INTERACTIONS OF CENTRAL TRANSMITTER-SUBSTANCES WITH NARCOTIC AND NARCOTIC-ANTAGONIST ANALGESICS

A Thesis submitted by

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We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time

- from *"Little Gidding"*

T.S. Eliot.

SUMMARY

Using the technique of intracerebroventricular (i.c.v.) microinjection in conscious mice and rats, the possible involvement of putative central nervous system transmitter-substances in the anti-nociceptive effects of morphine and the narcotic-antagonist analgesics nalorphine and pentazocine has been investigated. A number of nociceptive test procedures has been utilised, in an attempt to counter the deficiencies of any one test.

The results obtained provide strong evidence in favour of a central cholinergic system being involved in the production of the anti-nociceptive effects of morphine in both the mouse and the rat. Evidence is also presented which suggests that activity in a system involving 5-hydroxy-tryptamine in the brain is important in bringing about the anti-nociceptive action of morphine, and that of the cholinomimetic agent oxotremorine.

The catecholamines, dopamine and noradrenaline, would appear to have opposing functions with regard to the activity of morphine. Injections of dopamine i.c.v. enhance the anti-nociceptive properties of morphine, whilst injections of noradrenaline i.c.v. strongly antagonise morphine's action.

The picture with regard to the narcotic-antagonist analgesics is less clear, partly because of the inadequacies of the test methods available, and partly because the two agents chosen in this group (nalorphine and pentazocine) appear to possess quite different spectra of pharmacological activity, despite being classified in the same group.

Thus pentazocine resembles morphine in that it is antagonised by naloxone, whereas nalorphine is not, whilst on the other hand nalorphine appears to resemble morphine more than does pentazocine with regard to its

interactions with central transmitters. Thus nalorphine would appear to involve a central cholinergic system, whilst this is untrue of pentazocine.

The results presented are discussed in the light of the current status of acetylcholine, 5-hydroxytryptamine, noradrenaline and dopamine as neurohumoral agents in the central nervous system.

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SECTION ONE

INTRODUCTION

INTRODUCTION

1. THE NATURE OF PAIN

Pain is an extremely complex phenomenon, involving as it does reflex components at the level of the spinal cord, conscious perception of pain at the level of the cerebral cortex, and the evokation of secondary emotional responses at a subcortical level. There is no evidence to suggest that animals perceive pain in the same way as humans do, indeed there is no way of knowing if two humans perceive pain in the same way. Sherrington (1906) devised the term 'nociceptive' to describe the structures involved in the transmission of nervous impulses arising from damaging, or noxious, stimuli. This term is particularly appropriate when referring to tests in animals, and has been used throughout this thesis in that connection.

Basically, two theories for the peripheral generation of 'painful' (in man) or 'nociceptive' (in animals) impulses are extant. Firstly, the specificity theory states that pain is a specific modality, having its own specialised nerve-endings and conducting pathways (Sweet, 1959). Secondly, the pattern theory suggests that pain impulses reach the central nervous system (CNS) as a result of intense stimulation of any peripheral nerve-endings (Sinclair, 1955). Sherrington (1906) noted a lack of specificity in cutaneous nociceptive endings, in that stimulation of these receptors resulted in a sensation of pain when mechanical, thermal, chemical or electrical stimuli were used. The intensity of each stimulus was necessarily sufficient to threaten damage to the skin, however. Sherrington thus regarded injury as the 'adequate stimulus' for the evokation of pain.

More recently, a new theory has emerged, combining some of the points from both of the earlier models (Melzack & Wall, 1965). This theory suggests that pain impulses reach the higher centres having passed through a modulating system in the spinal cord which is responsible for controlling

the amount of sensory information passed on up the cord to the brain. This theory, the 'gate control theory' (Melzack & Wall, 1968a,b) suggests that the cells of the *substantia gelatinosa* function as a gate control system which 'opens' or 'closes' depending upon the balance of activity arising in the large, myelinated 'A'-fibres and the small, unmyelinated 'C'-fibres. It is suggested that activity in the 'C'-fibres holds the gate in a relatively open position, such that ongoing impulses pass up the cord. A sudden increase in sensory input to the cord results in a relative increase in 'A'-fibre activity over 'C'-fibre activity. However, if the high-intensity stimulus continues, the 'A'-fibres adapt more readily than the 'C'-fibres, and thus the gate is held in the open position whereby the activity carried in the 'C'-fibres passes through and on up the cord. Hence the amount of activity passing to the brain from the gate is controlled by the overall intensity of the incoming stimulus and also the balance of activity in the large and small diameter fibres. Thus this system allows spatial and temporal summation of incoming impulses, pain only resulting when such summation exceeds a particular level of intensity, when the impulses pass on up the cord to the higher centres to evoke the appropriate sensation.

It is well-known that descending influences from the higher centres are capable of modifying incoming sensory information. Thus Hagbarth and Kerr (1954) demonstrated a reduction in the size of recorded volleys ascending the cord following stimulation of a dorsal root, when the bulbar and midbrain reticular formation was stimulated (see also: Lundberg, 1964). Melzack and Wall (1965) incorporated this evidence in their theory by suggesting that such modulation of sensory information at a spinal level could take place through the gate control system.

Melzack and Wall (1965) also list the order of events following sudden, unexpected damage to the skin, as follows: (i) a startle response; (ii) a flexion reflex; (iii) postural readjustment; (iv) vocalisation; (v) orientation of the head and eyes to examine the damaged area; (vi) autonomic responses (see also: Gordonoff, 1959); (vii) evokation of past experience in similar situations and prediction of consequences of the stimulation; (viii) many other patterns of behaviour, such as rubbing the damaged area, *etc*. Thus the response to a painful (or, in animals, nociceptive) stimulus is highly complex, and cannot be explained in terms of a specific pain pathway, since it is obvious that many central systems are involved in the response, even when using a fairly simple animal test. Further, experiments involving lesions in various pathways in the CNS in attempts to relieve intractable pain have almost always met with only temporary success, suggesting the presence of multiple CNS pathways capable of transmitting pain impulses (Hageman & de Grood, 1970).

When analgesic drugs are used in man, at least part of their effectiveness is due to an impairment of man's ability to attach significance to the pain, rather than to an elevation of the threshold to the painful stimulus (Batterman & Himmelsbach, 1943; Beecher, 1957). In this connection, it is of interest to note that pain may be totally ignored by the severely injured in certain circumstances where their attention is focussed elsewhere (Beecher, 1959a). In animal testing, we cannot evaluate an agent for this type of effect directly, and therefore we must restrict ourselves to the measurement of effects which a drug has on some more-easily quantifiable aspect of behaviour following a nociceptive stimulus. This aspect will be discussed more fully in the section dealing with methods of evaluation of analgesic agents.

2. TYPES OF ANALGESIC AGENT

Analgesic agents may be divided into two basic groups, the so-called strong analgesics, characterised by morphine, and the antipyretic analgesics, characterised by aspirin (see: Martin, 1963; Randall, 1963; de Stevens, 1965). The strong analgesics include those agents possessing pharmacological properties similar to those of morphine (but not necessarily being of similar chemical structure) and related tc these are those compounds possessing similar chemical structure to morphine but differing in their precise pharmacological actions (*e.g.* the narcotic-antagonist analgesics). The antipyretic analgesics include the salicylates, pyrazolone derivatives and *p*-aminophenol derivatives.

The origins of both the groups of analgesics are ancient. Thus aspirin was accepted into medicine in about 1899, but the use of willow bark, containing salicin (a glycoside) goes back to ancient medicine (Denkewalter & Tishler, 1966). Opium was first used in Arabian medicine, and its use was recorded by Paracelsus in the 16th. century (Denkewalter & Tishler, 1966). Morphine was isolated from opium by Sertümer in 1805, and the drug was accepted into medical use by 1816 (Denkewalter & Tishler, 1966).

Since this thesis is concerned with narcotic and narcotic-antagonist analgesics, it is not proposed that the history and properties of the non-narcotic agents be further reviewed. However, more information may be obtained from the works of Randall (1963) and de Stevens (1965).

Narcotic analgesics

The term 'narcotic' is derived from the Greek, meaning that the agent is capable of producing stupor. The narcotic analgesics will produce stupor at high dosage, but they relieve pain without producing sleep. The

term 'narcotic analgesic' will be restricted in this thesis, to drugs in the class characterised by morphine.

As noted earlier, morphine was the first of the narcotic analgesics, being a naturally-occurring alkaloid; its isolation was followed by that of its methyl ether, codeine, in 1832 (*c.f.* Denkewalter & Tishler, 1966). Codeine is a much weaker analgesic than morphine, and is less likely to produce tolerance and dependence (see: May & Sargent, 1965). One of the first synthetic derivatives of morphine to be produced was the diacetyl derivative (diamorphine, heroin) which was found to be several times as potent as morphine as an analgesic, and also highly susceptible to abuse (see: May & Sargent, 1965). Subsequent to this, modification of the basic structure of morphine has led to the production of many hundreds of narcotic analgesics with wide spectra of activities, and further development in this direction has led to the narcotic-antagonist series of compounds (*c.f.* Martin, 1967). At this point it should also be mentioned that there are also a number of series of compounds which possess some or all of the properties of the narcotic analgesics whilst differing considerably from morphine in their chemical structure, such as pethidine, the methadone derivatives, and the prodines (see: Hardy & Howell, 1965).

Narcotic-antagonist analgesics

This group of compounds was developed as a result of the observation by Pohl (1915) that N-allylnorcodeine would antagonise the respiratory depressant effects of morphine and heroin. Following the synthesis of the N-allyl derivative of morphine itself (N-allylnormorphine, nalorphine: McCawley *et al.*, 1941) the properties of these substances were further investigated (Unna, 1943). Subsequently, a large number of agents with more or less similar properties has been synthesised (*c.f.* Martin, 1967).

The next section will proceed to describe the pharmacological properties of the narcotic and narcotic-antagonist groups of compounds.

Further information on the chemistry of the narcotic and narcotic-antagonist analgesics may be obtained from: Hardy and Howell, 1965; Harris, 1965; Martin, 1963, 1967; May and Sargent, 1965.

3. PHARMACOLOGICAL PROPERTIES OF NARCOTIC AND NARCOTIC-ANTAGONIST ANALGESICS

(a) The narcotic analgesics

The pharmacological properties of the narcotic analgesics have been widely studied, using a number of animal species and isolated organ preparations. Since the most important actions of these compounds involve the CNS, much of the work on the actions of the drugs has been directed at the CNS effects. For the purposes of this review, morphine has been used as the prototype 'agonist' analgesic (Kosterlitz & Watt, 1968).

(i) Action on the peripheral nervous system:— It seems unlikely that morphine has any action on the peripheral structures involved in the transmission of nociceptive stimuli, unlike aspirin (Lim, 1968, 1970). Some of the side-effects of morphine are brought about by an action on the peripheral nervous system, however: thus the reduction in gut motility is brought about by an inhibition of ACh release from the postganglionic parasympathetic nerve-endings (Paton, 1957), although a central effect may also be involved in this phenomenon.

(ii) Action on the spinal cord:— Takagi *et al.* (1955) demonstrated that both polysynaptic and monosynaptic discharges elicited by electrical stimulation of the sciatic nerve of the cat were suppressed by morphine, except in animals with low spinal cord sections. They interpreted these

findings (together with those of earlier workers) as indicating that morphine suppressed spinal reflexes by stimulating descending inhibitory pathways. This interpretation is supported by the results of experiments using spinalised rodents; when nociceptive stimuli were applied to the tail, morphine was considerably less effective in suppressing the withdrawal reflex than in intact animals (Bonnycastle *et al.*, 1963; Cook & Bonnycastle, 1951; Dewey *et al.*, 1969b; Irwin *et al.*, 1951; Weller & Sulman, 1971).

(iii) Action on the brain-stem:— The possibility of an enhancement of descending inhibitory influences arising from the brain-stem has been mentioned (Takagi *et al.*, 1955). However, as Martin (1963) has pointed out, other actions on the brain-stem appear to be dependent upon the species used in the study.

Ngai (1961) has shown that morphine produces a general depression of the respiratory centre, since the response to electrical stimulation, as well as that to raised CO_2 levels, is depressed. However, in the intact animal any effect on respiration must necessarily be the result of a combination of actions (Martin, 1963).

Effects of morphine on the cardiovascular system as a whole again complicate any attempt to study its particular actions on the brain-stem cardiovascular centres, and Martin (1963) was only able to conclude from the available evidence that morphine may depress 'certain aspects of brain stem vasomotor activity'.

The miotic effect of morphine is detectable in man, dog and rabbit (Martin, 1963), and most evidence suggests that increased activity of the

parasympathetic occulomotor nerve is responsible. McCrea *et al.* (1942) have shown that the degree of miosis produced is dependent upon the amount of light incident upon the retina, suggesting that morphine acts by enhancing the normal light reflex.

(iv) Action on the hypothalamus:— Morphine produces varying effects on body temperature, depending upon the species, dose and ambient temperature (Helfrich, 1934; Sloan *et al.*, 1962), probably *via* an action on the hypothalamic temperature-regulating centres (Lomax, 1967; Lotti, George & Lomax, 1965). (This action is examined again in greater detail in Results: Chapter Thirteen.)

Morphine has a pronounced antidiuretic effect in a number of species including man, dog, rabbit, rat and mouse (Martin, 1963). This phenomenon has been examined exhaustively in the dog by de Bodo (1944) who demonstrated that an intact hypothalamo-hypophyseal system was essential for the antidiuretic effect. It is probable that this action results from an increase in ADH release brought about by an effect on the supraoptic nuclei of the hypothalamus. This action of morphine is antagonised by nalorphine in the mouse (Inturrisi & Fujimoto, 1968a).

The increased release of ACTH and gonadotrophin by morphine is thought to be at least partly due to an effect on the hypothalamus (reviewed by Martin, 1963).

(v) Action on the cerebral cortex and ascending sensory pathways:— The ability of the cortex to respond to electrical stimulation is unimpaired by morphine (Wikler, 1950). Monnier *et al.* (1967) found that pethidine depressed systems involved with cortical activation, whilst non-narcotic

analgesics (although possessing an equivalent anti-nociceptive effect against tooth-pulp stimulation) enhanced cortical activation. Monnier *et al.* (1967) concluded that suppression of activation systems was, therefore, not a fundamental process to anti-nociceptive activity. However, it may still be involved to a significant extent in the clinical analgesic action of the narcotics. Carroll and Lim (1960) suggested that morphine most easily blocked synapses in the thalamus, followed by those in the brain-stem and spinal cord at higher dosage.

Sinitsin (1961: cited in Winter, 1965) stated that narcotic analgesics did not suppress conduction of impulses (derived from stimulation of the sciatic nerve) in the classical sensory pathways. However, small doses of narcotic did reduce responses in the projection systems from the thalamus to the associative areas of the cortex and within these areas of the cortex itself. The analgesics did not, apparently, depress the reticular formation itself, but disconnected it from the afferent somatosensory pathways. The effects on the associative systems may help to explain the observations of Batterman and Himmelsbach (1943) and Beecher (1957) that the sensation of pain is not necessarily removed by the analgesic agent, but that the significance of the pain is altered.

(vi) Tolerance and dependence development:— Tolerance has been defined by Seevers and Woods (1953) as 'cellular adaption to an alien chemical environment characterised by diminished biological response'. In other words, some change takes place in the body as a result of the presence of a drug, and in turn this change means that:

(a) larger doses of the drug must be administered in order to produce the same effect as before

or (b) the same dose of drug will produce progressively smaller responses

as tolerance develops.

Physical dependence has been defined by Seevers and Deneau (1963) as 'the state of latent hyperexcitability which develops in the cells of the central nervous system of higher mammals following frequent and prolonged administration of the morphine-like analgesics, alcohol, barbiturates and other depressants'. They go on to say that physical dependence 'becomes manifest subjectively and objectively as specific symptoms and signs, the abstinence syndrome or withdrawal illness, upon abrupt termination of drug administration; or with the morphine-like analgesics by administering the specific N-allyl substituted antagonists'. Psychological dependence is characterised by an irresistable desire to continue taking a drug because of the psychological effects which the drug has. This may occur with morphine (and related drugs: heroin is particularly liable in this respect), barbiturates, ethanol, cocaine, amphetamine and nicotine (Collier, 1966). Thus morphine gives rise to tolerance, and both psychological and physical dependence (the literature on this aspect of the pharmacology of morphine is abundant: *c.f.* reviews by Isbell & Fraser, 1950; Seevers & Deneau, 1963; Seevers & Woods, 1953; Wikler, 1950).

It is of particular interest to note that tolerance develops not only to the analgesic (and anti-nociceptive) actions of morphine (Seevers & Deneau, 1963) but also to the hypothermic (Lotti, Lomax & George, 1966) and the antidiuretic effects (Inturrisi & Fujimoto, 1968b).

Possible involvement of putative CNS transmitter substances in the anti-nociceptive actions of morphine, and in the genesis of tolerance and dependence will be discussed in a later part of the Introduction (see below: 5. Central transmitter substances in analgesia and anti-nociception).

(b) The narcotic-antagonist analgesics

As briefly noted earlier, the development of the narcotic-antagonist series of compounds came about as a result of the demonstration of antagonism of the depressant effects of morphine and heroin by N-allyl-norcodeine (Pohl, 1915), and later the full evaluation of nalorphine (Unna, 1943). It was not until 1954, however, that Lasagna and Beecher (1954) recognised that nalorphine, as well as antagonising the depressant effects of morphine (Unna, 1943) and precipitating the abstinence syndrome in individuals physically dependent on morphine (Wikler *et al.*, 1953), possessed quite a potent analgesic effect itself in man. This led to the synthesis of many compounds related chemically to morphine, but with modifications in their structure which produced antagonist properties (Martin, 1967). It has been recognised that many of these antagonists possess a degree of agonist (morphine-like) activity also (Martin, 1967), and the balance of agonist to antagonist activity may be quantified by determining the kinetic parameters of the drug/receptor interaction as described by Kosterlitz and Watt (1968). This work has led to the classification of naloxone (Blumberg *et al.*, 1961; Blumberg, Dayton & Wolf, 1966) as an almost purely antagonistic compound, with very little intrinsic agonist activity. This compound is, therefore, a very useful tool in studies involving narcotic analgesics.

With these points born in mind, the pharmacological properties of the narcotic-antagonist analgesics will be reviewed.

(i) Action on the peripheral nervous system.— As mentioned above, the narcotic analgesics prevent the release of ACh from postganglionic parasympathetic nerve-endings in the transmurally-stimulated guinea-pig small

intestine (Paton, 1957). The action of the narcotic-antagonist analgesics on this preparation depends upon the balance of agonist to antagonist activity (Kosterlitz & Watt, 1968). However, these latter workers have pointed out that non-specific effects on the release of ACh from the transmurally-stimulated preparation occur at high drug concentrations, in which case both the narcotic agonists and antagonists (including naloxone) reduce the release of ACh (Kosterlitz *et al.*, 1969).

Kosterlitz and Wallis (1964) found that morphine depressed transmission in the superior cervical ganglion, and that this effect was antagonised by nalorphine. Nalorphine depressed transmission in the preganglionic nerve fibre only at concentrations much higher than those encountered in the demonstration of its antagonistic effects.

(ii) Action on the spinal cord:— Wikler and Carter (1953) reported a slight depression of the flexor and crossed extensor reflexes by nalorphine in the chronic spinal dog, but could demonstrate no dose-effect relationship. Martin (1967) concluded from further evidence (Houde *et al.*, 1951) that the narcotic-antagonists as a group possessed 'certain neurophysiological and clinical actions that are similar to those produced by barbiturates, interneurone depressants and morphine'. He also pointed out the differing ceiling effects of the narcotic-antagonists on spinal reflex activity.

Nalorphine (Wikler & Carter, 1953) and naloxone (McClane & Martin, 1967) are both capable of antagonising the actions of morphine on spinal reflexes. Naloxone also antagonises the depressant action of the narcotic-antagonist analgesic cyclazocine on the flexor reflex of the chronic spinal dog (— an action presumably due to the agonist component of cyclazocine's activity)

(McClane & Martin, 1967).

(iii) Effects on the brain-stem:— Eckenhoff *et al.* (1952) demonstrated that nalorphine is capable of depressing respiration, as well as antagonising the depressant effects of morphine on respiration (Unna, 1943). Martin (1967) has noted that similar effects have been seen with a variety of narcotic antagonists including levallorphan, N-allylnordihydrocodeinone, pentazocine and cyclazocine, in man. This action is not generally seen in other species however (see: Martin, 1967).

The effects of the narcotic-antagonists on the cardiovascular system vary considerably from species to species and depend upon the conditions under which the experiment is carried out (*e.g.* anaesthesia, intact or decerebrate preparations). Nalorphine does, however, reverse the cardiovascular effects of opiates such as morphine (Eckenhoff *et al.*, 1952; Martin & Eisenman, 1962).

The ability of nalorphine to produce miosis in man was first reported by Wikler *et al.* (1953). Naloxone does not produce miosis in man, but antagonises this effect of morphine and cyclazocine (Jasinsky *et al.*, 1967), suggesting that the miotic effect is due to the agonist component of these compounds (Martin, 1967). The effects of the narcotic-antagonists on the pupils of animals vary from species to species, and the effect within a species is not necessarily consistent (see: Martin, 1967).

The actions of the antagonists on locomotor co-ordination are variable, nalorphine having no effect whilst a number of substituted benzomorphans are active, cyclazocine being the most potent (Harris & Pierson, 1964).

(iv) Action on the hypothalamus:— Nalorphine does not alter body temperature when injected systemically in dogs (Martin & Eades, 1961) but causes a fall in man (Wikler *et al.*, 1953). The hypothermia produced by morphine in rats is antagonised and converted to hyperthermia by nalorphine, the latter agent failing to modify body temperature when given alone (Lotti, Lomax & George, 1965b).

Nalorphine is devoid of any antidiuretic action, but antagonises that of morphine, which it does by reducing the quantity of ADH released by morphine (Winter *et al.*, 1954).

(v) Action on the cerebral cortex and ascending sensory pathways:— In the dog, nalorphine produces a sleep-like EEG (as does morphine) in the absence of behavioural depression (unlike morphine) (Wikler, 1952). In the rabbit, Goldstein and Aldunate (1960) detected an increase in EEG 'electrogenesis' (total integrated EEG output) with morphine or nalorphine, both agents inducing sedation. On the other hand, levallorphan produced an EEG arousal in the unanaesthetised rabbit, and enhanced activation on stimulation of the ascending reticular formation. Morphine and levorphanol have actions which are similar to one another but opposite to those of levallorphan (Gangloff & Monnier, 1957).

Nalorphine antagonises the action of morphine on potentials evoked in the midbrain and medulla by tooth-pulp stimulation in the dog (Chin & Domino, 1961). Levallorphan antagonises the EEG changes produced by morphine in the rabbit (Gangloff & Monnier, 1957).

(vi) Tolerance and dependence development:— Initial work suggested that the antagonist analgesics were devoid of addiction liability. Thus Isbell

(1956) reported that tolerance to the dysphoric effects of nalorphine occurred, although not to a sufficiently great extent to allow high dose-levels to be attained. Abrupt withdrawal did not result in the appearance of abstinence phenomena. Cochin and Axelrod (1959), however, demonstrated cross-tolerance between nalorphine and morphine in rats which had received nalorphine chronically.

Subsequently, Martin *et al.* (1965) clearly demonstrated that tolerance to cyclazocine develops in man, and there is cross-tolerance with nalorphine. Furthermore, a definite abstinence syndrome was noted upon withdrawal of cyclazocine (Martin *et al.*, 1965). Martin and Gorodetzky (1965) have extended these findings to show that nalorphine produces tolerance, cross-tolerance to cyclazocine, and the appearance of an abstinence syndrome upon withdrawal. Jasinsky *et al.* (1967) failed to observe any signs of abstinence upon withdrawal of naloxone following chronic dosage, which led Martin (1967) to say that it is the agonist components of the antagonist analgesics which are responsible for tolerance and dependence development. This subject has been further extended recently (Eddy & Martin, 1970).

Concommitant administration of nalorphine with chronically-injected morphine has been shown to prevent or reduce the development of tolerance to morphine in anti-nociceptive tests (Orahovats *et al.*, 1953) and in the body-temperature effects of morphine (Lomax & Kirkpatrick, 1969). Similarly, dependence on morphine in the monkey is prevented by administration of levallorphan with morphine (Seevers & Deneau, 1963).

The possible mechanisms by which tolerance and dependence might occur have been widely reviewed (Cochin, 1970; Collier, 1966; Martin, 1967, 1970; Seevers & Deneau, 1962, 1963; Seevers & Woods, 1953; Wikler, 1950), and it

is not proposed to discuss these further. The neurohumoral aspects of tolerance to and dependence upon these agents will be mentioned later (Introduction, part 5).

To summarise, the narcotic-antagonist analgesics possess a mixture of agonist and antagonist properties, the agonist effects resembling those of the narcotics themselves (such as morphine) whilst the antagonistic properties will antagonise the agonist effects of morphine. Tolerance develops to the agonistic part of the activity (whether the compound is classified as an 'agonist' or an 'antagonist analgesic'), as does physical dependence. However, it may be that the agonist actions of the antagonist analgesics are exerted *via* a different receptor to those of the agonist analgesics, because the dependence which develops to the antagonist analgesics is qualitatively different from that which develops to the agonist analgesics. Naloxone is an interesting and useful compound in that it possesses practically no agonist activity, and is, therefore, a useful pharmacological tool in studies on agonist and antagonist actions of narcotic analgesic drugs.

4. EVALUATION OF NARCOTIC AND NARCOTIC-ANTAGONIST ANALGESICS

The evaluation of narcotic and narcotic-antagonist analgesics may be classified under a number of headings. Basically, they may be evaluated for their abuse potential, for their analgesic (or anti-nociceptive) effects, and for their other, unwanted pharmacological (side-) effects. Further, these properties may be investigated in both man and animals.

(a) Evaluation in man

(i) Analgesic effect:— Many of the problems associated with the quantification of subjective states have been discussed by Beecher (1959a,b).

Beecher has stressed the usefulness of the evaluation of drugs for analgesic effect in pathological states, rather than experimentally-induced pain states in healthy subjects, since the former conditions involve the concommitant changes in mental state (anxiety & *c.*) associated with clinical pain. These changes are unlikely to be associated with experimentally-induced pain in a laboratory situation. In fact, clinically-useful doses of morphine are often not associated with any increase in pain threshold (Beecher, 1957; 1959b, p. 116). The usefulness of pathological pain has been stressed by Lasagna (1964), and Keele (1959) proposed a system using pathological pain whereby a scoring system is used which takes into account the adverse side-effects of the analgesic agent in order to give an overall estimate of the drug's usefulness.

Probably the most widely-used human experimental method is the radiant heat method developed by Hardy *et al.* (1940), whereby heat from a suitable source is focussed on a blackened area of the subject's forehead, for a set interval of time (usually 3 s); this is repeated, increasing the intensity of the beam until the pain threshold is reached. The procedure is then repeated in the presence of the drug under test. The usefulness of the method has been questioned by many groups working in this field (see: Beecher, 1959).

Other experimental methods used in man include the application of mechanical pressure to the ventral surface of the calf (Dundee & Moore, 1960) or to the soft tissue in front of the Achilles tendon (Burn, 1968). The originators of these methods claim adequate sensitivity and reproducability of results.

Probably the most promising experimental technique in man is the 'submaximum effort tourniquet' method (Beecher, 1968; Smith *et al.*, 1966).

The test involves exercising an ischaemic muscle, which leads to a buildup of toxic metabolites which in turn results in the production of pain which is said to resemble pathological pain in intensity and duration. Beecher (1968) was able to obtain precise dose-effect curves with P-values of 0.001.

(ii) Dependence liability:— Much of the work on physical-dependence liability has been carried out at the Addiction Research Centre, Lexington, Kentucky (Cochin, 1968; Halbach & Eddy, 1963). The procedures employed include the following (Fraser, 1966): (a) production of subjective (euphoric) effects like those produced by morphine, or of morphine-like behaviour patterns; (b) substitution of the test drug for morphine in subjects physically dependent on morphine in order to ascertain its ability to prevent the appearance of the abstinence syndrome; (c) production of direct physical dependence to the test drug by chronic administration in an increasing dose schedule over varying periods of time (60 + days), followed by abrupt withdrawal or nalorphine- (or naloxone-) induced abstinence to ascertain the degree of physical dependence development; (d) short-term intravenous preference tests carried out on a double-blind principle. These procedures may be used to study the abuse liability of both narcotic and narcotic-antagonist analgesics (Fraser & Rosenberg, 1964; Martin & Gorodetzky, 1965).

(b) Evaluation in animals

(i) Anti-nociceptive activity:— The literature dealing with the description and evaluation of anti-nociceptive testing methods has been reviewed comprehensively and criticised fully (Beecher, 1957; Goetzl, Burrill & Ivy, 1943; Jacob, 1966; Miller, 1948). Methods in current use may be classified according to the type of noxious stimulus employed.

(a) Mechanical: one of the most frequently-used tests in this category is that of Haffner (1929), as described and developed by Bianchi and Franceschini (1954). The test uses an artery clip, with jaws enclosed in rubber, which is applied to the base of the mouse's tail. A positive nociceptive effect is recorded if the mouse turns round and attempts to remove the clip. Absence of this response denotes a positive anti-nociceptive effect. The test is usually evaluated as a quantal assay.

Eddy (1932) applied steadily-increasing pressure to the tail of a cat, noting the pressure required to elicit an escape response. A similar method was described for the rat by Green *et al.* (1951), the end-point being vocalisation or a struggle response.

A method has been described by Randall & Selitto (1957) for the detection of non-narcotic analgesics. The threshold force necessary to elicit a struggle response when applied to the yeast-inflamed hind paw of a rat is the end-point. If the rat's foot is not initially inflamed, then the method is suitable for the evaluation of narcotic analgesics (Greindl & Preat, 1971).

The 'multiple toe-pinch' test was introduced by Collier *et al.* (1961) in order to increase the quantity of information obtained from one animal — a clip is placed in turn on each toe of a guinea-pig, the number of responses elicited being a measure of the nociceptive sensitivity of the animal.

(b) Thermal: probably the two most widely-used nociceptive tests fall into this category. The first of the two, which employs conducted heat, is usually referred to as the 'hot-plate test', and was first described by Woolfe and MacDonald (1944). Numerous modifications have been made to the apparatus and to the way in which the results are analysed (Chen & Beckman,

1951; Eddy & Leimbach, 1953; Eddy *et al.*, 1950; Jacob & Blosowsky, 1959; Janssen & Jageneau, 1957).

The second of the two employs radiant heat, and was a direct development of the technique used in humans by Hardy *et al.* (1940). It was first used in animals by D'Amour and Smith (1941), when heat was focussed on to the tail of a rat. Ercoli and Lewis (1945) have applied the stimulus to an area of shaved skin on the back of a rat. The response in both cases was a twitch of the area to which the heat was applied, or a generalised struggle response. Thorp (1946) described the use of guinea-pigs (skin) and rats (skin and tail). The methods mentioned above employed a lamp as the source of radiant heat, but Davies *et al.* (1946) used a hot resistance wire near the tails of rats, as did O'Dell *et al.* (1960) with the feet of mice. Bass and Vander Brook (1952) described what was probably the most refined of the tail-flick procedures: a lamp was used as the heat source, applied to rats' tails. Timing of the response was electronic, since each animal's tail occluded a slit above a photo-cell, such that when the animal flicked its tail the photo-cell was exposed to light from the lamp and the timer was stopped.

A later variation of the tail-flick test was first described by Ben-Bassat *et al.* (1959) for mice and by Janssen *et al.* (1963) for rats. This test has been termed the 'receptacle method'. The tail of the animal is immersed in water at 55-58°C, and the time noted until the tail is vigorously removed from the water. A full evaluation of the method has been carried out by Grotto and Sulman (1967).

(c) Chemical: the injection of an irritant substance into the peritoneal cavity of animals to provide a nociceptive stimulus was introduced by Siegmund *et al.* (1957). These workers used solutions of 2-phenyl-1,4-benzoquinone (phenylquinone). Phenylquinone injected i.p. produces a

characteristic syndrome, usually referred to as 'writhing', which consists of a stretching of the abdomen together with extension of the hind limbs.

Various other irritants have been used in both the mouse and rat, histamine, 5-hydroxytryptamine (5-HT), heparin, and the histamine-releasing compounds L-1935 and 48/80, and also dilute solutions of hydrochloric acid and sodium hydroxide (Eckhardt *et al.*, 1958). Phenylquinone was said by these authors to be the most useful agent, being less likely to lead to the occurrence of false positive results. Carroll and Lim (1958) investigated the mechanism by which the writhing was produced, and suggested that it involved brain-stem centres. Hendershot and Forsaith (1959) refined the method by introducing a 'graded response' evaluation of the data. The method has been used for assay purposes in rodents (Parkes & Pickens, 1965), and it has also been used in monkeys in order to study the narcotic-antagonist analgesics in primates (Pearl, Michel & Bohnet, 1969).

Emele and Shanaman (1963) used bradykinin rather than phenylquinone, injected i.p. in mice, on the grounds that the peptide has been implicated in the peripheral mediation of pain sensation following tissue injury. The syndrome is susceptible to both narcotic and anti-inflammatory analgesics. Burns *et al.* (1968) gave two injections of bradykinin i.p., 15 min apart, claiming that a more constant control response was thus obtained.

A further modifiaction of this method is that of Helfer and Jaques (1968), who have substituted arachidonic acid for phenylquinone. This substance may be injected more than once in the same animal, and no tachyphylaxis is observed, enabling time-course experiments to be carried out utilising the same animals throughout (Helfer & Jaques, 1970).

Braun *et al.* (1961) first reported the use of bradykinin injected

intra-arterially (i.a.) as a nociceptive stimulus in dogs. Lim *et al.* (1964) used this technique in cross-perfused pairs of dogs in order to demonstrate the peripheral action of aspirin.

Deffenu *et al.* (1966) injected bradykinin i.a. in rats, *via* an indwelling cannula in a carotid artery. This produced a characteristic rotation of the head to the injected side and elevation of the ipsilateral foreleg — a test which has been used to investigate the actions of the narcotic-antagonist analgesics (Blane, 1967; Botha *et al.*, 1969).

Two tests which employ chemical nociceptive agents may be further classified under the heading of 'functional impairment'. Pardo and Rodriguez (1968) injected formaldehyde solution into the knee joint of dogs, and then proceeded to measure the extent to which this impaired the use of the limb (by attaching a strain-gauge to the ankle joint). Langford *et al.* (1972) injected a yeast suspension into the hind feet of mice, and then used the reduction in spontaneous locomotor activity as the nociceptive effect.

(d) Electrical: Koll and Reffert (1938) were the first to employ electrical stimulation of the tooth-pulp of the dog as a nociceptive test. Goetzl *et al.* (1943) recommended this method, having reviewed the methods then available. The method has since been widely used in the dog and the guinea-pig (see: Radouco-Thomas *et al.*, 1967).

Electrical stimuli have been applied *via* electrodes to various parts of animals' bodies, including the rat scrotal sac (Macht & Macht, 1940) and the mouse tail (Grewal, 1952; Leslie & Nunn, 1968; Perrine *et al.*, 1972), in both cases the end-point usually being vocalisation. Other points of application include the rectum of the rat (Gibson *et al.*, 1955, whose second electrode was the cage floor; Collins *et al.*, 1964, whose second electrode

was placed s.c. in the tail). Again, vocalisation was the usual end-point in these tests.

Other tests involving electrical stimuli rely on some type of behavioural response as the end-point. Thus Garroll and Lim (1960) stimulated unrestrained rats with tail-electrodes, and noted three thresholds appearing as the shock intensity increased: first, movements of the hind quarters and tail; second, vocalisation during the stimulus; third, vocalisation which continued after cessation of the stimulus ('vocalisation after-discharge'). A similar test has been used by Charpentier (1962, 1968). The method has been further discussed with regard to its use in testing narcotic-antagonist analgesics by Hoffmeister and Kroneberg (1966), who suggested that vocalisation after-discharge is the result of activity in an affective reaction system (since it requires an intact thalamo-hypothalamo-rhinencephalic system) and may be analagous to the affective pain reaction in man.

The 'flinch-jump' test of Evans (1961, 1962) utilises a cage with a grid floor, through which rats are shocked electrically. On increasing the shock intensity, the rats first show a 'flinch' or startle reaction, followed at higher intensity by a 'jump' reaction, the latter presumably denoting aversion to the stimulus. Narcotic analgesics (and some non-analgesics) raise the 'jump' threshold whilst leaving the 'flinch' threshold intact.

Weiss and Laties (1958) shocked rats through the floor of their cages with steadily increasing current intensity. The rats were trained to press a lever, such that the intensity of the shock was reduced by a preset step each time the lever was pressed. The rats were thus able to maintain the shock intensity at a comfortable level. Administration of an analgesic

agent raised the intensity at which the animals maintained the stimulus. A similar procedure has been developed for use with monkeys (Weiss & Laties, 1964).

The efficacy of these methods will be discussed more fully in that part of the General Discussion dealing with the choice of methods used in this study.

(ii) Abuse potential:— An excellent review has appeared fairly recently on this subject (Cochin, 1968), and since the phenomena of tolerance and dependence have not been specifically studied in this thesis, it is not intended that this should be a comprehensive review.

It has been shown that rats seek morphine, in order to alleviate an abstinence syndrome arising after withdrawal from a regular chronic dosage schedule (Nichols *et al.*, 1956). This study, and many like it, relied on the animals drinking solutions of morphine or other drugs in preference to water. Later investigations have made use of operant-conditioning techniques whereby rats are trained to press a lever in order to obtain an intravenous (i.v.) injection of morphine, and the animals therefore control their own intake of morphine (Weeks, 1961, 1962; Weeks & Collins, 1964). A similar system has been used with monkeys (Weeks, 1964). The rate of self-injection is thought to indicate both a need to prevent the appearance of the abstinence syndrome (— an aversive situation) and also that the animal experiences a pleasant sensation (— a reward situation) which reinforces the lever-pressing (Cochin, 1968). This latter case is supported by the fact that rats abstinent for three weeks (at which stage the abstinence syndrome would have subsided completely) will drink morphine solution in preference to plain water (Nichols *et al.*, 1956). These studies indicate

that a degree of psychological dependence can be demonstrated in animals, and therefore new agents could be studied for dependence liability in animals.

The production of physical dependence has been studied in a number of species by giving the drug chronically on an increasing dosage regimen, then abruptly withdrawing the drug or precipitating abstinence by administering nalorphine or naloxone and observing the animal for signs of abstinence. Dependence has been demonstrated in the mouse following implantation of a morphine pellet (Maggiolo & Huidobro, 1961; Huidobro & Maggiolo, 1961; review of their work by Huidobro, 1967).

Tolerance to and dependence upon morphine in the rat were quantified by Martin *et al.* (1963). Nalorphine-induced abstinence in physically-dependent rats was studied by Kaymackalan and Woods (1956), both these studies involving chronic administration of the narcotic agent. Physical dependence and abstinence have been demonstrated in other species using similar methods of dependence production. Thus the rabbit and guinea-pig have been employed, but their relative expense, and the difficulty of nociceptive testing in these species render them less suitable than the rat for routine evaluation (Cochin, 1968).

The abstinence syndrome was demonstrated many years ago in the dog following the withdrawal of chronically-administered morphine (Eddy & Reid, 1934; Plant & Pierce, 1928). Martin and Eades (1961) devised a method whereby tolerance and dependence could be induced in dogs by infusing morphine intravenously over a period of 8 h, monitoring the degree of physical dependence by injecting challenge doses of nalorphine. This method was later adapted for use in the rat (Cox *et al.*, 1968).

The chronic spinal dog has been used to study the development of tolerance and dependence to the effects of narcotics on spinal reflexes (Martin & Eades, 1961). As has been pointed out by Cochin (1968), however, the difficulty in preparation and maintainence of these animals has precluded the wide use of the technique.

Monkeys have been used extensively to test new drugs for dependence liability, because of the predictive value with respect to man (Cochin, 1968). The methods used have been described by Dereau and Seevers (1964), and closely parallel those used in man (see: Cochin, 1968). The comparative pharmacology of morphine has been reviewed by Maynert (1967b). The techniques outlined above may be used to study the tolerance and dependence produced by both the narcotic and the narcotic-antagonist analgesics (Cochin, 1968; Cochin & Axelrod, 1959).

