted agent (Grossman, 1962).

1. THE EFFECTS OF NA, 5-HT AND CARBACHOL IMPLANTED INTO VARIOUS AREAS OF THE BRAIN

This study involved 15 of the cannulated animals. The stereotaxic co-ordinates for the various positions is given in Table 47. Fourteen of these positions are shown in Fig. 59, which is a composite of sagittal sections of rat brain taken between 0.5 and 2.0 mm lateral to the mid-line. Fig. 60 shows a diagram of a transverse section through the brain at A→1.6, showing the position of implantation in animal E 15. Due to an error during placement of the cannula, the implant in animal E 16 was in the reticular formation (RF) just above the arrow shaft in Fig. 60.

None of the 3 agents tested had any detectable effect on the nociceptive threshold in rats in any of the positions tried. However it was observed that animal E 4 (implant in the ventromedial hypothalamus) was stimulated to eat by the NA implant, and to drink by the carbachol implant. A fall in body temperature with carbachol and a rise with NA was seen in animals with implants in the anterior hypothalamus, AHA, (animals E 5 and E 1), and a fall was also seen with carbachol implanted in the preoptic area, POA, (animals E 2 and E 6). All these effects were apparent within 5-10 minutes of implantation. Animal E 12 showed very much reduced locomotor activity for about 45 minutes after the 5-HT implant.

Apart from these early effects, the implants had no detectable effects. Two clear days were allowed for recovery from each implant.

2. THE EFFECTS OF NA AND 5-HT ON THE ANTI-NOCICEPTIVE EFFECT OF MORPHINE

The same animals described above received a 6 mg/kg injection of morphine, subcutaneously, immediately followed by an implant of either 5-HT or NA. Two weeks later the procedure was repeated, each animal receiving the other agent, in combination with morphine treatment.

The nociceptive thresholds of all animals were within the standard error of the mean of a group of 5 animals given only morphine 6.0 mg/kg (that had not been fitted with cannulae). That is, the implants had no significant effect on the anti-nociceptive effects of morphine.

3. THE EFFECT OF NALOXONE ON THE ANTI-NOCICEPTIVE EFFECTS OF MORPHINE

Two groups of 5 animals were injected with morphine, 6.0 mg/kg subcutaneously, at time 0. Fifteen minutes later, one group was injected intraventricularly with 10 µg naloxone HCl, and one group with physiological saline. The effect on the nociceptive threshold is shown in Fig. 61 (Table 48). The naloxone completely abolished the elevation of nociceptive threshold caused by morphine.

A further 5 animals were cannulated for drug implants, and again the positions are represented in a composite sagittal section of the brain (Fig. 62) and the co-ordinates given in Table 49. Each animal

received 6.0 mg/kg morphine subcutaneously, immediately followed by an implant of naloxone. In those animals with the implants in the periventricular grey (E 24 and E 27) there was a reduction in the anti-nociceptive effect of morphine, although the morphine effect was not abolished (Table 50). In the other 3 animals with implants in the lateral preoptic area, the anterior hypothalamic area and the ventromedial hypothalamus, there was no detectable effect on the increase in nociceptive threshold produced by morphine.

4. DISCUSSION

Only a small number of animals were used in this study, and few responses to drug treatment were noted. It is possible that this was due to failure of the drugs to diffuse out of the end of the cannulae and into the tissues. This seems unlikely since at the end of the study pontamine sky blue was tamped into the end of the cannulae, and this diffused out without fail, leaving a blue stain in the tissues. Carbachol, 5-HT creatinine sulphate, and NA HCl and naloxone HCl are all highly soluble in saline and should have spread as easily as the dye. Also in a few cases, previously observed effects had been noted, indicating that the agents were diffusing into the tissues. The rise in temperature caused by NA has been seen by Kubikowski and Rewerski (1969) who also placed the drug into the anterior hypothalamic area (AHA). However, they also injected 5-HT into this area and observed small hypo- and hyperthermic effects, not seen in this study. The fall in body temperature caused by implantation of carbachol into the preoptic area (POA) has been reported by Lomax and Jenden (1966). The stimulation of eating and drinking by NA and carbachol respectively has been previously recorded by Grossman (1962), and it is interesting that he found that the most

sensitive areas in the brain were concentrated in a plane corresponding to A→5.4 to 5.8. The only animal in this study to show such tendencies was E 4, with an implant in the VMH at A→5.8.

The total inability of NA and 5-HT to change the nociceptive threshold could have been predicted from previous results, but the lack of effect of carbachol was surprising. One explanation might be that since the implantation was unilateral, the fibres on only one side of the brain were affected. Thus if the neural nociceptive pathways in this system were also unilateral only the impulses from the left or right foot would be blocked. To cover this eventuality, both feet were tested - with similar results. However, Metys, Wagner, Metysova and Hertz (1969) administered microinjections of carbachol into the mid-brain reticular formation, the septal area and the central grey region of the brains of rats in doses of about 0.3 μg, and they noted a significant increase in the nociceptive threshold. They also gave similar microinjections into the caudate nucleus and the dorsal hippocampus and found that about 20 x the dose was necessary to produce an anti-nociceptive effect. The present study did not cover the dorsal hippocampus or the caudate nucleus, but there was one implant in the septal area, one in the reticular formation, and 5 in the central grey matter - carbachol implanted into all 7 of these locations was without effect on the nociceptive threshold. The difference between the results in the present study and those of Metys et al (1969) is not easy to explain but may have been due to the different nociceptive stimuli used. Metys et al (1969) used an electrical stimulus and the one used in this study was produced by pressure. Also in the present study relatively large amounts of crystalline carbachol was placed in the

brain while Metys et al (1969) injected small volumes of carbachol in solution. This may have led to different distribution, and almost certainly will have resulted in different local concentrations of the drug. Further, the implants in this study may have been in slightly different areas of the brain structures concerned.

It thus appeared that, either the wrong sites had been selected, or bilateral implantation was required, or that following intraventricular injection, carbachol had its effects on a number of structures surrounding the ventricles and that its anti-nociceptive action was due to a combination of these effects.

The total lack of effect of NA and 5-HT in affecting the anti-nociceptive effect of morphine was also surprising. However it is likely that they lacked effect for one or more of the reasons given for the lack of effect of carbachol, in the previous paragraph. The situation with morphine was further investigated using the powerful antagonist of morphine, naloxone. This, when injected into the cerebral ventricles completely abolished the anti-nociceptive effect of morphine - again indicating a site of action around the ventricles. On implantation into the central grey matter (around both the 3rd ventricle and the aqueduct) a partial inhibition of the anti-nociceptive effect of morphine was seen. This partial effect lends support to the theory that bilateral implantation is necessary to obtain the full effect. However it is also possible that the partial inhibition by naloxone was due to it diffusing from the tissues into the ventricular system and thence to another site of action, for a high dose of approximately 50 μg was implanted. On the other hand our results are supported by Tsou and Jang (1964) who gave bilateral micro-injections of morphine into the brains of rabbits. They concluded that the main site of the anti-nociceptive action of morphine

lies in the central grey matter surrounding the 3rd ventricle and the aqueduct.

However it seems unlikely that the situation is so simple that morphine has its effects in one specific area. Poirier, Bouvier and Boucher (1968) and Lim (1968) pointed out that nervous pathways responsible for the transmission of pain are numerous and partially diffuse. It is likely that one can dissociate certain pathways that correspond to unilateral and contralateral representation of certain modalities of "pain" from other bilateral pathways that represent still further modalities.

In work involving lesions in the brain it would appear that a caudally placed lesion is more effective in causing a loss of "pain" impulses than is a more rostral lesion in the same pathway. However sustained analgesia to certain modalities of pain has been observed by placing relatively rostral lesions in the neospinothalamic (laterally situated) and the paleospinothalamic (medially situated) pain pathways (Poirier, Bouvier, Olivier & Boucher, 1968). Morphine may block one or both of these systems (Lim, 1968). In the present investigation, agents were only specifically placed in the vicinity of the paleospinothalamic pathways, although following intraventricular injection it is quite likely that agents reach both systems - via the 3rd ventricle the aqueduct and 4th ventricle for the paleo - and the lateral ventricle for the neospinothalamic pathway.

In summary it is apparent that no great conclusions can be drawn from this particular study, and this is due partially to the relatively small number of sites investigated. More work in this line involving the simultaneous application of agents to more than one site is more likely to yield significant information.

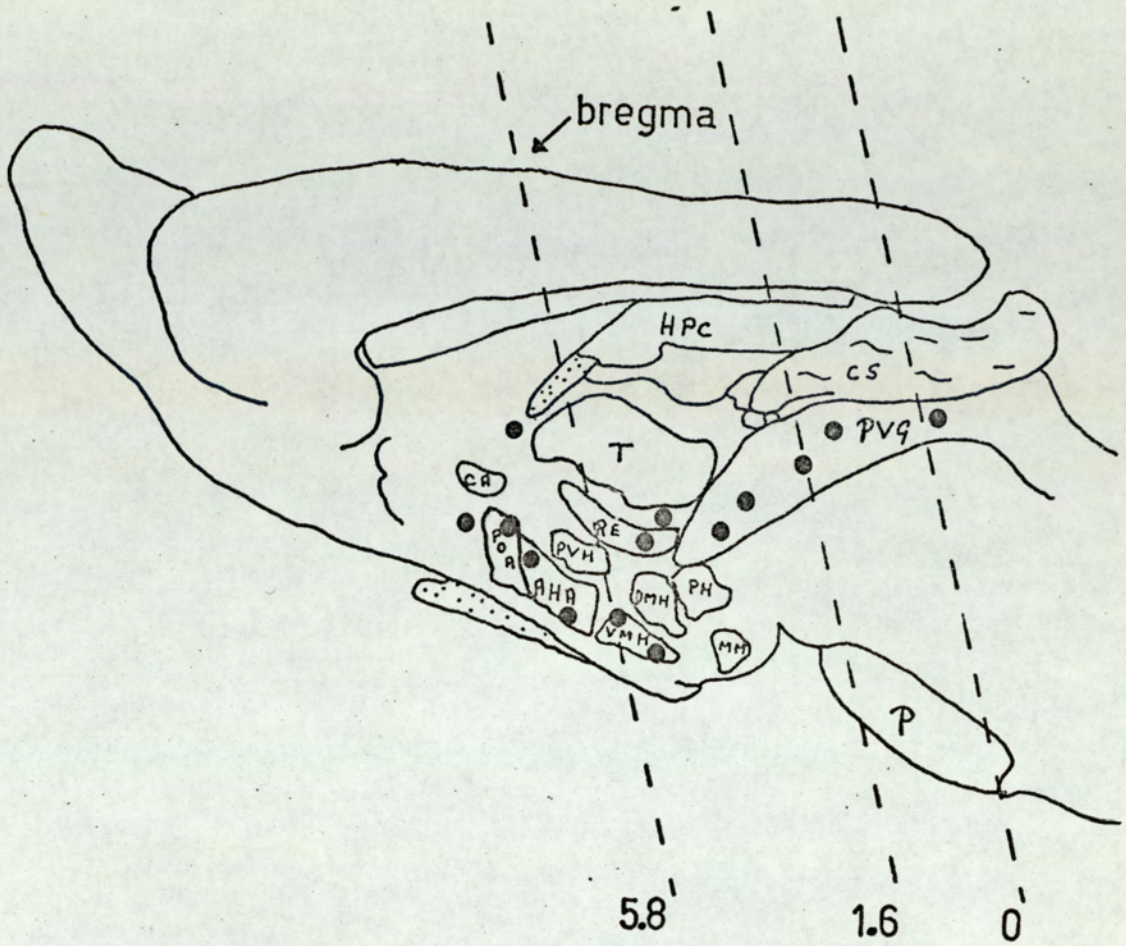

FIGURE 59 Diagram of a composite sagittal section of the rat brain showing the locations of the tips of cannulae used for implantation of drugs in solid form (see Table 51 for key). Stereotaxic co-ordinates given in Table 47.

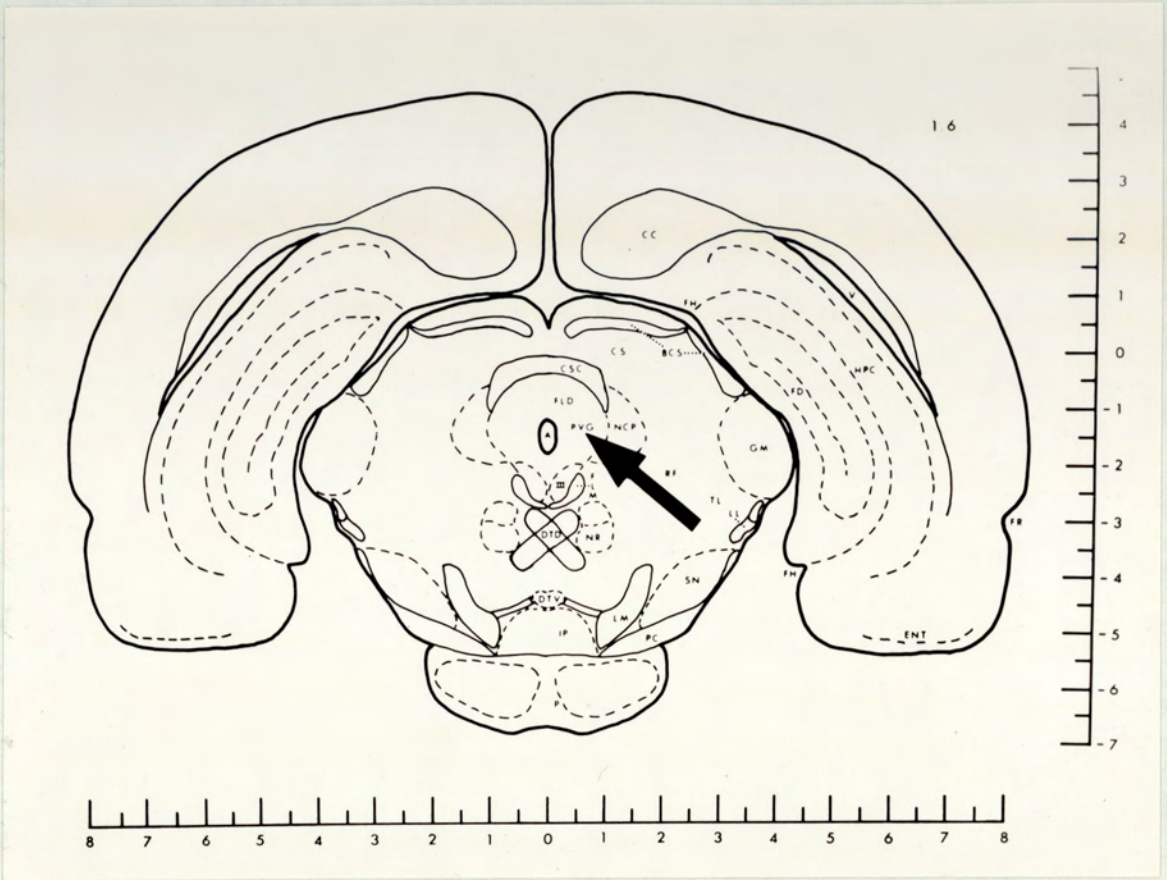

FIGURE 60 DIAGRAM T.S. RAT BRAIN AT A 1.6 (DE GROOT CO-ORDINATES)

The point of the arrow shows the position in the periaquaeductal grey of the cannula in rat E.15. The cannula in rat E. 16 was in the RF above the shaft of the arrow.
(see Table 51 for key).

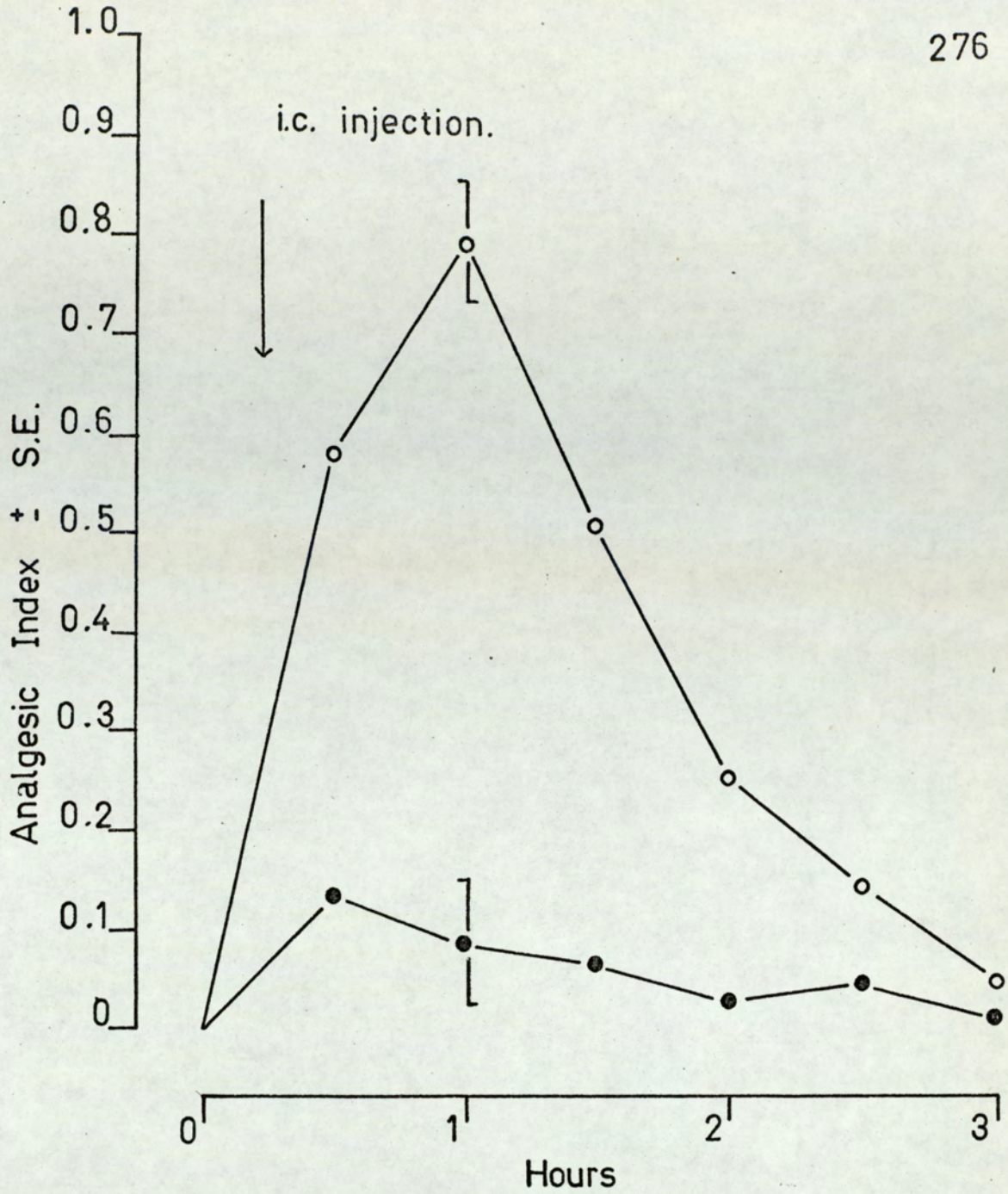

FIGURE 61 THE EFFECT OF INTRAVENTRICULAR NALOXONE ON THE ANTI-NOCICEPTIVE EFFECTS OF MORPHINE

2 groups of 5 animals received 6.0 mg/kg morphine at time 0. 15 minutes later 10 g naloxone HCl was injected into a lateral ventricle (at arrow) of one group (—●—) and 10 µl of saline into a lateral ventricle of the other (—o—). (see Table 48).