5. CENTRAL TRANSMITTER SUBSTANCES IN ANALGESIA AND ANTI-NOCICEPTION

(a) Acetylcholine

The possible involvement of a cholinergic system in the anti-nociceptive or analgesic effect of morphine was suggested several years before the presence of acetylcholine (ACh) in the CNS was demonstrated. Thus early work in this field was specifically aimed at demonstrating a peripheral mechanism of action. However, the research carried out prior to the implication of ACh as a central transmitter substance is worthy of review here. The rôle of ACh as a CNS transmitter substance has been reviewed extensively (see: Bradley, 1968; Gaddum, 1962; Hebb, 1970; McLennan, 1965).

Slaughter and Munsell (1940) demonstrated a potentiation of morphine's anti-nociceptive effect with neostigmine in the cat, using the tail-pressure

test of Eddy (1932). Atropine antagonised the action of morphine alone and in combination with neostigmine (Slaughter & Munsell, 1940). Similar results were obtained in the mouse with morphine and neostigmine, using the 'Straub tail' as the index of effect (Wramner, 1945).

In humans, using a modified Hardy-wolff-Goodell technique, Flodmark and Wramner (1945) potentiated and prolonged the analgesic effect of morphine with neostigmine. These observations were confirmed clinically by Slaughter (1950) who advocated the use of morphine/neostigmine combinations to combat severe pain. Further evidence was obtained in animals by Gordonoff (1959), who used physostigmine. Some workers have been unable to demonstrate this synergism, however, using a thermal stimulus in the guinea-pig (de Jongh, 1954), tooth-pulp stimulation in the guinea-pig (Frommel *et al.*, 1963) and electrical stimulation of the rat tail (Jóhannesson & Schou, 1963).

Morphine is capable of inhibiting cholinesterase (Bernheim & Bernheim, 1936), as are a number of other analgesic agents when tested *in vitro* (Wright & Sabine, 1943). The extent of inhibition depends upon the source of the enzyme, suggesting that this may be the reason for the selectivity of morphine for particular physiological systems. Investigations using a number of potent cholinesterase inhibitors have led to the suggestion, however, that cholinesterase inhibition does not play an important part in morphine's effect (Knoll *et al.*, 1951; Saxena, 1958; Szerb, 1957). Schaumann (1959) showed that the anti-cholinesterase activity of morphine *in vitro* was not confirmed *in vivo*, and that neostigmine was as good at potentiating morphine as physostigmine, despite not significantly inhibiting brain cholinesterase (whilst physostigmine did). Hano *et al.* (1964) detected no change in brain cholinesterase activity after single injection or chronic dosage of morphine. Dewey *et al.* (1969a) studied a number of

narcotic-antagonist analgesics and obtained no correlation between their ability to inhibit rat brain cholinesterase and their anti-nociceptive activity in that species or in man.

Knoll *et al.* (1953) suggested that neostigmine might act by displacing morphine from plasma binding sites, thus making more available to the brain. Other workers were unable to detect any difference in brain levels of morphine after neostigmine, however (Jóhannesson & Schou, 1963; Szerb & McCurdy, 1956).

Many studies have involved the measurement of the levels of endogenous ACh in the brain following acute or chronic morphine treatment. Thus large single doses of morphine in rodents produced a rise in whole-brain levels of ACh (Giarman & Pepeu, 1962; Hano *et al.*, 1964; Herken *et al.*, 1957). Tolerance to this action of morphine was seen in mice following chronic dosage (Hano *et al.*, 1964). However, Jóhannesson and Long (1964) could detect no change in rat brain ACh levels following acute or chronic morphine administration. Crossland and Slater (1968) noted a rise in 'bound' ACh and a fall in 'free' ACh in rat brain after morphine (classified according to the ease of extract from brain homogenates), the total quantity being elevated. On the other hand, Howes *et al.* (1969) reported that neither morphine nor the antagonist analgesics studied produced any clear-cut effects on ACh levels, whilst oxotremorine did increase the 'bound' ACh in a dose-dependent manner which correlated well with its activity in the tail-flick test.

Many workers have studied the release of ACh from brain, both *in vivo* and *in vitro*. *In vivo*, ACh release from the perfused lateral ventricle of the cat is suppressed by perfusing morphine, or by injecting it i.v. (Beleslin & Polak, 1965). Similar results were obtained by perfusing the

subarachnoid space at various levels up and down the cerebrospinal axis (Beleslin *et al.*, 1965). Jhamandas *et al.* (1970) measured ACh output from the cortex of cat cerebrum, and this was depressed by morphine. Nalorphine was found to be inactive alone, and to antagonise morphine, as was naloxone. Studying the release of ACh from cortical slices *in vitro*, morphine again suppressed release (Maynert, 1967a; Sharkawi & Schulman, 1969). Howes *et al.* (1970) reported that nalorphine, pentazocine and naloxone acted in a similar manner to morphine in reducing the synthesis of ACh in mouse cerebral cortex slices, whereas cyclazocine and oxotremorine increased it. It was concluded that the effects of these agents on brain ACh turnover were unrelated to their anti-nociceptive properties.

The locus of action of a possible central cholinergic component in anti-nociceptive activity has been studied. Thus various cholinomimetic agents have been shown to possess anti-nociceptive activity when injected *via* the intracerebroventricular (i.c.v.) route (Andreas & Oelssner, 1969; Metyš *et al.*, 1969a,b; Oelssner & Andreas, 1969) and directly into various areas of the brain substance (Andreas & Staib, 1971) although Tsou and Jang (1964) failed to detect an anti-nociceptive effect following i.c.v. injection of physostigmine in the rabbit. Dewey *et al.* (1969b) have localised the site of action of cholinergic anti-nociceptive agents to a supraspinal area in the mouse.

Thus the possible involvement of central cholinergic mechanisms in the anti-nociceptive activities of narcotic and narcotic-antagonist analgesics has been investigated using a number of techniques. All of the results point to the probability that a central cholinergic system is involved in the production of the anti-nociceptive effect, though whether this system is directly concerned with narcotic analgesic anti-nociceptive activity,

or whether it is simply a parallel system to that acted upon by the narcotics, is not clear (Ireson, 1970).

(b) The catecholamines

The presence of catecholamines in the brain was first demonstrated by von Euler (1946). Vogt (1954) subsequently mapped out the distribution of 'sympathin' and showed it to be uneven. The presence of dopamine (DA) was suggested by Montagu (1957), subsequently to be proved independantly by both von Euler (1958) and by Carlsson *et al.* (1958). The distribution of DA also was shown to be uneven (Bertler & Rosengren, 1959). The presence of the catecholamines in the CNS has been reviewed comprehensively (Bertler & Rosengren, 1959; Brodie *et al.*, 1959; Carlsson, 1959; Vogt, 1959). The possible rôle of these substances as synaptic transmitters within the CNS has also received considerable discussion (Bradley, 1968; Crossland, 1960, 1968; Hebb, 1970; McLennan, 1965). The CNS neurone systems containing catecholamines have been mapped out by Ungerstedt (1971).

However, prior to the discovery of catecholamines in the CNS, a possible peripheral involvement of adrenaline in morphine's activity was investigated, after Houssay *et al.* (1928) had demonstrated that morphine could release adrenaline from the adrenal medulla. Thus it was shown that the adrenal component of the anti-nociceptive activity of morphine was considerable (Friend & Harris, 1948; Gross *et al.*, 1948) and that catecholamines administered peripherally were anti-nociceptive (Colville & Chaplin, 1964; Contreras & Tamayo, 1966; Radouco-Thomas *et al.*, 1957) or potentiated the activity of morphine and other narcotic analgesics (Hurst & Davies, 1950; Radouco-Thomas *et al.*, 1957; Sigg *et al.*, 1958).

In man, the sympathomimetic amine amphetamine enhanced the effect of

morphine (Goetzl *et al.*, 1944; Ivy *et al.*, 1944) and of pethidine (Nickerson & Goodman, 1947).

Some reports, however, disagree with these findings, both in animal studies (Frommel *et al.*, 1963; Milošević, 1955) and in man (Isbell, cited by Wikler, 1950), the latter reporting a 'precipitous fall' in the nociceptive threshold when adrenaline was injected at the time of morphine's peak activity in the Hardy-Wolff-Goodell procedure.

The demonstration by Vogt (1954) that a number of centrally-acting drugs, including morphine, could lower brain levels of 'sympathin' stimulated many investigations into the way in which narcotic analgesics modify brain levels of the catecholamines, in the hope that more information might be gained as to the mode of action of the narcotic agents. If the catecholamines are present as synaptic transmitter-substances in the CNS, then interference with their synthesis, release or metabolism by drugs might offer an explanation for the effects of those drugs on brain function.

Many workers, using a variety of animal species, have reported reductions in whole-brain levels of noradrenaline (NA) following single (usually large) doses of morphine (Gunne, 1959, 1963; Quinn, Brodie & Shore, 1958; Segal & Deneau, 1962). However, other groups have failed to detect a change in brain NA levels following injection of single doses of morphine (Maynert, 1967a) in species other than the cat. Again, the effects of chronic morphine administration on brain catecholamines are variable. Thus Gunne (1959), using rats, reported that tolerance developed to the amine-depleting action of morphine, and that NA levels fell upon withdrawal of morphine. A similar fall upon withdrawal or nalorphine-induced abstinence in monkeys was noted by Segal and Deneau (1962). On the other hand, Sloan, Eisenmann *et al.* (1962) detected no change in rat brain NA during chronic

low-dose morphine treatment and withdrawal, when compared with control rats injected daily with saline. This result was substantiated by Maynert and Klingman (1962), using rats, rabbits and dogs. Chronic high dosage, however, raised brain NA levels above controls, and this increase was maintained during continuous morphine treatment, falling again upon nalorphine-induced abstinence only in animals displaying an excitatory abstinence syndrome (Maynert & Klingman, 1962). Sloan *et al.* (1963) obtained somewhat similar results, and the precipitous fall in rat brain NA upon nalorphine-induced abstinence was also noted by Maynert (1967a) in rats.

Thus there is much conflicting evidence concerning the effect of acute and chronic morphine administration on the metabolism of brain amines in animals. Gunne (1963) has reviewed the evidence up to that point. Laverty and Sharman (1965) examined the effects of a number of drugs on catecholamine levels in a number of species, and concluded that there was no simple correlation between the biochemical and behavioural effects of the drugs they tested. In a later study comparing morphine and levorphanol in rats, Akera and Brody (1968) concluded that alterations in brain levels of NA were peculiar to morphine itself, and played no part in the development of tolerance and dependence.

Rather than relying on the measurement of brain levels of catecholamines, many workers attempted to measure turnover-rates, since an increase in turnover is not necessarily reflected in changes in the concentration of that agent (if utilisation increases to keep pace with increased synthesis, or *vice versa*). Thus Gunne (1961, 1962, 1963) reported increased urinary excretion of adrenaline and NA during the first few days of chronic morphine treatment in rats and dogs, after which normal levels were resumed, only to

be elevated upon nalorphine-induced abstinence (only urinary adrenaline levels were increased by nalorphine in the chronically-morphinised dog: Gunne, 1962). More recently, using labelled ^{3}H-NA, Neal (1968) was unable to detect any change in brain NA turnover in rats during chronic administration and then withdrawal of morphine. Presumably this latter method of estimating NA turnover in brain is rather more precise than those involving measurement of urinary levels of NA and adrenaline, which must owe much of their content to adrenaline of adrenal medullary origin.

Brain dopamine (DA) seems to have received rather less attention than NA in the earlier studies. A fall in brain DA was detected in abstinent dogs only when the symptoms were "moderate or severe" (Gunne, 1963). No change in DA levels in the cat or rabbit were detectable upon acute morphine administration (Laverty & Sharman, 1965). However, more recent work has suggested that changes in brain DA turnover do occur: single doses of morphine (20.0 mg/kg) reduced the DA content of mouse brain (Takagi & Nakama, 1966). By inhibiting synthesis of brain catecholamines with H 44/68 (α-methyl-*p*-tyrosine methylester) Gunne *et al.* (1969) detected an accelerated depletion of DA following single morphine injections in rats, which they interpreted as an elevation in activity within the ascending DA neurone systems. Abrupt withdrawal resulted in decreased DA turnover, but an increased NA turnover, interpreted as an effect of stress, since all parts of the brain were involved (Gunne *et al.*, 1969). Brain levels of DA metabolites were measured by Fukui and Takagi (1972), and they concluded that an increase in activity in descending DA neurones as a result of morphine's effect increased brain levels of the metabolites. Nalorphine prevented this effect of morphine.

A second approach to the study of the involvement of brain

catecholamines in narcotic anti-nociceptive activity has been to modify the functional state of the amines by means of drugs and see if this in any way modifies the activity of the anti-nociceptive agent. In this context, reserpine was one of the first drugs to be used in this way. It causes the release and depletion of both catecholamines and 5-HT in the brain and in the periphery (Shore *et al.*, 1957a,b; review by Shore, 1962).

The antagonism of morphine's anti-nociceptive effect was first noted by Schneider (1954) using the tail-flick test in mice. The ability of reserpine to antagonise the anti-nociceptive action of morphine (and other narcotic analgesics) has since been reported by many workers, using a number of animal species, and a variety of testing methods (Colville & Chaplin, 1964; Contreras & Tamayo, 1966, 1967; Dewey *et al.*, 1968; Fennessy & Lee, 1970; Herold & Cahn, 1968; Medaković & Banić, 1963, 1964; Muñoz & Paeile, 1967; Radouco-Thomas *et al.*, 1957, 1959, 1967; Ross & Ashford, 1967; Schaumann, 1958; Schaumann & Kurbjuweit, 1961; Sparkes & Spencer, 1969, 1971; Verri *et al.*, 1967, 1968; Witkin *et al.*, 1960). Reserpine is also capable of antagonising other actions of morphine, such as psychomotor stimulation (Tripod *et al.*, 1954; Tripod & Gross, 1957) and lens opacity in rodents Weinstock & Marshall, 1968). Some workers, however, reported that reserpine potentiated the anti-nociceptive activity of morphine (Dandiya & Menon, 1963; Garcia Lémé & Rocha e Silva, 1961, 1963; Maj *et al.*, 1971; Tripod & Gross, 1957) and others could detect no modification of morphine's effect (Jóhannesson & Schou, 1963; Jóhannesson & Woods, 1964).

Tetrabenazine has been used in a similar manner to reserpine. This is a synthetic reserpine-like agent, which depletes catecholamines and 5-HT in the CNS whilst leaving peripheral amines virtually intact (Quinn, Shore & Brodie, 1958, 1959). Antagonism of the anti-nociceptive effect of

morphine by tetrabenazine has been reported (Muñoz & Paeile, 1967; Sethy *et al.* 1970; Takagi *et al.*, 1964).

Reserpine and tetrabenazine both antagonise the anti-nociceptive activity of morphine (in the hands of the majority of workers), but their effects are widespread in that NA, DA and 5-HT are depleted by these compounds. Thus work has been carried out in an attempt to control the experiments further and thus identify which of the amines is involved (or most involved) in morphine's effects. The catecholamines were implicated in a number of studies. Radouco-Thomas *et al.* (1957) restored the effect of pethidine after reserpine by giving NA peripherally. Similar results were obtained by using a monoamine oxidase (MAO) inhibitor plus the catecholamine precursor di-hydroxyphenylalanine (DOPA) (Radouco-Thomas *et al.*, 1967). Similarly, Takagi *et al.* (1964) reduced the effect of tetrabenazine with DOPA. This work has been further extended (Takagi & Ishida, 1965; Takagi & Nakama, 1968). Muñoz and Paeile (1967) were unable to reverse the effect of reserpine with DOPA plus a MAO inhibitor, but did potentiate the effect of morphine in normal animals with this mixture, as did Schaumann (1958).

In recent years, a number of compounds has become available which have effects on individual amines in the CNS, rather than affecting them all. Thus α-methyl-*m*-tyrosine (α-M*m*T) initially depletes NA, DA and 5-HT by inhibiting aromatic amino-acid decarboxylase; However, DA and 5-HT levels return to normal within 24 h, whilst NA levels remain depressed despite a normal route of synthesis to NA (Hess *et al.*, 1961a). The NA depletion has been explained in terms of interference with normal NA storage mechanisms (Hess *et al.*, 1961b; Udenfriend *et al.*, 1961), probably by the formation of metaraminol (Andén, 1964).

Now, antagonism of morphine by α-M*m*T has been reported in the rat

(Contreras & Tamayo, 1966) and in the mouse (Medaković & Banić, 1963, 1964). These latter workers could detect no effect in the rat, however. Rudzik and Mennear (1965) could detect no change in morphine's effect following α-*m*T. Pretreatment with a MAO inhibitor followed by α-*m*T resulted in potentiation of morphine's effect (Medaković & Banić, 1964).

The compound α-methyl-*p*-tyrosine (α-*p*T), often used as the methyl ester (compound H 44/68), is an inhibitor of tyrosine hydroxylase, and thus produces a depletion of both DA and NA (Spector *et al.*, 1965). This compound abolished the anti-nociceptive effect of morphine in the mouse and the rabbit (Verri *et al.*, 1967, 1968), and also antagonised the 'running fit' caused by levorphanol in mice (Hollinger, 1969).

A number of studies have involved the use of α-methylDOPA, which is an inhibitor of aromatic amino-acid decarboxylase (Smith, 1959, 1960), and as such depletes DA, NA and 5-HT (Hess *et al.*, 1961a,b). It also acts as a substrate for DOPA-decarboxylase itself, and is converted to α-methylNA, which is probably stored and released instead of NA itself (*c.f.* Stone & Porter, 1966, 1967, for reviews of this subject). The α-methylNA is itself immune to MAO (Blaschko *et al.*, 1937). α-methylDOPA raises the nociceptive threshold when given alone to rats and mice (Contreras & Tamayo, 1966; Ross & Ashford, 1967) and potentiates the effect of morphine (Contreras & Tamayo, 1966; Contreras *et al.*, 1969; Ross & Ashford, 1967).

Sodium diethyldithiocarbamate (DDC), the active reduction product of disulfiram, is an inhibitor of dopamine-β-hydroxylase (Goldstein & Contrera, 1962), and reduces brain concentrations of NA to almost undetectable levels (Goldstein & Nakajima, 1966), allowing the buildup of DA in the tissues (Goldstein *et al.*, 1964). However, recent work has suggested that DDC is rather less specific than was at first thought, and may in fact be

capable of reducing brain DA by inhibiting tyrosine hydroxylase (Goodchild, 1969). In mice, DDC, in doses sufficient to produce NA depletion, potentiated morphine (Zebrowska-Lupina *et al.*, 1971). In rats, DDC potentiated morphine in the hot-plate and tail-clip tests, but at a time soon after DDC injection when brain NA and DA levels were still normal, suggesting that its potentiating effect was not related to its effect on these amines (Watanabe *et al.*, 1969).

Drugs known to block peripheral adrenergic α and β receptors have been investigated. Thus the α-blocker tolazoline antagonises morphine's anti-nociceptive effect whilst dibenamine and phenoxybenzamine (also α-blockers) are ineffective (Contreras & Tamayo, 1966, 1967), as is phentolamine (Nott, 1968). The β-blocker propranolol also reduces the effect of morphine (Heller *et al.*, 1968). The adrenergic neurone blocking drug guanethidine antagonises morphine, whilst the related drug bretylium is without effect (Contreras & Tamayo, 1966, 1967).

Thus there is considerable evidence that catecholamines are involved in some way with the anti-nociceptive activity of morphine, but much of the evidence is contradictory, and with many species of animals being used and many nociceptive tests being used, it is difficult to detect any clear pattern of exactly in what way these amines are involved.

Again, some work has been carried out in an attempt to modify the production of tolerance and dependence by interfering with the normal function of amines in the CNS. Tetrabenazine thus prevents the development of tolerance, an effect which is reversed by pretreatment with DOPA (Takagi & Kuriki, 1969). Reserpine and tetrabenazine have also been reported to enhance the severity of the nalorphine-induced abstinence syndrome (Gunne,

1965; Maggiolo & Huidobro, 1962). MAO inhibitors have been reported to attenuate the abstinence syndrome in mice (Maggiolo & Huidobro, 1962) and to enhance it in dogs (Gunne, 1965). α-MmT (depleting NA) enhanced the abstinence syndrome in dogs, as did α-methylDOPA (Gunne, 1965) whilst in mice it caused an attenuation (Huidobro *et al.*, 1963). Yet again, with α-MpT, the abstinence syndrome in mice is attenuated (Huidobro *et al.*, 1963) whilst in rats it is potentiated (Mattila *et al.*, 1968). Using disulfiram pretreatment, the nalorphine-induced abstinence syndrome in rats was intensified (Mattila *et al.*, 1968).

Thus again, manipulating amine levels certainly does affect the morphine abstinence syndrome in animals, but there is little correlation of the results, certainly between species. However, in the mouse, the depleting agents attenuate the abstinence syndrome, whilst in the dog (and possibly the rat) the syndrome is enhanced by these agents. However, if the effects of this drug are brought about by depletion of amines, it is difficult to see why an MAO inhibitor which would be expected to raise levels of endogenous amines, should have the same effect on the abstinence syndrome as the depletors. The picture thus remains complicated.

(c) 5-HT

The presence of 5-HT in mammalian brain has been demonstrated (Twarog & Page, 1953), its distribution following that of NA (Amin *et al.*, 1954). The possible role of 5-HT as a transmitter substance in the CNS has been reviewed (Hebb, 1970; McLennan, 1965) and the neurone systems containing 5-HT have been mapped out (Ungerstedt, 1971). As was the case with the catecholamines, early work was centred upon attempts to measure changes in brain levels of 5-HT associated with administration of morphine, either acutely or chronically, followed by withdrawal or nalorphine-induced

abstinence.

The majority of workers in this field could detect no change in whole-brain 5-HT in a variety of species, either with single doses of morphine, during chronic treatment with morphine, or during withdrawal or nalorphine-induced abstinence (Gunne, 1963; Maynert *et al.*, 1962; Segal & Deneau, 1962; Sloan *et al.*, 1962, 1963; Sloan, Eisenman *et al.*, 1962; Yarbrough *et al.*, 1971). However, one recent report did note a fall in whole-brain 5-HT with an anti-nociceptive dose of morphine in the phenylquinone test in mice (Lee & Fennessy, 1970).

Later studies have attempted to measure 5-HT turnover in brain, utilising a variety of methods. One of the most common methods involves the injection of a MAO inhibitor, then the accumulation of 5-HT is assessed by measuring whole-brain levels of the amine, an increase in rate of accumulation indicating increased turnover. Using this method, an increase in turnover has been shown to develop with the development of tolerance (Loh *et al.*, 1969; Shen *et al.*, 1968, 1970; Way *et al.*, 1968) using the pellet implantation technique to produce tolerance and dependence. Similar results were obtained by Maruyama *et al.* (1971) employing a similar technique. However, a number of groups have attempted to repeat these experiments using similar techniques, and could detect no change in the rate of 5-HT turnover (Marshall & Grahame-Smith, 1970, 1971; Schechter *et al.*, 1972). Cheney *et al.* (1971) used a different method of measuring 5-HT turnover — that of measuring the incorporation of radioactive tryptophan into 5-HT. Again, they could detect no increase in turnover in tolerant and dependent mice.

The use of agents which modify the normal state of 5-HT in brain began with reserpine and tetrabenazine, the effects of which have been noted previously with regard to the catecholamines. The metabolic precursor of

5-HT, 5-hydroxytryptophan (5-HTP) has been shown to possess anti-nociceptive activity alone (Contreras & Tamayo, 1967; Contreras *et al.*, 1970) to be synergistic with morphine (Contreras & Tamayo, 1967; Dewey *et al.*, 1968, 1970; Sigg *et al.*, 1958) and to be capable of restoring morphine's activity (at least partially) after reserpine (Contreras & Tamayo, 1967; Garcia Lémé & Rocha e Silva, 1963). Sparkes and Spencer (1969, 1971) have reported that the anti-nociceptive activity of morphine is restored in reserpinised rats by injecting 5-HT intracerebroventricularly (i.c.v.).

The compound *p*-chlorophenylalanine (*p*-CPA) produces a depletion of 5-HT by inhibiting the enzyme tryptophan hydroxylase (Jequier *et al.*, 1967; Koe & Weissman, 1966, Weissman, 1967). Pretreatment with *p*-CPA has resulted in an hyper-nociceptive state in animals (Harvey *et al.*, 1968; Tenen, 1967) and an antagonism of the anti-nociceptive effect of morphine (Fennessy & Lee, 1970; Major & Pleuvry, 1971; Tenen, 1968). Several workers have attempted to prevent the development of tolerance to morphine by administering *p*-CPA concommitantly, thus preventing 5-HT biosynthesis. In general, those workers who detected an increase in 5-HT turnover with tolerance development were able to prevent or reduce tolerance development by giving *p*-CPA (Maruyama *et al.*, 1971; Shen *et al.*, 1968, 1970; Way *et al.*, 1968) whilst those who could not detect an increase in 5-HT turnover did not succeed in modifying tolerance or dependence development by giving *p*-CPA (Cheney & Goldstein, 1970; Marshall & Grahame-Smith, 1970, 1971).

Some other actions of morphine are modified by *p*-CPA: thus the reduction in spontaneous locomotor activity produced by morphine in rats is converted to an increase by *p*-CPA pretreatment (Eidelberg & Schwartz, 1970) and morphine hypothermia in rats is abolished by *p*-CPA (Haubrich & Blake, 1971).

A small number of investigations has been carried out involving the placing of lesions in the brain which modify the anti-nociceptive activity of morphine. Thus Harvey *et al.* (1968) placed lesions in the median forebrain bundle, which induced hyper-nociception, and were accompanied by a decrease in 5-HT of 76% in the telencephalon. Similarly, Samanin *et al.* (1970) placed lesions in the midbrain raphé system, a procedure which reduced levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), increased the sensitivity of the animals to noxious stimuli, and reduced the anti-nociceptive effect of morphine. As an extension of this work, Samanin and Valzelli (1971) were able to render a sub-effective dose of morphine active by stimulating electrically the dorsal median raphé nucleus, a procedure which itself did not alter the nociceptive threshold. The maximal anti-nociceptive effect occurred concurrently with the maximal increase in forebrain 5-HIAA levels.

Thus although the evidence for the involvement of 5-HT in the development of tolerance and dependence is conflicting, there seems good evidence that this substance is involved in the anti-nociceptive activity of morphine. The rôle of 5-HT in the effects of morphine has recently been reviewed by Way (1972).

6. THE PROJECT

It is apparent from this review of the literature that many studies have been undertaken in an attempt to implicate one or other of the putative CNS transmitter-substances in the anti-nociceptive and other actions of morphine. It is also apparent that a variety of different ways of approaching the problem has been used, such as: measurement of changes in brain amine levels or turnover rates following morphine administration; alteration of the functional states of these amines by the use of drugs; and

also, to a small extent, the placing of lesions in central neurone systems known to contain one or other of the amines.

There is evidence to show that NA, DA, 5-HT and ACh are involved in the anti-nociceptive effect of morphine. Similarly, however, for each of the above-mentioned substances, there is evidence to suggest that there is no involvement in the anti-nociceptive activity of morphine.

The present study was undertaken to clarify the situation at present prevailing in this field. We have tried to demonstrate the similarities in the anti-nociceptive actions of a narcotic analgesic (morphine) and a cholinomimetic agent (oxotremorine) to attempt to further substantiate the theory that a central cholinergic pathway is involved in the production of the anti-nociceptive effect of the narcotic analgesics. We have also studied the similarities of two narcotic-antagonist analgesics, nalorphine and pentazocine, since it is plain that they produce a somewhat different spectrum of activity to that of morphine itself, whilst sharing the major property of being analgesic in man. With each of these four agents we have altered the state of the central nervous system with respect to one or other of the postulated neurohumoral transmitters, in some cases by the use of drugs, in others by the injection of these substances themselves into the cerebral ventricles of the animals. By using this technique, we may produce a more direct effect on a particular neurone system than by using a pharmacological manipulation of the endogenous transmitters, a method which has yielded many conflicting reports from earlier studies. (The use of this technique and its advantages and disadvantages will be discussed more fully in the General Discussion.)

SECTION TWO

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

1. ANIMALS

(a) Mice

Mice used in experiments reported in this thesis were male albino mice of the Glaxo A_2G strain, initially supplied by Fisons Ltd. (Loughborough, Leicestershire) and subsequently from our own breeding stock. The animals were 18-22 g in weight at the time of the experiments.

(b) Rats

Rats used in experiments reported in this thesis were male Wistar rats, from our own breeding stock. Rats weighing 180-200 g were used for implantation of chronic indwelling cannula guides.

2. ANIMAL HUSBANDRY

(a) Mice

Subsequent to weaning, mice were kept in groups of 30-50 in polypropylene cages (supplied by Associated Crates, Stockport) measuring 20 × 13 × 6 inches. They were allowed free access to a conventional 41B cube diet (supplied by Pilsbury's Ltd., Birmingham) and to tap water. The mice were maintained thus in the animal house until they attained the required weight of 18-22 g, when they were transferred to an air-conditioned room, the temperature of which was maintained at 21° ± 0.5°C, with a relative humidity of 50-70%. The normal light/dark cycle was maintained.

Mice remained in this room for 24 hours prior to the commencement of an experiment, at which time they were distributed randomly into groups of ten, each group being housed in a polypropylene cage measuring 11 × 8 × 5 inches. Food and water were withdrawn at this time. Experiments were performed between 0900 and 2100 hours unless otherwise stated. Each animal was used in one experiment only.

(b) Rats

Subsequent to weaning, rats were kept in groups of five in polypropylene cages measuring 20 × 13 × 6 inches. They were allowed free access to a conventional 41B cube diet and to tap water. The animals were maintained thus until reaching a weight of 180-200 g, at which point they were operated upon to implant a chronic indwelling cerebro-ventricular cannula guide. Subsequent to this procedure they were housed individually, in similar cages to those mentioned above, and transferred to the air-conditioned room.

Rats remained in this room for the whole of their experimental period, usually 6-7 weeks post-cannulation, food and water being available *ad libitum* throughout this period. Experiments were performed between 0900 and 2100 hours unless otherwise stated.

3. SUBCUTANEOUS INJECTIONS IN MICE AND RATS

Subcutaneous (s.c.) injections were carried out in both mice and rats using 26 guage hypodermic needles attached to 1.0 ml tuberculin syringes. Injections were made in to the loose skin at the back of the neck, using injection volumes of 10.0 ml/kg (mice) or 1.0 ml/kg (rats). In experiments involving more than one subcutaneous injection in each animal, the second injection was given in to the loose skin of the flank, well away from the first site, to prevent any possibility of a chemical interaction between drugs at the injection site.

4. INTRAPERITONEAL INJECTIONS IN MICE AND RATS

Intraperitoneal (i.p.) injections were carried out in both mice and rats using 26 guage hypodermic needles attached to 1.0 ml tuberculin syringes. Injections were made by inserting the needle through the anterior

peritoneal wall, parallel to but to one side of the antero-posterior midline, directing the tip of the needle towards the diaphragm, but preventing it from penetrating too deeply. Injection volumes were 10.0 ml/kg (mice) or 1.0 ml/kg (rats).

5. INTRACEREBROVENTRICULAR INJECTION IN CONSCIOUS MICE

(a) Previous methods

A number of methods have been described which permit the introduction of liquids into the cerebral ventricles of conscious mice with the minimum of stress caused to the animal. The construction of a permanently-implanted cannula guide is unnecessary in the case of the mouse, since the skull is thin and lacking in calcification at the weight-range normally employed. Methods involving injection into the lateral ventricles are based on that originally described by Haley and McCormick (1957). Later modifications suggested by other authors have attempted to improve the accuracy of the method by various means (Clark, Vivonia & Baxter, 1968), but in our view they have only served to complicate an essentially simple procedure.

(b) Procedure

Although not elucidated fully in the original publication, the placing of the injection makes use of the fact that the skull is made up of a number of separate bones, joined together along the sutures by so-called "dovetail" joints (Fig. 1). Immobilising the animal by holding the loose skin at the back of the neck, the position of the sutures may be ascertained by feeling through the skin with the tip of a hypodermic needle. Needles used for this purpose were 27 gauge × 1/8" from the tip to the shoulder of the boss. The needle was drawn lightly forward along the saggital suture until the bregma was located, at which point the tip of the needle was moved laterally 1.0 to

1.5 mm along the coronal suture. There exists here a particularly large "dovetail" joint, which is easily detected with the tip of the needle. The needle was then pushed gently through the skull, maintaining the shaft perpendicular to the skull. The injection was then carried out using a 50 µl micro-syringe (Hamilton Corp., Whittier, California, supplied by Micromeasure N.V., the Hague, Netherlands) attached to the needle. A volume of 10.0 µl was normally used. The needle was then withdrawn and the animal returned to its cage. With practise, one animal could be injected and the syringe refilled within ten seconds.

(c) Dimensional trials

The placing of the injection was checked from time to time by injecting 10.0 µl of a solution of pontamine sky blue (50.0 mg/ml, in normal saline), a dye which is bound by protein. After varying time intervals the animals were killed by decapitation under light chloroform anaesthesia. The whole brain was then removed, and a check of the point of entry of the needle made by locating the hole in the skull and the corresponding hole in the brain surface, both of which were visible to the naked eye.

The external surfaces of the brain were examined for leakage of the dye, and in almost all cases this was observed in the region of the foramena of Luschka and Magendie, which are the passages linking the 4th. ventricle with the sub-arachnoid space in the region of the cisterna magna. Hence a blue coloration would be seen over the posterior aspect of the cerebellum and the dorsal surface of the medulla. In some cases the dye could be seen staining the blood vessels on the ventral surface of the brain, particularly when periods of greater than 15-20 minutes had elapsed between injecting the dye and killing the animal.

The extent of penetration of the ventricles themselves was determined by mounting the brains on a "Pelcool" freezing microtome stage, and freezing the whole brain. The stage was mounted on a sledge microtome (M.S.E., Ltd., Crawley, Sussex.), so that serial sections of the brain could be cut. The brain was mounted such that coronal sections were obtained, the extent of dye penetration being estimated by macroscopical examination of the cut surface. Photographs of the cut surface were taken, having allowed the preparation to thaw, as this gave greater definition of grey and white areas of the brain. A 4 × orange filter was employed in order to absorb the light reflected from the blue dye, thus giving the dyed areas a good black appearance on the panchromatic film used. Figures 2 and 3 show sections cut at various levels of the brain following injection of pontamine sky blue.

In all cases where the lateral ventricle had been penetrated by the needle, the dye was located in both lateral ventricles, the 3rd ventricle, aqueduct and 4th ventricle. In cases of non-penetration of the ventricle by the needle, it was evident that the pressure of the injection made into the brain substance itself caused a disruption of the surrounding tissue such that the ventricle nearest the site of injection became ruptured and the fluid gained access to the ventricles in this way. This occurred particularly when the needle entered the skull on the mid-line (*i.e.* at the bregma instead of just lateral to it), in which case the tip of the needle would rest just below the corpus callosum. The injected fluid would then cause a separation of the tissues underlying the corpus callosum from the fibre tract itself; in this manner the solution would spread laterally, penetrating to both lateral ventricles similtaneously. However, with continued use of the method the accuracy improved to an extent such that less than 5% of the injections were misplaced when tested by this method.

6. INTRACEREBROVENTRICULAR INJECTIONS IN CONSCIOUS RATS

(a) Previous methods

Two methods for direct injection without the use of a chronic indwelling cannula guide have been described by Valzelli (1964), and by Noble, Wurtman and Axelrod (1967). In the earlier method the needle was introduced through a suture in the side of the skull, with the animal under light ether anaesthesia. This has the advantage of rapidity and simplicity, but the accuracy of the injection must be questioned, and the anaesthetic may interfere pharmacologically with the effect of drugs being studied subsequently (Thuranszky *et al.*, 1966). The latter method involves light ether anaesthesia, during which a hole is trephined in the dorsal surface of the skull, using a sharp instrument, the skin being closed over the top. Injections can then be made simply by pressing a needle through the skin and into the cranium. The disadvantage of this method is again lack of dimensional control over the injection site.

Of the methods involving implantation of an indwelling cannula guide, the cannula of Hayden, Johnson and Maickel (1966) appeared simple to construct and implant, and amenable to subsequent modification to suit individual needs. A further simplification of this method has been carried out in our laboratories (Sparkes & Spencer, 1971), and the cannula guides used in this study are similar to those described by these authors.

(b) Construction

The cannula guides were fabricated from sheet perspex 6.35 mm (1/4 inch) thick, the final dimensions of each block being 6 × 7 × 6.35 mm. Each block was drilled through the centre to accept a 27 gauge needle, and one end of this hole was enlarged to a depth of 2.0 mm to accept a 6.0 mm length of 20 gauge stainless steel tubing, leaving 4.0 mm protruding (Fig. 4). This was

secured in place with epoxy cement ("Araldite", Ciba Ltd., Duxford, Cambridge), the curing of which was aided by heating to 50°C. This resulted in the flowing of the cement round the tubing, prior to its setting, to ensure a patent air-tight joint. The other end of the block was then drilled out to a depth of 3.0 mm, the resulting cavity being filled with a cold-setting silicone rubber solution (Silescol S.R. 300, Esco Rubber Ltd., London.). This was allowed to cure for at least seven days, after which a stilette, constructed of 20 gauge stainless steel wire, was passed down the centre of the cannula guide. Prior to implantation in the animal, the stilette was withdrawn and replaced a number of times in order to remove any swarf from the lumen of the guide; after implantation it remained in position except when injections were being carried out *via* the cannula guide, being replaced immediately after the injection had been carried out.

(c) Implantation of the cannula guide

Male Wistar rats weighing 180-200 g were used. It has been found that the skull does not grow sufficiently after this stage to either dislodge the cannula guide or significantly alter the position of the various brain structures with respect to the topographical features of the skull which are used in placing the cannula guide in the correct location. The use of rats of this age has also meant that at the end of their 6-7 week experimental life they were still fairly young and amenable to handling, whereas older animals tended to become averse to handling.

(i) The rats were anaesthetised using a 2:1 mixture of nitrous oxide:oxygen, and 3.5% halothane, the latter being reduced to 1.5% to maintain adequate anaesthesia.

(ii) The animal was then secured as shown in Fig. 5, by means of ear bars similar to those used in stereotaxic instruments.

(iii) The fur was shaved from the top of the head, and the area swabbed lightly with a 1% solution of chlorhexidine gluconate in 70% industrial methylated spirits.

(iv) Under the scalp, a subcutaneous injection was made of 0.3 ml of a solution containing 1.0% procaine and 0.04% adrenaline, in distilled water. This served two purposes: to give some degree of local anaesthesia for a period after the general anaesthetic had worn off; and to inhibit bleeding in the area of the surgical procedure.

(v) Two to three minutes were allowed for the injection to diffuse into the tissues. A mid-saggital incision was then made in the scalp and in the underlying connective tissue from the eyes to the ears, 2.0 to 2.5 cm long. The skin was retracted with small weighted hooks, and the underlying tissue cleared from the skull to each side. The surface of the skull was thoroughly dried with cotton wool.

(vi) Using a No. 2 round dental burr (in a dental drill) a hole was drilled in the position described in para. 6(e).

(vii) Three holes were then drilled around this, one in the frontal bone on the ipsilateral side to the injection site, one in the parietal bone on the ipsilateral side, and one in the contralateral parietal bone. These holes were enlarged slightly with the burr in order to accept small stainless steel screws, (No. 2113; Wiseman & Co., Ltd., London), which were duly screwed into place such that they were firmly embedded in the skull, but not protruding sufficiently within the cranium to compress the dura.

(viii) The cannula guide was then lowered into position between the three screws, such that the guide needle entered the hole prepared for it in the skull, the block resting firmly on the skull. Care was taken to ensure that the needle entered the brain perpendicular to the skull

surface.

(ix) Dental acrylic cement ("Sevriton", Amalgamated Dental, Ltd., London.) was then built up around the base of the perspex block and the three anchoring screws (Fig. 6).

(x) After the cement had hardened (2-3 min), the skin was sutured around the block with surgical silk, and a prophylactic injection of sodium penicillin G (48 mg) and streptomycin (40 mg) (Crystamycin Forte, Glaxo Laboratories Ltd., Greenford) given intraperitoneally, and the animal allowed to recover from the anaesthetic.

(xi) The animals were used for experimental purposes not sooner than five days after the implantation (Fig. 6).

(d) Injections

Injections were carried out by holding the animal steady with one hand, and removing the stilette from the cannula guide with a pair of forceps. The injection cannula was constructed from a 27 gauge needle, 12.0 mm from tip to the shoulder of the boss, with an acute bevel on the point. This was attached to a 50.0 µl Hamilton microsyringe, and was inserted down the lumen of the cannula guide until the boss rested on the top surface of the rubber plug. An injection volume of 10.0 µl was then administered, the needle withdrawn and the stilette replaced. The animal was then returned to its cage.