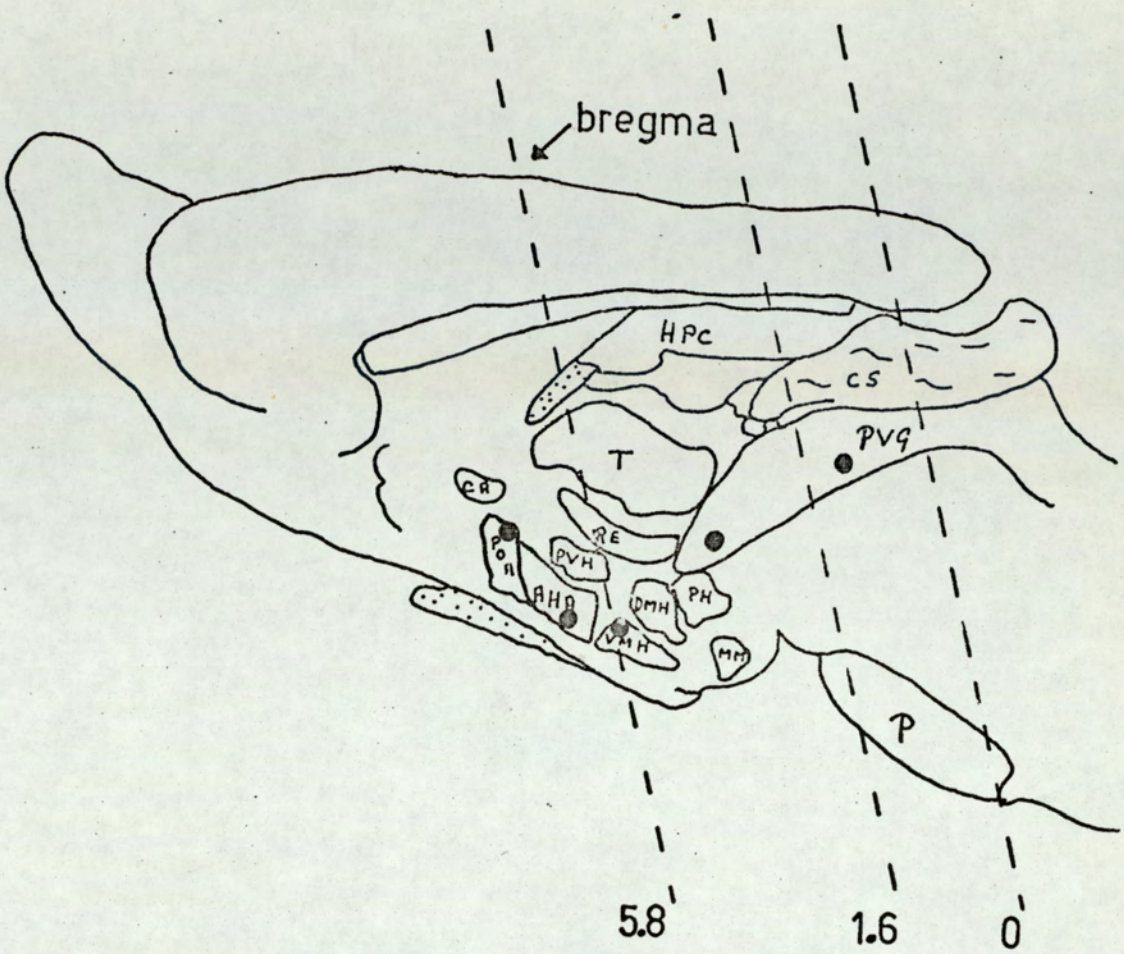

FIGURE 62 Diagram of a composite sagittal section of the rat brain showing the locations of the tips of cannulae used for implantation of drugs in solid form (see Table 52 for key). Stereotaxic co-ordinates given in Table 49.

TABLE 47

THE STEREOTAXIC CO-ORDINATES FOR THE CANNULA TIP LOCATIONS SHOWN IN FIG. 59

Animal No.	Anterior + Posterior -	Horizontal (left)	Vertical	Position
E 6	+ 9.0	1.0	- 2.0	Anterior to POA
E 2	+ 8.0	1.0	- 2.0	POA
E 5	+ 7.0	1.0	+ 1.8	AHA
E 1	+ 6.6	1.0	- 2.4	AHA
E 7	+ 6.8	1.0	+ 2.5	LS
E 4	+ 5.8	1.0	- 3.5	VMH
E 9	+ 5.0	1.0	- 4.0	VMH
E 3	+ 4.6	0.5	- 2.0	RE
E 11	+ 4.2	0.5	- 1.0	RE
E 17	+ 3.8	0.5	- 1.5	PVG
E 15	+ 1.6	0.5	- 1.5	PVG
E 16	+ 1.6	2.5	- 2.0	RF
E 18	+ 1.0	0.5	- 1.0	PVG
E 14	- 0.2	0.5	- 1.5	PVG

TABLE 48

THE EFFECT OF INTRAVENTRICULAR NALOXONE ON THE ANTI-NOCICEPTIVE EFFECTS OF MORPHINE

(see Fig. 61)

	Analgesic index ± Standard Error					
Time (Hours)	0.5	1.0	1.5	2.0	2.5	3.0
Morphine 6.0 mg/kg s.c. Saline 10.0 µl i.c.	0.580 ± 0.091	0.792 ± 0.062	0.502 ± 0.071	0.254 ± 0.063	0.143 ± 0.042	0.046 ± 0.061
Morphine 6.0 mg/kg s.c. Naloxone 10.0 µg i.c.	0.142 ± 0.082	0.090 ± 0.061	0.074 ± 0.020	0.042 ± 0.041	0.068 ± 0.046	0.014 ± 0.054

TABLE 49

STERIOTAXIC CO-ORDINATES FOR THE CANNULA TIP LOCATIONS SHOWN IN FIG. 62

Animal No.	Anterior + Posterior -	Horizontal (left)	Vertical	Position
E 21	+ 7.4	1.5	- 1.5	POA
E 22	+ 6.0	1.0	- 2.5	AHA
E 29	+ 5.2	0.5	- 4.0	VMH
E 27	+ 3.8	0.5	- 2.0	PVG
E 24	+ 1.0	0.5	- 1.8	PVG

TABLE 50

THE EFFECT OF INTRACEREBRAL IMPLANTATION OF NALOXONE HCl ON THE ANTI-NOCICEPTIVE EFFECT ON MORPHINE

Animals E 24 and E 27 (stereotaxic co-ordinates in Table 49) were injected with 6.0 mg/kg morphine. Immediately following this injection implants of naloxone HCl were placed in the periventricular grey (PVG). The nociceptive thresholds were determined at the times indicated.

	Analgesic index ± Standard Error					
Time (Hours)	0.5	1.0	1.5	2.0	2.5	3.0
Morphine 6.0 mg/kg s.c. (5 animals)	0.502 ± 0.062	0.826 ± 0.094	0.492 ± 0.074	0.204 ± 0.062	0.120 ± 0.040	0.014 ± 0.062
Morphine 6.0 mg/kg s.c. Naloxone i.c. E 27	0.276	0.142	0.186	0.094	0.112	0.017
Morphine 6.0 mg/kg s.c. Naloxone i.c. E 24	0.324	0.214	0.251	0.211	0.094	0.012

TABLE 51 Key to Figs. 58, 59 and 60.

AHA	Anterior area of the hypothalamus
BCS	Brachium of the superior colliculus
CA	Anterior commissure
CC	Corpus callosum
CS	Superior colliculus
CSC	Commissure of superior colliculus
DMH	Dorsomedial nucleus of the hypothalamus
DTD	Decussation of Meynert; Decussatio Tegmenti dorsalis
DTV	Decussation of Forel; Decussatio tegmenti ventralis
ENT	Entorhinal cortex
FD	Dentate gyrus
FH	Hippocanpal fissure
FLD	Dorsal fasciculus of Schutz; Dorsal longitudinal bundle
FR	Rhinal fissure
GM	Medial geniculate body
HPC	Hippocampus
IP	Interpeduncular nucleus
LL	Lateral leminiscus
LM	Medial leminiscus
LS	Lateral septal nucleus
MM	Medial mamillary nucleus
NCP	Bed nucleus of the posterior commissure
P	Pons
PC	Cerebral peduncle
PH	Posterior nucleus of the hypothalamus
POA	Lateral preoptic area

Table 51 continued

PVG	Central grey substance (periventricular grey)
PVH	Paraventricular nucleus of the hypothalamus
RE	Nucleus reuniens thalami
RF	Reticular formation of mesencephalon
T	Thalamus
TL	Lateral tegmental nucleus
VMH	Ventromedial nucleus of the hypothalamus

CHAPTER NINE

THE EFFECTS OF INTRAVENTRICULARLY ADMINISTERED AGENTS ON BLOOD PRESSURE

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page no.

THE EFFECTS OF INTRAVENTRICULARLY ADMINISTERED AGENTS ON BLOOD PRESSURE

Intraventricular injections of various agents have been reported to have effects on blood pressure in a number of species (see Section 1, Chapter 4). It was considered possible that some of the effects previously reported in this study could have been caused by alterations in blood pressure. This chapter describes the effects of some agents injected into the cerebral ventricles of both conscious and unconscious rats.

1. THE EFFECTS ON BLOOD PRESSURE OF NA INJECTED INTO THE VENTRICLES OF ANAESTHETISED RATS

Rats that had been cannulated 2 or more days previously were anaesthetised with pentobarbitone sodium, 60 mg/kg, (Nembutal, 1.0 ml/kg) injected intraperitoneally. They were then set up as described in Section 2, (paragraph 5) to record blood pressure. Fig. 63 shows a photograph of a typical trace in which the pressure response to 0.2 µg of NA given intravenously is shown, followed by a depression in the blood pressure due to 10 µg NA injected into a lateral ventricle. However the trace also shows that a repeat injection of 10 µg NA into the ventricle 5 minutes following the first, had no effect on the blood pressure. It was found consistently that the second intraventricular dose of NA was without effect on blood pressure, even when the time interval between the doses was as much as 4 hours. Smaller doses (0.001 - 2.5 µg) had no detectable effect on the blood pressure and higher first doses (20-80 µg) either caused depression of blood pressure, or caused a small prolonged rise that appeared 2-5 minutes after injection.

However a similar depression of blood pressure could be shown if the animal's head was not clamped or if the cannula was not inserted with care. Thus gross disturbances of the animal could cause a depression in the blood pressure. This depression in response to a disturbance could usually be repeated once, though to a lesser degree, but never more than that. The effect is shown in Fig. 64 where the cannula was rapidly inserted into the guide. The same figure shows that injection of 20 µl of physiological saline (10 µl greater than that used as the drug vehicle) after recovery from the initial depression had no significant effect on the blood pressure. Volumes of up to 50 µl were tried and shown to have no effect.

If the spinal cord of the animal was sectioned at C2 and then left for 1 hour before the first injection, then the depressor effect to cannula insertion of NA was not seen. However the basal blood pressure was very much lower in these animals. Occasionally a small pressor response to NA appeared in response to intraventricular NA in the C2 sectioned animal.

2. THE EFFECT ON BLOOD PRESSURE OF CARBACHOL AND 5-HT INJECTED INTO THE CEREBRAL VENTRICLES OF ANAESTHETISED RATS

Doses of 5-50 µg of 5-HT injected into the ventricles usually had no detectable effects on the blood pressure, but occasionally very small pressor effects were seen such as illustrated in Fig. 65. Intraventricular injection of carbachol caused a dose related decrease in blood pressure (see Fig. 66) which could be prevented by pretreatment at -30 minutes with 1.0 mg/kg hyoscine. However atropine methonitrate (5.0 mg/kg) pretreatment at -30 minutes did not have an appreciable effect on this depressor effect, suggesting a central site of action for carbachol.

3. THE EFFECTS ON BLOOD PRESSURE OF NA, 5-HT AND CARBACHOL INJECTED INTO THE CEREBRAL VENTRICLES OF CONSCIOUS RATS

Animals were prepared as described in Section 2, for the recording of blood pressure in conscious animals. Some time before recording pressure, the animals were placed in the box shown in Section 2, Fig. 8. After 1-2 hours when the rats were familiar with their new environment, the chronic aortic cannulae were connected up for blood pressure recording. The resting blood pressure so obtained was usually 10-20 mm/Hg higher than that shown by animals anaesthetised with pentobarbitone sodium. It was essential that such experiments were conducted in a quiet room for noise - such as people entering the room and talking (as shown in Fig. 67) - caused wild fluctuations in the blood pressure. Before injection of an agent, a cannula attached to a length of polyethylene tubing was charged with a solution of that agent, and placed into the ventricles. The animal was then given 3 minutes to settle down and the injection given from outside the cage. Similarly, intravenous injections were administered remotely, via the chronic cannula in the jugular vein.

NA (0.001 - 40 μg), 5-HT (5-20 μg) and carbachol (0.5 - 2 μg) had no significant effects on the blood pressure of the conscious rat (the carbachol dose was considerably lower than that given in the anaesthetised animals due to the toxic manifestations shown in conscious animals). Fig. 68 shows the effects of such intraventricular injections.

These conscious animal preparations were extremely insensitive to intravenous injections of agents known to possess blood pressure effects. This is demonstrated in Fig. 69 where 10 μg of NA was injected into a rat weighing 297 g (approximately 35 μg/kg).

Considerable irregularities in the pulse rythm can be seen lasting for some 2 minutes, but there was very little elevation of blood pressure. The depressor response to carbachol given intravenously was also smaller than that seen in anaesthetised animals.

4. DISCUSSION

The results presented here would indicate that there was very little immediate leakage from the ventricular system into the peripheral blood supply. If there had been any such appreciable leakage, it is likely that pressor responses would have been seen in either the normal or the C2 sectioned, anaesthetised animals after intraventricular injection of NA. When large doses of NA were used in C2 sectioned animals, small pressor responses were seen but these were normally delayed; it seems likely that they were due to leakage into the peripheral vascular system. Cardiovascular responses to intraventricular NA have been recorded in cats by other workers. Slight depressor responses to doses of 12, 20 and 50 μg were seen in pentobarbitone-anaesthetised cats by Nashold, Mannarino and Wunderlich (1962), whereas Share and Melville (1963) noted only sinus bradycardia in the pentobarbitone-anaesthetised cat in response to 40 or 80 μg of intraventricular NA. In the chloralose-anaesthetised cat, Gagnon and Melville (1966) reported that small intraventricular doses (0.01 - 0.1 μg) of NA caused cardiovascular excitation, whilst higher doses caused cardiovascular depression.

In view of these results, doses as low as 0.001 μg were tried in this study and were without detectable effect. Any systematic study of the observed depression due to intraventricular NA was thwarted by the inability to reproduce the effects in each animal. In this respect it is interesting to note that the cardiovascular responses following intraventricular nicotine were not reproducible

in chloralose-anaesthetised cats, if the interval between injections was less than 40 minutes (Armitage & Hall, 1967b). However from the present study it would appear that in the rat more than 4 hours are required for recovery from the initial injection of NA. The depressor effects, caused by intraventricular NA or major (environmental) disturbances of the animals, were similar to one another in that they could not usually be repeated. It is possible that the effects were due to increased pressure in the ventricular system. However this seem unlikely since volumes of up to 50 µl of saline injected into the ventricles had no detectable effect on blood pressure. Yet, in cats and dogs an elevation of the blood pressure has been reported in response to increased intracranial fluid pressure (Hancock & Hilton, 1967).

In the present study intraventricular injection of 5-HT into the anaesthetised rat had no detectable effect on blood pressure, and a similar lack of effect has been reported by Share and Melville (1963) in the chloralose-anaesthetised cat.

A depression in blood pressue due to intraventricular carbachol has been reported in the chloralose-anaesthetised cat. Injections of 40 µg carbachol into the lateral ventricles of these cats produced a large fall in blood pressure that was accompanied by a fall in heart rate. These effects were reversed by hyoscine pretreatment but not by atropine methonitrate pretreatment (Armitage & Hall, 1967a). Thus the effects in the cat were very similar to those seen here in the pentobarbitone-anaesthetised rat. However, Armitage and Hall did see a component of the carbachol-induced depression that was not abolished by hyoscine pretreatment. This they suggested was due to a nicotinic action of carbachol. This hyoscine-insensitive component was not seen in the present experiments in the rat.

The recording of blood pressure in conscious animals was commenced in the hope that the responses to intraventricular agents seen in the anaesthetised animals would also be seen in the conscious animals, thus allowing a more prolonged study. However the reflex systems involved in the maintenance of a steady blood pressure seem to be remarkably strong in the conscious rat. It should be pointed out that Lambert and Lang (1970) have observed that intraventricular injections of bradykinin cause pressor responses in the conscious rat. However such injections also cause strong behavioural stimulation (including vocalisation and violent movement) which may to some extent account for the observed pressor responses. The resting blood pressure reported by these authors was around 100 mm Hg (about 50 mm less than ours) which would have facilitated the demonstration of pressor responses. 5-HT was also without effect on blood pressure, following intraventricular injection into the conscious rat. However Correalle (1954) reported that subcutaneous injections of 0.4 mg/kg 5-HT caused a fall in blood pressure and cutaneous vasodilation in the conscious rat. Though the effects of peripheral administration of 5-HT are not examined in the present study the effects seen by Correalle (1954) may have been due to peripheral effects, and that in our experiments there was not sufficient leakage from the ventricles to cause similar changes.

In conclusion, the results of this chapter would indicate that following intraventricular injection there was very little immediate leakage of solutions from the ventricular system into the peripheral circulation. Also, the effects of intraventricular injections of NA and 5-HT and carbachol (in doses similar to those used in the preceding chapters) did not have any significant effects on the blood pressure of conscious animals.

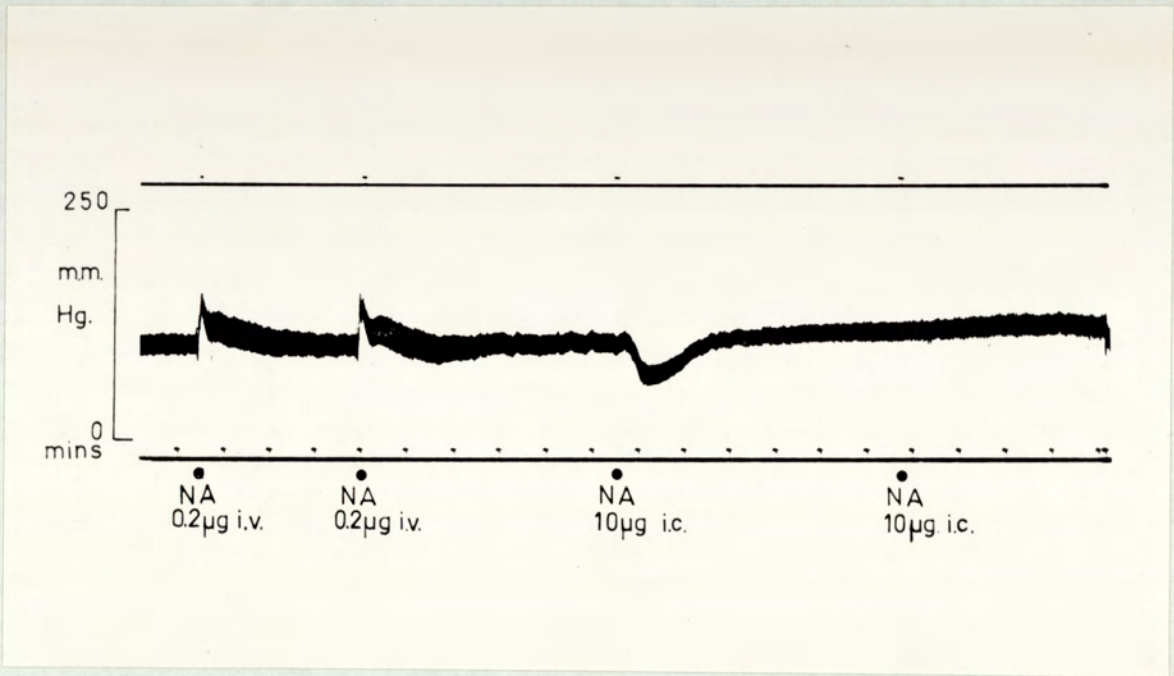

FIGURE 63 ANAESTHETISED RAT BLOOD PRESSURE PREPARATION

The record shows the effect of NA on aortic blood pressure (mm Hg), when injected both intravenously (i.v.) and intraventricularly (i.c.).