(e) Co-ordinates

Initially, the co-ordinates employed by Hayden *et al.* (1966) were employed in placing the guide, using dividers preset to the distances required. The use of dividers was found to be cumbersome, however, and subsequently the position was estimated without recourse to instruments.

The point of entry of the cannula guide was 2.5 mm lateral and 0.9 mm caudal to the bregma. In early experiments using this site of injection, some unusual results were noted, and there was often a leak-back of injected fluid through the cannula guide after removal of the injection cannula and prior to replacement of the stilette. Subsequent examination of dissected brains following dye injection (para. 6(f), below) showed the injection site to be too far lateral, resulting in an injection into the brain tissue to one side of the ventricle. This would account for the leaking back of the injected fluid under pressure, and the apparent lack of pharmacological effect of the injected substances.

Subsequently, we decided to devise our own siting for the guide, relying on our knowledge of the shape and position of the ventricles gained from examination of dissected brains. The site chosen was exactly analagous to the injection site in the mouse (para. 5(b)), in that it lay on the coronal suture, about 2.0 mm lateral to the bregma (Fig. 7: top diagram). Again, the exact distance laterally was judged by eye. Following the adoption of this procedure, some 100 animals have been implanted, and in no case has the test injection of dye not entered the lateral ventricle at the specified point.

The top edge of the lateral ventricle lies some 5-6 mm from the top surface of the skull at this point; since the tubing on the bottom of the cannula guide projects 4.0 mm below the perspex block, there remains 1-2 mm of tissue between the tip of the guide and the ventricle. The 12.0 mm injection cannula projects below the tip of the cannula guide for a distance of 1-2 mm, which is sufficient to pass through the remaining brain tissue into the ventricular space.

(f) Dimensional trials

Correct positioning of the cannula guide was checked in all experimental animals after experiments had been completed, using a procedure similar to that adopted in mice (para. 5(c)). A trial injection of 10.0 µl of a solution containing 50.0 mg/ml pontamine sky blue in normal saline was carried out *via* the implanted cannula guide, the animal then being immediately sacrificed by decapitation under chloroform anaesthesia. The fresh brain was dissected out carefully. In nearly all cases some staining by the dye could be seen at the base of the cerebellum, indicating passage through the ventricular system and into the cistema magna *via* the foramena of Luschka and Maganedie.

The brain was mounted on the freezing stage of the microtome, and serial coronal plane sections cut in order to check the exact position of the cannula guide, and the extent of penetration of the ventricular system by the test injection. Penetration of the lateral ventricle in the position indicated in Figure 7 (lower diagram) was noted in all animals subsequent to the adoption of the revised co-ordinates mentioned earlier (para. 6(e)). The dye was distributed throughout the ventricular system, except in some cases where hydrocephalus had developed (see Results: Chapter One) when a more restricted distribution was sometimes noted. Penetration of the lateral ventricle is illustrated in Figure 8.

7. DRUGS AND VEHICLES USED

A list of drugs used, their salts, and their suppliers may be found in Appendix B, Section 5 of this thesis. All doses noted in the text refer to the base or acid drug, except LSD-25, where doses refer to the tartrate.

All agents injected *via* the i.c.v. route were dissolved in sterile,

pyrogen-free normal saline ("Sterivac", Allen & Hanbury's Ltd.). All drugs injected subcutaneously or intraperitoneally were dissolved in normal saline, with the exception of the following:

Reserpine: the solid was dissolved in 1 drop of lactic acid plus 2 drops of absolute alcohol, with the aid of warming under a hot tap. The resulting solution was diluted to final volume with distilled water, again aided by warming.

Tetrabenazine: the solid was dissolved in 2 drops of 95% ethanol plus 5 to 6 drops of N/10 hydrochloric acid. The resulting solution was then diluted with normal saline to within 5.0 ml of the required volume, and the pH adjusted to neutrality (litmus) by the addition of N/10 sodium hydroxide solution. Normal saline was then used to make up to the final volume.

Phenylquinone: a solution was prepared as detailed below in Methods, para. 9(d).

Procaine and adrenaline solution (Methods, para. 6(c)) was prepared in distilled water.

Penicillin G and streptomycin injection (Methods, para. 6(c)) was prepared in distilled water.

8. MEASUREMENT OF CORE TEMPERATURE IN CONSCIOUS MICE

The method described by Brittain and Spencer (1964) was employed. A needle-type thermocouple probe was inserted 3 to 4 cm down the oesophagus of the mouse whilst holding the animal by the loose skin at the back of the neck. The probe rested thus with the thermocouple in the region of the cardia of the stomach, to give a true reading of core temperature. The thermocouple was attached to a direct-reading electric thermometer (Light Laboratories,

Brighton) calibrated from 15° to 45°C in 0.2°C divisions. The probe was held in place until a steady reading was obtained, usually within 5 seconds of its introduction into the oesophagus.

9. MEASUREMENT OF CHANGES IN THE NOCICEPTIVE THRESHOLDS OF MICE

(a) Methods available

An outline of methods available has been given in the Introduction.

(b) The Hot-plate test

A copper can, eight inches in diameter, was suspended in a water bath such that the bottom of the can was well below the surface level of the water surrounding it in the bath. The water in the bath was maintained thermostatically at 61°C ± 0.5°C, being circulated *via* a pump to ensure an even temperature. Thus a higher plate temperature than that normally employed has been used, since as suggested by Jacob and Blosowsky (1959), this gives more reproducable control readings than the more usual 55°C or thereabouts suggested by other authors (Woolfe & MacDonald, 1944; Chen & Beckman, 1951).

The mice were placed on the copper surface of the base of the can (in pairs, to reduce the effect of the strange environment on their behaviour) and the time taken for each to exhibit a characteristic reaction — lifting the front or hind paws to lick them — was measured using a stopwatch. If the animals did not react within 30 seconds of being put on the hot-plate, they were removed, and a time of 30 seconds was recorded.

Animals were tested once only on the hot-plate, at a time interval after drug administration dependent on the time of peak activity of the drug, determined in a preliminary experiment. Single exposure to the stimulus was

favoured because on second and subsequent exposures to the hot-plate, animals tended to jump out of the can almost immediately on being placed in it (except where drugs interfered with locomotor activity to such an extent that the animals were incapable of doing so) rather than showing the usual paw-licking response. (This jumping response in mice has been previously reported by Jacob, 1966.) There would, therefore, appear to be a significant degree of learning involved in this test, and in fact certain authors have suggested that this may play a part in the apparent development of tolerance to narcotic analgesics in rats when this test is used as the nociceptive challenge (Kayan, Woods & Mitchell, 1969; Adams *et al.*, 1969). These authors make it clear that the previous exposure to the hot-plate must be in the presence of morphine, however. Conversly, Tamayo and Contreras (1970) claimed that there was no "drug-test interaction" in the hot-plate test when mice were used as experimental animals, but they did detect one in the tail-clip test.

The mean reaction times of animals in groups of ten were calculated, together with their standard errors, and significant differences between the group means were calculated using Students "t"-test. Standard errors were not calculated for groups where greater than 50% of the animals recorded 30 second reaction times✖. In experiments where ED_{50} values were calculated, the dose/response curves were plotted using quantal data. These data were obtained by measuring reaction times in the normal way, then calculating the "% anti-nociceptive effect" in each group by counting the number of animals whose reaction times exceeded a preset figure, usually 2 × the saline treated control group mean, and expressing this as a % of the total number of animals in that group. The data thus obtained were treated statistically using the method of Litchfield and Wilcoxon (1949).

✖ See note, p. 64.

(c) The Tail-flick test

The mice were placed in individual glass containers with their tail protruding, such that the tail rested in a groove on a wooden board. The heat-source used was low-voltage projector lamp with a built-in concave mirror, which focussed the light/heat on the tail of the mouse. A shutter was interposed between the lamp and the tail, enabling the stimulus to be interrupted at will, whilst maintaining the heat source at a constant temperature.

Having positioned the lamp over a mouse's tail, the shutter was opened and a stopwatch started. The shutter was closed and the watch stopped when the mouse flicked its tail out of the beam. Mice were all tested prior to any drug treatment, and the current supply to the lamp adjusted to give reaction times of between 3.5 to 4.5 seconds. In practise, once this had been adjusted to 4.5 ampéres, it required no further alteration. However, occasionally a mouse would have a control time lying outside the range quoted above, in which case it would be discarded. Following drug treatment, if any mouse had not reacted within ten seconds, the stimulus was interrupted and a reaction time of ten seconds recorded. Animals were used in groups of eight or ten, and if 50% or greater of the animals in a group exceeded this cutoff time, then no standard error could be calculated for the mean reaction time of that group.* Mean group reaction times were compared using Students "t"-test. The test was normally carried out on the same mice at intervals after drug treatment, to produce time curves of the drug effects. In experiments where dose/response curves were plotted, the individual reaction times were measured as before, then a "% anti-nociceptive effect" for each group was calculated by expressing as a percentage of the total number of animals in that group the number of animals whose reaction times had exceeded a preset figure, usually 1.5 × the control mean reaction time of the group in question

* See note, p. 64.

(Haslett, 1963). The quantal data thus obtained were subjected to treatment by the method of Litchfield and Wilcoxon (1949).

(d) The Phenylquinone test

The solution of phenylquinone was prepared by dissolving 20.0 mg of phenylquinone (2-phenyl-1,4-benzoquinone) in 4.0 ml of absolute alcohol, with the aid of warming, then diluting to a final volume of 100.0 ml with distilled water, also with the aid of warming. The solution was stored in a stoppered cylinder and kept in a closed cupboard, since it deteriorates on exposure to light and air. Solutions prepared for longer than three hours were not used. The dose injected was 0.2 ml of the above solution per 20.0 g body weight intraperitoneally, a dose of 2.0 mg/kg phenylquinone. Five mice were injected at once, then being placed in a partitioned cage such that each could be observed individually. The number of writhes each mouse made in the period 5-15 minutes post-injection was then counted by means of hand-tallies. Two such groups of five animals constituted a single drug-treated group of ten animals, for which the mean number of writhes (and the standard error of the mean) was calculated, from the individual totals. Mean figures for groups were compared using Students "t"-test.

When dose/response curves were plotted, the quantal data were obtained by expressing as a percentage the number of animals in a group of ten which did not writhe during the ten minute observation period following the phenylquinone injection. These data were subjected to analysis by the method of Litchfield and Wilcoxon (1949).

Mice were injected only once with phenylquinone, since it is known to exhibit tachyphylaxis (Helfer & Jacques, 1970). The challenge dose was given to coincide with the peak of activity of the drug under investigation, as

determined by a previous experiment.

10. MEASUREMENT OF CHANGES IN THE NOCICEPTIVE THRESHOLD OF RATS

(a) Methods available

An outline of the testing methods available in this field has been given in the Introduction.

(b) The Foot-pressure test

The method employed uses a mechanical stimulus, and is similar to that described by Randall and Selitto (1957) for detection of anti-inflammatory analgesics. The apparatus used was commercially available (The Analgesy-meter: Hugo Basile, Milan; supplied by Arnold R. Horwell, Ltd., London) and is illustrated in Figure 10. The rat was supported by the operator, such that one of the hind feet of the rat rested on the pedestal, G. The teflon cone E was then lowered gently into position on the top of the foot by allowing the arm C to pivot down about the fulcrum, F. The operator then actuated the motor, M, by pressing a foot switch. The motor drove the helically-ground shaft A, which in turn carried the weight B along the arm C, away from the fulcrum (F), the extent of travel being indicated by a pointer moving over the scale S. By virtue of its moment about the fulcrum, the moving weight exerted a steadily increasing downward force on the rat's foot, *via* the teflon cone. The rat then removed its foot by a vigorous movement, the foot slipping easily from beneath the cone. At this point the motor was stopped, and the load necessary to cause the rat to withdraw its foot calculated by reading off the distance travelled by the weight. The scale corresponds to loads of 0-250 g, but this range may be extended by the addition of extra weights to B. A cutoff load of 500 g has been employed since larger loads were found to cause permanent damage to the feet.

Due to the variation in control readings obtained from individual rats, an initial control threshold load was obtained for each rat prior to drug treatment. Following administration of the drug, the new threshold values for load were obtained at various time intervals. In order to relate these values to the individual control readings, the "Anti-nociceptive Index" (A.I.) was calculated for each reading subsequent to drug treatment, as follows (after Cox, Ginsburg & Osman, 1968):

Control load: T_0

Load after drug treatment: T_1

Cutoff load: 500 g

$$\text{Then A.I.} = \frac{T_1 - T_0}{500 - T_0}$$

An animal reaching the cutoff load would therefore have an A.I. of 1.00.

Rats were treated in groups of five, and the individual A.I. for each rat calculated at the various time intervals after drug treatment. From these, mean group A.I.'s were obtained, together with the standard errors of the means. A standard error was not calculated if three or more rats in one group had A.I.'s of 1.00*. Group means were compared using Students "t"-test.

In experiments involving dose/response curves, the threshold loads and individual A.I.'s were obtained as before, then the "% anti-nociceptive effect" for each group was calculated by expressing, as a percentage of the total number of animals in that group, the number of animals with A.I.'s greater than 0.75. The data thus obtained were subjected to treatment by the method of Litchfield and Wilcoxon (1949).

*Note: It should be pointed out that the calculation of 'standard errors' of means which include one or more cutoff values is not strictly statistically correct. Attention has been drawn to the use of such data in figures by means of an asterisk.

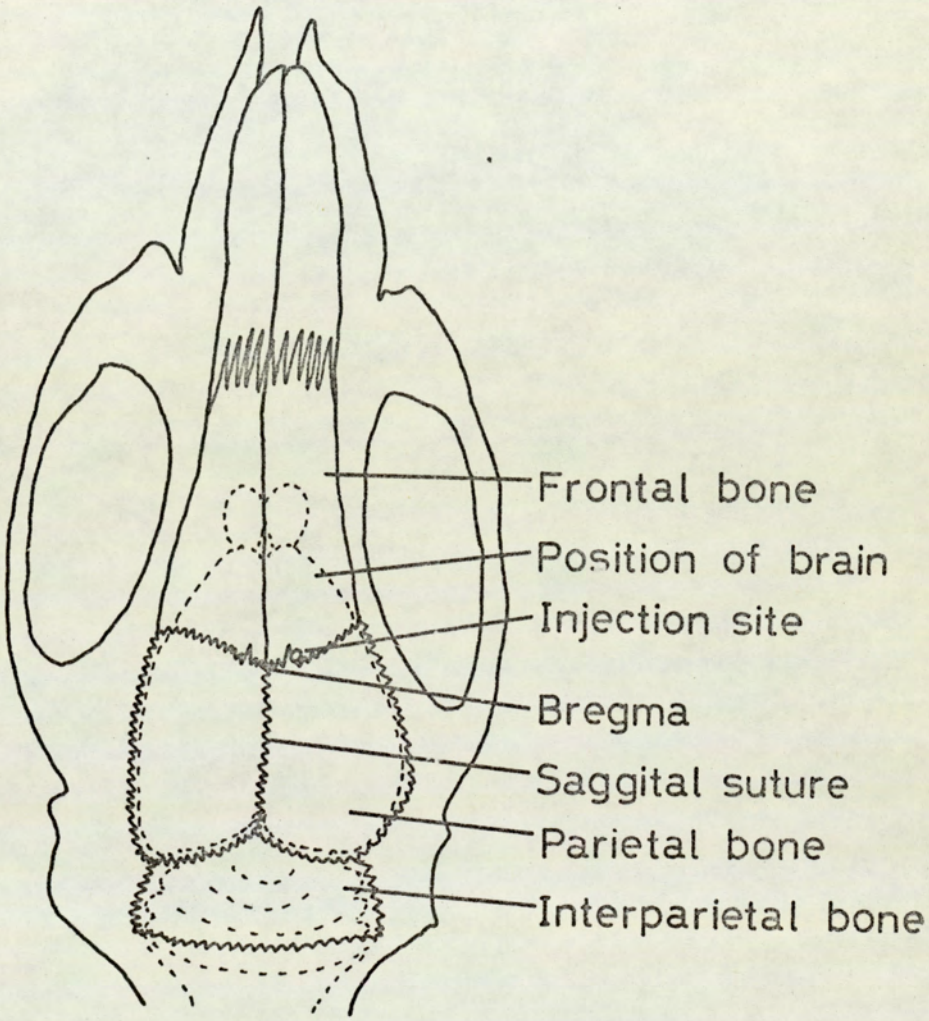

FIGURE 1: DIAGRAM OF RODENTS SKULL

Shown are the main cranial bones and sutures, and their relationship to the brain and the site of i.c.v. injection. These features are common to the rat and mouse.

FIGURE 2: PHOTOGRAPH SHOWING INJECTION TRACT IN MOUSE BRAIN

The photograph shows a fresh brain in coronal section, following injection of 10.0 µl of a 5% solution of pontamine sky blue into the right lateral ventricle. (This corresponds to the lower diagram in Fig. 7.)

(a)

(b)

(c)

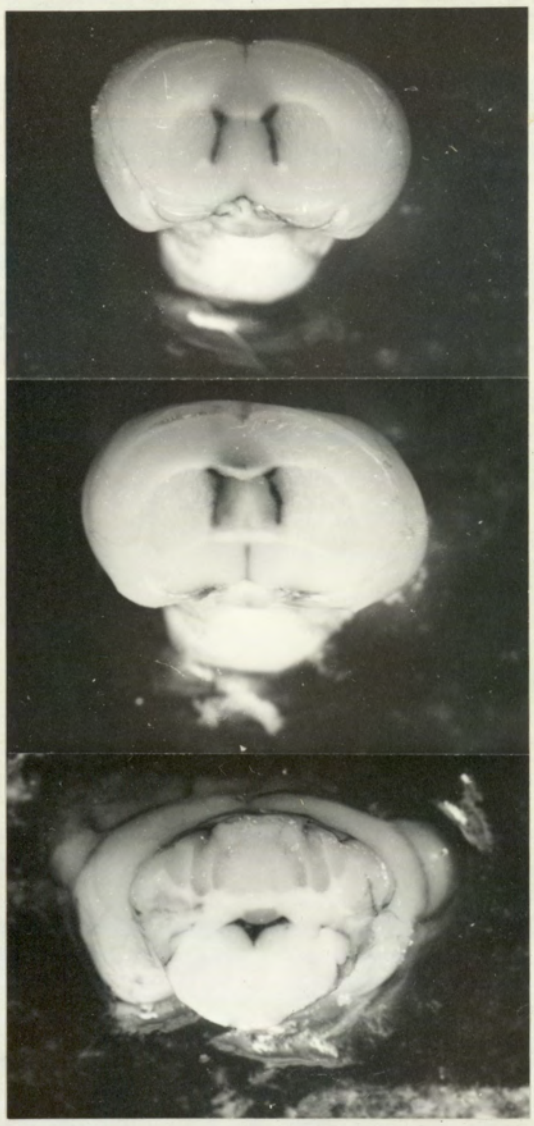

FIGURE 3: SECTIONS OF MOUSE BRAIN SHOWING DISTRIBUTION OF INJECTION

The photographs show fresh brain in coronal section, after the injection of 10.0 µl of a 5% solution of pontamine sky blue into the right lateral ventricle.

(a) Lateral ventricles rostral to injection site.

(b) Lateral ventricles and third ventricle caudal to injection site.

(c) 4th. ventricle.

Magnification × 5.

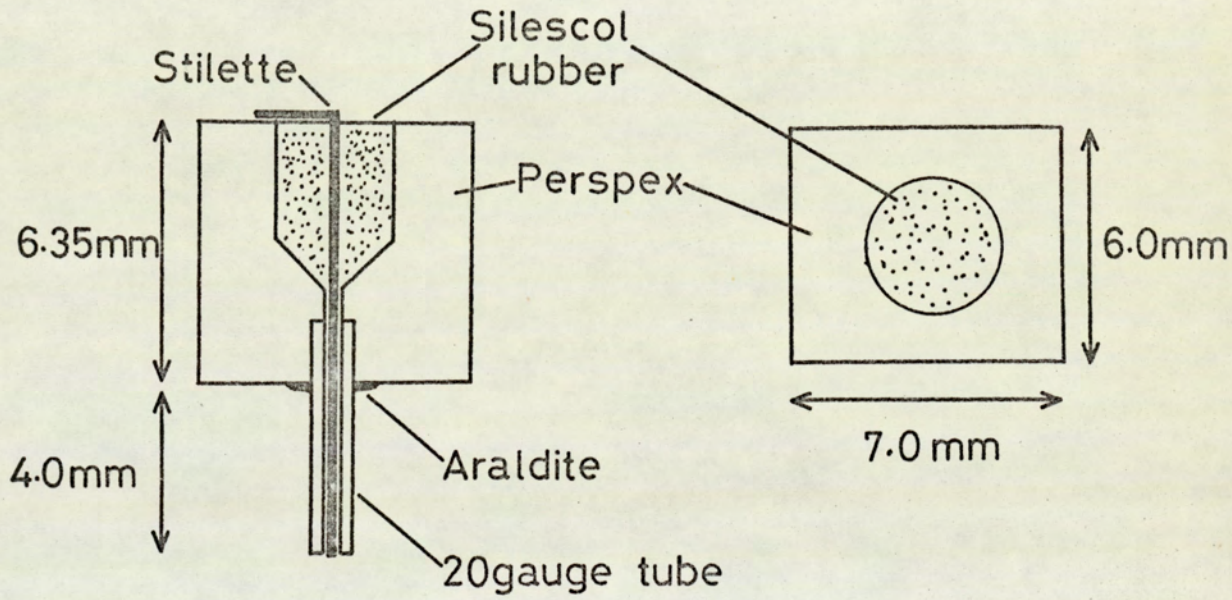

FIGURE 4: DIAGRAM OF CANNULA GUIDE USED IN RATS

Showing vertical section and plan view.

Scale: 5 mm:1 mm.

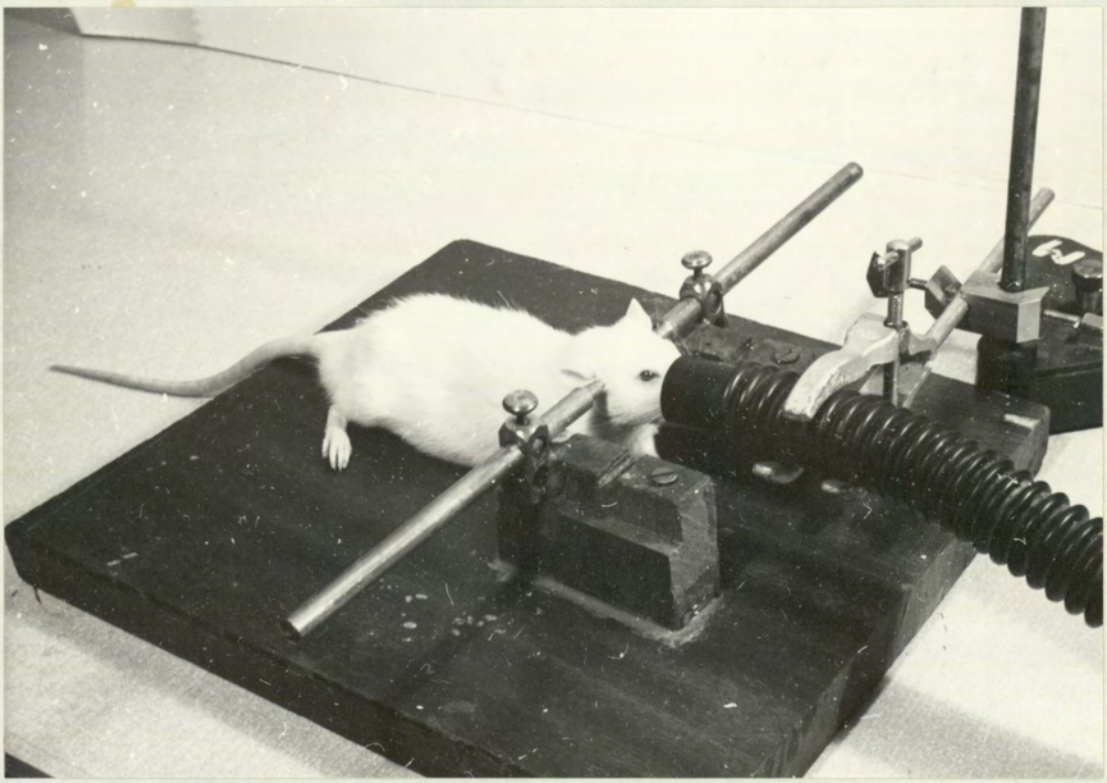

FIGURE 5: PHOTOGRAPH OF APPARATUS USED TO HOLD RATS DURING IMPLANTATION OF CANNULA GUIDES

The corrugated tube supplies the anaesthetic gases from a Boyle's apparatus.

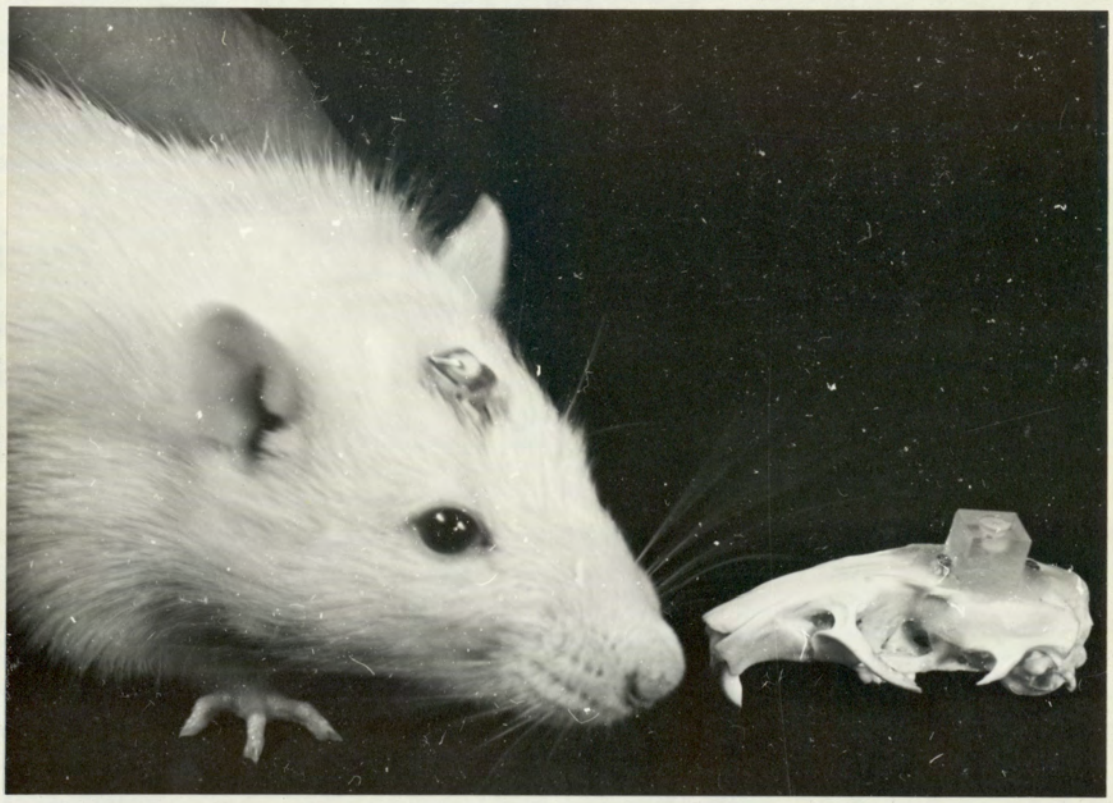

FIGURE 6: PHOTOGRAPH OF A CONSCIOUS RAT 3 WEEKS POST-CANNULATION

Also shown is a skull from a similar rat with cannula guide implanted.

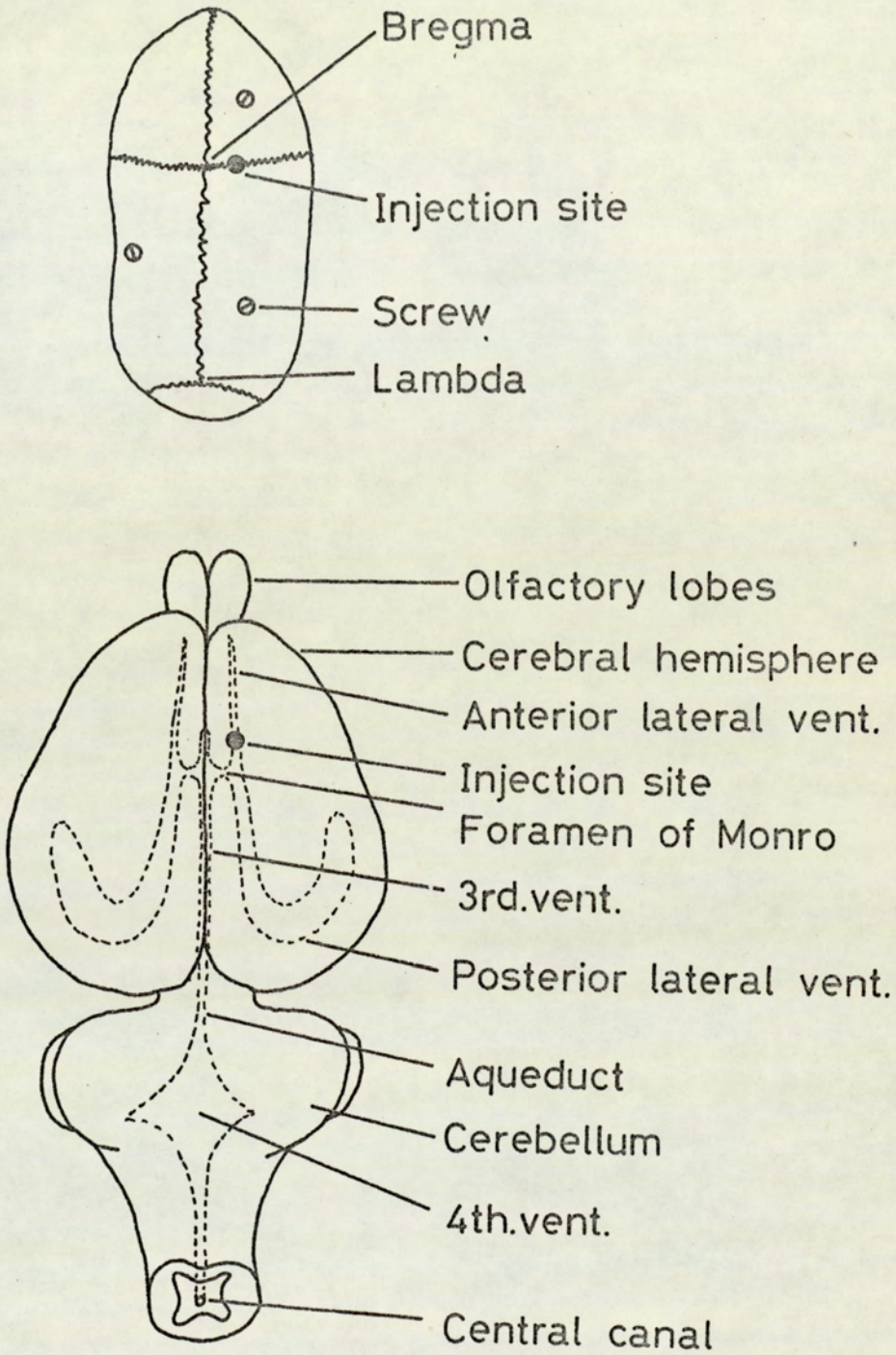

FIGURE 7: DIAGRAMS SHOWING POSITION OF I.C.V. INJECTION SITE IN THE RAT

The upper diagram shows the position of the injection site and anchoring screws with respect to the skull sutures.

The lower diagram shows the approximate configuration of the cerebral ventricular system and the point at which the injection cannula penetrates the right lateral ventricle.

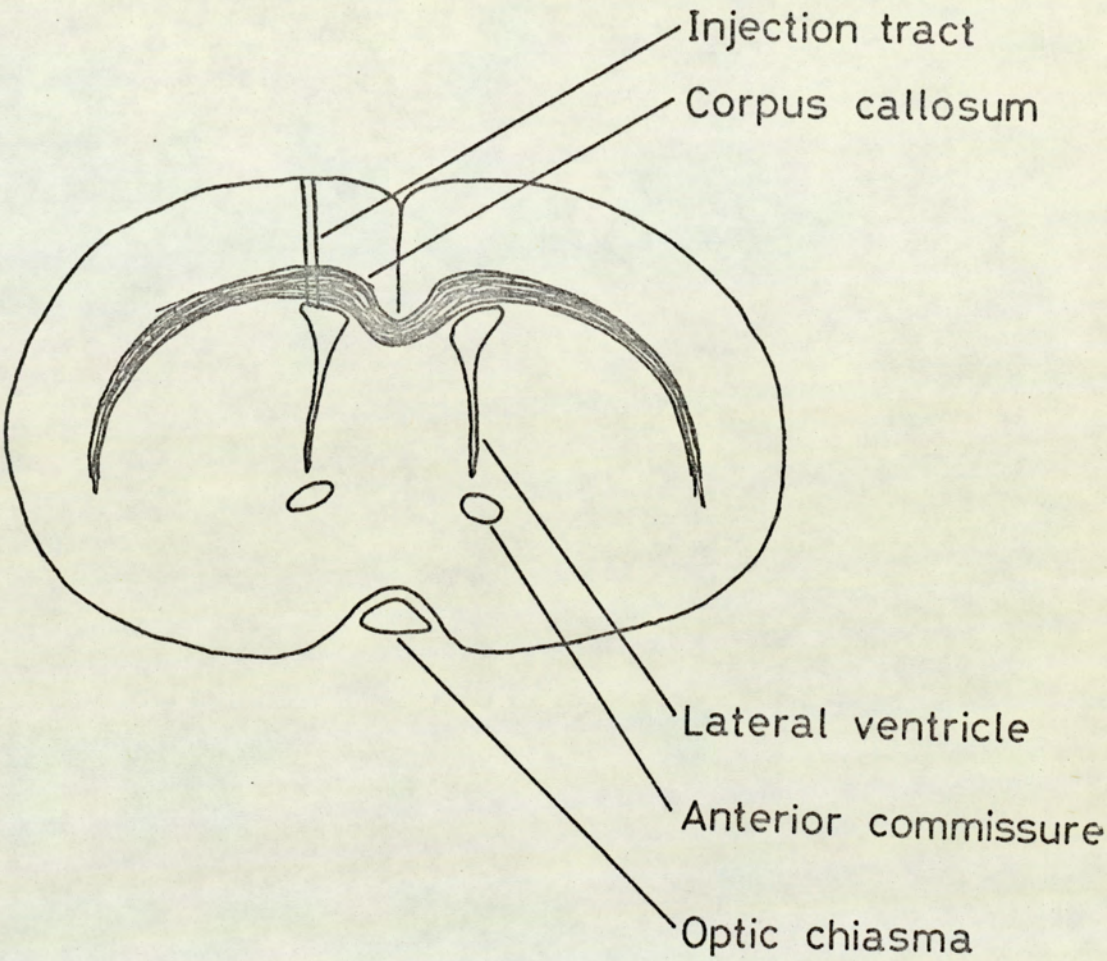

FIGURE 8: DIAGRAM OF CORONAL SECTION OF RAT BRAIN AT POSITION OF INJECTION

This site corresponds to the section shown of the mouse brain in Fig. 2.

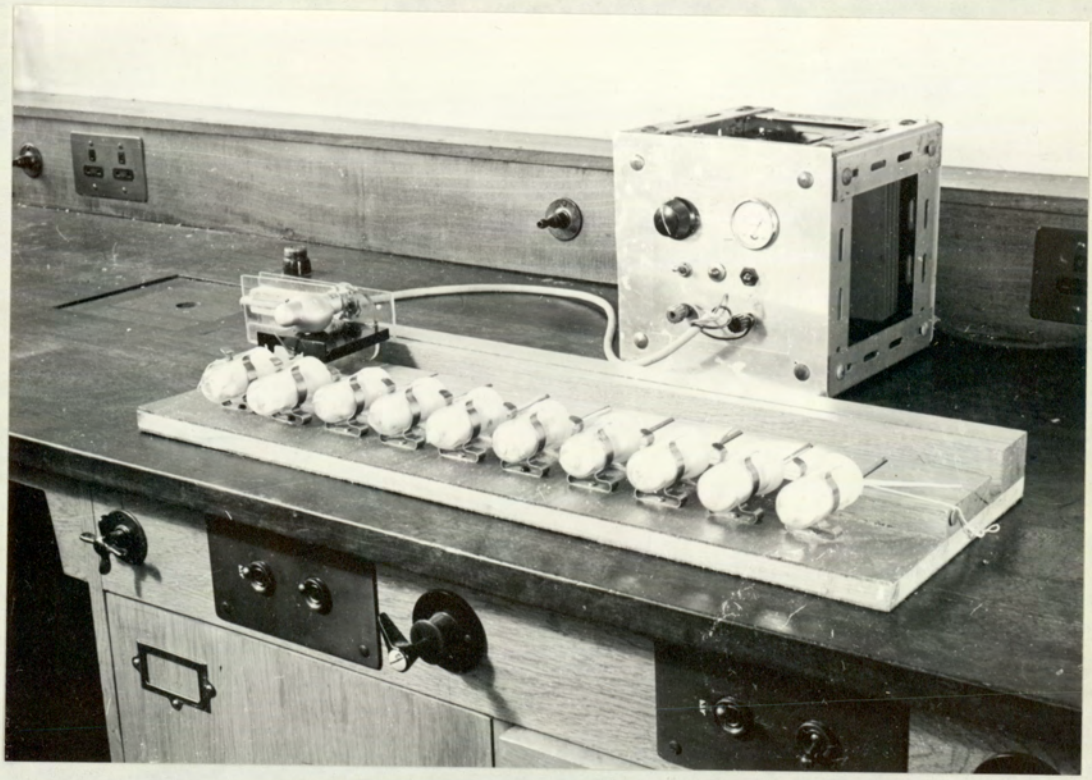

FIGURE 9: PHOTOGRAPH OF TAIL-FLICK APPARATUS USED TO MEASURE NOCICEPTIVE THRESHOLD IN MICE

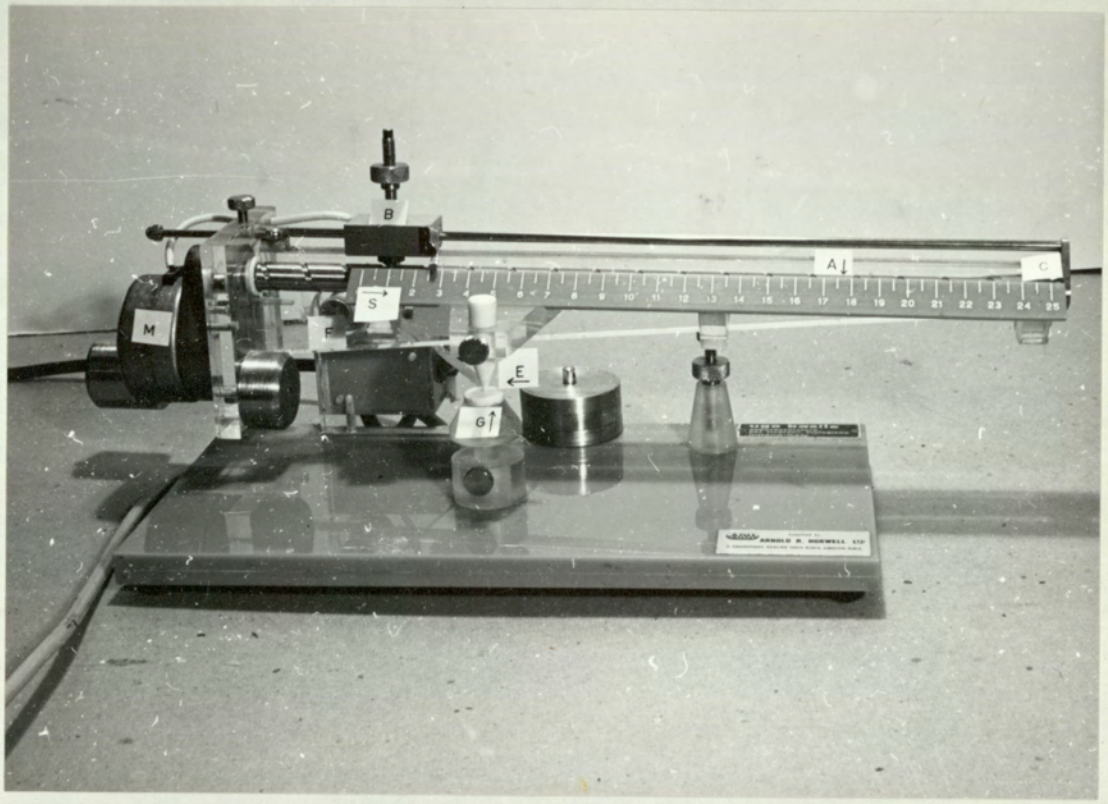

FIGURE 10: PHOTOGRAPH OF FOOT-PRESSURE APPARATUS USED TO MEASURE NOCICEPTIVE THRESHOLD IN RATS

(See text for key: para. 10(b)).