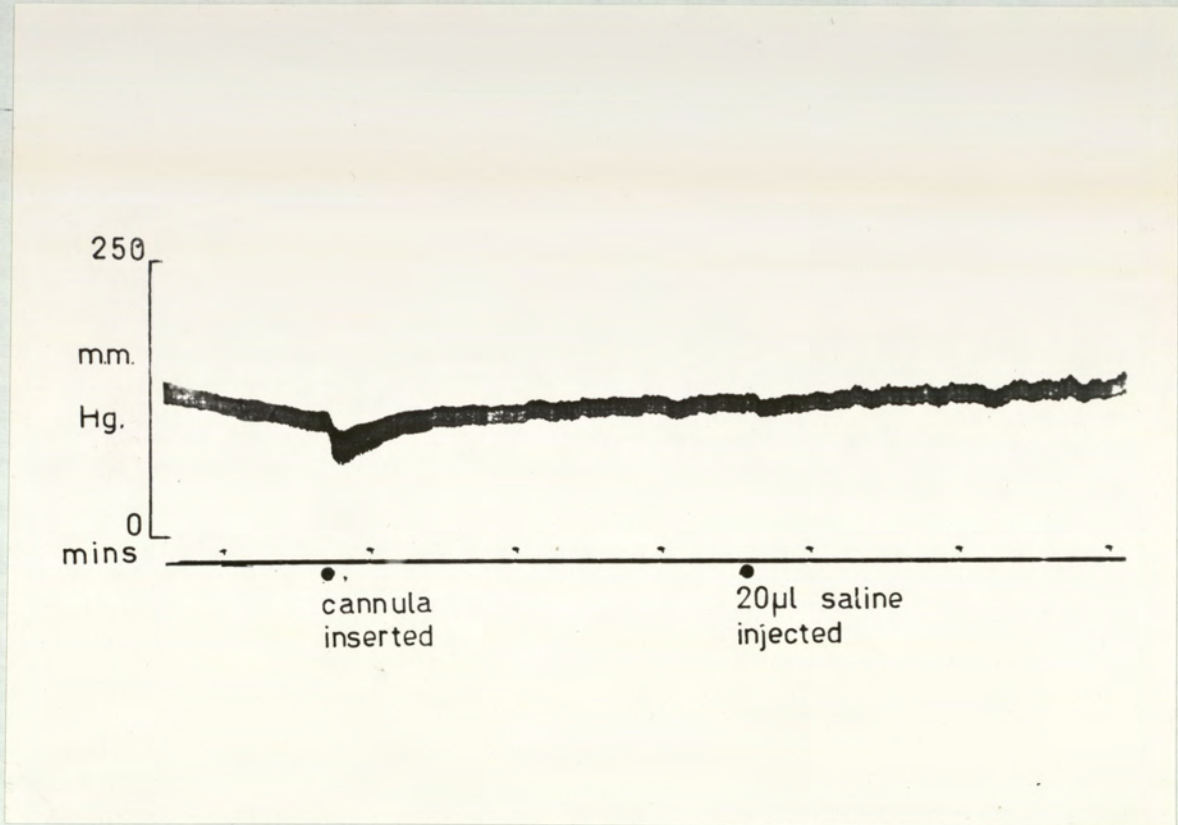

FIGURE 64 ANAESTHETISED RAT BLOOD PRESSURE PREPARATION

The record shows the effect on blood pressure (mm Hg) of rapid insertion of an intraventricular cannula down a cannula guide, and the subsequent injection of 20 µl saline 3 minutes later.

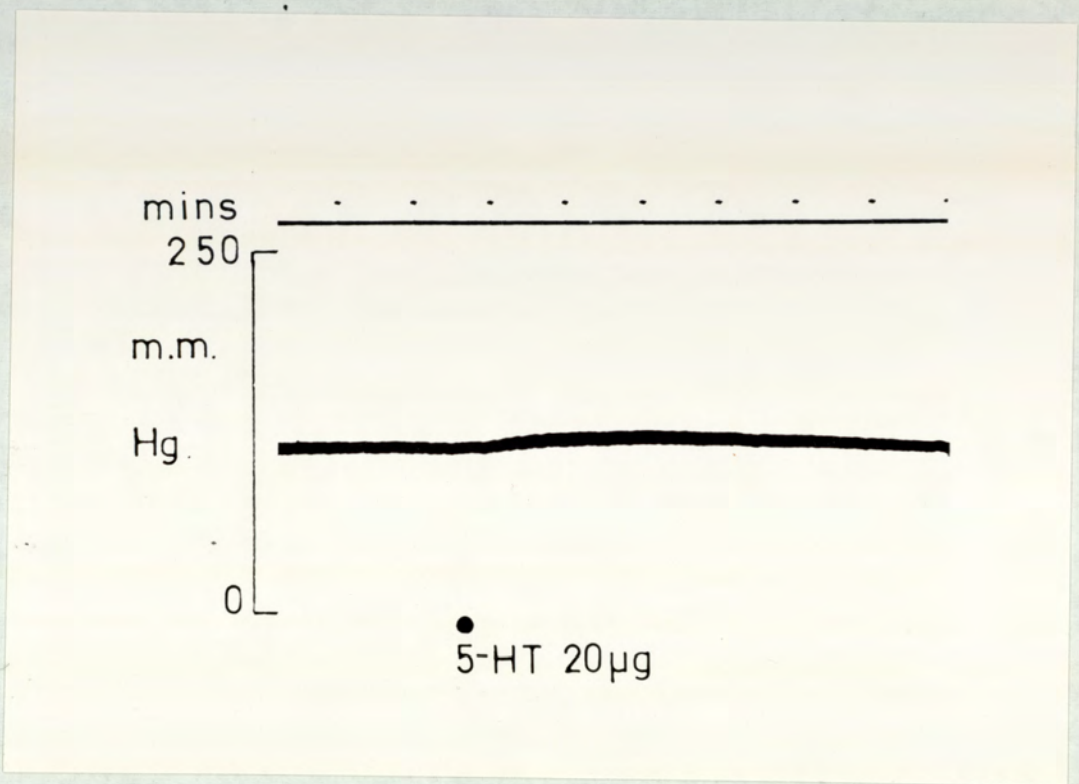

FIGURE 65 ANAESTHETISED RAT BLOOD PRESSURE PREPARATION

The record shows the effect on blood pressure (mm Hg) of a 20 µg injection of 5-HT into the cerebral ventricles.

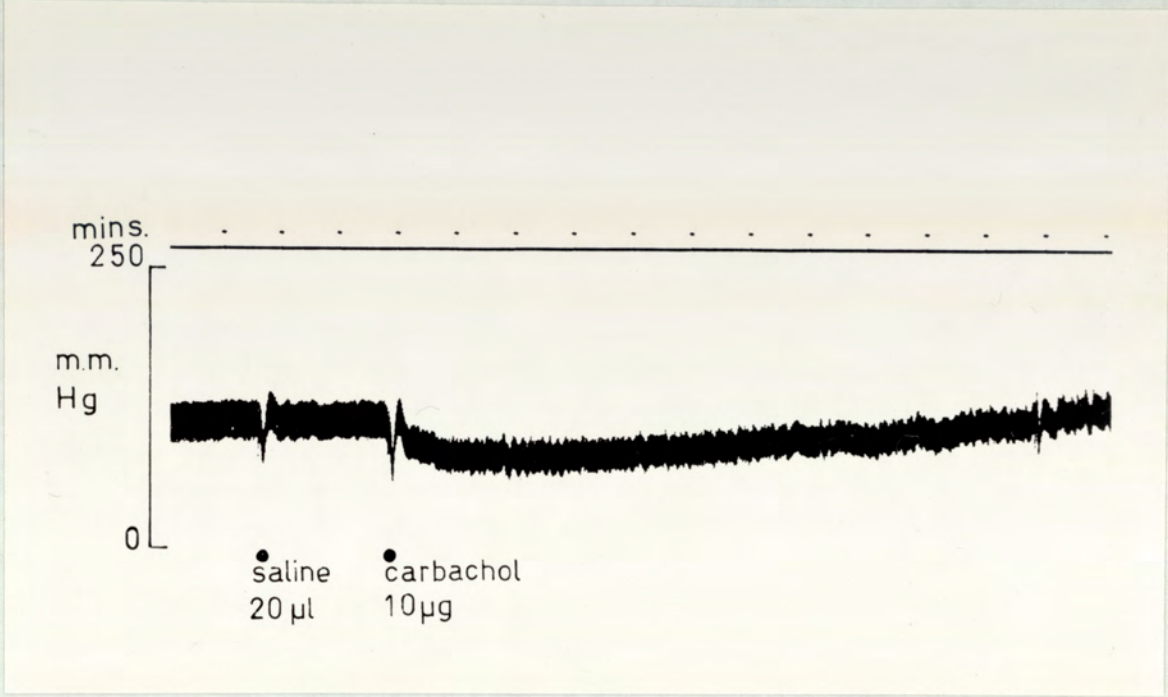

FIGURE 66 ANAESTHETISED RAT BLOOD PRESSURE PREPARATION

The record shows the effect of an intraventricular injection of 20 µl of physiological saline followed by a 10 µg injection of carbachol dissolved in 10 µl saline.

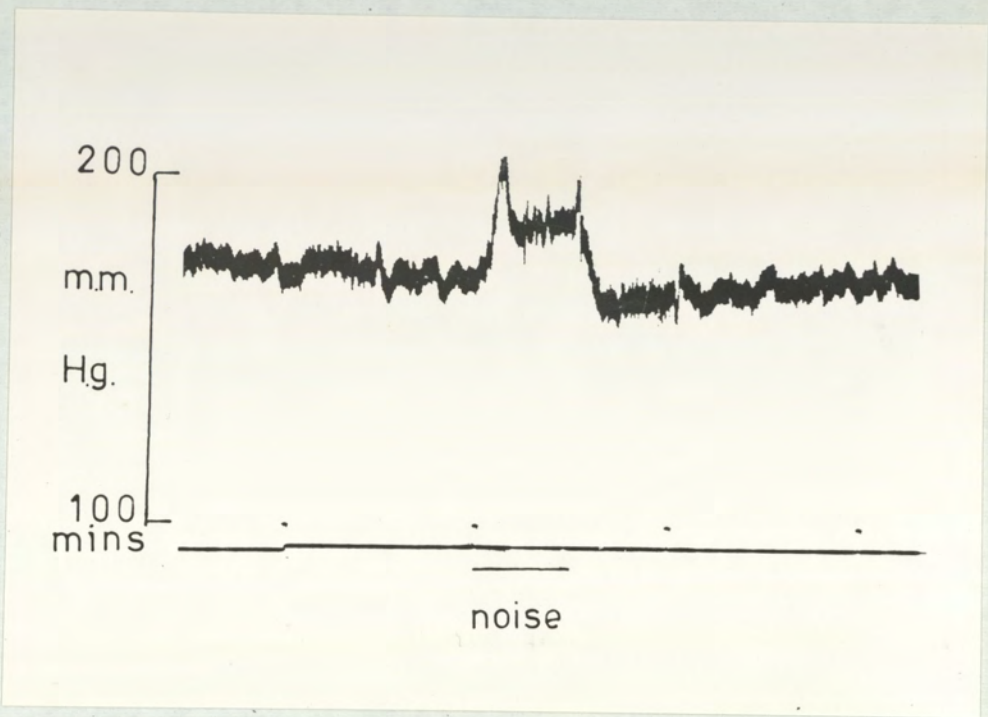

FIGURE 67 CONSCIOUS RAT BLOOD PRESSURE PREPARATION

The record shows the effect on blood pressure of noise in the form of people entering the experimental area and talking.

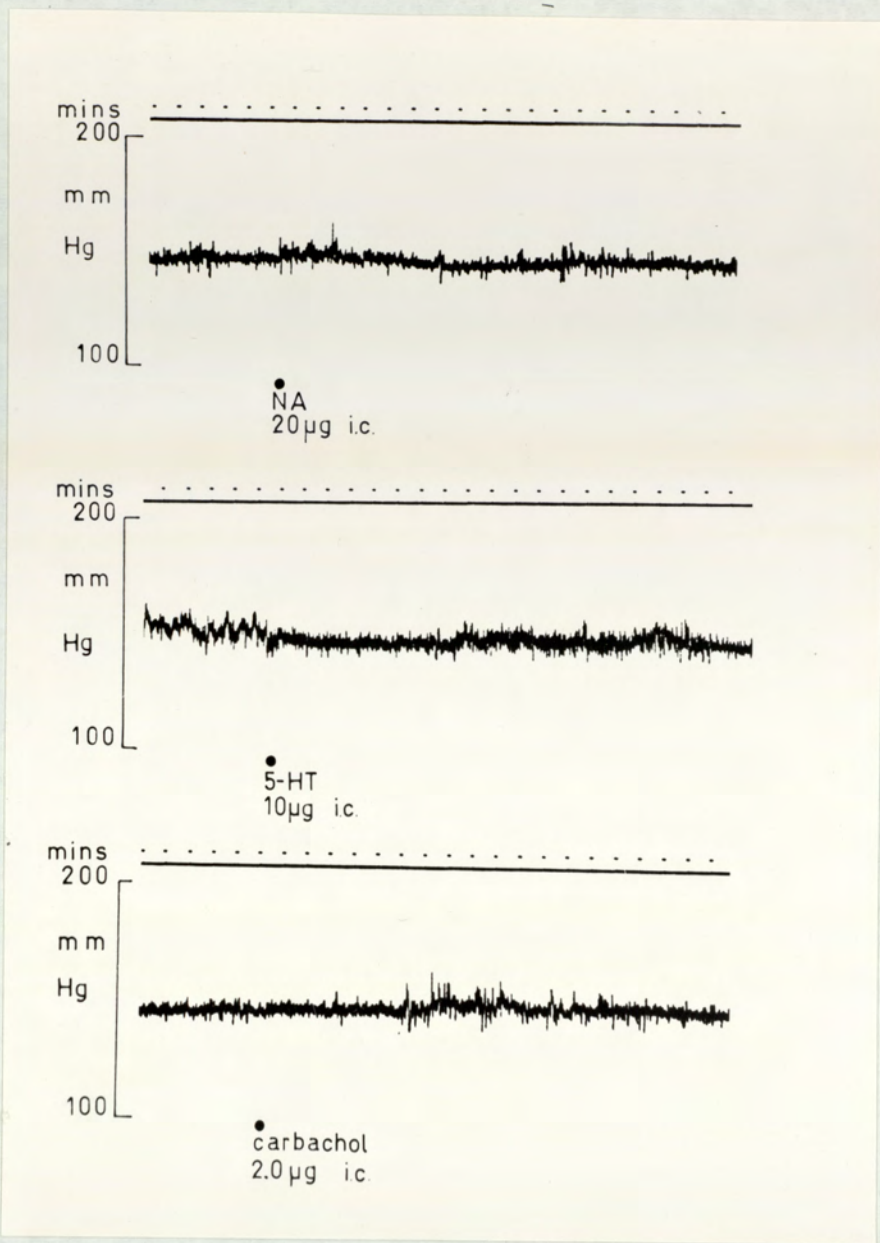

FIGURE 68 CONSCIOUS RAT BLOOD PRESSURE PREPARATION

The record shows the effects on blood pressure of intraventricular injections of NA 20 µg (upper trace). 5-HT 10 µg (middle trace); and carbachol 2.0 µg (lower trace).

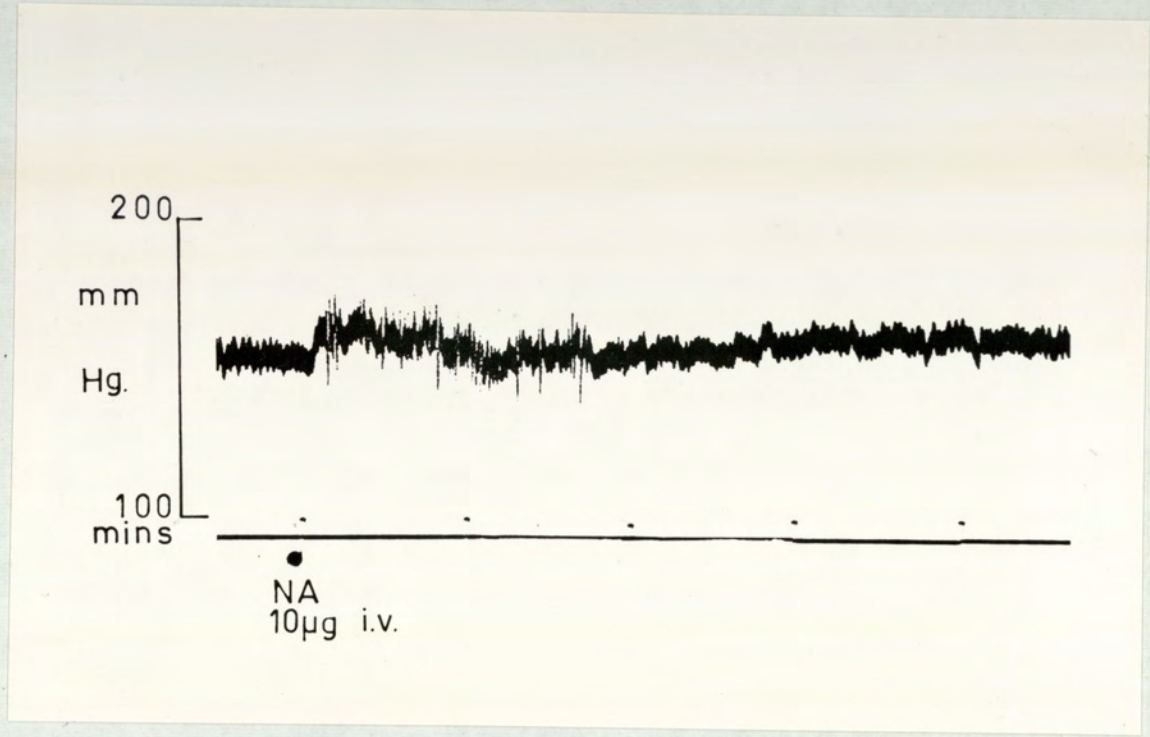

FIGURE 69 CONSCIOUS RAT BLOOD PRESSURE PREPARATION

The record shows the effect on blood pressure of an intravenous injection of 10 µg NA (approximately 35 µg/kg).

SECTION FOUR

GENERAL DISCUSSION

GENERAL DISCUSSION

When drugs are given by conventional routes of administration, for example orally or by intravenous or intraperitoneal injection, etc., the observable pharmacological effects may be due to actions upon both central and peripheral systems. Yet, in man, these drugs may be used essentially for supposed central actions. There are also a large number of drugs which fail to gain access to the brain because of their minimal (and often unpredictable) penetration of the blood-brain barrier, (see Introduction, Chapter 2).

Intraventricular injections were used in this study for two reasons: first, it would markedly encourage entry into the brain of a number of agents which by conventional routes would fail to gain access: secondly, it would limit to the brain the effects of any agent given by this route - particularly important in the case of those agents, for example noradrenaline, acetylcholine, whose profound peripheral effects confuse or obscure quite important central pharmacological actions.

This limitation of effect to the brain is achieved by the injection of very small quantities of agents into the ventricular spaces. Thus, although the intraventricular concentrations achieved are sufficient to affect the local nervous tissues, any agent that may get returned to the peripheral blood stream, along with the CSF, is diluted by the large volume of blood, and its effects become minimal.

Previous workers have injected a greater variety of agents into various areas of the brains of several species of animal, and a great number of effects have been observed. In the present study, it was decided to limit the agents administered to either substances that have been proposed as possible neurotransmitters in the brain or to

agents that are known to affect these transmitters. Thus the use of this route of administration allowed the study of the effects of altered central levels of transmitters, both on their own and in the presence of other agents.

A number of methods have been described for the administration of agents into the rat brain (see Introduction, Chapter 3). Some have involved the use of conscious and some the use of unconscious animals. In order to study behavioural and associated effects the use of a conscious animal was essential. The majority of techniques using the conscious animal involve the implantation of a cannula guide into the brain, which is secured to the skull. Injections can then be made via a cannula placed through the cannula guide. Other methods of giving agents into the ventricular spaces have been described. An example is the method of Valzelli (1964) in which agents are injected directly into the cerebral ventricles of lightly anaesthetised rats, using a simple syringe plus needle. Although such a technique would appear to be extremely simple, it has disadvantages. For example, animals can only be used once, thereby eliminating the possibility of using them as their own controls. Another disadvantage is the need to use some form of anaesthetic or immobilising agent which would almost certainly affect the results. The various cannula guides described in the literature are all similar in principle though they vary in their complexity of construction. The method of Hayden, Johnson and Maickel (1966) involved the use of readily available materials (perspex sheet, stainless steel tube and a cold-setting rubber solution) and relatively large numbers of these could be constructed without the use of sophisticated equipment or specialised engineering skills. The original design of Hayden et al has been modified in the present study to suit the local conditions, and has proved to be a highly satisfactory

method of introducing agents into the cerebral ventricles of rats.

The rat was chosen as the experimental animal for 3 main reasons: first, it is a relatively cheap animal; secondly, because of its size and extreme adaptability it requires much less space and is considerably easier to look after and handle than are cats, dogs, etc.; thirdly, altered central transmitter levels in the rat had been extensively studied using drugs, but relatively little had been done by the application of such agents into the cerebral ventricles.