SECTION THREE

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

PART A

EFFECTS OF TECHNIQUES EMPLOYED

CHAPTER ONE

EFFECTS OF I.C.V. INJECTION TECHNIQUE

1. EFFECTS OF I.C.V. INJECTION IN CONSCIOUS MICE

(a) Effect of needle insertion

The insertion of the injection needle through the skull and into the brain substance, and subsequent withdrawal without injecting any fluid into the ventricles, was not accompanied by any obvious change in the behaviour of the animal. Mice thus treated were indistinguishable from untreated animals immediately following the experimental procedure.

(b) Effect of injection of sterile apyrogenic saline solution on behaviour

The effects of various volumes of injection have been studied, ranging from 1.0 µl to 20.0 µl (the originators of the method used volumes of 10.0 µl to 50.0 µl: Haley & McCormick, 1957). Volumes of less than 5.0 µl produced no obvious reaction in the mice. Volumes in excess of this produced a characteristic syndrome, however, which may have been brought about by the volume of the injection causing an increase in intracranial pressure.

The syndrome was characterised by an initial 'stunned' posture, with exophthalmos, lasting for one to two seconds following withdrawal of the needle. The animal then reared up on its hind legs and proceeded to vigorously wipe its muzzle with its forepaws. This movement was accompanied by vocalisation in some animals. This activity lasted for a period of two to four seconds, at the end of which the animal resumed its normal posture, although spontaneous motor activity would be absent at this stage. If the animal was nudged with the finger at this time it would move away, but then resume the former quiescent state.

The duration of this latter stage was greater the larger the volume of injection used, lasting for a maximum of three to four minutes with the 10.0 µl injection volume which was that normally employed. After this point,

injected animals could not be distinguished from untreated ones. Animals thus injected appeared to suffer no permanent damage as a result of the injection, as some of the injected animals were retained under observation in the laboratory for several weeks post-injection, and they remained apparently healthy throughout this period.

Injection of vehicles other than sterile, apyrogenic 0.9% w/v sodium chloride solution was not carried out, since this was the only vehicle used to inject drugs into the cerebral ventricles of the conscious mouse.

(c) *Effects of injection of sterile apyrogenic saline solution on nociceptive threshold*

The injection of 10.0 µl saline i.c.v. at various intervals before testing had no measurable effect on the nociceptive threshold in mice as determined by the hot plate test or the tail flick test. However, in the phenylquinone test, a significant reduction in the number of writhes elicited by a challenge dose of phenylquinone was noted when saline was injected i.c.v. at various intervals before the phenylquinone (Fig. 11). A significant reduction in the number of writhes was noted when the saline was injected up to 20 minutes before the phenylquinone (*i.e.* 30 minutes before the centre point of the ten minute counting period). This effect of the i.c.v. injection was not significant, though still apparent, when the time interval between the i.c.v. and phenylquinone injections was extended to 40 minutes, and was marginal at 60 minutes (Fig. 11).

2. EFFECTS OF IMPLANTATION OF AN I.C.V. CANNULA GUIDE INTO THE LATERAL VENTRICLE OF THE RAT

Subsequent to full recovery from the effect of the anaesthetic, implanted rats were initially hyper-irritable, and resisted handling by

struggling and by vocalisation. This effect was most noticeable during the first 24 hours following the operation, diminishing rapidly thereafter until after three to four days the rats became docile and easily handled.

It was necessary to house implanted rats individually, since they tended to be violent toward one another if housed together. This is believed to be due to interference with the perspex cannula guide by another animal during mutual grooming, leading to bouts of violence.

3. EFFECTS OF I.C.V. INJECTIONS IN CONSCIOUS RATS, USING PREVIOUSLY IMPLANTED CANNULA GUIDES

(a) Effect of insertion of injection cannula

Rats showed no sign of being aware of the insertion of the injection cannula through the cannula guide to penetrate the one to two millimetres of tissue between the tip of the guide and the ventricular cavity. Similarly, no reaction was observed on removal of the cannula and replacement of the stilette. Throughout the whole procedure the animal remained quiet and still, while being lightly restrained by one hand of the experimenter.

(b) Effect of injection of sterile apyrogenic saline solution on behaviour

The volume of injection used in all experiments was 10.0 µl, which penetrated, usually, the whole of the cerebral ventricular space, as described with respect to dye injection studies in Section 2. In normal rats, 10.0 µl of saline produced no overt changes in behaviour. Occasionally the injection was followed immediately by a short period, lasting two to four seconds, during which the rat twitched its vibrissae.

In a few cases, in rats which had been cannulated for three to four weeks and which had received i.c.v. injections twice weekly during this

period, injection of saline produced a violent reaction in the animal. This took the form of struggling and vocalisation. In many such cases there was a leakback of clear fluid from the cannula guide on withdrawal of the injection cannula. In a small proportion of rats this syndrome manifested itself at times other than during i.c.v. injection, often taking the form of violent activity within the cage, running back and forth *etc.* In other cases, animals tended to spend long periods reared up on their hind legs, as far as was possible within the confines of the cage. In more severe cases the animal would be immobile on the floor of the cage, usually on one side, being unable to gain its feet. These animals vocalised feebly when approached. In nearly all cases of the syndrome described above, persistent piloerection was noted, giving the animals a generally unhealthy appearance.

In most cases animals exhibiting any of the above symptoms were killed by decapitation under light chloroform anaesthesia. Unfortunately, this could not always be carried out at the onset of the syndrome (for instance if the development occurred rapidly over a weekend) which is why we have been able to study and describe the later stages of the syndrome.

As with all cannulated animals, these rats were each injected with 10.0 µl of pontamine sky blue before being sacrificed, their brains being removed and sectioned subsequently, as described in Section 2. In nearly all cases, animals exhibiting any of the symptoms as described above had hydrocephali, varying in size not always proportionate to the severity of the syndrome shown before death. These accumulations of fluid were always associated with the ventricles, and were almost always confined to the ipsilateral side to the placement of the cannula guide, *i.e.* the right lateral ventricle. In only a small percentage of cases was there any enlargement of other of the ventricles, in which case only the third

ventricle around the area of the foramen of Monro was affected.

The enlargement of the lateral ventricle was visible from observations of the whole brain, when the right hemisphere would be notably larger than the left. Following injection of dye the blue coloration was often visible below the cerebral cortex of the right hemisphere. This increase in space occupied by the cerebrospinal fluid (csf) often resulted in displacement of neural structures bordering the ventricle, being particularly noticeable where structures are clearly defined, *e.g.* the corpus callosum.

(c) Effect of injection of sterile apyrogenic saline solution on nociceptive threshold

Injection of 10.0 µl saline i.c.v. in the rat was never seen to alter the nociceptive threshold significantly, when the method of Randall and Selitto was used to determine the threshold.

4. DISCUSSION

Insertion of the i.c.v. injection needle without making an injection in mice produced no overt behavioural effect. It seems unlikely therefore that the small (presumably, as far as macroscopical investigation allows us to say) area of tissue damaged by needle entry would be sufficient to account for the behavioural changes noted following injection. The lateral ventricle at the point of entry of the needle lies directly below the corpus callosum (Zeman & Innes, 1963), hence the needle penetrates only the cortex and the corpus callosum. Thomas (1966), using the rat, has shown that quite large bilateral parietal lobe cortical ablation is necessary before any behavioural impairment is detectable, using a 'closed field' intelligence test as the test situation. The damage caused to the corpus callosum is thought unlikely to impair C.N.S. function to any great degree, since only a small proportion

of this, a large structure, is visibly damaged by the injection needle. These foregoing comments would apply to the rat with respect to the implantation of the cannula guide.

The effects of increased intracranial pressure have been studied by a number of authors, mainly with respect to its effects on cerebral blood flow. Shulman and Verdier (1967) increased the csf pressure of anaesthetised dogs to values as high as 400 mm Hg (normal readings are around 10 to 15 mm Hg). Such increases in pressure caused no alteration in cerebral blood flow, indicating that a homeostatic mechanism operates in order to keep cerebral blood flow constant whatever the changes in csf pressure. The results of Shulman and Verdier show that this is brought about by changes in resistance to blood flow in the precapillary and postcapillary vessels.

The transient behavioural changes associated with injection of volumes of 5-20 µl normal saline in mice are due apparently to a short-lived increase in csf pressure brought about by the injected volume. This is supported by the fact that the larger the volume injected, the longer the period before recovery from these effects. Also, if the injection is carried out over an extended period, the resulting syndrome is less severe. This latter procedure is difficult, however, as it entails restraining the mouse for a longer period and maintaining the needle in the correct orientation with respect to the skull.

No evidence is available on the mechanism by which increased csf pressure brings about the behavioural changes noted, but it seems likely that the pressure causes some deformation of neural tissue with a resultant modification in the rate and/or pattern of firing of neurones. However, the fact that the initial effects of the injection are seen as generally well-

co-ordinated movements (see this chapter, 1(b)) suggests that generalised (convulsive) activity of the C.N.S. does not play a part in this response. Electroencephalographic analysis of this syndrome would prove of great value and interest.

The fact that a simple i.c.v. injection of saline is capable of reducing substantially the number of writhes produced by a challenge dose of phenylquinone supports the concept of a central mechanism being involved in the genesis of the writhing movements, or possibly in the suppression of them. Unfortunately, since the effect is significant during the period 20-40 minutes post i.c.v. injection, this has meant that the i.c.v. route could not usefully be employed in the investigation of the activity of substances in the phenylquinone test. As will be demonstrated in later chapters, the substances given by the i.c.v. route in this study have without exception shown their peak activity well within this 40 minute period.

The fact that the reduction in the number of writhes is noticeable up to an hour following the i.c.v. injection is in itself interesting from the point of view of the effects of the injection on the animal. This shows that although the gross behavioural effects of the injection are not detectable beyond three to four minutes after the injection, there is a clearly demonstrable effect on the activity evoked by phenylquinone injection. Hence the effects of the injection itself may not be as transient as would appear from observation of the gross behaviour of the animal. It should be noted, however, that this was the only experimental nociceptive procedure reported in this thesis in which i.c.v. injection of saline had any detectable effect. When using other test procedures, saline controls were usually included in each experiment.

As stated earlier, (this chapter, 3(b)), injection of 10.0 µl normal

saline into the ventricles of the conscious rat had no overt behavioural effects, although the rapid movement of the vibrissae which was sometimes observed may be an indication of an effect similar to that seen in mice, but of a lower intensity. A lesser effect would be expected because of the larger fluid space in the rat's brain, into which we have injected the same volume of fluid as in the mouse.

The occurrence of hydrocephalus in rats following i.c.v. infusion was noted by Goodrich *et al.* (1969). They infused artificial csf into a lateral ventricle of conscious rats at a rate of 3.0 µl/minute, a rate said to be comparable to normal csf production in rats (estimated to be 2.2 µl ± 0.3 µl/min in a study by Cserr, 1965). Non-communicating hydrocephalus in the lateral ventricle into which the infusions were made was found to develop in some rats after three or four infusions, and in only 30% of these animals did a subsequent infusion penetrate to the contralateral ventricle. By contrast, our results suggest that although hydrocephalus develops on the ipsilateral side to the injection, the connections to the 3rd and contralateral ventricles remain patent, as shown by studies with injected dye. As was the case with Goodrich *et al.*, development of hydrocephalus was seen in only a proportion of cannulated animals, in our case animals which had been receiving two injections per week for at least three weeks. Other animals, however, received two injections per week for up to six or seven weeks, and remained perfectly healthy up to this stage, at which point they had reached the end of their useful life because of growth and difficulty in handling.

The reason for the development of the hydrocephali in some animals and not others is not clear. That it is connected with the injection of fluid rather than the presence of the cannula guide is suggested by the fact that we have never observed hydrocephalus in animals which have had guides

implanted but no injections made through them. Goodrich *et al.* (1969) suggested that small blood clots or other debris may obstruct the normal circulation of csf, but this explanation is untenable in our case since we have shown that subsequent injections into a hydrocephalic lateral ventricle penetrate to the other parts of the ventricular system.

One possible explanation may be that the sudden increase in intra-ventricular pressure on the side of the injection may bring about an irreversible change in the choroid plexus of that ventricle, which after a number of injections begins to over-produce csf. This may involve a homeostatic mechanism, such as that described by Shulman and Verdier (1967). A factor arguing against this is that the increased pressure brought about by greater production of csf on the ipsilateral side would communicate itself to the other parts of the ventricular system, and not give rise to a unilateral hydrocephalus.

The normal rate of csf circulation is slow, and the sudden volume/pressure increase in the ipsilateral ventricle brought about by the injection may distend the ventricle mechanically, and the increased csf production may reflect a mechanism whereby the fluid is over-produced in an attempt to 'fill-up' the distended space subsequent to a number of injections. This latter suggestion is not entirely refuted by the results of Goodrich *et al.* (1966) because, although their infusion pressure was low, and their infusion rate comparable to that of normal csf production, their figure for csf production (Cserr, 1965) refers to total production throughout the whole of the ventricular system. Their infusion of 3.0 µl/min into one of the lateral ventricles compares with the figure for total csf production of 2.2 µl/min. It should be remembered that slightly less than half the total normal production emanates from the single lateral ventricle. Hence the quantity of

artificial csf entering the lateral venticle in those experiments is approaching twice the normal production. In this case the critical factor would appear to be the rate at which the excess fluid can pass through the foramen of Monro into the third ventricle.

A further suggestion is that an ionic imbalance of some sort in the cannulated ventricle following numerous injections gives rise to over-production of csf in an attempt to restore the correct ionic balance. However, Goodrich *et al.* (1966) used artificial csf for their infusions, and it is still difficult to see why (or how) such an increase in production could lead to enlargement of one part of what is a freely-interconnected system throughout which any changes in pressure would be expected to communicate instantaneously, since the spaces are liquid-filled.

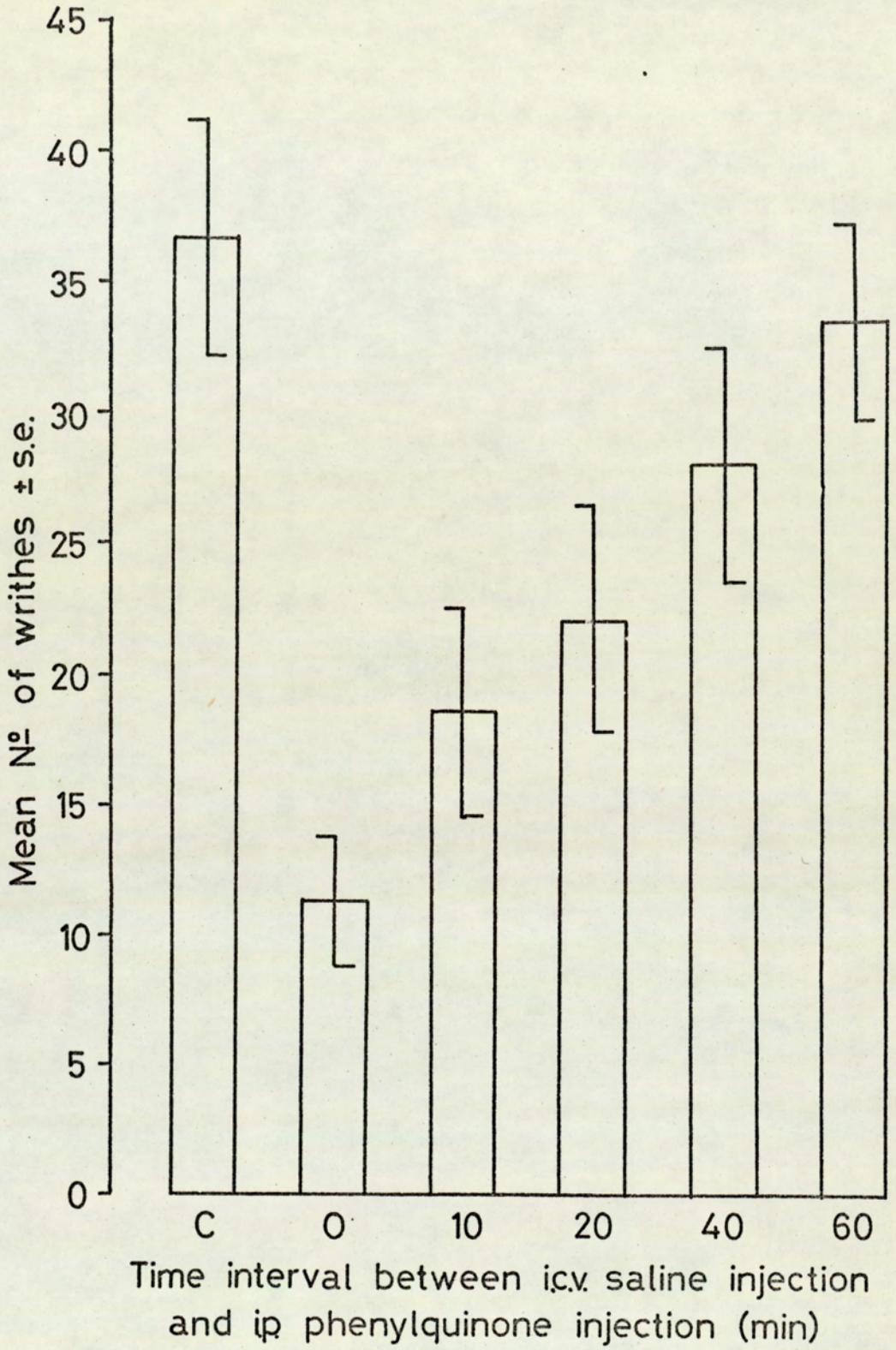

FIGURE 11: EFFECT OF I.C.V. SALINE ON PHENYLQUINONE WRITHES IN MICE

Saline (10.0 µl) was injected i.c.v. at various time intervals before a challenge dose of phenylquinone, 2.0 mg/kg i.p. The column labelled "C" represents the group which received no i.c.v. injection. (n = 10 for each group).

PART B

EFFECTS OF INDIVIDUAL AGENTS ON THE NOCICEPTIVE THRESHOLD

CHAPTER TWO

ANTI-NOCICEPTIVE ACTIVITY OF NARCOTIC AND NARCOTIC-ANTAGONIST COMPOUNDS

1. EFFECT OF MORPHINE IN THE HOT-PLATE TEST

(a) Peripherally-administered morphine

When injected subcutaneously (s.c.), morphine produced a dose-dependent rise in the nociceptive threshold of mice, as determined by an increase in reaction time to the thermal stimulus of the hot-plate (Fig. 12: r.h. plot). When the data were analysed by the method of Litchfield and Wilcoxon (1949), the ED_{50} was 4.35 mg/kg (limits: 2.42 to 7.82 mg/kg). The end-point of licking the front paws remained unaltered, the morphine merely prolonging the lapse of time between the initiation of the stimulus and the occurrence of the end-point. The threshold was determined 40 minutes after the injection of morphine. (The peak effect of morphine in the tail-flick test was between 40 and 60 minutes after s.c. injection — see para. 3, this chapter.)

(b) Centrally-administered morphine

In order to exclude, as far as practicable, the possibility that a peripheral action of morphine may be involved in the suppression of the response to a heat stimulus applied to the feet, the effect of morphine was determined following its i.c.v. administration. The hot-plate test was carried out 15 min after the i.c.v. injection of saline (controls) or morphine (at four dose-levels). The doses given were calculated on a µg/mouse basis, but have been expressed as X µg/kg body weight in the graphical representation of the results to allow for easy comparison with the plot obtained with subcutaneously-administered morphine (Fig. 12). (This calculation assumed that each mouse weighed 20.0 g. In fact they were chosen from within the range 18-22 g, the error involved being, therefore, small.) The slopes of the dose-response curves following s.c. and i.c.v. morphine do not differ significantly from each other. The ED_{50} following

i.c.v. morphine, calculated from the line, was 5.75 µg/kg (limits: 3.20 to 10.35 µg/kg); thus, morphine was approximately 760 times more potent when given by the i.c.v. route than by the s.c. route.

2. EFFECTS OF NALORPHINE, PENTAZOCINE AND NALOXONE IN THE HOT-PLATE TEST

Subcutaneously-administered nalorphine (15.0 mg/kg), pentazocine (20.0-75.0 mg/kg) and naloxone (20.0 mg/kg) had no effect on the nociceptive threshold of the mouse as determined by the hot-plate test. The action of pentazocine was also investigated following i.c.v. injection at doses of 5.0 to 100.0 µg per mouse. (These experiments were carried out after the suggestion of Paalzow (1969) that the lack of anti-nociceptive effect of pentazocine was due to rapid metabolism of the lactate salt. It was our opinion that injection *via* the i.c.v. route might circumvent the site of any such rapid metabolism.) No increase in reaction time was observed following this procedure, except at the 50.0 and 100.0 µg dose levels, when a significant increase was detected 15 min after injection. However, those animals receiving 100.0 µg pentazocine displayed a tendency to having clonic convulsions when handled, suggesting that the higher doses studied were approaching toxic levels.

3. EFFECT OF MORPHINE IN THE TAIL-FLICK TEST

(a) Peripherally-administered morphine

When injected s.c., morphine produced an increase in reaction time, as measured by the tail-flick test, which varied with time after the injection, as shown in Fig. 13. A statistically significant difference ($P<0.05$) was observed between the group receiving morphine and the saline control group. The peak effect occurred at 40-60 min post-injection, the effect being only marginal at 120 min. In this particular experiment, the action of morphine

was fairly rapidly terminated at two hours post-injection. This was not true for all experiments; occasionally the activity was still marked at two hours, but on no occasion did it persist three hours after injection. The activity of morphine in this test varied considerably from day to day, and for this reason it was important to insure that experiments were properly controlled when morphine was investigated. A dose of 5.0 mg/kg was the smallest which consistently gave a significant increase in tail-flick reaction time.

The increase in reaction time produced by morphine varied in a dose-dependent manner, as is shown by Fig. 14 (r.h. plot), using quantal data obtained when the test was carried out 20 min after s.c. injection. The data yielded an ED_{50} of 8.0 mg/kg (limits: 6.25 to 10.22 mg/kg).

(b) Centrally-administered morphine

The time-course of the anti-nociceptive activity of morphine in the tail-flick test was studied following the i.c.v. injection of 1.5 µg per mouse (Fig. 15). Comparison with the results of s.c.-administered morphine (Fig. 13) shows that the peak effect is reached rather more rapidly following the central route of administration, and in the examples illustrated the effect following i.c.v. injection is somewhat prolonged. In view of the variation in the level of activity of morphine in this test, however, it would be unwise to draw any conclusion from such a comparison, although the peak activity was always reached more rapidly following i.c.v. administration.

Using the i.c.v. route, the anti-nociceptive action of morphine was again found to be dose-dependent, the quantal data being plotted on the l.h. side of Fig. 14. Analysis of the data provided a figure of 47.5 µg/kg for the ED_{50} (limits: 26.5 to 85.5 µg/kg). Data for the dose-response curve

was obtained by carrying out the test 30 min after the i.c.v. injection. Comparison of the ED_{50}'s for the i.c.v. and s.c. routes in this test reveal that morphine is some 169 times as potent when given *via* the i.c.v. route. (In this particular comparison, the s.c. ED_{50} was obtained somewhat before the occurrence of peak activity, and therefore the s.c. ED_{50} is rather larger than it would be had the determination been carried out at the peak. Hence the factor of 169 times is rather larger than it might have been.) The slopes of the two lines do not differ from each other to a significant extent, nor do they differ significantly from the slopes of the plots obtained following morphine administration (by s.c. or i.c.v. route) in the hot-plate test (Fig. 12).

4. EFFECTS OF NALORPHINE, PENTAZOCINE AND NALOXONE IN THE TAIL-FLICK TEST

(a) Nalorphine

Nalorphine produced a marginal increase in tail-flick reaction time following s.c. injection. This was significant statistically ($P<0.05$) at a dose of 5 mg/kg, the effect having its peak at about 60 min after injection. Reaction times had returned to control levels within two hours of administration of the drug. However, a low "ceiling effect" was demonstrable with this drug, because higher doses (up to 20.0 mg/kg) produced no greater rise in nociceptive threshold than did a dose of 5.0 mg/kg (Fig. 16).

(b) Pentazocine

Pentazocine behaved similarly to nalorphine in this test, giving a marginal but significant rise in nociceptive threshold at doses of 10.0 to 40.0 mg/kg s.c. Again, there was no increase in effect with the larger doses. The peak activity was seen at 40-60 min after injection, the effect being no longer detectable two hours after injection (Fig. 17).

(c) Naloxone

Naloxone, given in doses of 5.0 to 20.0 mg/kg s.c., produced no increase in tail-flick reaction time at any time following s.c. injection.

5. EFFECT OF MORPHINE IN THE PHENYLQUINONE TEST

(a) Peripherally-administered morphine

The number of animals exhibiting writhing movements following a challenge dose of phenylquinone was reduced in a dose-dependent manner by pretreatment with morphine, injected s.c. A dose-response curve was obtained (Fig. 18, r.h. plot), by injecting the phenylquinone 40 min after the morphine test-dose, such that the mid-point of the ten minute observation period occurred 50 min after the morphine injection. The ED_{50} obtained from these results was 0.90 mg/kg (limits: 0.64 to 1.26 mg/kg).

(b) Centrally-administered morphine

A dose-response curve was obtained to i.c.v. morphine in the phenylquinone test. It has been shown (Chapter One) that i.c.v. saline significantly reduces the number of animals writhing following a challenge dose of phenylquinone; nevertheless, since the effect of the i.c.v. injection will be uniform for each group tested, it was considered of value to obtain a dose-response curve to morphine given by this route. The phenylquinone was injected 20 min after the i.c.v. injection, so that the ten minute counting period was centred on a point 30 min after the morphine i.c.v. injection. When carried out in this manner, a linear dose-response curve was obtained (Fig. 18, l.h. plot) giving an ED_{50} of 8.5 µg/kg (limits: 4.15 to 16.5 µg/kg). The dose-response curves following s.c. and i.c.v. administration of morphine were found not to differ significantly in slope. The morphine was, therefore, approximately 100 times more potent when given by the i.c.v.

route than when given by the s.c. route.

6. EFFECT OF NALORPHINE IN THE PHENYLQUINONE TEST

Subcutaneously-administered nalorphine antagonised phenylquinone-induced writhing, in a dose-dependent manner. This contrasts with the effects in other tests. The phenylquinone was injected 20 min after the nalorphine, so that the ten minute observation period was centred around a point 30 min after the nalorphine injection. A dose-response curve was obtained (Fig. 19, l.h. plot), which yielded an ED_{50} value of 52.0 mg/kg (limits: 31.5 to 85.8 mg/kg). The slope of this curve did not differ significantly from that obtained for s.c. morphine in this test.

7. EFFECT OF PENTAZOCINE IN THE PHENYLQUINONE TEST

Pentazocine, injected subcutaneously, reduced phenylquinone writhing in a dose-dependent manner (Fig. 19, r.h. plot) and the resultant ED_{50} value was 4.8 mg/kg (limits: 2.53 to 9.22 mg/kg). The slope of the dose-response curve to pentazocine was not significantly different from those of nalorphine or morphine injected s.c. in this test.

8. EFFECT OF NALOXONE IN THE PHENYLQUINONE TEST

Doses of naloxone up to 50.0 mg/kg produced no reduction in the number of writhes elicited by phenylquinone as compared with control animals. At 100 mg/kg naloxone reduced the number of writhes significantly, 20% of the animals entirely failing to writhe. The proportion of animals failing to writhe was greater at still higher doses. However, at doses in excess of 100.0 mg/kg, animals exhibited little or no spontaneous locomotor activity; 250 mg/kg doses produced clonic convulsions when the animals were handled, suggesting that these doses were approaching toxic levels.

9. EFFECT OF MORPHINE IN THE FOOT-PRESSURE TEST

The time-course of morphine's anti-nociceptive activity in the rat was determined using the foot-pressure test, with a dose of 5.0 mg/kg given by the subcutaneous route (Fig. 20). The maximal effect occurred at 30-60 min, and was still prominent two hours after injection. At three hours, the effect was less marked, though still statistically significant, while at four hours the effect was no longer significant. There was some variation in peak intensity and duration of action of morphine in this test from day to day (as has been noted in the mouse tail-flick test: this chapter, para. 3(a)). For this reason, experiments were always specifically controlled.

Using a 30 min pretreatment time, a dose-response curve was obtained for s.c. morphine (Fig. 21). From this line, the s.c. ED_{50} was calculated to be 3.20 mg/kg (limits: 2.13 to 4.80 mg/kg).

10. EFFECTS OF NALORPHINE, PENTAZOCINE AND NALOXONE IN THE FOOT-PRESSURE TEST

Nalorphine (up to 15.0 mg/kg s.c.), pentazocine (up to 20.0 mg/kg s.c.) and naloxone (up to 20.0 mg/kg s.c.) had no effect on the nociceptive threshold of the rat in the foot-pressure test; measurements were made at a variety of times up to four hours following injection.

11. DISCUSSION

Many of the observations made in this chapter were not new, but were necessary for a number of reasons. Firstly, we had to become familiar with the various testing methods used in such a study of the interactions between these compounds and other substances. Secondly, it was necessary to know exactly how these compounds themselves behaved in the various tests used, and also to compare the results obtained here with those of other workers

who have been involved in a similar field. Thirdly, the strains of animals being used and the particular environmental conditions under which our experiments were carried out may have caused variations in our results as compared with those of others.

A variety of testing methods has been used in an attempt to compensate for the weaknesses and/or deficiencies of each individual method. (This subject has been discussed more fully in the Introduction.)

The potency of morphine in the hot-plate test was rather more consistent from day to day than in the tail-flick test, but it is difficult to suggest a suitable reason for the more marked variation in the latter test. It may be connected with the state of various body functions which vary from day to day (such as stress levels — although animals were treated to exactly similar conditions and procedures as far as was possible). It is well-known that the levels of various endogenous substances in the mouse brain vary in a circadian rhythm (*e.g.* 5-HT: Albrecht *et al.*, 1956), and these variations may play a part in the observed inconsistency of the morphine response (Morris & Lutsch, 1967, 1969).

We have shown morphine to be active in very small doses, and in a dose-dependent manner, when injected i.c.v. in the mouse, in all three test situations used. This suggests a supraspinal site of action for morphine in all three tests. It is difficult to suggest a more exact locus of action than this, because of the limitations of the intraventricular injection technique. However, there is considerable evidence from other sources to suggest a periventricular site of action for morphine. Miller and Elliott (1955), using peripherally-administered ^{14}C-morphine, correlated peak brain levels with peak anti-nociceptive activity, as measured by a tail-flick

method in the rat. They reported that morphine became concentrated in the choroid plexus and the ventricles. Tsou and Jang (1964) using a radiant heat test method in the rabbit, suggested a locus of action for morphine in the periventricular grey matter of the 3rd ventricle. Dewey *et al.* (1969b), using spinalised mice, showed that although morphine was still capable of prolonging the tail-flick reaction time, much larger doses were required than in intact mice. Also, a low ceiling effect to morphine was reported in spinal mice. Pentazocine was shown to be partially effective in spinal animals at high dosage (60 mg/kg: producing only 17% anti-nociceptive effect) whereas the compound was only marginally active in intact mice. Dewey *et al.* (1969b) used these results as a justification of the use of the tail-flick test in the investigation of (apparently) supraspinal mechanisms of action of narcotic and narcotic-antagonist drugs.

In a more recent paper, Weller and Sulman (1971) used decerebrate mice in the hot-plate test and in a modified tail-flick test (using hot-water as the heat stimulus — Ben-Bassat *et al.*, 1959). They found that transection of the brain tangential to the superior colliculi considerably reduced the efficacy of morphine in the tail-flick test, but did not alter it in the hot-plate test. This suggested a supramesencephalic site of action for morphine in the tail-flick test, but a more caudal site in the hot-plate test. Herz *et al.* (1970), using the rabbit tooth-pulp test (which is not strictly comparable to any of the test situations used in the present study) localised the site of morphine action in the region of the aqueduct-4th ventricle, following microinjection into localised areas of the ventricular system. Further evidence in support of this site of action was provided by Albus *et al.* (1970).

Another point of interest stemming from the work of Herz *et al.* (1970)

is their observation of a longer delay between i.c.v. injection and appearance of the anti-nociceptive effect than the delay following s.c. injection. This suggested that morphine may have to penetrate some distance from the ventricular spaces to reach its site of action. This is difficult to reconcile with the results of Miller and Elliott (1955), which suggested that morphine reaches its highest concentrations in the csf and the areas of the brain immediately surrounding the ventricular spaces. This in turn makes it seem likely that morphine may reach its site of action in the brain *via* the csf. Our own results suggest that morphine acts more rapidly when injected i.c.v. than s.c., at least in the tail-flick test. However, the discrepancy between our results and those of Herz *et al.* may involve a species and/or test disparity.

Examination of the potency ratios of morphine i.c.v./s.c. suggests that morphine is between 100 and 1,000 times as potent on a body weight basis when given by the i.c.v. route than by the s.c. route. It would be necessary to carry out further ED_{50} determinations in order to be able to confirm any differences between the tests used, on this basis, but this aspect will be discussed more fully in the discussion to Chapter Three.

The results obtained with the narcotic-antagonist analgesics in this study confirm those obtained by other workers. We have found nalorphine and pentazocine to be without effect in the hot-plate test (Archer & Harris, 1965; Harris & Pierson, 1964), and to be capable of producing only a small rise in the nociceptive threshold of mice as measured by the tail-flick test. Hart and McCawley (1944) initially suggested that nalorphine was as effective in the rat tail-flick test as morphine, but this was later refuted by a number of workers including Harris and Pierson (1964) who stated that a marginal type of response was typical of the narcotic-antagonist analgesics. Archer

and Harris (1965) suggested that the tail-flick and hot-plate procedures were, in fact, more useful in detecting dependence liability than in predicting analgesic effect.

Paalzow (1969) suggested that pentazocine was not active in the usual tests because of its rapid metabolism, a suggestion which was supported by the fact that when injected in an oily emulsion, it became active in Paalzow's test. It was not found to be active, following i.c.v. injection, in the hot-plate test, which tends to rule out the possibility that rapid peripheral metabolism might be responsible for its lack of activity in this test.

The efficacy of nalorphine and pentazocine in the phenylquinone and related tests has been repeatedly demonstrated using rodents, (Blumberg *et al.*, 1965; Collier & Schneider, 1969; Pearl *et al.*, 1968; Taber & Greenhouse, 1964) although Pearl *et al.* (1969) were unable to reverse the syndrome following i.p. phenylquinone in monkeys, using nalorphine. In the present study, the dose-response curves of nalorphine and pentazocine did not differ significantly from each other, nor did they differ from that of morphine. However, Taber *et al.* (1964) using phenylquinone as the noxious stimulus, produced a series of dose-response curves for the antagonists nalorphine, cyclazocine and pentazocine which did not differ in slope, whilst that of morphine did differ from these three. It is possible that the present curves have been plotted from insufficient data to be able to detect any slight differences in slope.

The inactivity of naloxone as an anti-nociceptive agent in all four of the test procedures used in the present study (with the exception of the very high doses used in the phenylquinone test) is in accordance with the available evidence, which suggests that naloxone is a pure antagonist (Blumberg *et al.*, 1961; Kosterlitz & Watt, 1968).

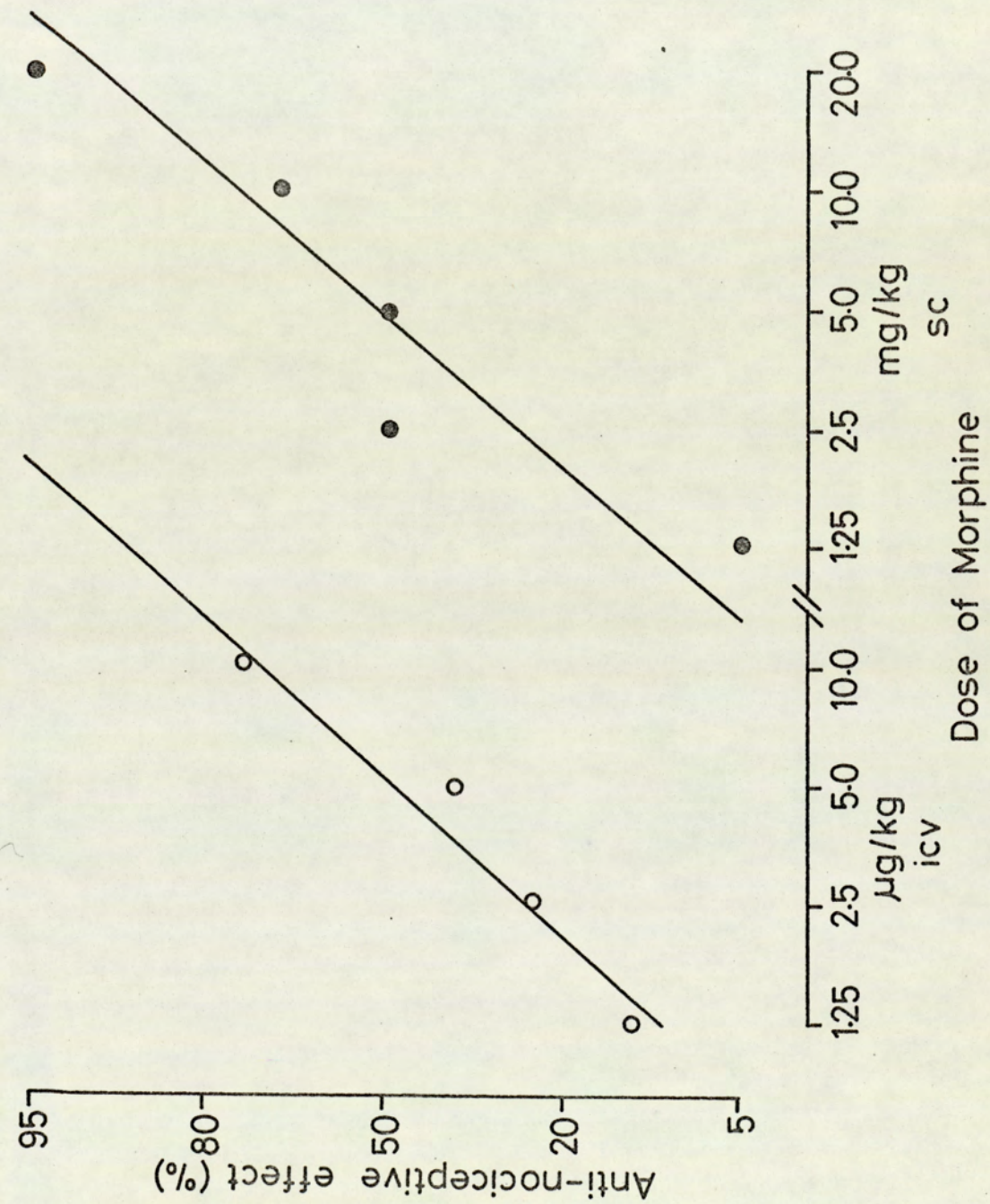

FIGURE 12: DOSE-RESPONSE CURVES TO MORPHINE IN THE HOT-PLATE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose on a log. scale, for i.c.v. (O—O) and s.c. (●—●) routes of administration. The slopes of the lines do not differ significantly when tested by the method of Litchfield & Wilcoxon (1949). (n = 8 for each i.c.v. dose plotted; n = 10 for each s.c. dose plotted.)

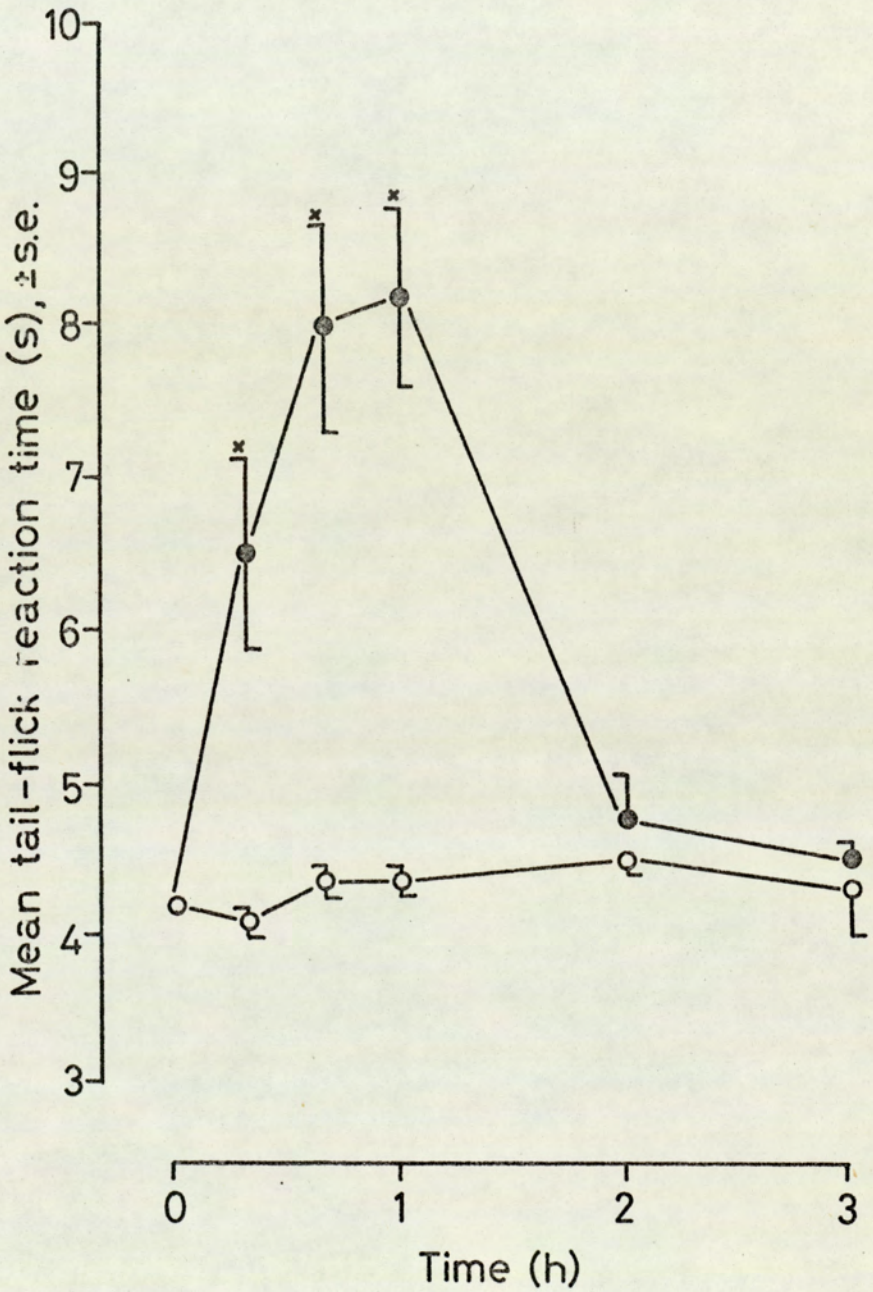

FIGURE 13: TIME-COURSE OF THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE TAIL-FLICK TEST

Groups of ten mice received either saline (O—O) 10.0 ml/kg or morphine (●—●) 5.0 mg/kg subcutaneously at 0, their reaction times being determined at various times thereafter.

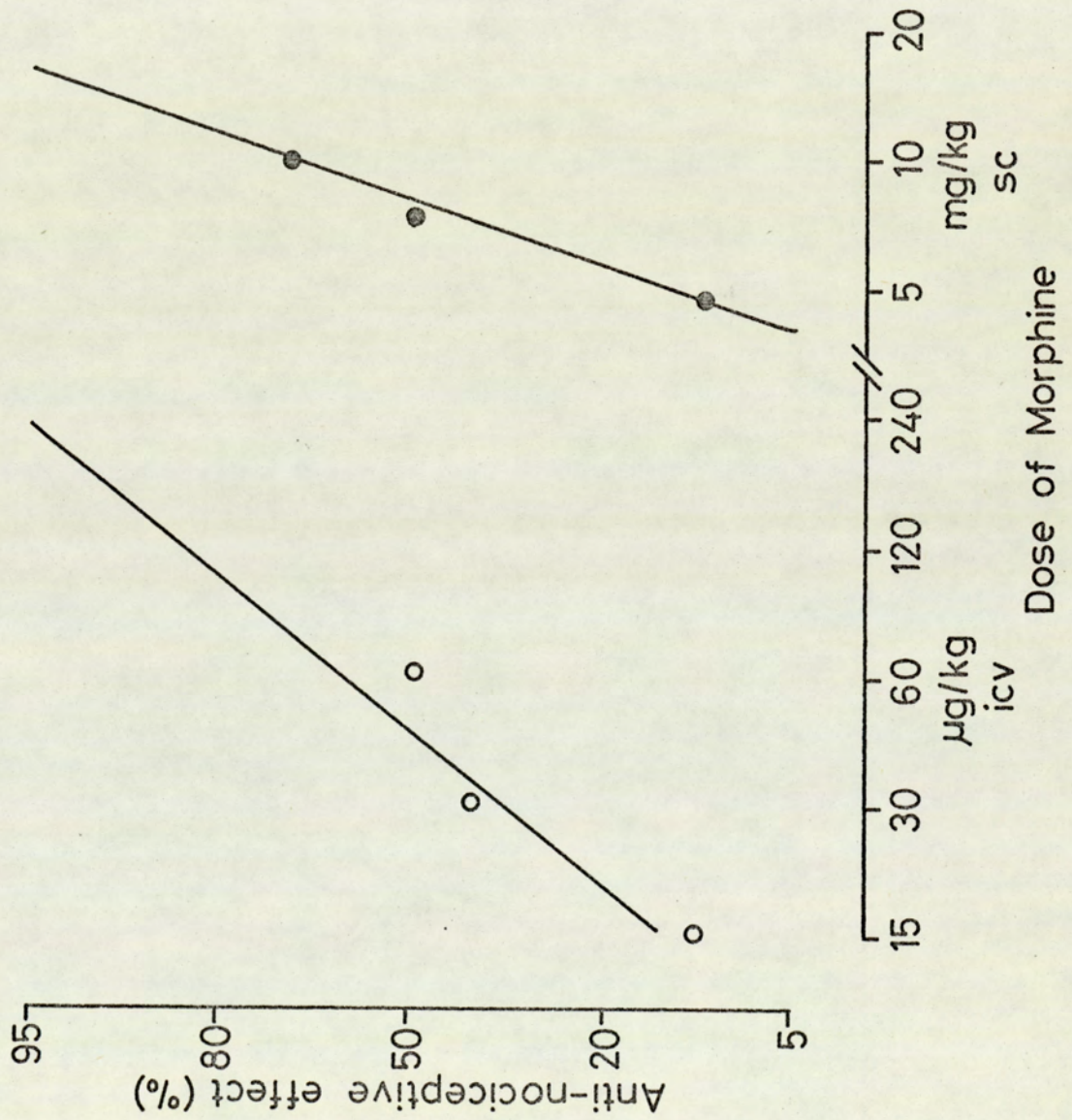

FIGURE 14: DOSE-RESPONSE CURVES TO MORPHINE IN THE TAIL-FLICK TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose on a log. scale, for i.c.v. (O—O) and s.c. (●—●) routes of administration. The slopes of the lines do not differ significantly. (n = 10 for each dose plotted.)

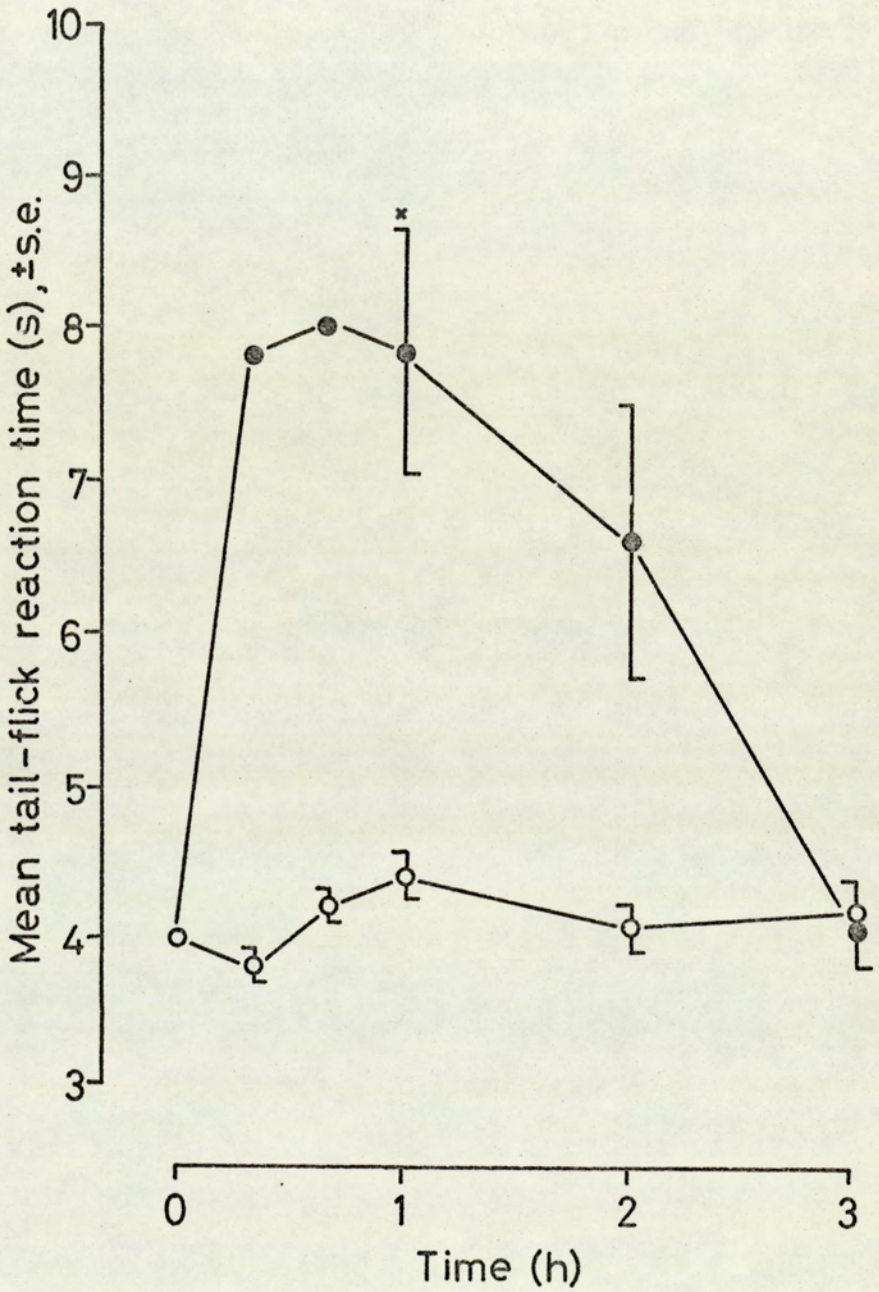

FIGURE 15: TIME-COURSE OF THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE FOLLOWING I.C.V. INJECTION IN THE TAIL-FLICK TEST

Groups of ten mice received either saline (O—O) 10.0 µl i.c.v. or morphine (●—●) 1.5 µg i.c.v. at 0, their reaction times being determined at various times thereafter.

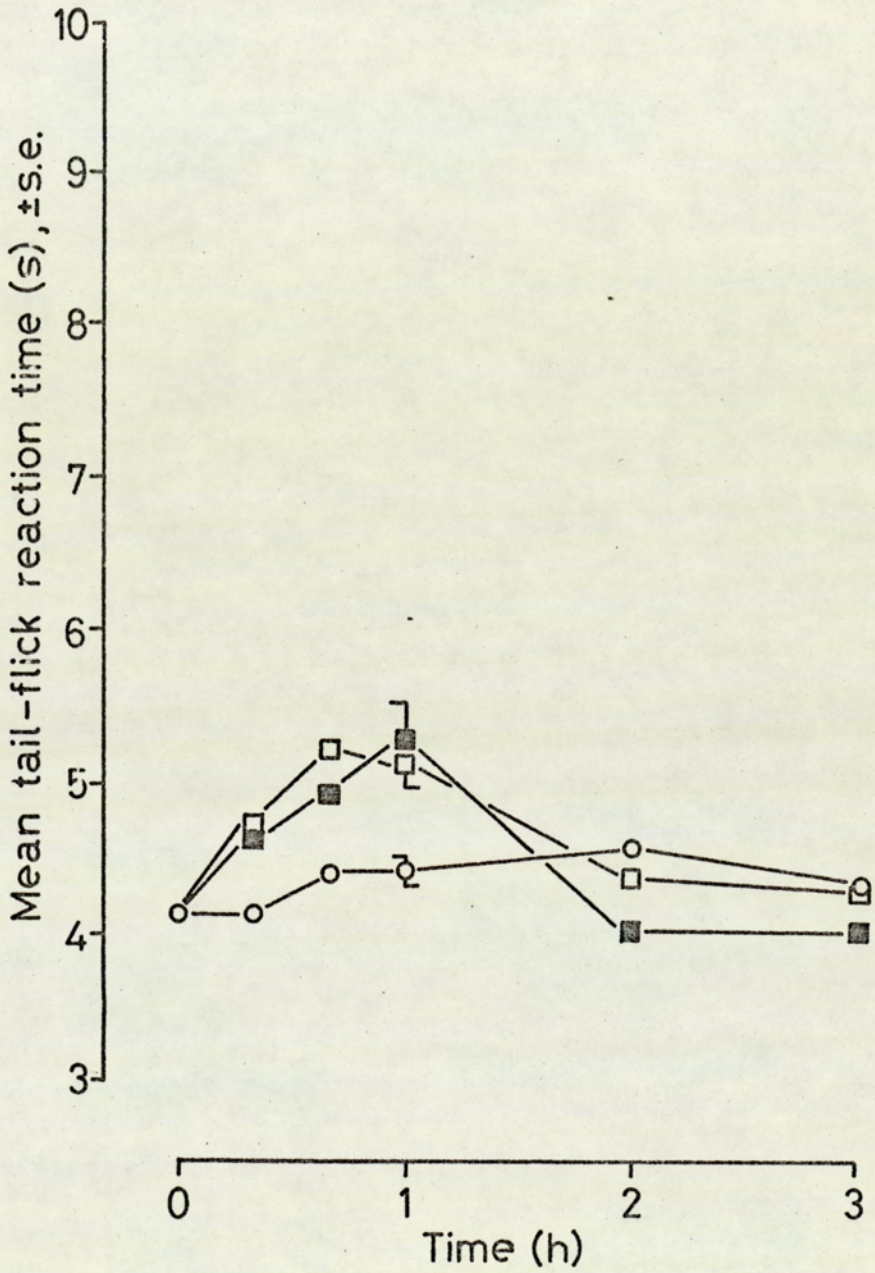

FIGURE 16: EFFECT OF NALORPHINE IN THE TAIL-FLICK TEST

Groups of ten mice received saline 10.0 ml/kg, (O—O), nalorphine, 5.0 mg/kg (□——□) or nalorphine, 20.0 mg/kg (■——■) subcutaneously at 0, their reaction times being determined at various times thereafter.

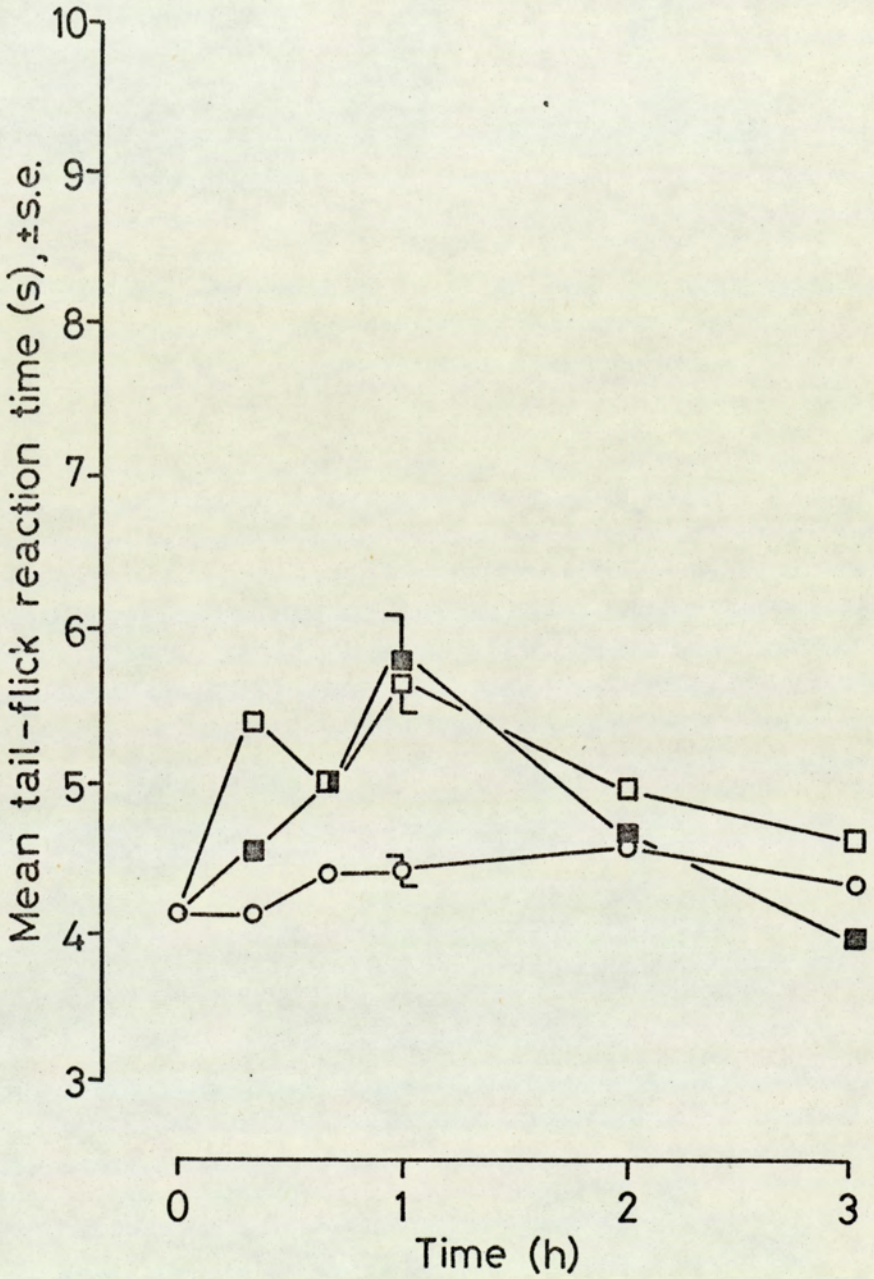

FIGURE 17: EFFECT OF PENTAZOCINE IN THE TAIL-FLICK TEST

Groups of ten mice received saline, 10.0 ml/kg (O—O), pentazocine, 10.0 mg/kg (□——□) or pentazocine, 40.0 mg/kg (■——■), subcutaneously at 0, their reaction times being determined at various times thereafter.

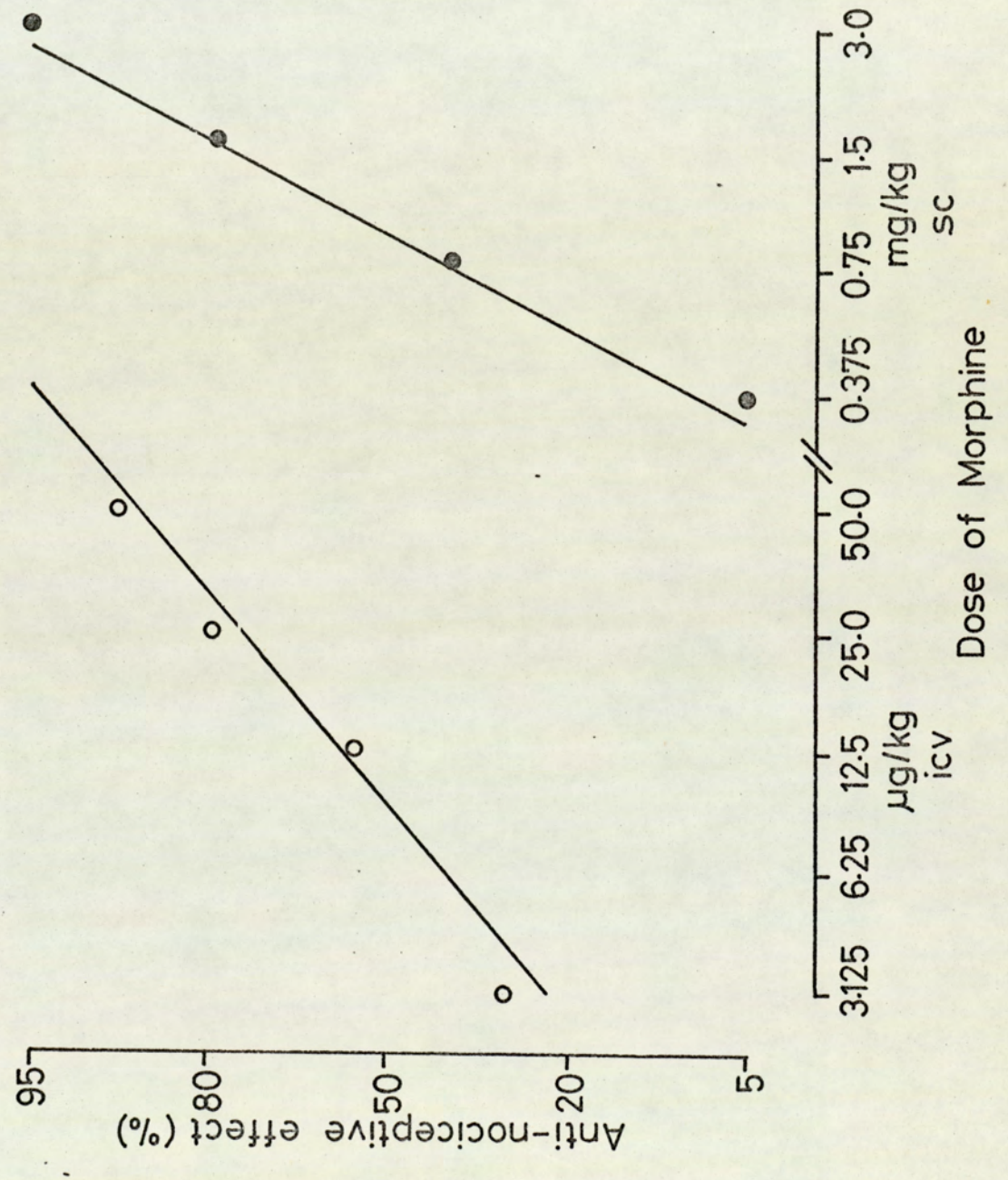

FIGURE 18: DOSE-RESPONSE CURVES TO MORPHINE IN THE PHENYLQUINONE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose on a log. scale for i.c.v. (O—O) and s.c. (●—●) routes of administration. The slopes of the lines do not differ significantly. (n = 10 for each dose plotted.)

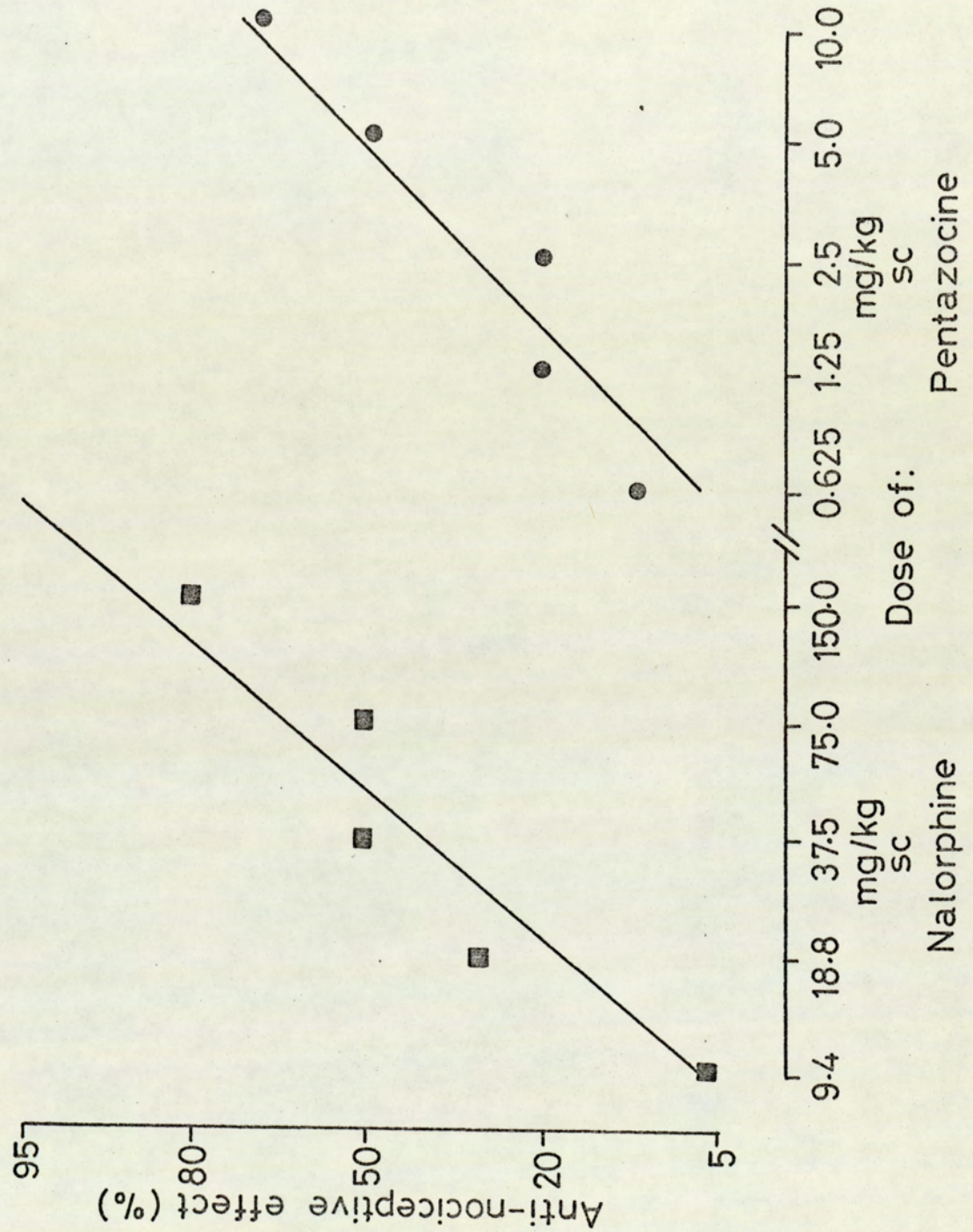

FIGURE 19: DOSE-RESPONSE CURVES FOR NALORPHINE AND PENTAZOCINE IN THE PHENYLQUINONE TEST

Percent anti-nociceptive activity is plotted on a probability scale against dose on a log. scale, for nalorphine (■—■) and pentazocine (●—●), both given by s.c. injection. The slopes of the lines do not differ significantly from each other or from that of morphine in this test (Fig. 18). (n = 10 for each dose plotted.)

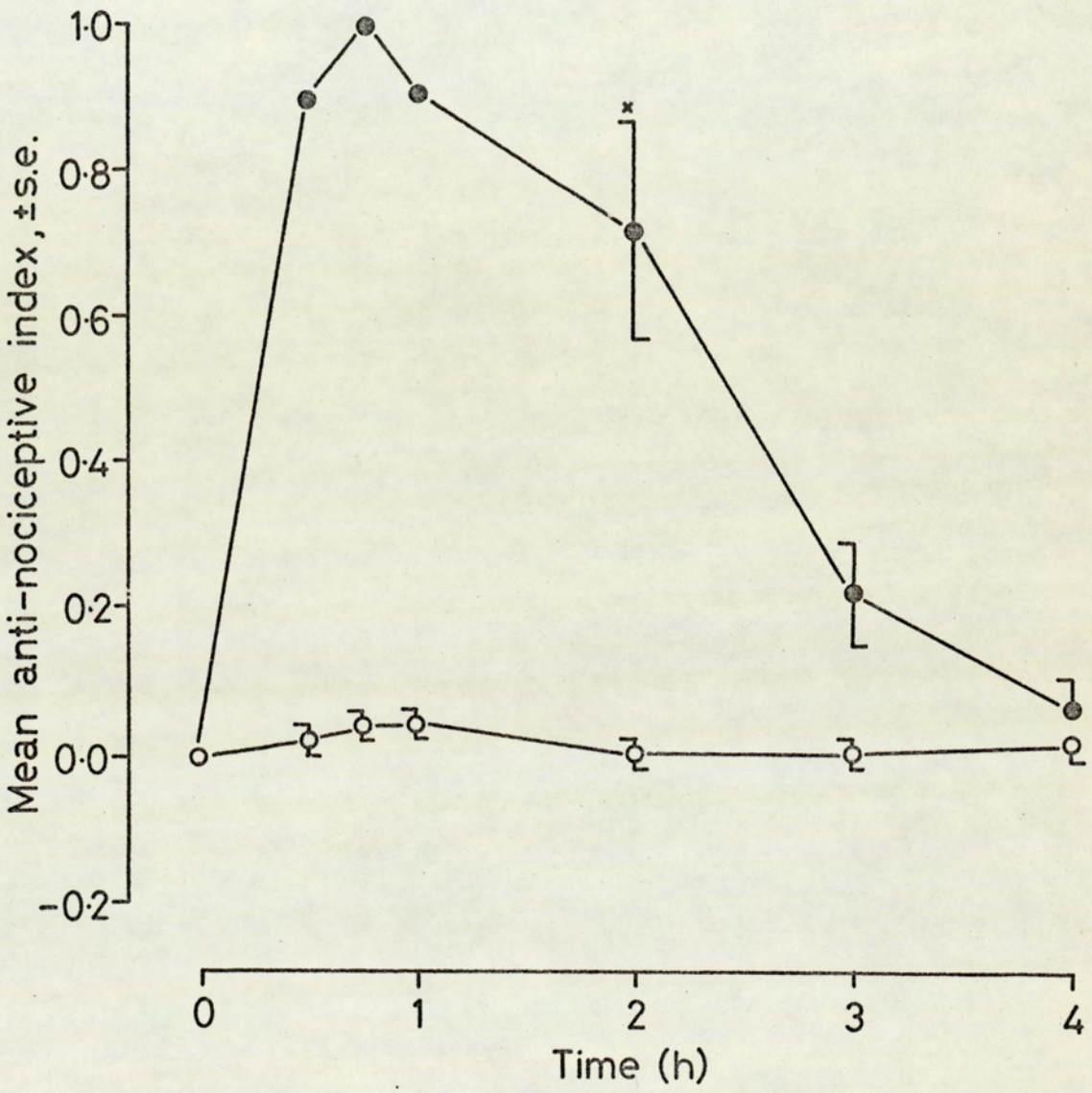

FIGURE 20: TIME-COURSE OF THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE FOOT-PRESSURE TEST

Groups of five rats received either saline (O—O), 1.0 ml/kg or morphine (●—●), 5.0 mg/kg subcutaneously at 0, their nociceptive thresholds being determined at various times thereafter.

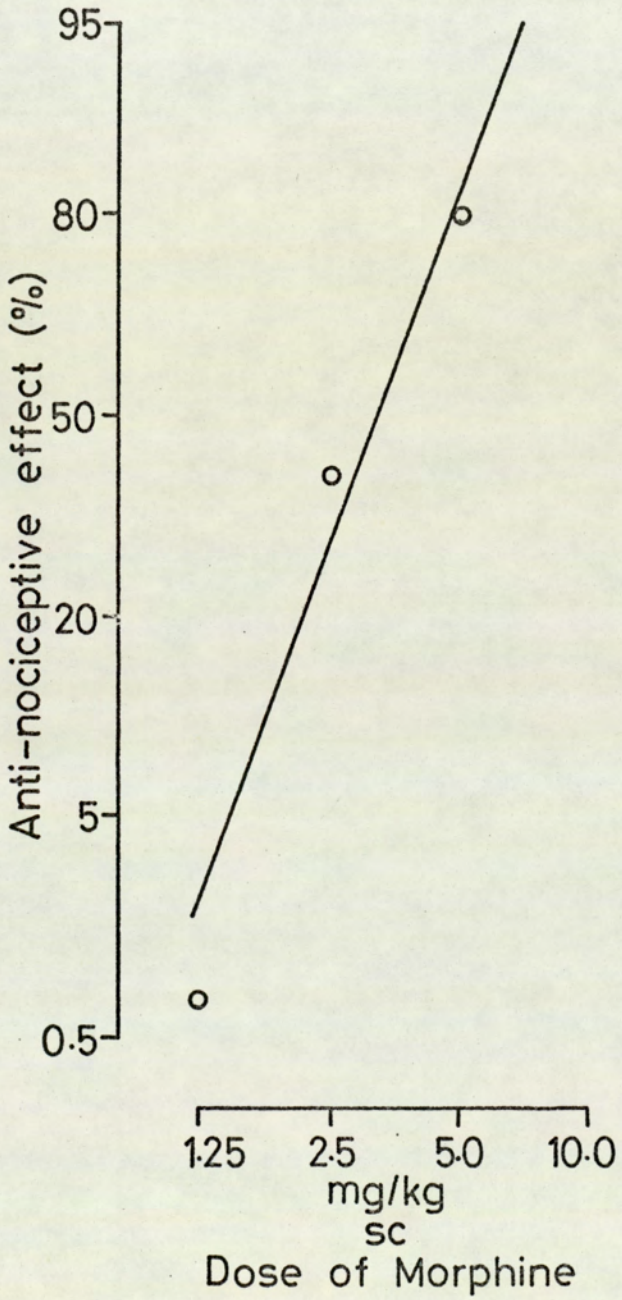

FIGURE 21: DOSE-RESPONSE CURVE TO MORPHINE IN THE FOOT-PRESSURE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of morphine (s.c.) on a log. scale.
(n = 5 for each dose plotted.)

CHAPTER THREE

ANTI-NOCICEPTIVE ACTIVITY OF OTHER AGENTS

1. INTRODUCTION

A variety of pharmacologically-active compounds has been reported to possess anti-nociceptive activity in one or more of the widely-used animal tests. For instance, substances known to possess cholinomimetic activity have been reported to be anti-nociceptive in the mouse hot-plate test (*e.g.* tremorine: van Eick & Bock, 1971; oxotremorine: Handley & Spencer, 1969), the mouse tail-flick test (*e.g.* oxotremorine: Harris, Dewey & Howes, 1968; Harris *et al.*, 1969) and the mouse tail-flick test (tremorine: Chen, 1958). As far as adrenergic compounds are concerned, less evidence is available, but Handley and Spencer (1969) reported noradrenaline to be an effective anti-nociceptive agent in the mouse hot-plate test following i.c.v. administration.

In order that cholinergic and adrenergic compounds, and 5-HT might be used in this study to modify (hopefully) the activities of the narcotic and narcotic-antagonist analgesics, it was necessary to examine carefully the activity of these various modifying agents in the nociceptive tests which were available to us. The results of these experiments are reported in this chapter.

2. EFFECT OF OXOTREMORINE IN THE HOT-PLATE TEST

(a) Peripherally-administered oxotremorine

The anti-nociceptive activity of oxotremorine following s.c. injection was determined in the hot-plate test 60 min after its injection, preliminary experiments having shown the peak activity in this test to be 40 to 60 min post-injection. The anti-nociceptive activity was dose-dependent, the dose-response curve being plotted in Fig. 22 (r.h. plot). Analysis of the data yielded an ED_{50} of 63.0 µg/kg (limits: 39.0 to 101.0 µg/kg). The slope of

the oxotremorine dose-response curve did not differ significantly from that for morphine in this test (Fig. 12). The doses of oxotremorine required to produce these anti-nociceptive effects were very low, and caused no tremor in mice, (although some peripheral signs of cholinergic stimulation were detected, such as salivation and diarrhoea). Hypothermia was marked (5.0°C below controls) at the doses used (up to 0.1 mg/kg), the peak of hypothermia occurring at about 45 min after s.c. injection of oxotremorine. Hence there is a possibility that the hypothermic effect caused an alteration in the nociceptive threshold, as measured by application of a thermal stimulus.

(b) Centrally-administered oxotremorine

In order to rule out, as far as possible, a peripheral site for the anti-nociceptive action of oxotremorine, a dose-response curve was obtained for i.c.v.-administered oxotremorine, the test being carried out 15 minutes after injection. Again, the anti-nociceptive effect was dose-dependent, the dose-response curve (illustrated in Fig. 22, l.h. plot) yielding an ED_{50} value of 2.5 µg/kg (limits: 1.25 to 5.0 µg/kg). The slope of the curve did not differ significantly from the slope for s.c. administered oxotremorine in this test. Oxotremorine was approximately 25 times as potent when given by the i.c.v. route as compared with the s.c. route.

At the doses used in this experiment, some hypothermia was noted, the degree of hypothermia approximating to that seen for equivalent anti-nociceptive doses following s.c. oxotremorine.

3. ANTI-NOCICEPTIVE EFFECT OF PHYSOSTIGMINE IN THE HOT-PLATE TEST

Subcutaneously-administered physostigmine (up to 20.0 µg/kg) was not significantly anti-nociceptive in the hot-plate test. However, i.c.v. physostigmine (0.5 to 1.0 µg) did produce a marginal but significant

lengthening of the hot-plate reaction time. The effect appeared to exhibit a low ceiling effect in this test.

4. EFFECT OF 5-HT IN THE HOT-PLATE TEST

5-HT, in doses up to 20.0 µg i.c.v. (equivalent to 1.0 mg/kg) had no effect on the reaction time of mice in the hot-plate test. The animals appeared to be in a hyper-exciteable state with the higher doses, however, tending to jump out of the restraining cylinder, and to exhibit the leg-shaking activity described by Weller and Sulman (1971) prior to lifting the front paws and licking them.

5. EFFECT OF CATECHOLAMINES AND RESERPINE IN THE HOT-PLATE TEST

Intracerebroventricularly-administered noradrenaline (NA) produced a dose-dependent increase in the hot-plate reaction time of mice (Fig. 23). The dose-response curve was obtained by carrying out the test 15 min after i.c.v. injection of NA, and yielded an ED_{50} of 0.74 µg (limits: 0.70 to 0.79 µg), which corresponds to a figure of 37.0 µg/kg (limits: 35.0 to 39.5 µg/kg).

Reserpine (1.0 to 2.0 mg/kg, i.p.) injected 2 or 24 hours before determining the hot-plate reaction time delayed the appearance of the end-point in this test. Hypothermia of 1° to 2°C was apparent at 2 hours, and 6° to 8°C at 24 hours after reserpine. The animals were also extremely depressed, exhibiting ptosis, hunched posture, and complete lack of spontaneous motor activity.

6. EFFECTS OF OXOTREMORINE IN THE TAIL-FLICK TEST

Oxotremorine, given by subcutaneous injection, produced an increase in

tail-flick reaction time in mice, at doses of 0.01 to 0.50 mg/kg. The time-course of this effect is shown in Fig. 24, using a dose of 0.05 mg/kg s.c. Also depicted in Fig. 24 is the time-course of the effect following i.c.v. oxotremorine, a dose of 0.05 µg being employed.

It will be apparent that the peak activity following i.c.v. injection occurs at 20 min after injection, whilst that following s.c. injection occurs at about 40 min. This, together with the lower dose of oxotremorine required *via* the i.c.v. route, suggests a central site of action, since the drug may reach the central site more rapidly following i.c.v. administration.

Fig. 25 shows the dose-dependent nature of the increase in reaction time for both s.c. and i.c.v. oxotremorine. The slopes of the lines do not differ significantly from each other. The i.c.v. ED_{50} (calculated from the line) was 2.65 µg/kg (limits: 1.65 to 4.25 µg/kg). The s.c. ED_{50} was 0.05 mg/kg (limits: 0.03 to 0.08 mg/kg). Hence the oxotremorine was approximately 20 times as potent following i.c.v. injection as compared to s.c. injection. The ED_{50} determinations were carried out 30 min after injection for both the i.c.v. and s.c. routes.