In the first chapter of results, various behavioural effects of agents injected into the cerebral ventricles were described. It was noted that these effects were not always the same as those reported by other authors using different species and methods. Recently Herman (1970) has reported the effects of NA on behaviour, when injected into the cerebral ventricles of conscious rats. He found that doses of 10 µg NA increased exploratory activity in animals with low normal levels of activity, and had no effect on animals with medium or high normal levels. He also found that doses of 50 and 100 µg increased locomotor activity and 200 µg was necessary to produce the stupor seen in the present study. All doses used in the present study (10-80 µg) produced stupor and reduction in activity along with muscular relaxation and hyperphagia - effects apparently not seen by Herman. Excitation was only seen when NA was infused into the ventricles over long periods. These differences are difficult to explain except on the basis of methodology. The technique for intraventricular injection used by Herman, consisted of drilling a hole in the anaesthetised animals' skulls 14 hours before the intraventricular injection. Injection was made into the conscious animal through this hole without the use of a cannula guide. In the present study this technique was attempted a few times in acute

anaesthetised experiments, but there was always a certain amount of leakage of blood and fluid back up the cannula tract. Thus Herman's observations may have been due to effects of NA on brain structures other than those surrounding the ventricles (i.e. on structures around the cannula tract) or by effects of the agents reaching the peripheral blood supply. This possibility is supported by the fact that in the present study, doses of NA of 160 μg or greater were lethal, whereas Herman's animals tolerated doses of 200 μg.

There are a number of reports of the effects of intraventricular administration of agents on body temperature, and initially it was decided to re-investigate and further examine these effects in the rat. However considerable amount of trouble was encountered with control animals, the temperatures of which were invariably elevated following intraventricular injection of physiological saline, despite its being sterile and apyrogenic. Subsequent investigations led to the conclusion that endogenous materials were collecting in the cannula guide, and were responsible for this rise. In this respect, it is interesting to note that following our suggestion, Feldberg, Myers and Veale (1970) have recorded recently in unanaesthetised cats a long-lasting late rise in temperature which appeared after infusions of solutions from the lateral cerebral ventricles to the cisterna magna. They too concluded that this was due to some unknown pyrogenic factor, present in the lumen of the cannula, being washed into the ventricles. An even greater similarity between these results and those in the present study was that this rise could be abolished if the perfusion cannula protruded 1 mm beyond the tip of the cannula guide. They also reported that if either sodium chloride or combined sodium chloride + potassium chloride solutions were used as perfusing fluids, an increase

in temperature was seen that could be abolished by the addition of calcium salts to the medium. In the present study, sterile apyrogenic sodium chloride solution (physiological saline) injected into the ventricles of rats had no detectable effect on body temperature when the final design of cannula guide etc. was used.

In the literature there are a number of reports of an elevation of the nociceptive threshold following the intraventricular injection of NA (see Introduction, Chapter 3). This effect has also been observed in this Department in mice (Handley & Spencer, 1969). In the present study however the effect did not appear in rats following either intraventricular injection or infusion of NA. Instead infusion of 5-HT produced a transient period in which the nociceptive threshold appeared to be elevated. These results along with the known effects of morphine in elevating the nociceptive threshold and its known effects on NA, 5-HT and ACh, initiated the investigation into the inter-relationships between these agents.

Before we could commence a systematic investigation into changes in the nociceptive threshold, a simple method of applying a mild nociceptive stimulus had to be selected. There are a number of methods available involving mainly the use of chemical irritants, heat, electric shock or pressure. Again a simple test was required which would not permanently damage the animals so that they could serve as their own controls, and be used in further experiments after a few days recovery period. Methods involving the use of chemical irritants injected into the peritoneal cavity were eliminated on the basis that they would not be suitable for use more than once. Although electrical stimulation techniques are widely used, it can be difficult to interpret the results (see Winter, 1965). In the present study the application of

heat to the tail was investigated first. A beam of light was focused on the rat tail, and the time taken for the tail to flick was measured. There was great variation in the data, as found previously by Green, Young and Godfrey (1951), and in a number of rats blisters appeared later, with the tail sloughing off about 24 hours after the experiment. It seems likely that this was due to the necessity of using relatively old rats for the cannulation, animals in which the tails were fairly 'scaly' and tough. Pressure methods affect deep pain receptors and thus would not be affected to the same degree by the thickness of the skin, nor be associated with the same degree of tissue damage. A commercially available apparatus (described in Section 2, paragraph 10), using the Randall and Selitto (1957) foot-pressure technique, was easy to use and in our hands produced consistent data. In contrast to the results of Green, Young and Godfrey (1951), who used pressure on the tail, it has been found that least variance in the results was obtained if the struggle response rather than the squeak response was used. A number of the rats used here were reluctant to squeak at all, though they would struggle vigorously.

Using the foot-pressure system the effects of NA, 5-HT and ACh on the nociceptive threshold were then examined. 5-HT in the doses used had no anti-nociceptive activity when injected into the cerebral ventricles, but NA gave the animals the appearance of being more sensitive to nociceptive stimuli. This effect has been observed in man, in that intravenous infusion of NA increased sensitivity to painful stimuli - infusion of adrenaline on the other hand caused mild analgesia (Dundee, Black & Nicholl, 1962). In the present study ACh caused a very brief and erratic elevation of the threshold on intraventricular injection, and the cholinomimetics oxotremorine and carbachol had powerful anti-nociceptive effects when administered in this way.

NA, 5-HT and ACh were also infused into the ventricles and again NA gave animals the appearance of being super-sensitive to nociceptive stimuli. 5-HT produced a transient elevation of the threshold after prolonged infusion. ACh elevated the threshold for as long as it was infused, there being a remarkably rapid recovery at the end of the infusion. The depressant effects of 5-HT and stimulatory effects of NA are interesting in view of the results obtained in this study with morphine and associated drugs. Tenen (1967) administered p-chlorophenylalanine (a central 5-HT depletor) to rats and noted an increase in sensitivity to nociceptive stimuli, which he suggested may have been due to depletion of 5-HT from the brain. This would fit any theory that suggested that the nociceptive threshold was held in a balance by the relative levels of NA and 5-HT in the brain.

The interactions of intraventricular NA and 5-HT with the anti-nociceptive effects of morphine were then investigated. It was found that intraventricular 5-HT potentiated morphine whilst NA inhibited morphine's effect. These results are supported by other authors using other methods of altering the central NA and 5-HT levels in the rat. Recently it has been reported that lesions in the mid-brain raphé region of the rat reduced the levels of brain 5-HT and decreased the anti-nociceptive activity of morphine in these animals. Further, in some lesioned rats an increase in the sensitivity to nociceptive stimuli was seen (Samanin, Gumulka & Valzelli, 1970). This again supports the theory that the anti-nociceptive effect of morphine is in some way dependent on the presence of 5-HT and that the nociceptive threshold may be held in a balance by the relative levels of NA and 5-HT. Sheard and Agajanian (1967) noted that neural release of 5-HT from the mid-brain raphé region, induced by electrical stimulation of

various areas of the brain, was accompanied by a concomitant rise in body temperature. It would be interesting to see if stimulation of such centres potentiates the effects of morphine.

Morphine is known to have a number of effects on both central and peripheral cholinergic systems (for refs. see Section 3, Chapter 5). Cholinomimetic agents have been found to possess anti-nociceptive effects both in this study and elsewhere. This led to the theory that morphine might mediate its anti-nociceptive action via ACh, the relative levels of NA and 5-HT modulating the effect. If such a theory was to hold, it might be expected that there would be a relationship between the activities of various narcotic agonists and antagonists and their effects on brain levels of these transmitters. Recently Howes, Harris and Dewey (1970) have reported that there is no relationship between the turnover of ACh in mouse brain and the activities of nalorphine, naloxone, pentazocine and cyclazocine as anti-nociceptive agents. They all had similar effects on ACh turnover, indicating that there may be a relationship between these agents and ACh that is not related to their anti-nociceptive activity. Further Herz (1968) has reported that ACh has a generally depressant effect causing loss of both conditioned responses as well as increasing the nociceptive threshold.

Thus, although these agents all have similar effects on brain ACh, it may be that their different anti-nociceptive potencies are brought about by different actions on brain NA and 5-HT. Nalorphine, pentazocine and naloxone were found not to have anti-nociceptive activities in this study and this is usually found to be the case for these agents in animal tests. However intraventricular injection of 5-HT into animals treated with nalorphine produced a brief elevation

in the nociceptive threshold. This effect was not seen with pentazocine or naloxone. Kosterlitz and Watt (1968) found that nalorphine and morphine both had considerable agonist activity in relaxing the electrically-stimulated guinea-pig ileum, whilst pentazocine had considerably less and naloxone virtually none. Thus it may be that the 5-HT in some way either potentiated the agonist activity of morphine and nalorphine or removed the antagonist activity that they both have (Kosterlitz & Watt, 1968), the agonist activity of both pentazocine and naloxone being too weak to show in this situation. It is suggested that more work be carried out on this system using a wider range of narcotic agonists and antagonists, since it might produce a screening technique for estimating the analgesic potency of these agents using an animal model, or even for predicting narcotic or non-narcotic activity.

In a further chapter the effects of intracerebrally-administered NA and 5-HT were studied on the production of acute tolerance in rats. From the limited amount of work done it is suggested tentatively that 5-HT may be involved in the production of tolerance. The work indicates that on prolonged administration of morphine, the production of 5-HT ~~is in some way progressively blocked, or its production~~ might in some way be reduced - since intraventricular injection of 5-HT following the production of acute tolerance induces a brief elevation of the nociceptive threshold, not seen in normal animals. In 1965, Collier proposed a theory to explain tolerance and physical dependence, in which interaction of the drug with a chemical transmitter substance was postulated. He suggested that morphine may block 5-HT on nerve cells resulting in the induction of extra 5-HT receptors. Following the withdrawal of morphine, there would be a larger than normal number of receptors available, resulting in excessive pharmacological reactions. The present experiments suggest that the presence of active 5-HT

is necessary for morphine to exert its effect, and in such situations a large intraventricular dose of 5-HT temporarily overcomes morphine tolerance and restores its antinociceptive effect. Thus, in terms of Collier's suggestion, the injected 5-HT may be exerting its effect by temporarily overcoming the morphine block.