7. EFFECTS OF PHYSOSTIGMINE AND CARBACHOL IN THE TAIL-FLICK TEST

An injection of ACh i.c.v. was considered in order to increase central cholinergic activity. However, ACh is so rapidly metabolised by cholinesterase when injected i.c.v., it is practically useless for such a purpose (Dikshit, 1934; Sparkes, C.G., personal communication). Carbachol was therefore investigated in view of its known immunity to choline esterase. Also, the cholinesterase inhibitor physostigmine was studied, in the hope that it would bring about an increase in endogenous ACh in the region of the post-synaptic membranes normally sensitive to ACh.

The anti-nociceptive activity of carbachol was investigated following its i.c.v. injection. There was a small anti-nociceptive effect, the time-course of which was characterised by an initial small peak 20 min after injection, followed by a rather prolonged return to control values (Fig. 26). Doses of 0.05 µg to 0.5 µg were used, but at none of these doses was the increase in reaction time observed significant (level of significance taken: $P<0.05$). In fact, the increase seen was similar for all the dose-levels investigated and was clearly not dose-dependent.

Physostigmine, on the other hand, at doses of 2.0 and 3.0 µg, produced a significant increase in reaction time in this test (Fig. 26). The peak of activity occurred at about 20 min after i.c.v. injection. Three micrograms of physostigmine i.c.v. produced a peak effect comparable to 5.0 mg/kg morphine s.c. and 1.5 µg morphine i.c.v., although the effect of physostigmine was rather shorter in duration than that of morphine.

8. EFFECT OF 5-HT AND LSD-25 IN THE TAIL-FLICK TEST

Doses of 5-HT below 10.0 µg i.c.v. produced no anti-nociceptive effect in the tail-flick test. However, doses of 10.0 to 20.0 µg produced a characteristic, short-lived but significant increase in reaction time (Fig. 26). Some effect was detectable as early as five minutes after injection, and the maximal effect occurred with a sharp peak at 25 min after injection. At 45 min, the reaction times had returned to the control (saline treated) level. Doses in excess of 20.0 µg i.c.v. produced no greater anti-nociceptive effect, but at these higher levels some toxic manifestations were observed. These took the form of an exaggerated hyperexcitability, occasionally leading to death following a tonic extensor spasm.

The 5-HT used in all the experiments reported in this work was supplied

as the creatinine sulphate complex. In order to exclude the possibility that the creatinine sulphate might be responsible for the observed anti-nociceptive effect, an equivalent quantity of creatinine sulphate, 13.0 µg creatinine sulphate, (corresponding to 10.0 µg 5-HT) was injected i.c.v. into a group of mice. No change in the nociceptive threshold was observed.

LSD-25, which was without effect in this test (up to 0.05 mg/kg s.c. and 0.05 µg i.c.v.) antagonised the anti-nociceptive effect of i.c.v. 5-HT (20.0 µg), when the LSD-25 was injected s.c. 15 min prior to the 5-HT injection. This suggested that 5-HT was the active moeity.

9. EFFECT OF CATECHOLAMINES AND RESERPINE IN THE TAIL-FLICK TEST

Using the tail-flick test, i.c.v. noradrenaline (0.05 to 20.0 µg) produced no alteration in the nociceptive threshold of mice, although some depression of locomotor activity was observed at higher doses (5.0 to 20.0 µg). Doses of noradrenaline up to 2.0 mg/kg s.c. had no effect on the nociceptive threshold.

Dopamine, 5.0 to 200.0 µg, given by the i.c.v. route had little effect on the nociceptive threshold, although doses of 50.0 µg and above tended to produce a slight hyperalgesia 20-30 min after injection; however, this was not always significant. Doses of 100.0 to 200.0 µg produced ataxia in the animals, with lowered body posture. Up to 10.0 mg/kg dopamine s.c. had no effect on the nociceptive threshold.

Reserpine, 1.0 to 2.0 mg/kg i.p., had no effect on the nociceptive threshold up to three hours after injection. However, at 18 to 24 h after injection, the tail-flick reaction time tended to be somewhat shortened when compared with saline-treated control animals, indicating an increased sensitivity to the noxious stimulus. The reduction in reaction time was of

the order of 1.0 s, and was statistically significant in most experiments.

Tetrabenazine, 40.0 mg/kg i.p., produced a similar increase in sensitivity to the noxious stimulus as did reserpine, which was significant five hours after the injection. The reduction in reaction time was of the order of 0.5 s.

10. EFFECT OF OXOTREMORINE IN THE PHENYLQUINONE TEST

Oxotremorine s.c. proved potent at reducing the number of writhes elicited by a challenge dose of phenylquinone. The effect was dose-dependent, analysis of the data (Fig. 27) giving an ED_{50} value of 34.0 µg/kg (limits: 21.0 to 54.0 µg/kg). The phenylquinone was injected 20 min after the oxotremorine, such that the ten minute observation period was centred around a point 30 min after the oxotremorine injection, corresponding with the peak activity of this agent in mice as demonstrated in the tail-flick test.

11. EFFECT OF LSD-25 IN THE PHENYLQUINONE TEST

LSD-25 (5.0 to 40.0 µg/kg) was administered to mice *via* the s.c. route, 20 min prior to a challenge dose of phenylquinone, i.p. Doses of 10.0 µg/kg and above significantly reduced the number of phenylquinone-induced writhes. This made LSD-25 inappropriate in this test, as a pharmacological tool to block the effects of endogenous or exogenous 5-HT.

12. EFFECT OF OXOTREMORINE IN THE RAT FOOT-PRESSURE TEST

Oxotremorine (0.01 to 0.20 mg/kg) given s.c. to rats produced a dose-dependent rise in the nociceptive threshold. Fig. 28 shows the time-course of oxotremorine activity, with a dose of 0.1 mg/kg s.c. At the doses used, some central nervous system depression was apparent, and peripheral effects were quite marked, particularly salivation and the production of red tears.

Hypothermia was not marked at the doses normally employed in subsequent experiments (0.01 to 0.05 mg/kg), though at higher doses it was significant (between 1.0 and 2.0°C). In most subsequent experiments the peripheral effects of oxotremorine were blocked by pretreatment with atropine methonitrate, 0.5 mg/kg s.c., a dose which was without effect on the anti-nociceptive activity of oxotremorine (see Results: Chapter Ten).

The peak anti-nociceptive activity of oxotremorine was rapidly attained after the injection, the effect terminating very quickly also, such that the nociceptive threshold had returned to normal within two hours of the injection.

The anti-nociceptive activity of oxotremorine in the rat is dose-dependent, the dose-response curve obtained 30 min after s.c. injection (Fig. 29) giving an ED_{50} of 0.07 mg/kg (limits: 0.027 to 0.182 mg/kg). The slope of the line is not significantly different to that obtained for morphine (Chapter Two, Fig. 21).

13. EFFECT OF PHYSOSTIGMINE IN THE RAT FOOT-PRESSURE TEST

Physostigmine (1.0 to 10.0 µg) injected i.c.v. produced a brief elevation of the anti-nociceptive index. Doses of 1.0 to 2.5 µg did not consistently produce an effect, but doses in excess of 2.5 µg did (Fig. 30 shows the effect of 2.5 µg physostigmine i.c.v.). The elevation of anti-nociceptive index was usually short-lived, with a peak at 15 min post-injection, the index being back to zero after a further 15 min. A ceiling effect was apparent, since doses of 5.0 and 10.0 µg had similar activity, which was only marginally greater than that produced by 2.5 µg as in Fig. 30.

14. EFFECTS OF 5-HT, LSD-25, CATECHOLAMINES AND RESERPINE IN THE RAT FOOT-PRESSURE TEST

5-HT (5.0 to 20.0 µg) was injected i.c.v. in rats. The lowest dose was without effect on the nociceptive threshold in the foot-pressure test. Doses of 10.0 to 20.0 µg i.c.v. produced a brief anti-nociceptive effect, which varied considerably from day to day, and was always of brief duration (Fig. 30). In fact, in character this effect both qualitatively and quantitatively resembled that of i.c.v. physostigmine in the rat (Fig. 30), and that of i.c.v. 5-HT in the mouse (this Chapter, para. 8; Fig. 26). As in the mouse, doses of 10.0 and 20.0 µg of 5-HT produced similar increases in nociceptive threshold, indicating a low ceiling effect.

LSD-25 was without effect on the nociceptive threshold of rats (1.0 µg i.c.v.), as it had been in mice in the tail-flick test.

Injections of noradrenaline (up to 20.0 µg i.c.v.) induced no alterations in nociceptive sensitivity of rats, although doses of 10.0 to 20.0 µg produced considerable behavioural depression, with lowered body posture, flaccidity of skeletal musculature, and weak vocalisation. All these effects followed a similar time-course, beginning about five, and continuing for between 15 and 30 minutes after injection.

As in the mouse, dopamine (5.0 to 200.0 µg i.c.v.) produced no significant alteration in the nociceptive threshold of rats. The 200.0 µg dose produced some hyperalgesia, detectable at 15 and 30 min after injection, but this effect was not significant when analysed statistically. Surprisingly, these large doses of dopamine produced no obvious signs of pharmacological activity as far as gross behaviour was concerned.

Reserpine (1.0 to 5.0 mg/kg i.p.) produced no alteration in the

nociceptive threshold of rats when given 18 h prior to carrying out the foot-pressure test. At this stage some hypothermia was detectable (1.5 to 2.0°C) together with the usual signs of reserpine depression — lowered body posture, lack of spontaneous movement, ptosis and piloerection.

15. DISCUSSION

The results reported in this chapter confirm the potency of oxotremorine as an anti-nociceptive agent, in all four of the test situations employed (three in the mouse, one in the rat). The activity of oxotremorine has been widely reported in the literature, both in the tests used in this study and in other tests. Initial experiments made use of the precursor of oxotremorine, tremorine, which Chen (1958) demonstrated as possessing anti-nociceptive activity using the tail-clip test. Some recent work has also involved the use of tremorine (van Eick & Bock, 1971). It has been shown, however, that the pharmacological actions of tremorine are brought about by a metabolite which is formed in the liver (Welch & Kocsis, 1961) later identified as oxotremorine, and subsequently synthesised (Cho *et al.*, 1961).

Since that date, oxotremorine has been widely used as a central and peripheral cholinomimetic, and its anti-nociceptive properties have been examined in some detail (George *et al.*, 1962; Handley & Spencer, 1969; Harris, Dewey & Howes, 1968; Harris *et al.*, 1969). Chen (1958) reported tremorine's activity in the tail-clip test to be slower in onset than that of morphine, and to be considerably longer-lasting. The results reported here suggest the action of oxotremorine to be more rapid in onset than that of morphine, and to be of shorter duration. This disparity no doubt reflects the time taken for the tremorine (in Chen's study) to be metabolised to oxotremorine after administration of the former compound. Similarly rapid onset of action for oxotremorine was reported by Haslett (1963). The rapid

disappearance of oxotremorine activity in the rat reflects the short half-life of the compound in that species, as compared with the mouse (Hammer *et al.*, 1968).

Since oxotremorine has the property of causing a considerable degree of hypothermia, it is conceivable that this phenomenon may contribute, at least in part, to the anti-nociceptive effect. Particularly in those methods which employ heat as the noxious stimulus, it is logical to expect that an alteration in body temperature might alter the sensitivity of that body to externally-applied heat. The crucial factor would appear to be skin temperature (Cox & Weinstock, 1964). Winter and Flataker, (1953) using a radiant heat stimulus demonstrated that a morphine-induced fall in skin temperature of the dog resulted in a rise in the nociceptive threshold, the two being, apparently, in a cause/effect relationship. However, in the rat, the nociceptive threshold was raised by morphine despite a *rise* in skin temperature, again using a radiant heat test. The fact that reserpine was active in the hot-plate test, and also causes a considerable degree of hypothermia in mice suggests that a fall in body temperature may be important in that test, but probably not in the tail-flick or foot-pressure tests (Cox & Weinstock, 1964). (See Results: Chapter Thirteen, for further discussion of this point.)

Physostigmine has been examined widely as an anti-nociceptive agent, more from the point of view of an adjunct to morphine (for which, see Chapter Five) than as an agent in itself. Oelssner and Andreas (1969) reported it to be effective after s.c. administration in the tail-flick test, the writhing test (using acetic acid i.p. as the challenge) and an electrical stimulation test. They also reported neostigmine and pyridostigmine to be active following i.c.v. injection, although these authors concluded that the

observed activity was unrelated to their ability to inhibit cholinesterase *in vitro*. Ireson (1969; 1970) found s.c. physostigmine to be an active anti-nociceptive agent in the phenylquinone test, but to be inactive in an electroshock test. Physostigmine has also been reported to be active in experimentally-induced pain in man, using the Hardy-Wolff-Goodell (1940) technique (Flodmark & Wramner, 1945; Gross *et al.*, 1948).

Some conflict is apparent in this field, however, as Chen (1958) failed to detect any anti-nociceptive effect with physostigmine in the mouse tail-clip test. This failure to demonstrate anti-nociceptive activity may be a function of the short duration of action of physostigmine, however, as Chen did not state clearly how long after the injection the test of activity was carried out, or whether more than one test was performed.

A comparison of the ED_{50}'s for oxotremorine following peripheral and central administration is of interest when the potency ratios of morphine given by these two routes are also studied. In the tail-flick test, morphine was 169 times as potent when injected i.c.v. than when injected s.c. By comparison, oxotremorine was only 20 times as potent by the i.c.v. route. It is well-known that morphine has a relatively low lipid solubility (Cube *et al.*, 1970) and this almost certainly accounts for its much greater potency when given by the i.c.v. route. In fact Cube *et al.* (1970) found it to be almost 900 times as potent using the rabbit tooth-pulp test. They concluded that substances of low lipid solubility suffer impeded entry to their central nervous site of action following peripheral administration. Oxotremorine, on the other hand, appears to gain easy access to the central nervous system following peripheral administration. George *et al.* (1962) found i.c.v. oxotremorine to be 10 times as potent as intravenous oxotremorine in inducing tremor in the rabbit. This agrees quite well with our figure of 20 times

potency. The slight discrepency is explained by the facts that:

(a) they were observing a different parameter, which may have involved a different site of action to the anti-nociceptive effect

(b) they used intravenous rather than s.c. injection as their peripheral site

and (c) there would be a greater dilution of drug in the comparatively larger volume of csf in the rabbit.

Similarly, in the hot-plate test, morphine was found to be 760 times as potent when given by the i.c.v. route, whereas oxotremorine was only 25 times as potent. Again, therefore, the relative lipid-insolubility of morphine has apparently reduced its ability to penetrate to its central site of action after peripheral injection. It is interesting that the potency ratio of i.c.v. to s.c. oxotremorine is very similar in the two tests (20 times and 25 times for the tail-flick and hot-plate tests respectively) whereas these for morphine in the two tests differ considerably (169 times and 760 times respectively). This may point to a different site or mechanism of action for morphine in the hot-plate test, a site (or sites) which may be more easily reached following i.c.v. injection than the site (or sites) at which it exerts its activity in the tail flick test.

It seems certain that there exists a difference in the way in which morphine acts in the two tests to which we have been referring. Although both tests employ heat as the noxious stimulus, the end-points which we observe are quite different, and this may explain some of the disparity between them. The end-point for the tail-flick test is a simple reflex movement, which may be elicited in spinal animals (Dewey *et al.*, 1969b) and in animals with transections at the superior collicular level (Weller & Sulman, 1971). It is clear from the results of these studies, however, that

morphine has considerably less anti-nociceptive effect in this test in both spinal and decerebrate animals than in intact ones. Its site of action (in the test) is, therefore, certainly supraspinal and probably rostral to the superior colliculi.

In the hot-plate test, a high degree of motor co-ordination is necessary on the part of the mouse to enable it to lift its paws and lick them (Nott, 1968). Although the overall response to a heat stimulus of this nature is not delayed by decerebration, the pattern of the elicited response is altered (Weller & Sulman, 1971). Instead of lifting and licking the front paws, decerebrate mice tended to lick their hind paws, and also tended to jump out of the restraining cylinder less often in response to the stimulus. Morphine however, remained just as effective in the decerebrate mice as it was in intact mice on the hot-plate. These results clearly point to a difference in the site of action of morphine in the hot-plate and tail-flick tests. The results of Dewey *et al.* (1969b), also show that oxotremorine is markedly less effective in spinal mice when tested by the tail-flick method. Unfortunately, there is to date no evidence as to the efficacy of oxotremorine in decerebrate mice in the hot-plate test.

A further complication to the morphine/oxotremorine comparison in the various tests is that oxotremorine is roughly equipotent (by s.c. injection) in the tail-flick, hot-plate and the phenylquinone test (ED_{50}'s: 0.05 mg/kg, 0.06 mg/kg and 0.03 mg/kg respectively), whereas morphine, although roughly equipotent in the tail-flick and hot-plate tests (ED_{50}'s: 8.0 mg/kg and 4.35 mg/kg, respectively), was considerably more potent in the phenylquinone test (ED_{50}, 0.9 mg/kg). Again, no explanation is readily available for this anomaly, but it appears from a comparison of all the results that oxotremorine may exert its anti-nociceptive action at a common site in all of the test

situations compared here, but that morphine may act at a number of sites in the central nervous system, depending on the type of noxious stimulus used and the response which is thereby elicited from the animal.

With regard to the site of action of oxotremorine and other cholinomimetics, Metyš *et al.* (1969b) have suggested that it may be a periventricular site, following experiments in which they used a variety of substances in the rat, rabbit and mouse (they used thermal, mechanical, chemical and electrical means to elicit the nociceptive responses). These workers concluded that, because of the rapid onset of the anti-nociceptive effect following i.c.v. injection, a periventricular site (or sites) was likely to be involved. They extended their work by using microinjections into the brain tissue itself, and concluded from this that the most likely sites were the septal area and the mesencephalon, although involvement of the hypothalamus and medial thalamic structures could not be entirely ruled out. This work may be compared with that of Herz *et al.* (1970) with regard to morphine, as discussed previously (Discussion to Chapter Two).

Noradrenaline is active only in the hot-plate test, following i.c.v. injection. It has no activity when evaluated in the other tests used in this study. The anti-nociceptive effect detected here confirms the earlier findings of Handley and Spencer (1969) who, conversly, failed to detect any activity following s.c. noradrenaline in this test. The behaviour of noradrenaline in this one test only again adds support to the hypothesis that the hot-plate test (or, possibly, the reaction elicited in the animal) differs from the tail-flick (mouse) and foot-pressure (rat) tests. Noradrenaline exhibited no anti-nociceptive activity in either of the latter tests. Although one employs a heat stimulus and the other a mechanical stimulus, the reaction elicited in these two tests is similar, in that it would appear

to be a simple reflex withdrawal (initially at least, although a more co-ordinated movement may be detected after the initial reaction).

There have been many reports that i.c.v. catecholamines were capable of inducing analgesia or anaesthesia (reviewed by Rothballer, 1959). However, that author came to the conclusion, following his own experiments, that the effects seen may be non-specific, brought about by the use of large doses. It was suggested that large doses of adrenaline (in that case) may block normally excitatory adrenergic neurones by the presence of a large excess of transmitter at the receptor site (Abrahams & Pickford, 1956). Rothballer reported that he had never noted analgesia in the absence of stupor, in his own work using cats. A number of reports have recently suggested that central administration of catecholamines may, in fact, be capable of eliciting excitatory responses and central activation, in cats (Cordeau *et al.*, 1971) and in rats (Segal & Mandell, 1970). The result in the hot-plate test reported in this study is therefore more difficult to interpret, since the doses which raised the nociceptive threshold in the mouse were well below those necessary to induce signs of ataxia or stupor, whilst no overt signs of excitation were observed either.

It has been suggested that 5-HT has a transmitter rôle in the central nervous system with regard to the affective component of the perception of noxious stimuli (Herold & Cahn, 1968). In this light, our results showing 5-HT to have a brief anti-nociceptive action in the tail-flick and foot-pressure tests following i.c.v. injection are difficult to explain. However, a number of other workers have implicated 5-HT in the anti-nociceptive action of morphine (Contreras & Tamayo, 1967; Sparkes & Spencer, 1971; Tenen, 1968), and Tsou and Jang (1964) found 5-HT itself to possess anti-nociceptive activity when injected i.c.v. into rabbits. Saarnivaara (1969a) found

peripherally-administered 5-HT to be synergistic with morphine, but suggested that 5-HT in the brain acted as a pain transmitter. Saarnivaara (1969a) obtained a potentiation of morphine with *p*-chloro-phenylalanine (a 5-HT depletor; Koe & Weissman, 1966), in direct opposition to the results of Tenen (1967) who obtained an increase in pain sensitivity with *p*-chloro-phenylalanine. This discrepancy may be the result of a species difference, Saarnivaara using rabbits and Tenen rats, although both workers employed an electrical test method. Possibly, 5-HT may have opposing functions at different levels of the CNS.

LSD-25 was investigated with a view to its use as an antagonist of 5-HT (Gaddum, 1953; Giarman & Freedman, 1965); that is, the endogenous and i.c.v. injected amine. However, LSD-25 had been reported previously to be capable of blocking phenylquinone-induced writhing (Brittain *et al.*, 1963; Eckhardt *et al.*, 1958) and these results have been confirmed by us. 5-HT release may, in fact, be implicated in the genesis of writhing produced by irritant chemicals. LSD-25 is not, therefore, suitable for use as a pharmacological tool in the phenylquinone test. However, since it shows no anti-nociceptive activity in the tail-flick or foot-pressure tests, and since it is capable of blocking the anti-nociceptive activity of i.c.v. 5-HT in the tail-flick test, its use as a 5-HT blocker in these tests clearly is acceptable.

Reserpine is known to deplete both central and peripheral neuronal stores of 5-HT and catecholamines (Brodie *et al.*, 1957; Holzbauer & Vogt, 1956) by blockade of the pump mechanism which concentrates the monoamines in the storage granules (Carlsson *et al.*, 1963). It therefore appeared to be a useful tool in the study of the interaction of morphine and other substances with these monoamines. However, its wide spectrum of activity with regard to these endogenous substances somewhat reduces its usefulness. Also, there

existed conflicting reports as to its behaviour in various of the nociceptive tests. For instance, anti-nociceptive activity was reported by Garcia Lémé and Rocha e Silva (1961; 1963) using the hot-plate test, and by Ross and Ashford (1967), using the same test. These authors found the peak effect to be at 24-48 h (Ross & Ashford) or 48-72 h (Garcia Lémé & Rocha e Silva). Other workers have reported that reserpine pretreatment enhanced the anti-nociceptive effect of morphine (Dandiya & Menon, 1963; Ross & Ashford, 1967; Tripod & Gross, 1957). This potentiation was observed in the hot-plate test (Dandiya & Menon; Ross & Ashford) and also in the mouse tail-flick test (Tripod & Gross). However, the majority of opinion suggests no anti-nociceptive activity for reserpine, and an antagonism of morphine's effect (Medaković & Banić, 1963, 1964; Radouco-Thomas *et al.*, 1959; Ross & Ashford, 1967; Schneider, 1954; Sigg *et al.*, 1958; Takagi *et al.*, 1964; Verri *et al.*, 1967, 1968). It is of interest to note that with only one exception (the results of Tripod and Gross, 1957, in the tail-flick test) the only situation where reserpine has been found to possess anti-nociceptive activity and to potentiate morphine is the hot-plate test. This led Nott (1968) to conclude that the apparent anti-nociceptive activity of reserpine in this test is a result of its disruption of locomotor activity, a view which is supported by the present results.

Reserpine has not been used in this study in the phenylquinone test. Since reserpine itself releases peripheral and central amines from storage sites (Brodie *et al.*, 1957; Holzbauer & Vogt, 1956), and the release of 5-HT has been implicated in the aetiology of phenylquinone-induced writhing (Eckhardt *et al.*, 1958), it was considered that little would be gained by attempting this exercise.

Tetrabenazine has been used in a small number of experiments, since it

has been reported that it depletes only central stores of amines whilst leaving peripheral stores largely intact (Pletscher, 1957; Pletscher *et al.*, 1958). Takagi *et al.* (1964) reported that this compound had similar activity to reserpine in the tail-clip test in mice, in that it antagonised morphine whilst having no activity itself.

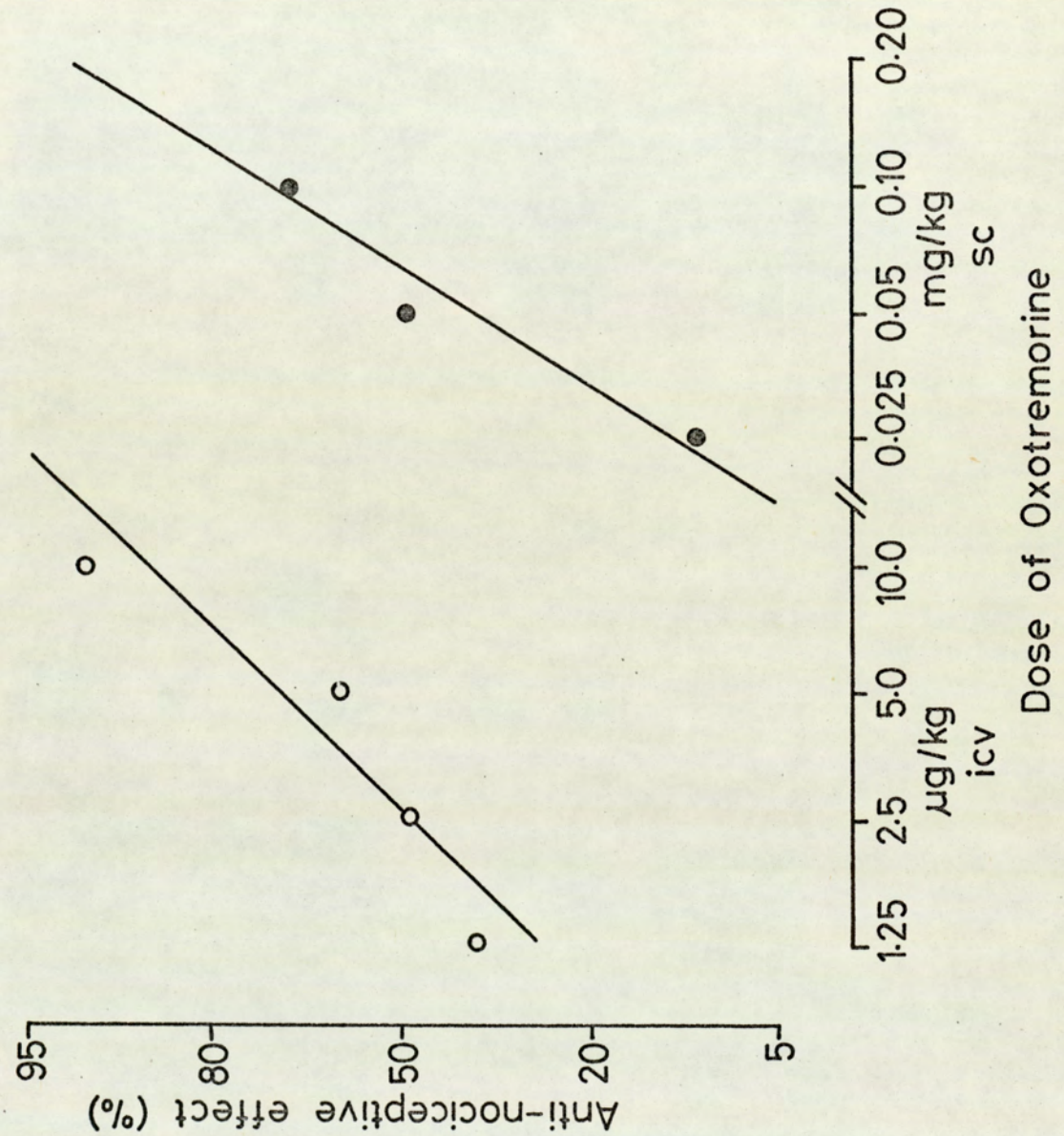

FIGURE 22: DOSE-RESPONSE CURVES TO OXOTREMORINE IN THE HOT-PLATE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of oxotremorine on a log scale, for i.c.v. (O—O) and s.c. (●—●) routes of administration. The slopes do not differ significantly. (n = 8 for each i.c.v. dose plotted; n = 10 for each s.c. dose plotted).

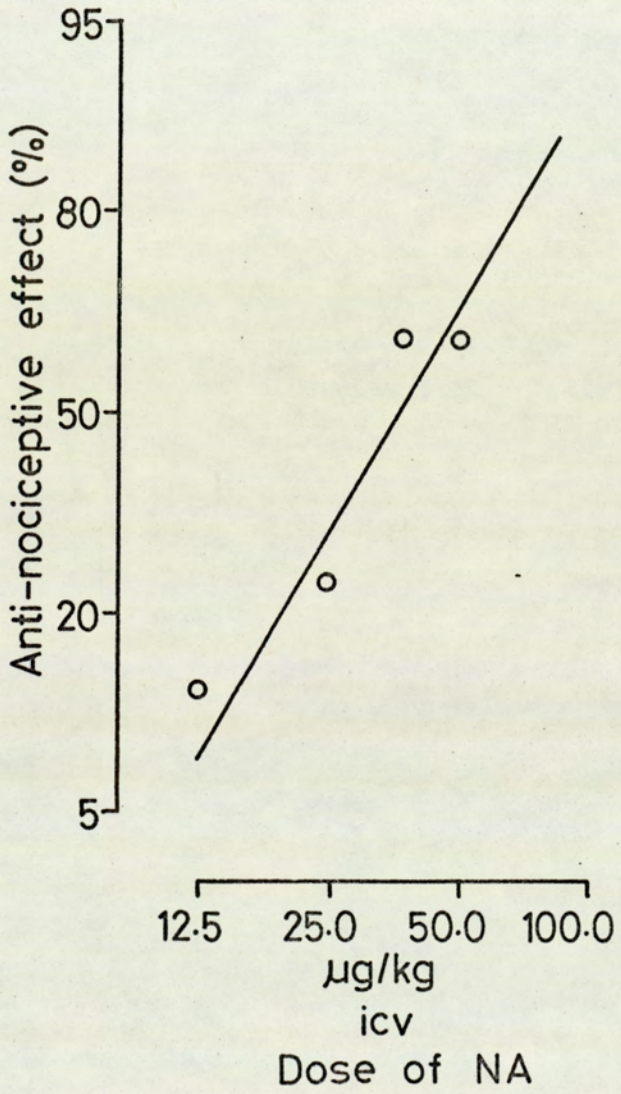

FIGURE 23: DOSE-RESPONSE CURVE TO NORADRENALINE IN THE HOT-PLATE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of NA, given by i.c.v. injection, on a log scale. (n = 8 for each dose plotted.)

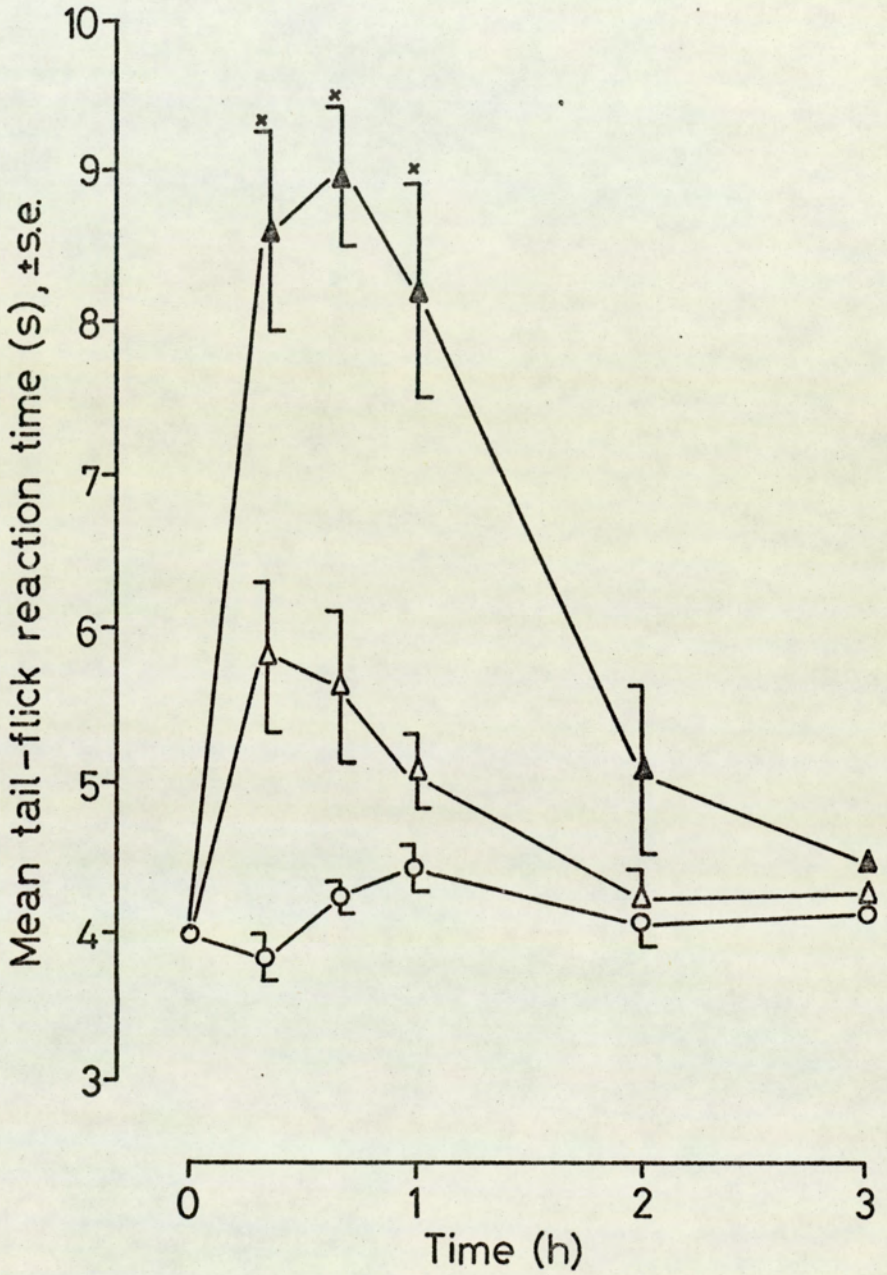

FIGURE 24: TIME-COURSE OF THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE TAIL-FLICK TEST

Groups of ten mice received either saline, 10.0 µl i.c.v. (O—O), oxotremorine, 0.05 µg i.c.v. (Δ—Δ) or oxotremorine, 0.05 mg/kg s.c. (▲—▲) at 0, their reaction times being determined at various times thereafter.

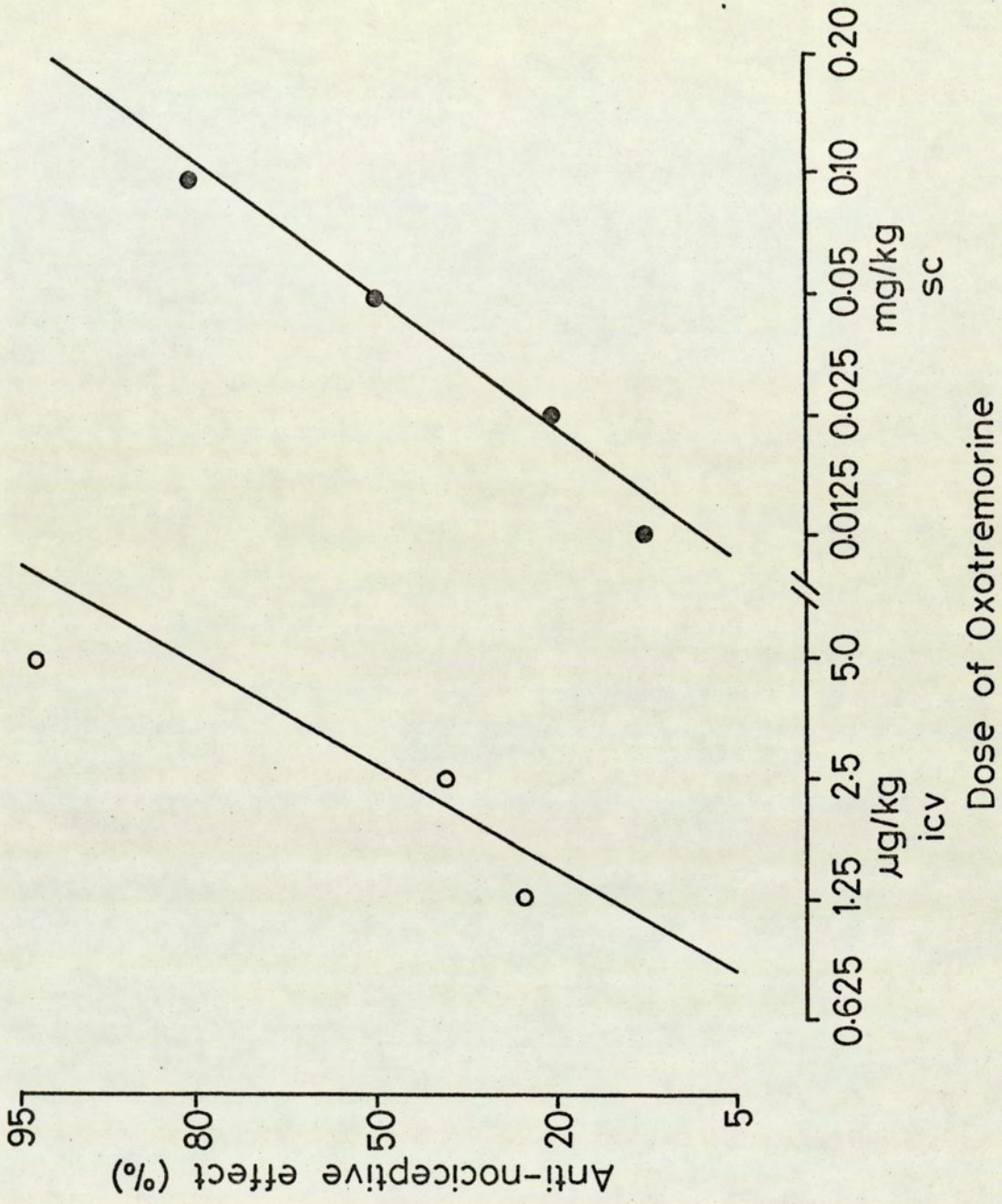

FIGURE 25: DOSE-RESPONSE CURVES TO OXOTREMORINE IN THE TAIL-FLICK TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of oxotremorine on a log scale for i.c.v. (O—O) and s.c. (●—●) routes of administration. The slopes do not differ significantly. (n = 8 for each i.c.v. dose plotted; n = 10 for each s.c. dose plotted.)

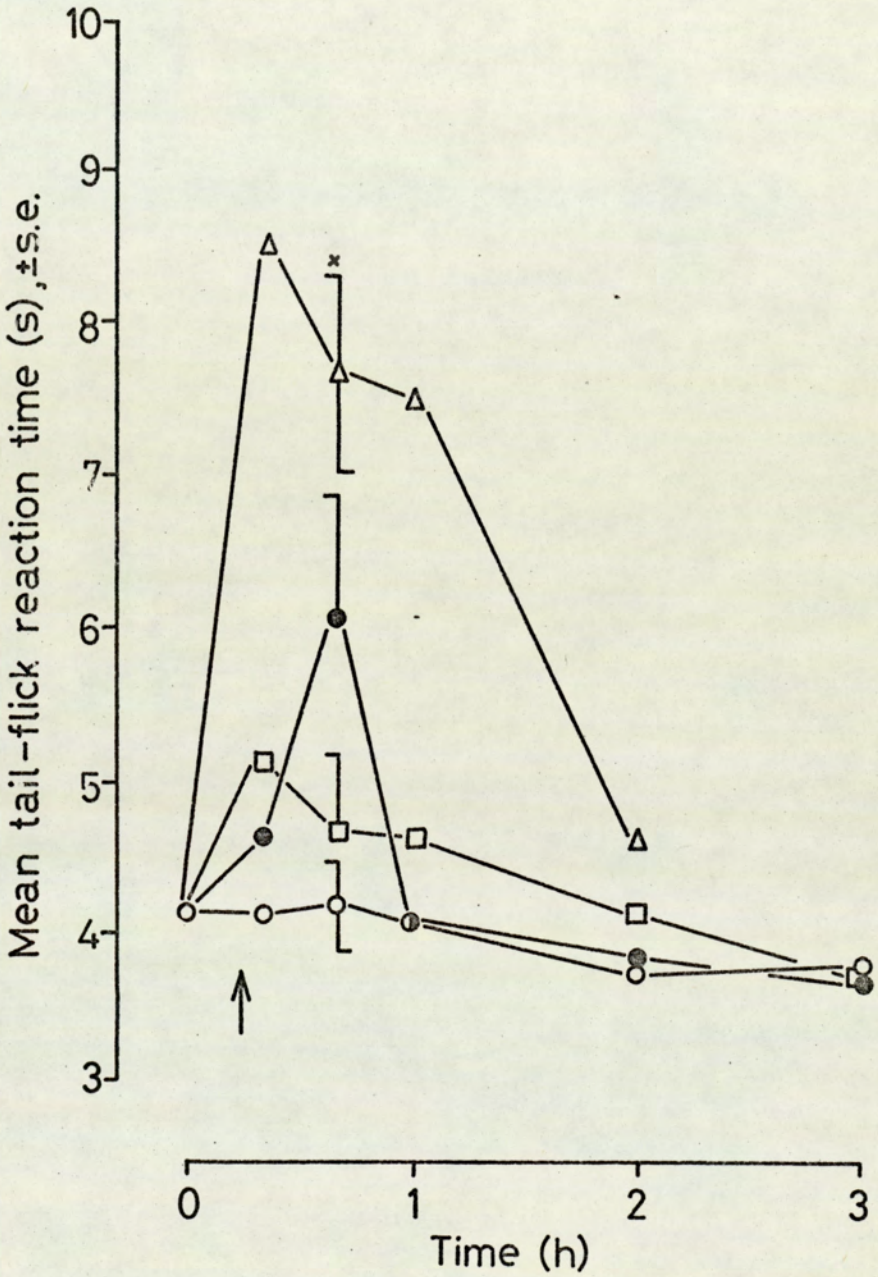

FIGURE 26: ANTI-NOCICEPTIVE ACTIVITY OF 5-HT, CARBACHOL AND PHYSOSTIGMINE AFTER I.C.V. INJECTION IN THE TAIL-FLICK TEST

Groups of five mice received saline, 10.0 µl i.c.v. (O—O) or 5-HT, 10.0 µg i.c.v. (●—●) at the arrow; groups of ten mice received carbachol, 0.2 µg i.c.v. (□—□) or physostigmine, 3.0 µg i.c.v. (Δ—Δ) at 0. Reaction times were determined for all groups at various times after injection.

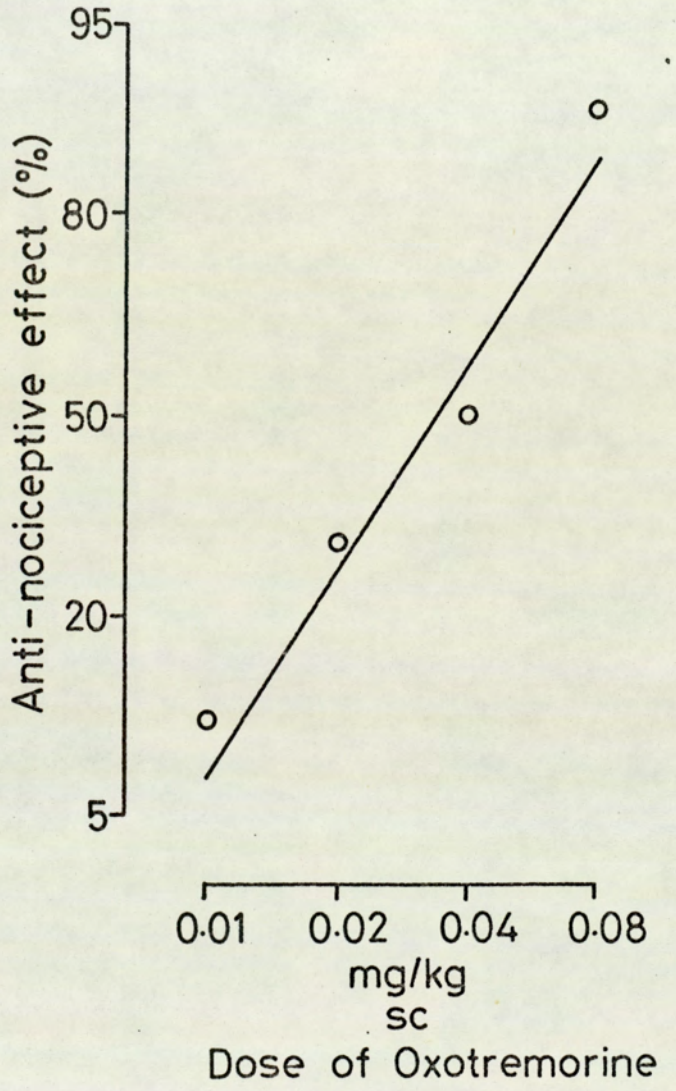

FIGURE 27: DOSE-RESPONSE CURVE TO OXOTREMORINE IN THE PHENYLQUINONE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of oxotremorine (by s.c. inj.) on a log scale. (n = 10 for each dose plotted.)

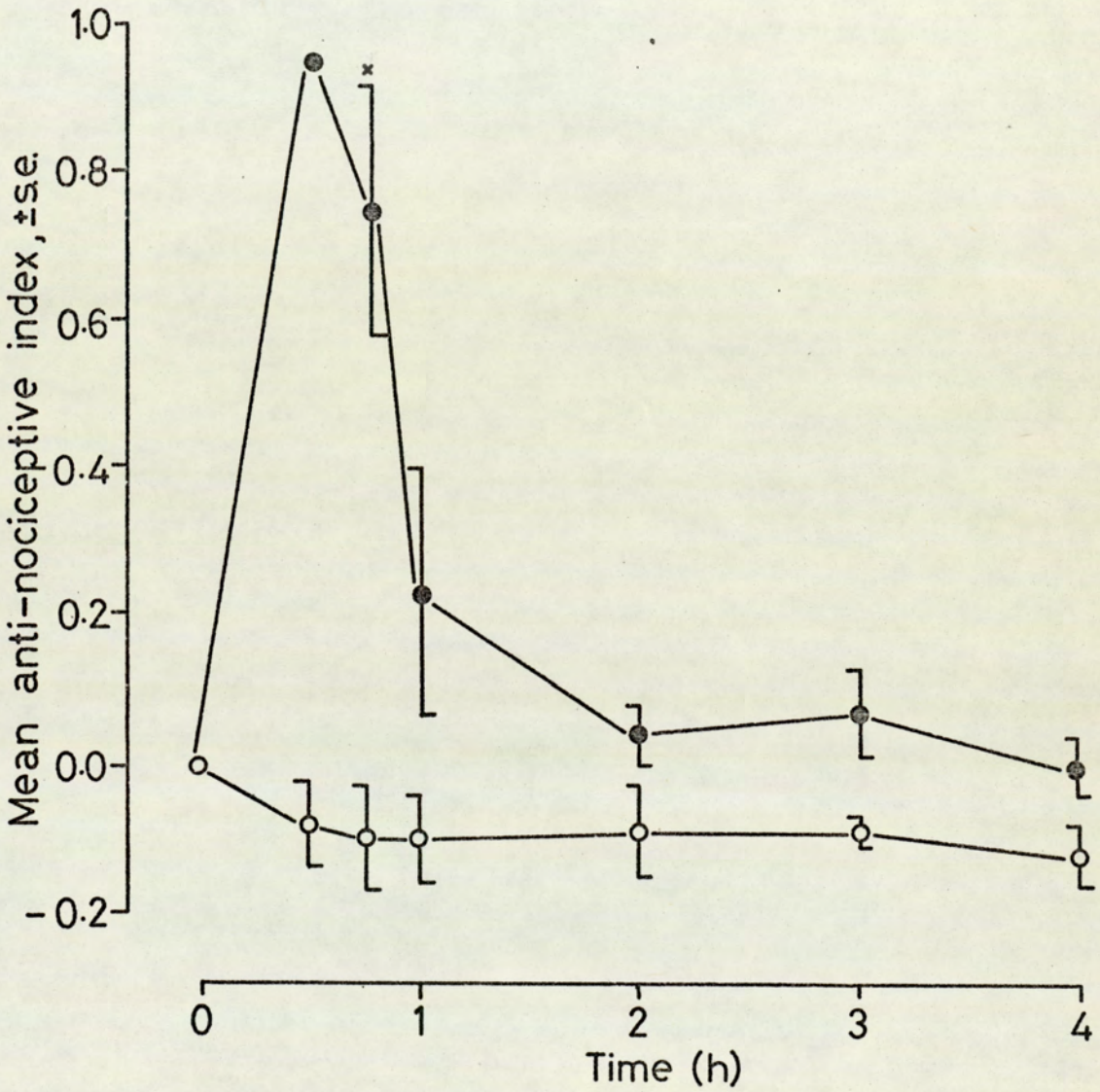

FIGURE 28: TIME-COURSE OF THE ANTI-NOCICEPTIVE EFFECT OF OXOTREMORINE IN THE FOOT-PRESSURE TEST

Groups of five rats received either saline, 1.0 ml/kg s.c. (O—O) or oxotremorine, 0.1 mg/kg s.c. (●—●) at 0, their nociceptive thresholds being determined at various times thereafter.

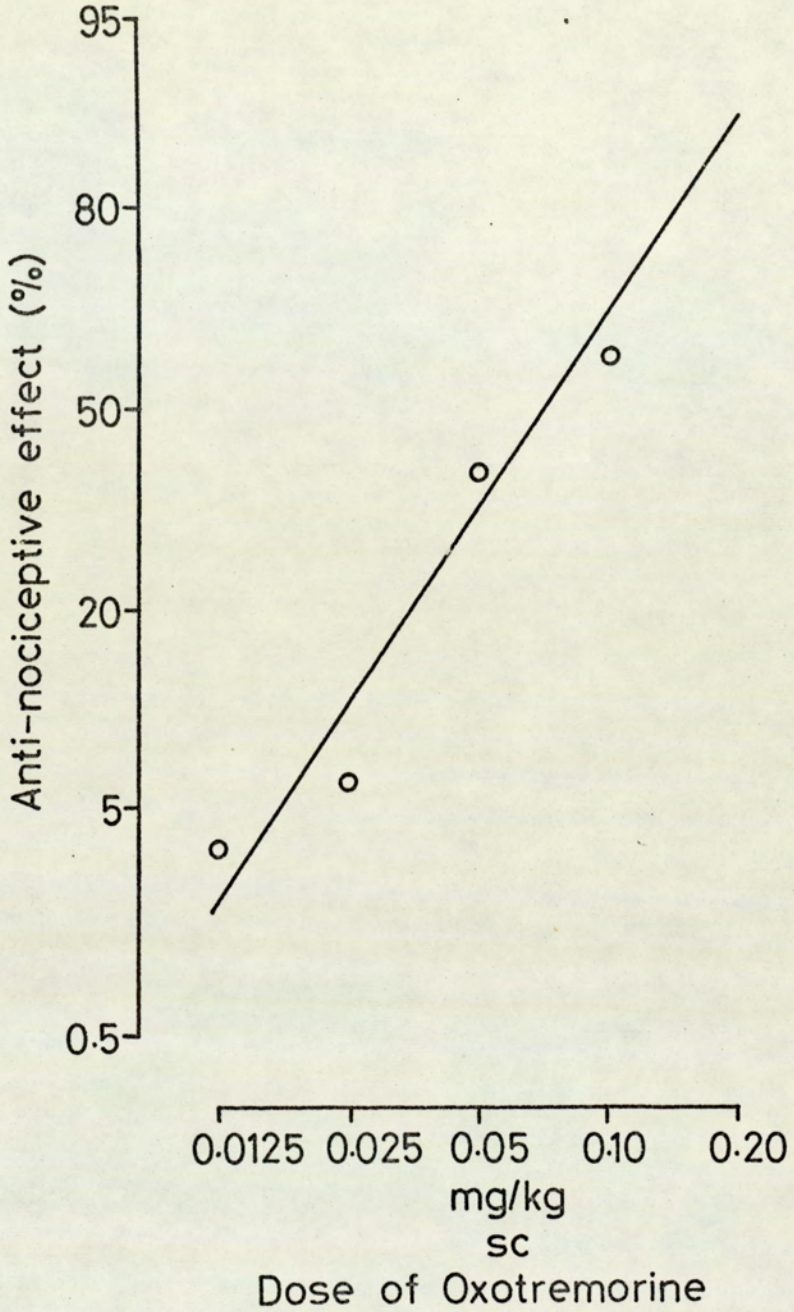

FIGURE 29: DOSE-RESPONSE CURVE TO OXOTREMORINE IN THE FOOT-PRESSURE TEST

Percent anti-nociceptive effect is plotted on a probability scale vs. dose of oxotremorine (s.c. injection) on a log-scale (n = 5 for each dose plotted).

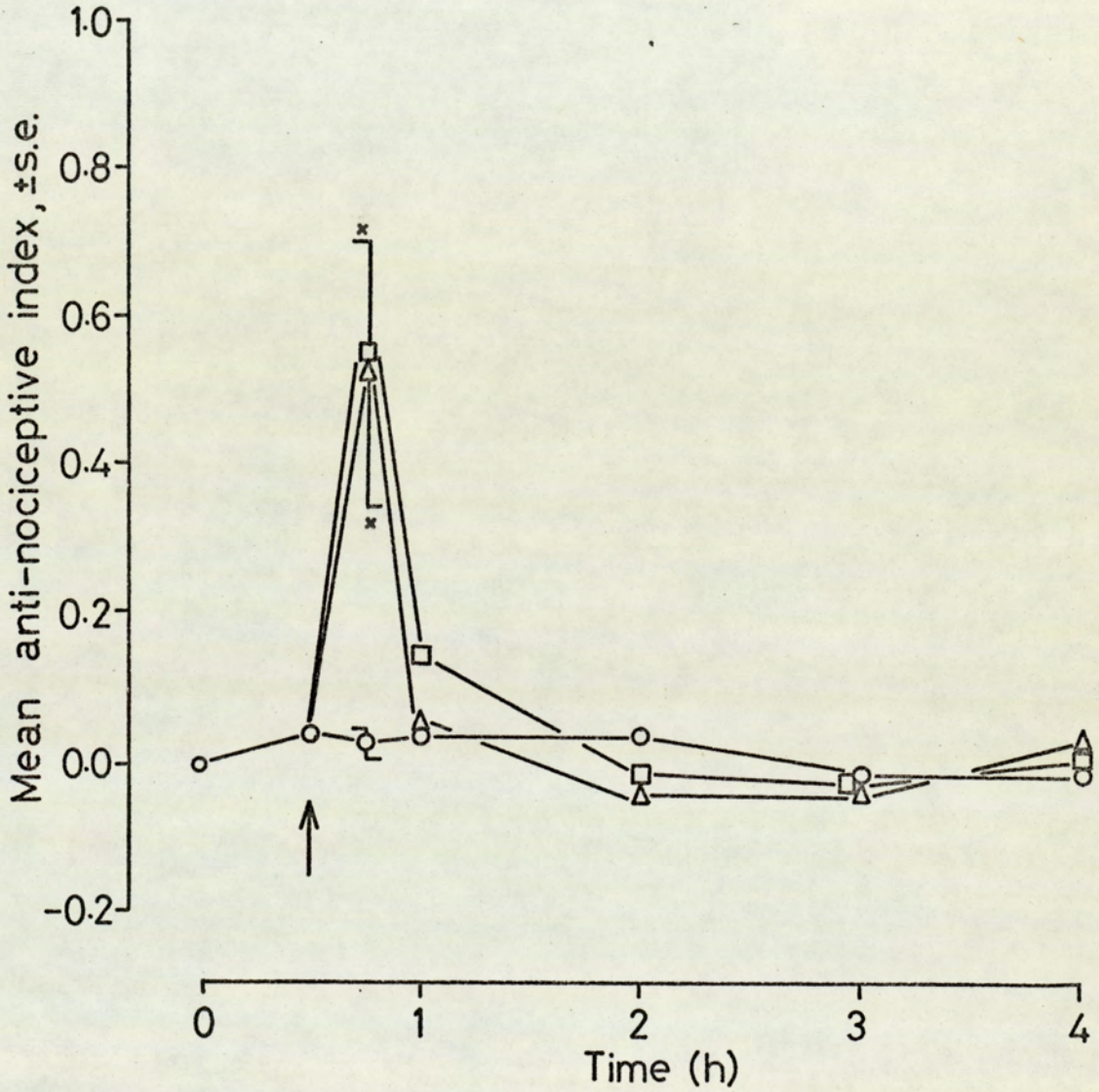

FIGURE 30: ANTI-NOCICEPTIVE EFFECTS OF PHYSOSTIGMINE AND 5-HT GIVEN BY I.C.V. INJECTION, IN THE FOOT-PRESSURE TEST

Groups of five rats received saline, 10.0 µl i.c.v., (O—O) physostigmine, 2.5 µg i.c.v. (□—□) or 5-HT, 20.0 µg i.c.v., (Δ—Δ) at the arrow, their nociceptive thresholds being determined at various times thereafter. The arrow is at +30 min.

PART C

ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AS MODIFIED BY OTHER AGENTS

CHAPTER FOUR

ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AS MODIFIED BY THE NARCOTIC ANTAGONIST, NALOXONE

1. INTRODUCTION

The experiments reported in this chapter were carried out to substantiate the belief that the effects of morphine in the test situations used here were brought about by a specific action on the central nervous system, rather than by any non-specific depressant effects. Kosterlitz *et al.* (1969) have shown that morphine (and, indeed the narcotic-antagonist, naloxone) have such non-specific depressant effects when applied in high concentration to the cat superior cervical ganglion and to the longitudinal muscle of guinea-pig ileum. The characteristics of these effects differ from those of the specific inhibitory activity of morphine on these same preparations when applied at considerably lower concentrations, so that these authors have suggested that two criteria for a specific action of morphine would be that the drug was acting at low concentration and that the effect so produced was blocked by a pure antagonist, (which itself was devoid of activity).

Such a pure antagonist is naloxone (Blumberg *et al.*, 1961). Kosterlitz and Watt (1968), using the guinea-pig isolated ileum to determine the kinetic parameters of a number of narcotic agonist and antagonist drugs, concluded that in this preparation at least naloxone has no agonist activity, but is a potent antagonist. Blumberg, Wolf and Dayton (1965), using the phenyl-quinone test in rats and mice, showed naloxone to have no anti-nociceptive activity following s.c. injection, a result which has been supported by our own work (Chapter Two), and naloxone has therefore been used as a "pure" antagonist in our study.

2. THE HOT-PLATE TEST

Naloxone, 1.0 µg i.c.v., injected 15 minutes before the animals were tested on the hot-plate, abolished completely the anti-nociceptive effect of

10.0 mg/kg morphine, which had been injected 60 minutes before the hot-plate test. Therefore, i.c.v. naloxone was able to abolish the anti-nociceptive effect of morphine which, as was shown in Chapter Two, would have been fully developed at the time of the naloxone injection.

3. THE TAIL-FLICK TEST

In this experiment, the naloxone was injected i.c.v. fifteen minutes before the injection of morphine, s.c. The time-course of the anti-nociceptive effect was then followed for three hours subsequent to the morphine injection. The results are shown in Fig. 31. Naloxone, 1.0 µg i.c.v., completely abolished the anti-nociceptive effect of a dose of morphine, 5.0 mg/kg s.c.

4. THE PHENYLQUINONE TEST

In this experiment, naloxone was injected by the s.c. route, 20 minutes prior to the morphine s.c. injection. The challenge dose of phenylquinone was then injected i.p. 20 minutes after the morphine injection, such that the centre of the ten minute counting period was 30 minutes after the morphine injection. Naloxone (10.0 mg/kg s.c.) entirely abolished the anti-nociceptive activity of morphine (1.0 mg/kg s.c.) in this test.

5. DISCUSSION

The results presented here show that in the hot-plate, tail-flick and phenylquinone tests, morphine is acting as a true narcotic agonist in bringing about its anti-nociceptive effects, because it is blocked in all the tests by naloxone, thus fulfilling the criterion suggested by Kosterlitz *et al.* (1969).

In addition, we have added further evidence to the postulation that

morphine is acting at supraspinal site of action, since its anti-nociceptive activity is blocked by i.c.v. administration of naloxone. The results also show that naloxone, when administered by this route, is capable of abolishing morphine anti-nociception which is already established — the antagonist does not have to be administered before the agonist.

The (apparent) equal ease with which naloxone blocks morphine's anti-nociceptive activity in both the hot-plate and tail-flick tests is interesting in view of the suggestion made earlier (see Discussion, Chapter Three) that morphine may be acting at different sites in the central nervous system in these two tests. The results with naloxone would suggest that even if two sites of action are involved, they both respond to the same type of morphine activity, which is blocked by the specific antagonist naloxone.

Results reported in this chapter support the view that we are indeed studying the specific actions of morphine in the various anti-nociceptive tests used.

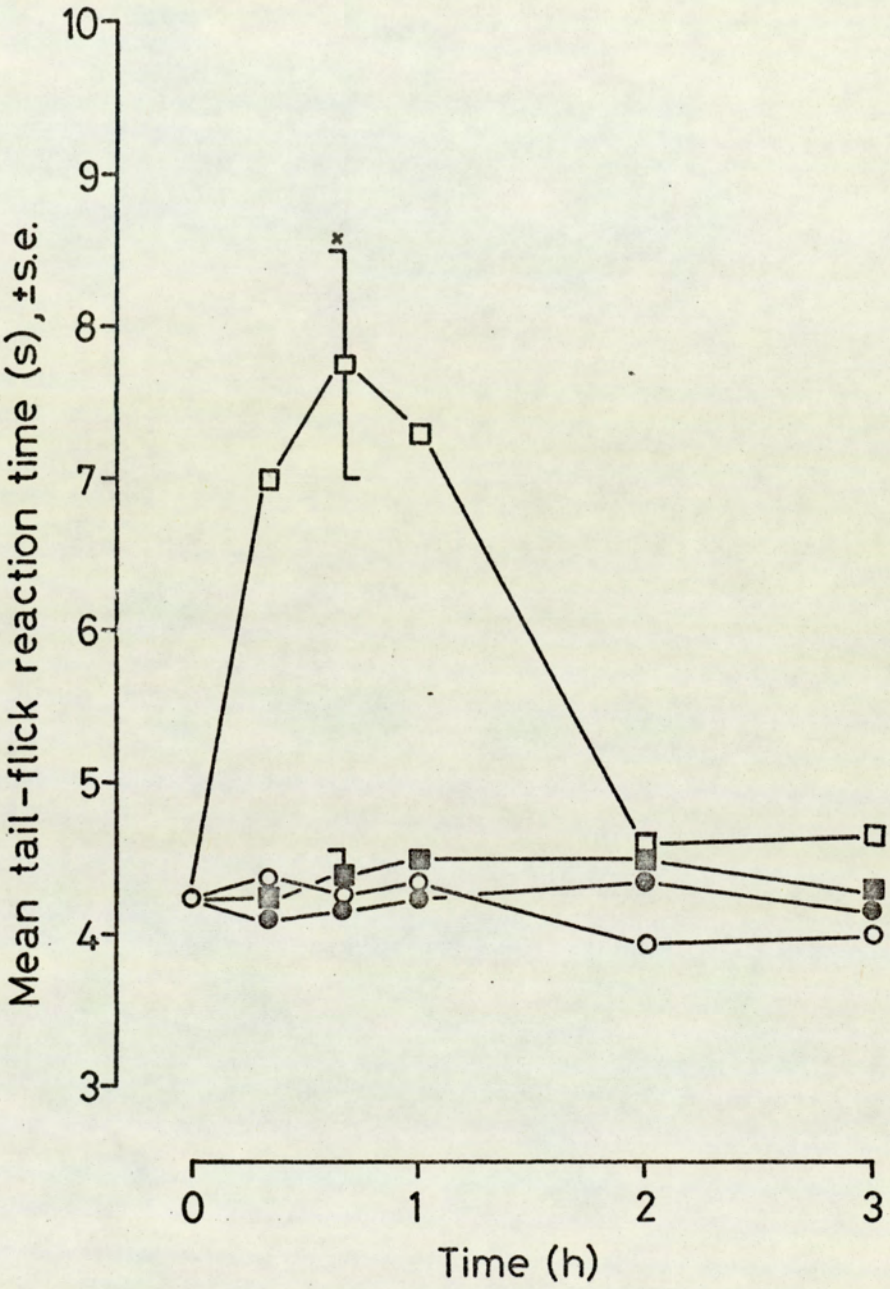

FIGURE 31: EFFECT OF I.C.V. NALOXONE ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

○—○: Saline, 10.0 µl i.c.v., at -15 min + saline, 10.0 ml/kg s.c., at 0.

●—●: Naloxone, 1.0 µg i.c.v., at -15 min + saline, 10.0 ml/kg s.c., at 0.

□—□: Saline, 10.0 µl i.c.v., at -15 min + morphine, 5.0 mg/kg s.c., at 0.

■—■: Naloxone, 1.0 µg i.c.v., at -15 min + morphine, 5.0 mg/kg s.c., at 0.

CHAPTER FIVE

ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AS MODIFIED BY CHOLINERGIC AND ANTI-CHOLINERGIC COMPOUNDS

1. INTRODUCTION

The possibility that morphine may act *via* a cholinergic mechanism, at least in part, has been suggested by numerous workers. Slaughter and Munsell (1940), using a mechanical compression test in cats (Eddy, 1932), demonstrated a synergism between morphine and neostigmine, and an antagonism between atropine and morphine or morphine/neostigmine combinations. Flodmark and Wramner (1945), measuring the pain threshold in man with the Hardy-Wolff-Goodell technique (1940), also detected a potentiation and prolongation of morphine's effect with neostigmine. Wramner (1945) confirmed this synergism with regard to the Straub tail reaction in mice, and reported that atropine antagonised the reaction produced by morphine or morphine/neostigmine combinations. Morphine/cholinergic combinations have been further studied by Christensen and Gross (1948) and by Slaughter (1950) in man.

The question arose as to what cholinergic systems might be involved in such an interaction. Houssay *et al.* (1928) had been examining the metabolic actions of morphine, and had presented evidence to suggest that morphine stimulated the release of adrenaline from the adrenal medulla. Ivy *et al.* (1944) had later shown that peripherally-administered adrenaline was analgesic in man and anti-nociceptive in dogs. The results of Houssay *et al.* (1928) and Ivy *et al.* (1944) led Harris and Friend (1947) to suggest that the cholinesterase inhibitors employed by Slaughter and Munsell (1940) and other workers were causing an increase in the release of adrenaline from the adrenals brought about by the morphine. Harris and Friend (1947) were able to show that morphine was less effective in adreno-medullectomised rats than in sham-operated animals. (This work was later presented in a fuller form: Friend & Harris, 1948.) These workers used a mechanical compression test in rats, the end-point used being vocalisation. Similar results were obtained

using adrenalectomised mice by Szerb and Jacob (1951).

Various other theories have been advanced as to the exact relationship between morphine and cholinesterase, particularly so following the demonstration that morphine itself was capable of inhibiting serum cholinesterase *in vitro* (Wright & Sabine, 1943); however, this ability was not confirmed *in vivo* (Schaumann, 1959). This apparent lack of correlation was further added to when some workers failed to demonstrate a synergism between cholinesterase inhibitors and morphine. Shaw and Bentley (1952) in fact demonstrated an antagonism, in that physostigmine aroused dogs which had been depressed by morphine. De Jongh (1954) failed to show a morphine/physostigmine synergism using a thermal noxious stimulus in the guinea-pig. A further point of contention was exposed by Knoll *et al.* (1951), who were able to demonstrate synergism between morphine and a variety of reversible cholinesterase inhibitors, such as physostigmine and neostigmine, but could detect no interaction when the irreversible cholinesterase inhibitor tetraethylpyrophosphate (TEPP) was substituted. These workers later suggested (Knoll *et al.*, 1953) that neostigmine in fact displaced morphine from protein binding sites in plasma, thus giving higher brain levels of the drug. Szerb and McCurdy (1956), however, could measure no increase in brain levels of morphine in the presence of neostigmine.

The presence of acetylcholine (ACh) in the central nervous system (Feldberg & Vogt, 1948; review by Hebb, 1970) as a possible neurohumoral transmitter substance, has added to the available sites where a narcotic/cholinergic interaction might take place. We have further examined the possible interaction between morphine and cholinergic systems in the hope of bringing new evidence to light on the mode of action of morphine.

The majority of experiments reported in this chapter have been carried out using the mouse tail-flick test, for a number of reasons: (i) the test is easily carried out and allows quite a large number of animals (and, therefore, different interactions) to be dealt with at once (ii) the test produces consistent results within any one group of animals (iii) the day to day consistency of drug effects is good (with the possible exception of morphine: Results: Chapter Two) (iv) the availability of time-course studies with this test provides more information per group of animals than other tests used, and (v) this test is (apparently — see General Discussion) the least likely to suffer from the occurrence of spurious results (see also Discussion to Results: Chapter Three). However, some experiments have been carried out using the hot-plate, phenylquinone and foot-pressure tests, in order to be able to compare results in these tests with those obtained using the tail-flick test.

2. THE MOUSE HOT-PLATE TEST

A combination of morphine, 5.0 mg/kg s.c., and physostigmine, 1.0 µg i.c.v., produced an anti-nociceptive effect significantly greater than that produced by either agent alone at these doses (Fig. 32). The effect of the combination was approximately 2 × that of either agent alone. This dose of physostigmine alone produced an anti-nociceptive effect of about 40%, and it was difficult to produce a potentiation of morphine in this test using a dose of physostigmine which was itself inactive. Thus the effect may be simply additive rather than true potentiation. The effect of 5.0 mg/kg s.c. morphine plus 1.0 µg i.c.v. physostigmine was equivalent to 10.0 mg/kg s.c. morphine.

3. THE TAIL-FLICK TEST

(a) Physostigmine

I.c.v. physostigmine (1.0 µg), a dose which did not alter the nociceptive threshold in this test, produced a highly significant increase in the activity of morphine (2.5 mg/kg s.c.). The potentiating effect was apparent for some 45 min after the injection of physostigmine (Fig. 33).

(b) Carbachol

The results obtained using a combination of carbachol i.c.v. and morphine s.c. were anomalous. It has been reported in Chapter Three that carbachol injected alone, i.c.v., was capable of raising the nociceptive threshold in this test, though apparently not in a dose-dependent manner. In combination with morphine, the activity of carbachol was erratic from day to day, with the result that a significant potentiation of morphine was not obtained consistently, and there was considerable variation in effect within any given test group.

(c) Atropine sulphate

Atropine, 10.0 mg/kg s.c., injected 15 min before morphine, 5.0 mg/kg s.c. initially had no significant effect on the anti-nociceptive activity of morphine. However, at 60 and 120 min after the morphine injection, some attenuation of the effect could be observed, although this was not statistically significant (Fig. 34). Reducing the dose of atropine to 2.0 mg/kg s.c. resulted in a much greater attenuation of morphine's effect, the anti-nociceptive activity of the atropine/morphine combination being approximately 1/5 that of morphine alone (Fig. 34).

When morphine was injected i.c.v. (1.5 µg), however, both high (20.0

mg/kg s.c.) and low (2.0 mg/kg) doses of atropine sulphate blocked morphine to a similar extent, equivalent to an 80% block at its maximal (Fig. 35). Taken over the total time-course of the effect, i.c.v. morphine appeared to be blocked less fully than did s.c. morphine with the smaller (2.0 mg/kg) dose of atropine.

When atropine was injected i.c.v., 0.5 µg, it attenuated the activity of morphine (5.0 mg/kg s.c.) by about 60% (Fig. 36), which was approximately equivalent to the attenuation observed with the dose of 2.0 mg/kg s.c. of atropine. Higher doses of atropine given i.c.v. (5.0 µg and 10.0 µg) produced no significant attenuation of the anti-nociceptive effect of morphine.

(d) Atropine methonitrate (methylatropine)

Methylatropine (2.0 mg/kg s.c.) injected 15 min before morphine produced a small attenuation (<10%) of the anti-nociceptive effect of morphine (5.0 mg/kg s.c.), which was not statistically significant (Fig. 37). A dose of methylatropine of 10.0 mg/kg s.c. produced the rather unexpected result of an attenuation of the anti-nociceptive activity of morphine, to the extent of about 40%, which effect was statistically significant (Fig. 37).

When injected i.c.v., methylatropine, 0.5 µg, had no effect on the anti-nociceptive activity of morphine, but a dose of 2.0 µg i.c.v. did result in a significant reduction (by about 50%) of morphine's effect (Fig. 38) when injected 15 min before the morphine (5.0 mg/kg s.c.).

When a small dose of methylatropine (2.0 mg/kg s.c.) was injected 15 min prior to i.c.v. morphine (1.5 µg) no alteration of the anti-nociceptive

effect of morphine was noted. However, 20.0 mg/kg s.c. methylatropine produced a small but not significant reduction (about 15%) in morphine's effect.

(e) Hyoscine

Hyoscine (1.0 mg/kg s.c.) injected 15 min before morphine (5.0 mg/kg s.c.) produced a reduction of about 70% in the anti-nociceptive activity of morphine (Fig. 39). This effect was highly statistically significant. Hyoscine alone did not alter the nociceptive threshold. Hyoscine (10.0 mg/kg s.c. and 20.0 mg/kg s.c.) produced a similar attenuation of morphine's anti-nociceptive effect, which was only marginally greater than that produced by 1.0 mg/kg s.c.

Thus hyoscine reduces the anti-nociceptive activity of morphine by about 70%, but is unable to completely abolish the effect in this test.

4. THE PHENYLQUINONE TEST

Hyoscine alone (1.0 mg/kg s.c.) produced a 30% increase in the number of writhes induced in mice by a challenge dose of phenylquinone. Also, a significant reduction (about 40%) of the anti-nociceptive effect of morphine in this test was brought about by this dose of hyoscine (Table 1).

5. THE RAT FOOT-PRESSURE TEST

Physostigmine, 1.0 µg i.c.v., produced a large potentiation of the anti-nociceptive effect of morphine, 1.25 mg/kg s.c., when injected 30 min after the morphine (Fig. 40). The value of the anti-nociceptive index was doubled. However, the statistical significance of the effect could not be determined as three of the five animals in the test group passed the cutoff

load without responding to the noxious stimulus. However, it is obvious from the figure (Fig. 40) that a large potentiation of morphine's effect has occurred.

6. DISCUSSION

The fact that physostigmine and neostigmine are capable of potentiating the anti-nociceptive activity of narcotic analgesics in a variety of species is well-known (Flodmark & Wramner, 1945; Ireson, 1969, 1970; Knoll *et al.*, 1957; Saxena, 1958; Schaumann, 1959; Slaughter & Munsell, 1940; Szerb, 1957; Wramner, 1945). However, the mechanism of the synergism is open to some doubt. A number of workers have attempted to demonstrate a similar synergism with one or other of the irreversible cholinesterase inhibitors (for instance: di-isopropylfluorophosphonate, DFP: Saxena, 1958; tetraethylpyrophosphate, TEPP: Knoll *et al.*, 1951; Szerb, 1957) but without success. This suggested the possibility that physostigmine and its congeners did not owe their potentiating ability (of morphine) to their inhibition of cholinesterase. Szerb (1957) suggested that, in the case of neostigmine, the potentiation of morphine was brought about by direct stimulation of cholinergic receptors by neostigmine, an effect earlier demonstrated by Burgen and Chipman (1952).

Knoll *et al.* (1953) postulated that neostigmine had the ability to displace morphine from protein binding sites in plasma, thereby giving rise to effectively higher blood levels of (free) morphine, which would in turn lead to higher brain levels. Jóhannesson and Schou (1963), and Szerb and McCurdy (1956) were unable to detect raised brain levels of morphine in neostigmine-treated animals, however. In fact Jóhannesson and Schou (1963) were one group of a number who failed to detect any synergism between neostigmine and morphine (Frommel *et al.*, 1963; de Jongh, 1954). The last-

named author in fact reported an antagonism. It is interesting to note, however, that both Frommel *et al.* (1963) and de Jongh (1954) used a radiant heat nociceptive test in the guinea-pig, which indicates a possible species variation between guinea-pigs and rodents (as used in the present study).

That even some synergism with neostigmine is detectable is of interest, since this compound has a quaternary nitrogen atom, and might not, therefore, be expected to gain ready access to the central nervous system. The possibility remains, therefore, that the effects seen by many of the workers mentioned here were connected with a peripheral effect of the drug, possibly the release of adrenal adrenaline as suggested by Friend and Harris (1948). We consider the possible peripheral effects of physostigmine have been largely eliminated in the present study by the use of the i.c.v. injection technique.

The possibility that morphine is acting, at least in part, through a stimulation of central cholinergic mechanisms is somewhat strengthened by the results presented here of the synergism between physostigmine and morphine. Attempts to block the anti-nociceptive activity of morphine with anti-cholinergic drugs have given mixed results. Knoll and Komlós (1951) reported that atropine potentiated morphine. The weight of evidence, however, is that atropine antagonises morphine (Christensen & Gross, 1948; Dundee *et al.*, 1961; Moore & Dundee, 1962), results which have been confirmed in the present study, with some qualification.

The fact that larger doses of atropine fail to antagonise morphine whilst smaller doses do may possibly be explained by the recent work of Polak (1971). *In vitro*, atropine causes the release of ACh from rat cortex slices. This is thought to be due to blockade of presynaptic ACh receptors, which when stimulated by released ACh, prevent the release of further ACh. In the

presence of atropine these receptors can no longer function, with the result that more and more ACh is released. It may be that the larger doses of atropine which we have used give rise to just such a build-up of ACh, which counteracts the post-synaptic block which we assume is responsible for the antagonism of morphine with the lower doses of atropine. It should be noted, however, that in the present study, hyoscine antagonised the anti-nociceptive action of morphine at all doses studied. It is possible that, in contrast to atropine, no dose-level of hyoscine can block the presynaptic receptors postulated by Polak (1971), and hence will cause no release of ACh in the brain.

The effects of atropine which we have observed are almost certainly central in origin, since the antagonism of morphine was observed following atropine injected *via* the i.c.v. route. It is possible that the failure to block morphine with higher doses of atropine (given s.c.) was the result of a peripheral effect, possibly resulting from an alteration in the biological distribution of morphine in the presence of atropine. Such an alteration has recently been demonstrated for oxotremorine with atropine (Karlén *et al.*, 1971). However, this possibility has been largely discounted since high doses of atropine injected i.c.v. also failed to block morphine, suggesting a central locus of action for the phenomenon.

Subcutaneous doses of methylatropine (containing the same quantity of atropine base as had been used to block morphine when injected as the sulphate) did not produce any blockade of the anti-nociceptive activity of morphine. However, if the dose of methylatropine was increased, (for example, to 10.0 mg/kg) then some degree of blockade was obtainable, although this did not approach that obtained with a low dose (2.0 mg/kg s.c.) of the sulphate. This result suggests that sufficient of the quaternary derivative

does cross the blood-brain barrier after peripheral administration of a large dose to have some central effects. Methylatropine injected i.c.v. produced an antagonism of the anti-nociceptive activity of morphine, but at a higher dose than was necessary to achieve an antagonism with the tertiary atropine salt. These results suggest that the antagonism by atropine is a result of an interaction at a central site, removed a small distance from the ventricular spaces. The apparently lower potency of methylatropine than atropine sulphate, despite using the i.c.v. route, is then explained on the basis of greater difficulty of penetration of the quaternary molecule from the csf (Albanus *et al.*, 1969).

The direct effects of morphine on brain ACh have been studied both *in vitro* and *in vivo*. Jóhannesson and Long (1964) could detect no difference in whole-brain levels of ACh in rats given single doses of morphine. However, other workers have reported increased levels of ACh in whole-brain after single doses of morphine (Giarman & Pepeu, 1962; Hano *et al.*, 1964; Herken *et al.*, 1957). Jhamandas *et al.*, (1970) measured the outflow of ACh into perspex cylinders attached to the cerebral cortex of anaesthetised cats, and found the outflow to be reduced by morphine, whether applied locally to the cortex or injected systemically.

Further evidence that morphine retards the release of ACh from the cortex has been obtained using *in vitro* preparations (Maynert, 1967a; Sharkawi & Schulman, 1969). Howes *et al.* (1970) showed that synthesis of ACh in cortical slices was reduced by morphine, which is probably a function of a buildup of ACh intraneuronally, as a result of reduced release brought about by morphine. Such a buildup of ACh at the sites of synthesis could inhibit synthesis (Sharkawi, 1970).