Morphine, besides having analgesic actions in man also has euphoric and other side effects. Similarly nalorphine, which is analgesic in man has disturbing side effects of central origin, which render it unsuitable for clinical use (Halbach, 1968). When used in man nalorphine produces dependence similar to that produced by cyclazocine though the withdrawal symptoms produced by these agents appears to be qualitatively different from that produced by morphine (Martin & Gorodetzky, 1965). However pentazocine does not have such side effects nor does it have a strong tendency to produce dependence (Halbach, 1968). Disturbance in central 5-HT levels may be the means whereby dependence is induced and may also be the means by which the euphoric and other disturbances are induced. Thus if those narcotic agents that do not produce dependence in man, do not affect central 5-HT pathways, intraventricular injection of 5-HT may produce a simple acute test for dependence liability, since in this test both morphine and nalorphine were affected and pentazocine and naloxone (which is also without effect in man) were not. Tolerance liability and dependence liability seem to be linked and this test may therefore also produce a test for both tolerance and dependence liability. However the evidence in this report in no way ~~supports~~ confirms such a hypothesis and considerably more work needs to be carried out in this field.

It must be remembered that other factors have effects on acute tolerance. Acute tolerance is inhibited by administration of the

protein inhibitor actinomycin D (Cox, Ginsburg & Osman, 1968). More recently Cox and Osman (1970) have demonstrated that a number of protein synthesis inhibitors reduce the acquisition of acute tolerance in rats. Some of these agents were active only when given intracerebrally and their results suggested that the synthesis of new ribonucleic acid and protein in the brain is a feature of the development of tolerance to morphine in rats. However Loh, Shen and Leong Way (1969) reported that the protein synthesis inhibitor cyclohexamide blocked the increase in 5-HT synthesis they had previously reported to accompany the development of morphine tolerance in mice (Shen, Loh & Leong Way, 1968). Thus 5-HT has again been implicated in morphine tolerance.

It is possible that the effects seen in the present study are due not to direct effects of the various intracerebrally-injected agents on receptors to morphine in the brain. Instead, the effects may be due to an indirect action on other substances. For example, it has already been suggested that the anti-morphine effect of NA may be due to a general elevation in nociceptive sensitivity. A further possibility is that the effects are due in part to an action on the hypothalamo-neurohypophysial neuro-secretory system. Neuro-secretory material is formed in the perikarya of both the supraoptic and paraventricular nuclei of the hypothalamus. Acute administration of morphine causes this neurosecretory material to be more actively discharged along the axons into the posterior pituitary (Ray & Gosh, 1969). These results are in keeping with the anti-diuretic effects of morphine reported by Winter, Gaffney and Flataker (1954). In 1967, George suggested that stimulation of the adrenals by morphine was due to adrenocorticotrophic hormone (ACTH) release from the ~~posterior~~ anterior pituitary. This again would be in keeping with the results of Ray and Gosh (1969). Further, chronic administration of morphine blocks the secretion of ACTH from the ~~posterior~~ anterior

pituitary (George, 1967) and also causes a congestion of neurosecretory material in the supraoptic and paraventricular nuclei (Ray & Gosh, 1970). This is of importance since both cortisone and ACTH have marked inhibitory effects on the anti-nociceptive effects of morphine (Winter & Flataker, 1951). Further, as long ago as 1920, Lewis (see George, 1967) observed that adrenalectomised rats were four to five hundred times more sensitive to morphine than normal controls. Thus, agents affecting the neurohypophysial neuro-secretory system, and thus affecting the release of ACTH and subsequently the release of corticosterone from the adrenals, could have effects on the anti-nociceptive action of morphine. George (1967) suggested that NA released from the adrenals may be involved in the feedback mechanisms controlling the release of corticosteroids from the adrenal glands. Further work is therefore necessary to eliminate the possibility that intraventricularly administered NA and 5-HT have effects on this or similar systems. However, it does seem unlikely that interference with such a mechanism could produce such a profound and rapid alteration in the nociceptive threshold.

In chapter 7 the effects of intraventricular injections of 5-HT and NA on the anti-nociceptive effects of a salicylate were investigated. It was found that 5-HT had no significant effect but that NA reduced the anti-nociceptive effect of salicylate in the inflamed foot. The results indicated the possibility that aspirin may exert at least part of its anti-nociceptive effect via central mechanisms. This conclusion has been supported recently by Cranston, Luff, Rawlins & Rosendorf (1970), who injected and infused sodium salicylate both intravenously and intraventricularly into rabbits. They found that the salicylate when given by either route antagonised the febrile response to intravenous endotoxin. From their results they concluded that the salicylate had at least part of its anti-febrile action in the central nervous system. Although these experiments were on the effects of salicylate

on body temperature, it would be constructive to conduct similar experiments designed to study the anti-nociceptive effects of salicylate acting upon the central nervous system.

The final chapter concerned the effects on blood pressure of intraventricular injection of some of the agents used in this study. These experiments were conducted initially in the anaesthetised animal with the intention of looking for any signs of leakage from the ventricles to the peripheral circulation. The rationale behind this was that the cardiovascular system is very sensitive to even small doses of NA, and it was assumed that any leakage from the brain would cause fluctuations in the blood pressure of anaesthetised animals. That NA caused a depression in blood pressure was surprising. The effect did not appear to be an artefact though it could only be elicited once in any one anaesthetised animal and was not seen at all in conscious animals. To examine the effect further would have involved the use of a very large number of animals, and the quest was therefore abandoned. However the investigation into the effects of drugs on blood pressure confirmed that there would appear to be very little leakage of injected substances from the ventricles. Thus it is unlikely that the effects seen following intraventricular injection of the agents used in this study were due to actions on peripheral systems. Further, from the experiments performed in the conscious animal, it was demonstrated that fluctuations in blood pressure that might have upset an animal's behaviour were not caused by these injections.

POSSIBLE IMPLICATIONS

Interactions between agents having an effect on cholinergic systems and narcotic analgesia have been reported previously.

Potentiation of morphine analgesia by prostigmine has been shown in cats by Slaughter and Munsell (1940) and Abaza and Gregoire (1952). Flodmark and Wramner (1945) have demonstrated that neostigmine alone is capable of increasing the pain threshold in man. However, whether or not these potentiating effects on morphine are due to a direct action on cholinergic systems has been questioned since Komlos and Komlos-Szasz (1954) found evidence to suggest that neostigmine potentiated analgesic drugs by inhibiting those enzymes that normally inactivate the drug in the liver. However, Dundee, Nicholl and Moore (1961) have shown that both atropine and hyoscine inhibit pethidine analgesia in humans, hyoscine being considerably more potent than atropine. Also Wolff, Hardy and Goodell (1940) found clinically a combination of morphine and hyoscine to be less effective than morphine alone. Thus it would seem that drugs with cholinomimetic or anti-cholinergic activity can effect the activity of analgesic agents in man. This effect has been confirmed in the present study in rats.

It seems clear from clinical reports that the quality of analgesia produced by pentazocine is different to that produced by morphine (Fraser & Rosenberg, 1964) - indicating that perhaps of the many receptors or pathways affected by morphine, one at least is unaffected by pentazocine. Herein may be the reason why pentazocine does not produce dependence nor is subject to tolerance.

From the work in this thesis, and that of previous authors, it seems possible that cholinergic and tryptaminergic pathways may exert an inhibitory influence on the transmission of pain impulses ascending the spinal cord into and beyond the thalamus, whereas adrenergic pathways may be involved directly in the transmission (instead of inhibition) of pain sensation. The action of narcotic analgesics may be to stimulate the cholinergic and/or tryptaminergic pathways or antagonise the

adrenergic ones. It is here where a difference between the experimental properties of morphine and pentazocine arises, since neither the adrenergic nor tryptaminergic pathways appear to be affected by pentazocine. It seems possible that there is a cholinergic inhibition of the more simple (objective) pain pathways whilst the adrenergic and tryptaminergic (working in opposition to one another) are involved in the psychological reaction to pain, a factor perhaps not well controlled by pentazocine and which is the basis of the dependence and tolerance exhibited by morphine.

The recent suggestion of Martin (1970) that there may be duality in the pathways conducting pain sensation (one morphine-sensitive; one morphine-insensitive) is a satisfying one since it explains most of the known facts about both experimental and clinical dependence and tolerance, whilst the question of pathway hypertrophy is reasonably compatible with some of Collier's remarks (1965) invoking the conversion of silent to active receptors. Both theories (Collier's and Martin's) however are difficult to reconcile with some of the present experimental findings. We might in contrast postulate that in a dual system, both pathways are morphine-sensitive, only one of which is subject to hypertrophy or atrophy. Thus, objective pain sensation transmission may be suppressed by a cholinergic system activated by both morphine and pentazocine, not subject to hypertrophy nor atrophy. In contrast, a parallel system activated by an adrenergic link and inhibited by a tryptaminergic one, may be a) unaffected by pentazocine, b) exquisitely sensitive to morphine, and c) be the seat of dependence and/or tolerance through a shift in the ratio of active to silent receptors.

S E C T I O N F I V E

APPENDICES

DRUGS AND VEHICLES USED

All drugs were dissolved in normal saline except where otherwise stated. Doses quoted are in terms of free base except where the salt is quoted in full.

Acetylcholine chloride	- Sigma
Adrenaline bitartrate	- BDH
Aspirin (Acid Acetylsal B.P.)	- BDH Suspended in N. Saline using a small quantity of gum acacia.
Atropine methonitrate	- BDH
DL-DOPA	- Sigma Suspended in N. Saline, stirred before withdrawal of each dose.
Dopamine hydrochloride	- Sigma
Halothane B.P.	- I.C.I. (Fluothane) Inhalation with N_2O and O_2
Heparin	- Evans Medical
5-hydroxytryptamine creatinine sulphate complex	- Sigma
Hyoscine hydrobromide	- BDH
LSD-25	- Sandoz (Delysid)
Methysergide hydrogen-maleate	- Sandoz
Morphine hydrochloride	- BDH
Nalorphine hydrobromide	- Burroughs Welcome (Lethidrone)
Naloxone hydrochloride	- Endo Laboratories Inc.

Noradrenaline hydrochloride	-	Sigma
Oxotremorine oxalate	-	Supplied as a gift to Dr. P.S.J. Spencer by Dr. Nils Sterner of A.B. Ferrosan, Malmo, Sweden.
Pentazocine lactate	-	Winthrop
Pentobarbitone sodium	-	Abbott (Nembutal)
Phentolamine methansulphonate	-	Ciba (Rogitine)
Reserpine	-	Halewood Chemicals Limited
Tetrabenazine	-	Hoffman La-Roche Dissolved in 3 drops of lactic acid and 0.8 ml of 100% ethanol - made to half of final volume with distilled water and the pH adjusted to 4.5 by addition of aqueous solution of sodium bicarbonate - made to final volume with distilled water.

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