However, the results described above in intact animals (where whole-

brain levels of ACh were measured following single doses of morphine) are open to question with regard to their relevance to the anti-nociceptive action of morphine. In the majority of cases the doses of morphine used were many times the anti-nociceptive ED_{50} (Giarman & Pepeu, 1962, used 50.0 mg/kg in rats, and commented that a 47% increase in whole-brain ACh was accompanied by "severe depression"; Hano *et al.*, 1964, used 100.0 mg/kg, in mice). Also, the concentrations of morphine employed in the *in vitro* experiments (of the order of 10^{-3} M in the incubation medium) are within the range of concentrations shown by Kosterlitz *et al.*, (1969) to exhibit non-specific effects on a variety of tissues and preparations. That non-specific actions were being observed is supported by the following observations: Maynert (1967a) observed no blockade of this effect of morphine with nalorphine, and Howes *et al.* (1970) found nalorphine and naloxone to behave similarly to morphine.

Thus the possibility remains that at least part of the anti-nociceptive activity of morphine is brought about by a release of ACh in the central nervous system. That the effect of morphine could not be totally abolished by hyoscine suggests that morphine does not act entirely through a muscarinic mechanism.

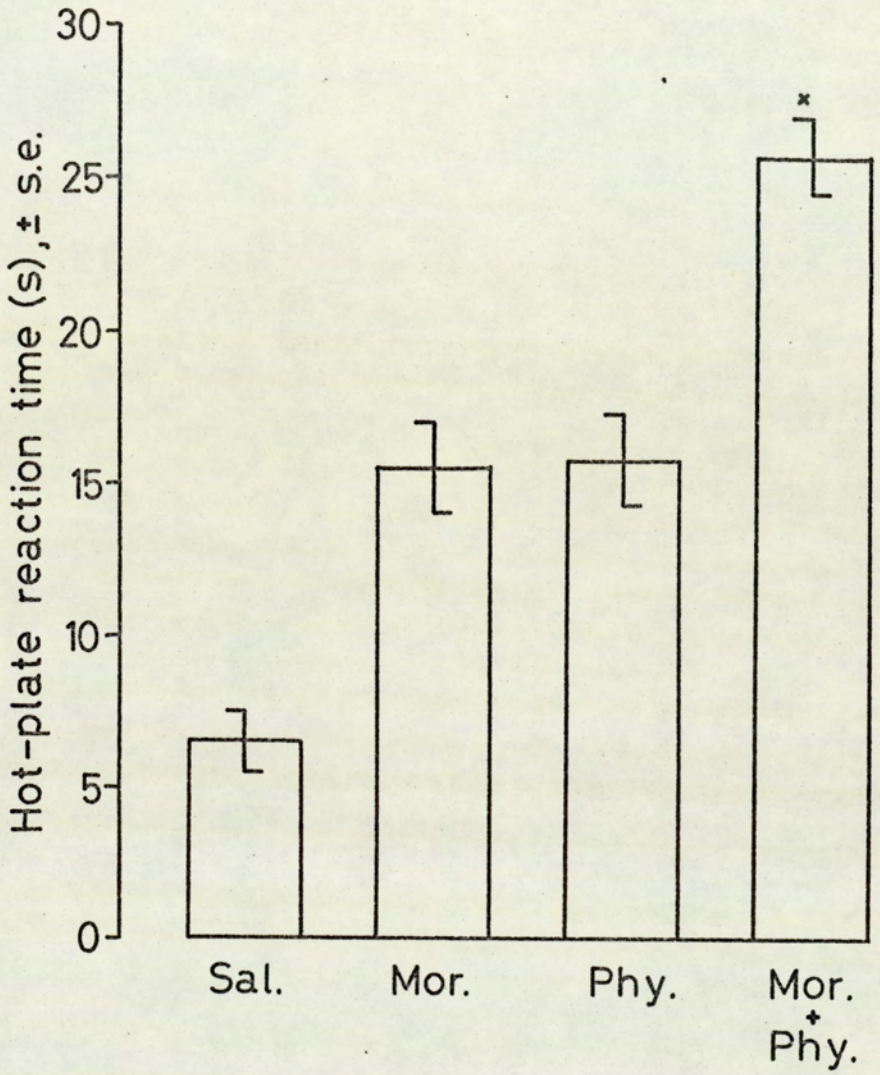

FIGURE 32: EFFECT OF PHYSOSTIGMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE HOT-PLATE TEST

Groups of ten mice were injected as follows:

Sal.: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at +50 min.

Mor.: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at +50 min.

Phy.: Saline, 10.0 ml/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at +50 min.

Mor. + phy.: Morphine, 5.0 mg/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at +50 min.

For all groups, the hot-plate test was carried out at +60 min.

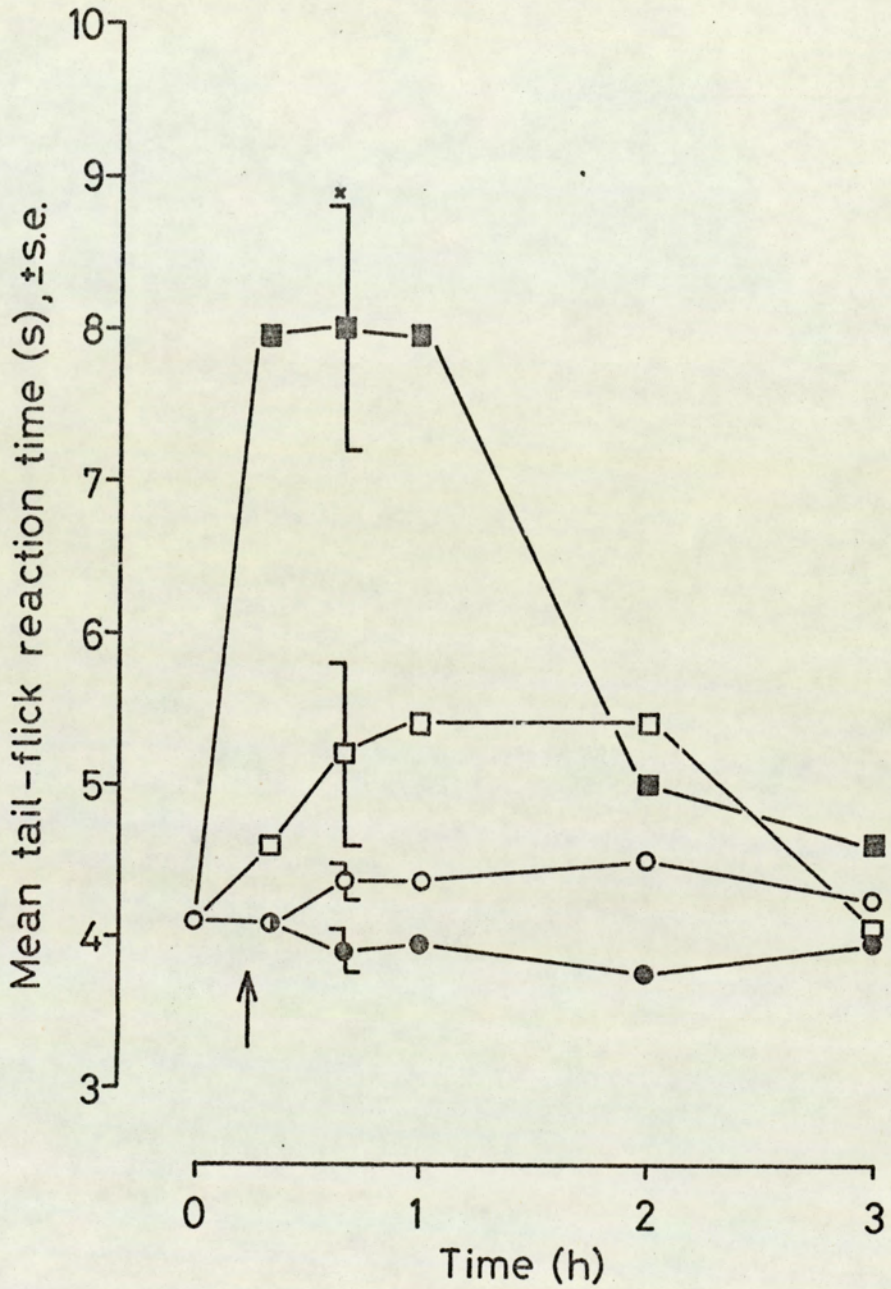

FIGURE 33: EFFECT OF PHYSOSTIGMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

□—□: Morphine, 2.5 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

■—■: Morphine, 2.5 mg/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v at the arrow.

The arrow is at +15 min.

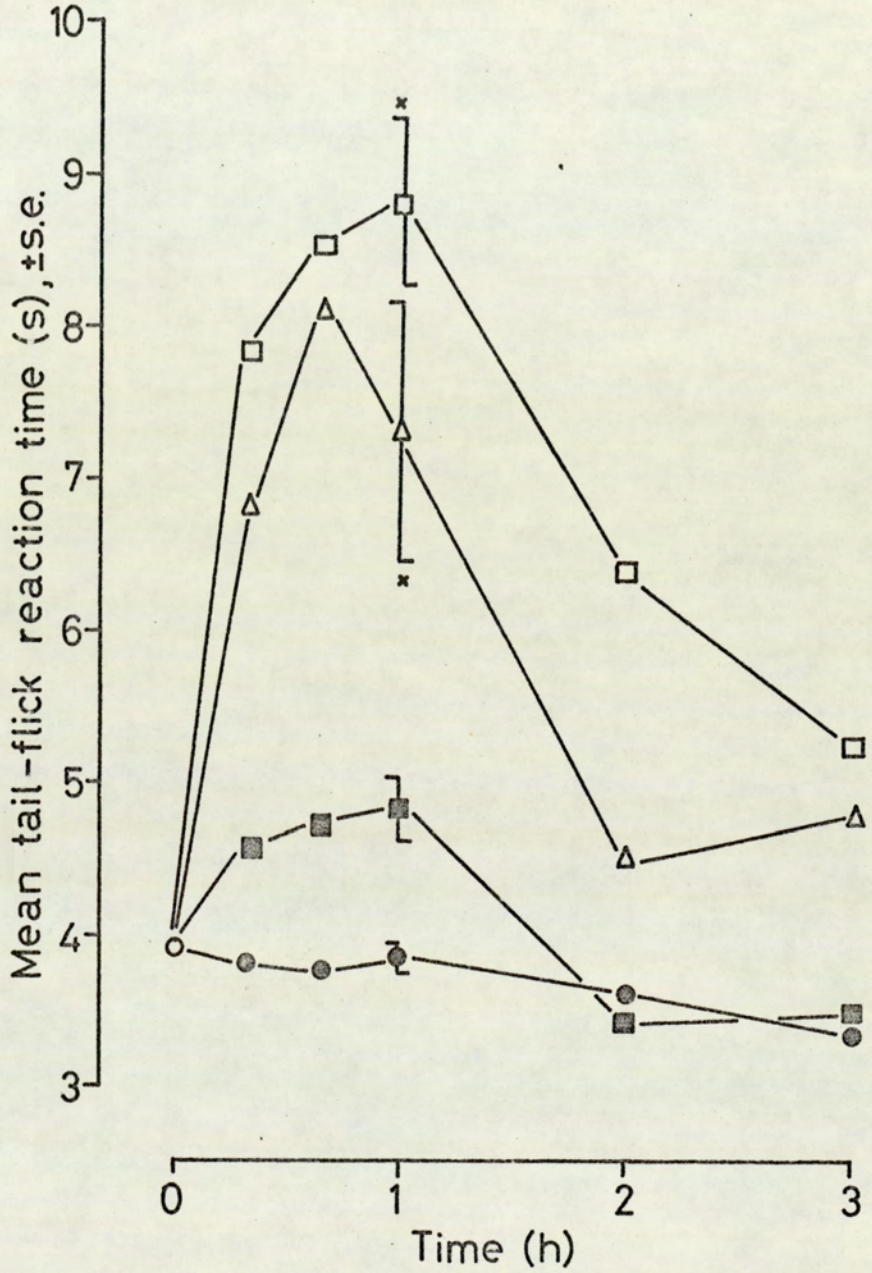

FIGURE 34: EFFECT OF ATROPINE SULPHATE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST: MORPHINE INJECTED S.C.

Groups of ten mice received the following:

●—●: Atropine sulphate, 10.0 mg/kg s.c. at -15 min + saline, 10.0 ml/kg s.c. at 0.

□—□: Saline, 10.0 ml/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

■—■: Atropine sulphate, 2.0 mg/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

Δ—Δ: Atropine sulphate, 10.0 mg/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

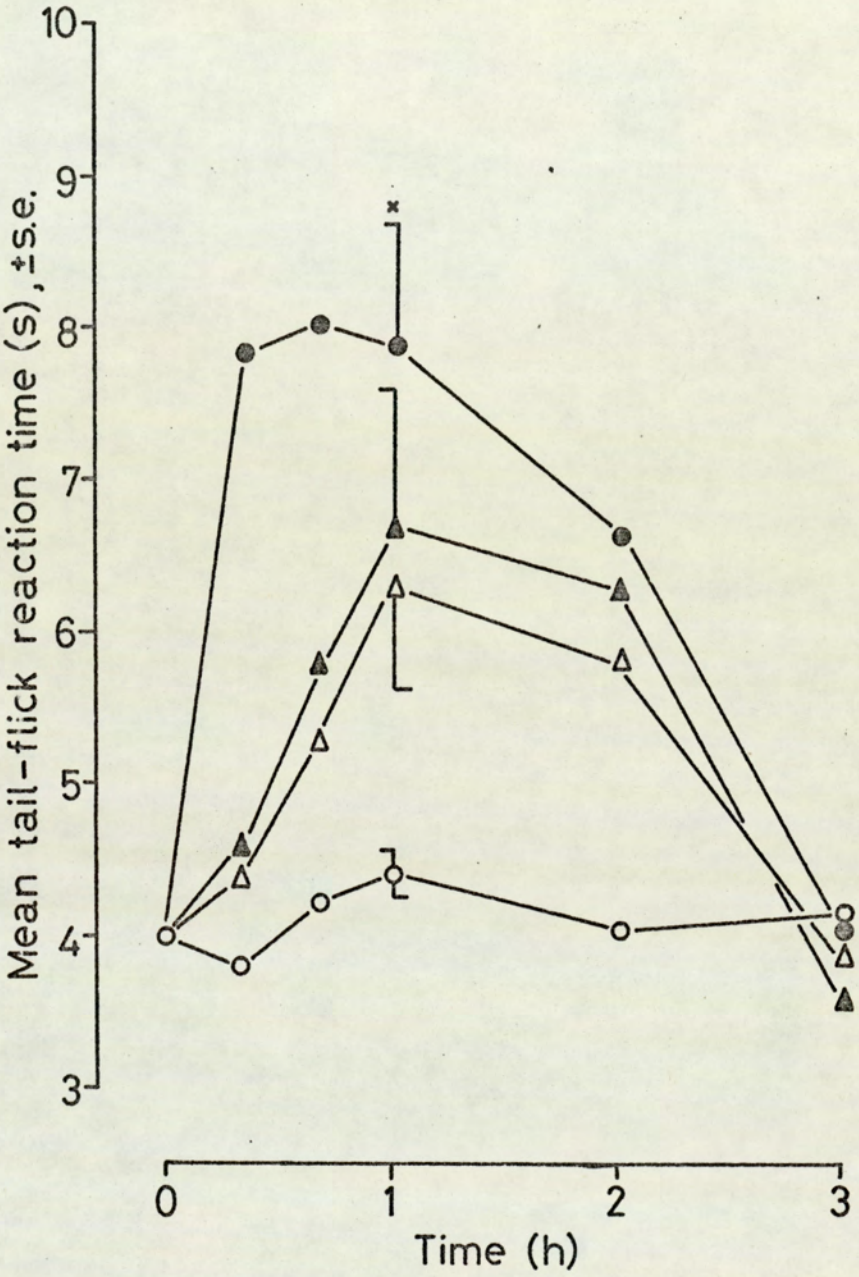

FIGURE 35: EFFECT OF ATROPINE SULPHATE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST: MORPHINE INJECTED I.C.V.

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at -15 min + saline, 10.0 µl i.c.v. at 0.

●—●: Saline, 10.0 ml/kg s.c. at -15 min + morphine, 1.5 µg i.c.v. at 0.

Δ—Δ: Atropine sulphate, 2.0 mg/kg s.c. at -15 min + morphine, 1.5 µg i.c.v. at 0.

▲—▲: Atropine sulphate, 20.0 mg/kg s.c. at -15 min + morphine, 1.5 µg i.c.v. at 0.

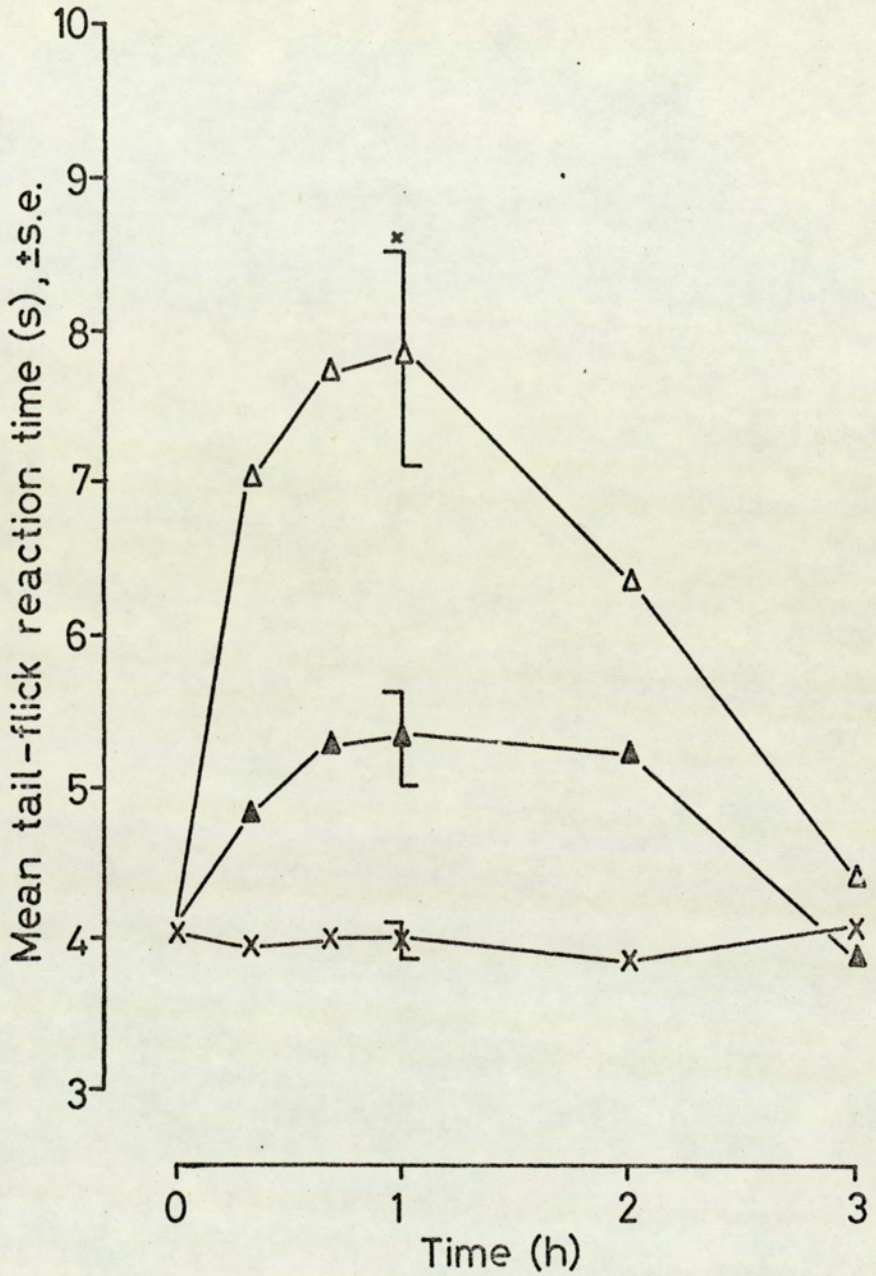

FIGURE 36: EFFECT OF ATROPINE SULPHATE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

X—X: Atropine sulphate, 0.5 µg i.c.v. at -15 min + saline, 10.0 ml/kg s.c. at 0.

Δ—Δ: Saline, 10.0 µl i.c.v. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

▲—▲: Atropine sulphate, 0.5 µg i.c.v. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

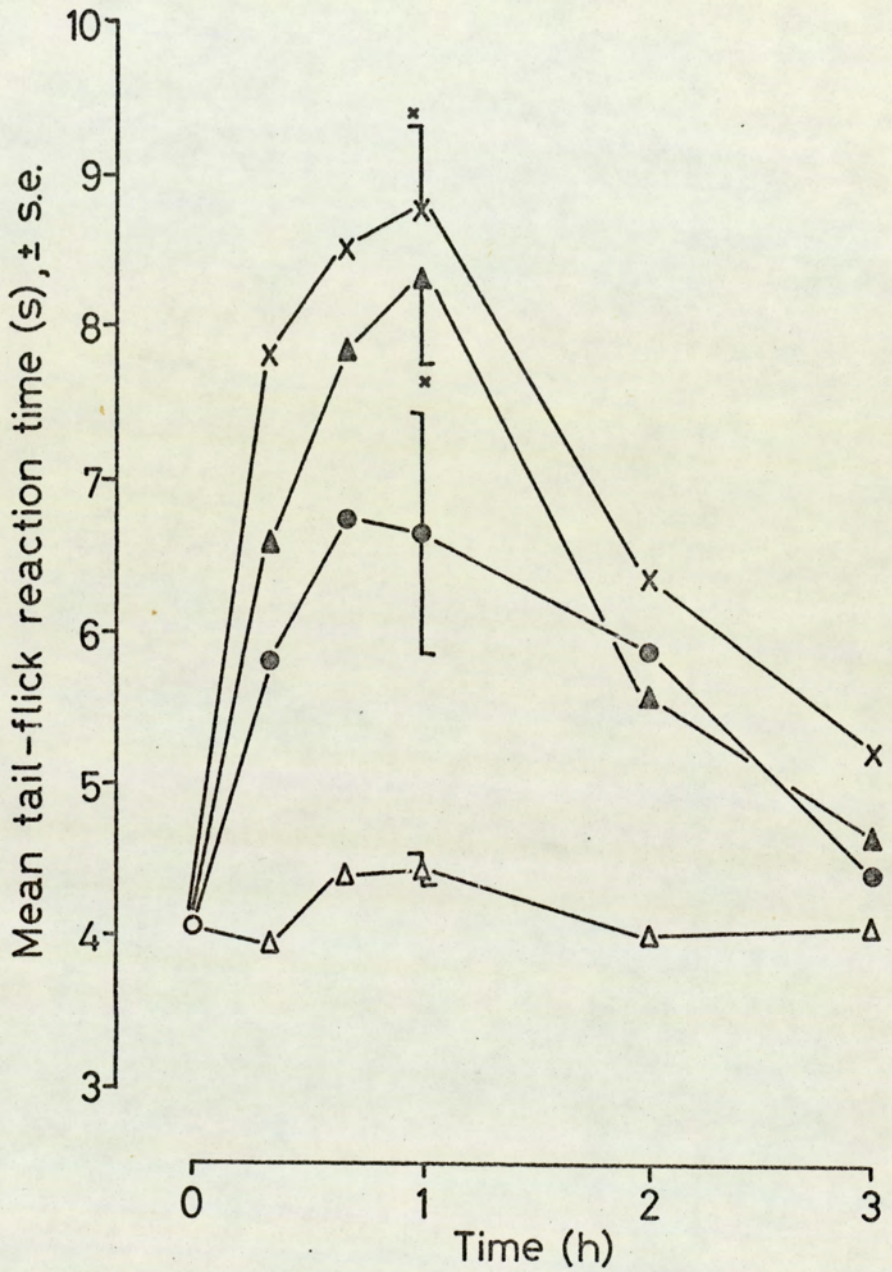

FIGURE 37: EFFECT OF ATROPINE METHONITRATE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

Δ—Δ: Atropine methonitrate, 2.0 mg/kg s.c. at -15 min + saline, 10.0 ml/kg s.c. at 0.

X—X: Saline, 10.0 ml/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

▲—▲: Atropine methonitrate, 2.0 mg/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

●—●: Atropine methonitrate, 10.0 mg/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

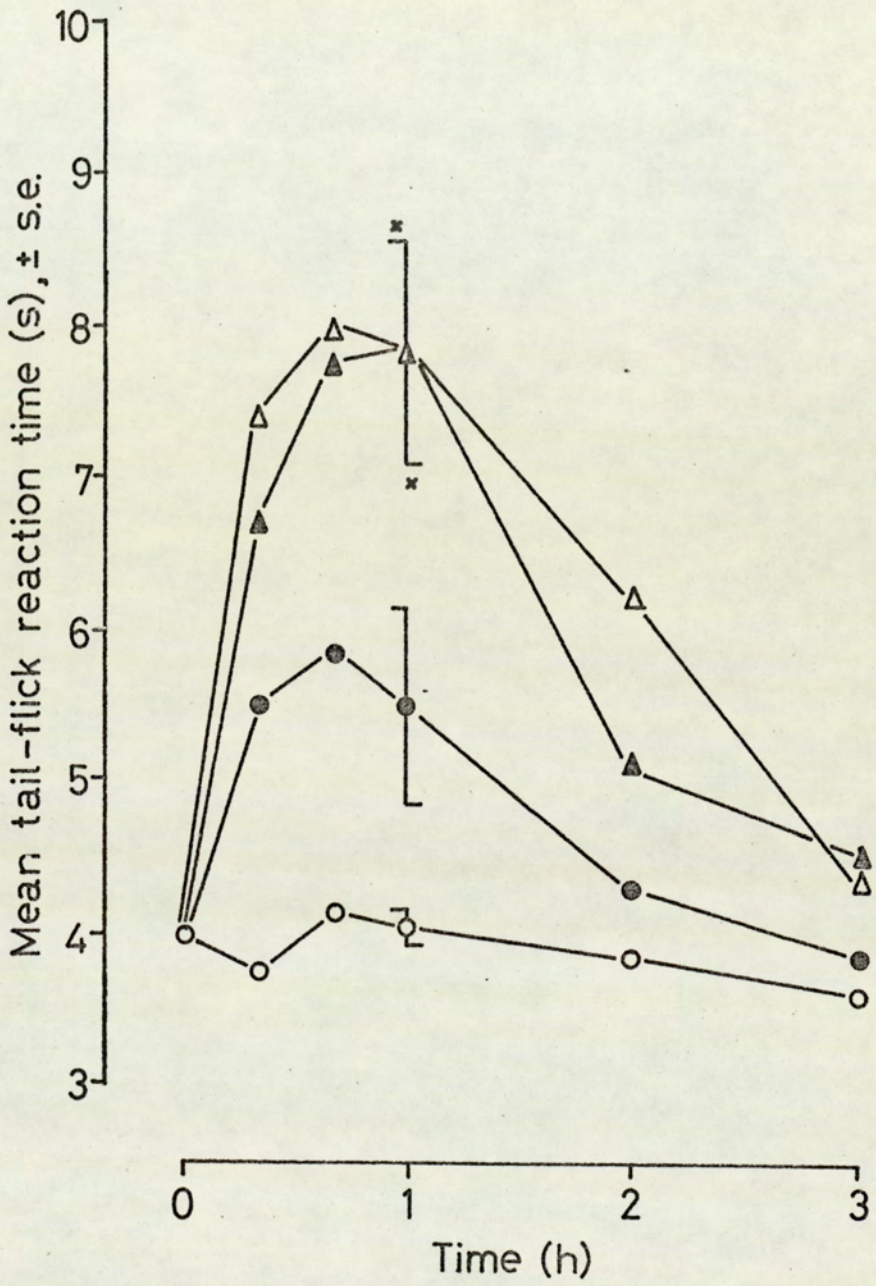

FIGURE 38: EFFECT OF ATROPINE METHONITRATE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

O—O: Atropine methonitrate, 2.0 µg i.c.v. at -15 min + saline, 10.0 ml/kg s.c. at 0.

Δ—Δ: Saline, 10.0 µl i.c.v. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

▲—▲: Atropine methonitrate, 0.5 µg i.c.v. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

●—●: Atropine methonitrate, 2.0 µg i.c.v. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

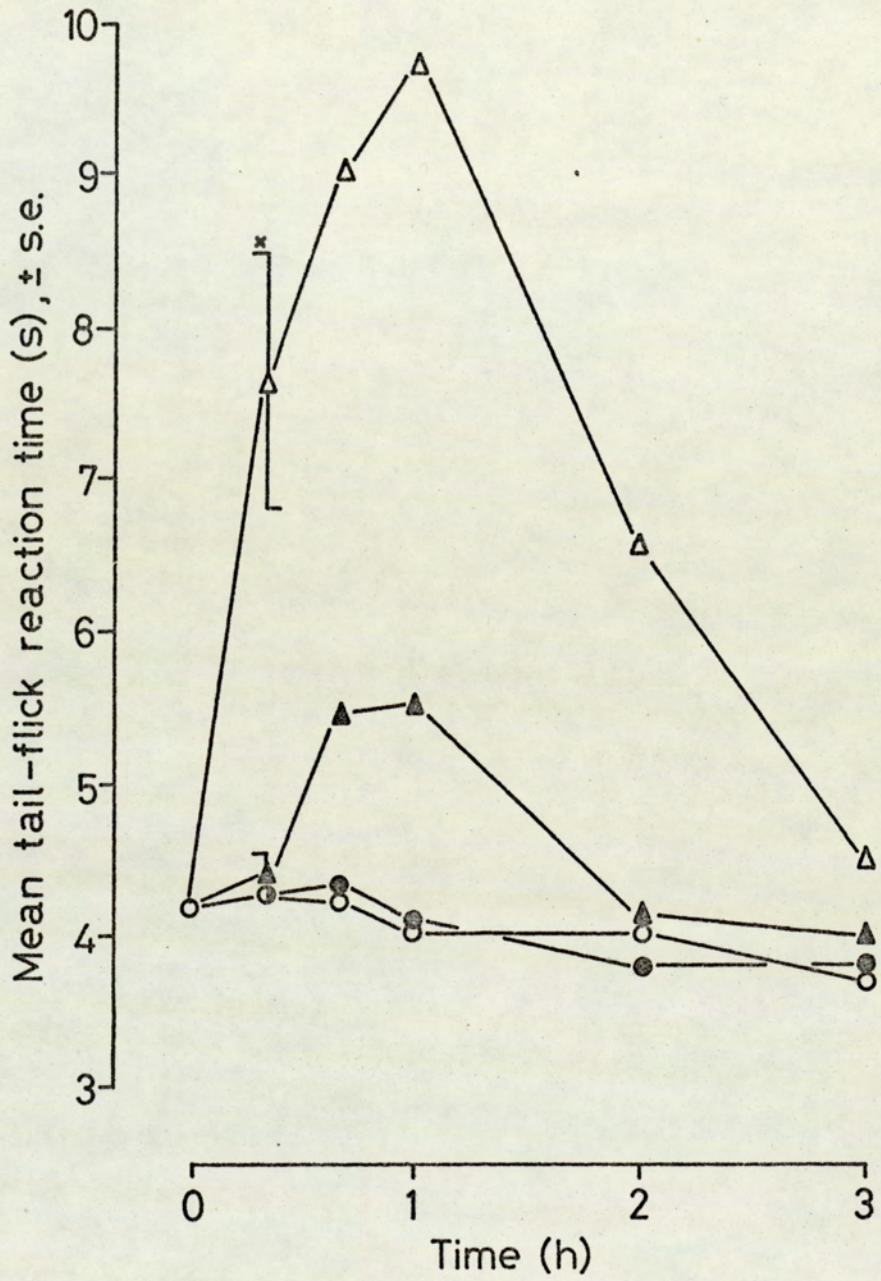

FIGURE 39: EFFECT OF HYOSCINE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at -15 min + saline, 10.0 ml/kg s.c. at 0.

●—●: Hyoscine, 1.0 mg/kg s.c. at -15 min + saline, 10.0 ml/kg s.c. at 0.

Δ—Δ: Saline, 10.0 ml/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

▲—▲: Hyoscine, 1.0 mg/kg s.c. at -15 min + morphine, 5.0 mg/kg s.c. at 0.

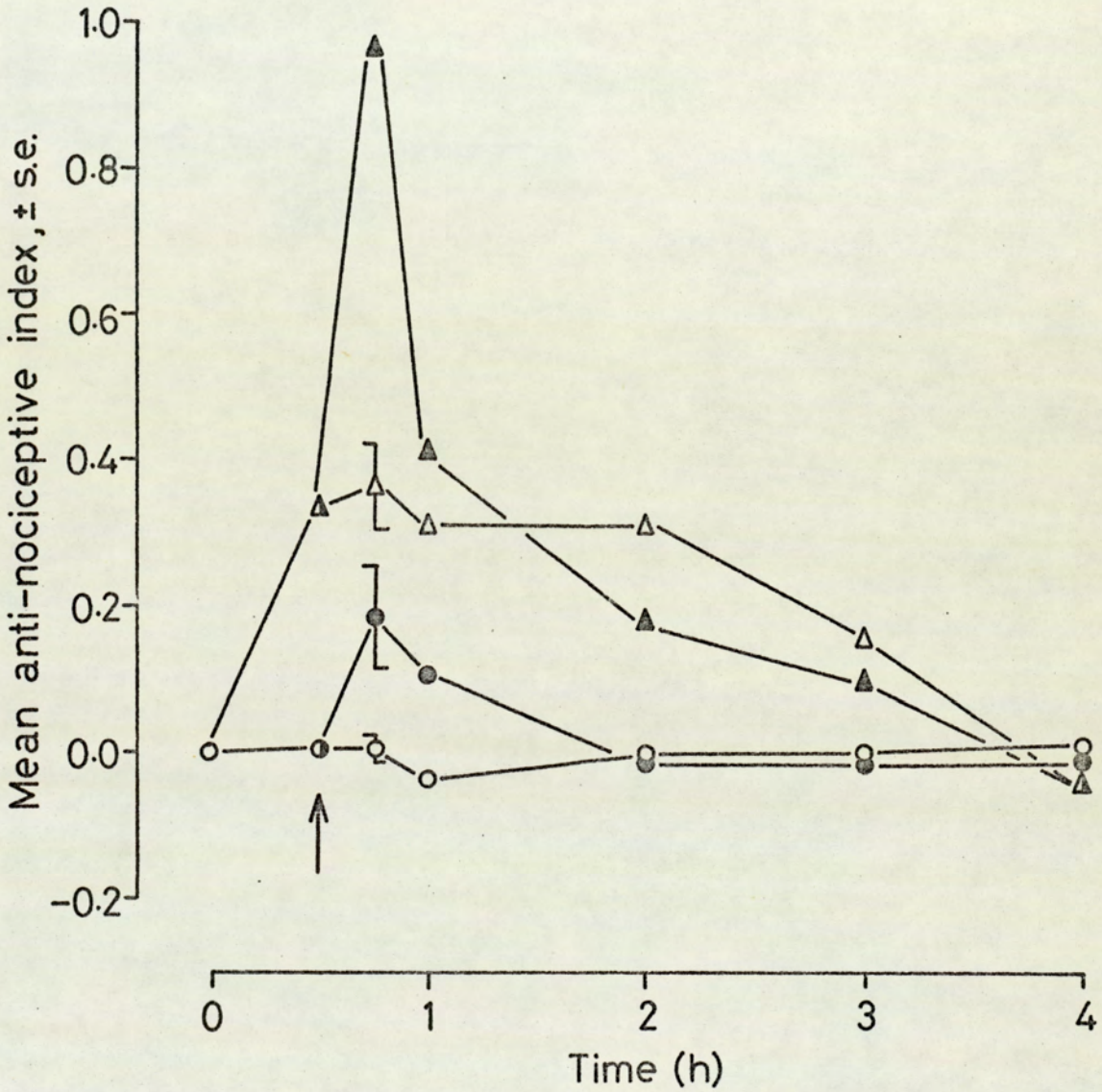

FIGURE 40: EFFECT OF PHYSOSTIGMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

O—O: Saline, 1.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 1.0 ml/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 1.25 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 1.25 mg/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

Treatment prior to phenylquinone, 2.0 mg/kg i.p.	Mean No of writhes ± s.e.
Saline 10.0 ml/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	32.3 ±3.3
Hyoscine 1.0 mg/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	43.2 ±4.3
Saline 10.0 ml/kg s.c. at -40 min + morphine 1.0 mg/kg s.c. at -20 min	4.6 ±1.9
Hyoscine 1.0 mg/kg s.c. at -40 min + morphine 1.0 mg/kg s.c. at -20 min	13.9 ±3.7

TABLE 1: EFFECT OF S.C. HYOSCINE ON MORPHINE ANTI-NOCICEPTION IN THE PHENYLQUINONE TEST

Groups of five mice were injected as detailed in the table.

CHAPTER SIX

ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AS MODIFIED BY ADRENERGIC AND ANTI-ADRENERGIC COMPOUNDS

1. INTRODUCTION

The possible involvement of a peripheral adrenergic mechanism in narcotic analgesia has been discussed briefly in the proceding chapter (Chapter Five). The demonstration of the presence and uneven distribution of noradrenaline in brain, together with the fact that morphine (albeit in large doses) could reduce the levels of hypothalamic noradrenaline (Vogt, 1954), stimulated research into a possible functional interaction between these two substances in the CNS.

One of the first (and, subsequently, most widely used) "tools" to be used in the study of this interaction was reserpine, since its ability to deplete both central and peripheral stores of monoamines is well defined. (Shore *et al.*, 1957a, and see review by Shore, 1962.) Reserpine has been found by most workers to attenuate the anti-nociceptive effect of morphine and related drugs in a variety of tests and species (Colville & Chaplin, 1964; Contreras & Tamayo, 1966; Dewey *et al.*, 1968, 1970; Fennessy & Lee, 1970; Medaković & Banić, 1963, 1964, 1969; Muñoz & Paeile, 1967; Radouco-Thomas *et al.*, 1957, 1959, 1967; Schaumann, 1958; Schneider, 1954; Sigg *et al.*, 1958; Sparkes & Spencer, 1969, 1971; Takagi *et al.*, 1964; Verri *et al.*, 1967, 1968). Nott (1968) found reserpine to be antagonistic to morphine in the tail-flick test but synergistic in the hot-plate test; Ross & Ashford (1967) detected antagonism in the tail-clip test and potentiation in the hot-plate test. In some cases, however, only a potentiation of morphine by reserpine was reported, despite the use of several types of nociceptive test (Dandiya & Menon, 1963; Garcia Lémé & Rocha e Silva, 1961; Maj *et al.*, 1971).

Because of the widespread effects of reserpine (and of the related compound tetrabenazine, which also can attenuate morphine's anti-nociceptive activity; see: Muñoz & Paeile, 1970; Sethy *et al.*, 1970; Takagi & Ishida,

1965; Takagi & Nakama 1968; Takagi *et al.*, 1964, 1968), a number of more specific agents have come into use which apparently deplete only one or two monoamines in brain. Thus α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase (Spector, 1966; Spector *et al.*, 1965) has been used to lower dopamine and noradrenaline levels in brain in an attempt to clarify whether the effects seen following reserpine were due to an action on catecholamines or on 5-HT. In fact, α-methyl-*p*-tyrosine has been reported to potentiate (Maj *et al.*, 1971), have no effect on (Fennessy & Lee, 1970), and even to attenuate (Verri *et al.*, 1967, 1968) the anti-nociceptive activity of morphine!

The related compound α-methyl-*m*-tyrosine was initially thought to deplete catecholamines and 5-HT by inhibiting the enzyme aromatic amino-acid decarboxylase (Hess *et al.*, 1961a,b), and was later found to prevent the binding of freshly-synthesised noradrenaline (Hess, 1962). This latter effect was explained by Carlsson and Lindqvist (1962) as a replacement of noradrenaline by metaraminol, the decarboxylated, β-hydroxylated product of α-methyl-*m*-tyrosine itself. The anti-nociceptive effect of morphine was unaltered by pretreatment with α-methyl-*m*-tyrosine (Medaković & Banić, 1963, 1964). However, Muñoz and Paeile (1967) reported that the metabolic product metaraminol was antagonistic to morphine. Another inhibitor of aromatic amino-acid decarboxylase, α-methyldopa (Spector, 1966), which is probably decarboxylated and β-hydroxylated to α-methylnoradrenaline *in vivo* (Carlsson & Lindqvist, 1962), enhanced morphine anti-nociceptive activity (Contreras & Tamayo, 1966; Contreras *et al.*, 1967, 1969) and reversed the attenuating effect of reserpine on morphine (Ross & Ashford, 1967).

Because of the confusion from results of previous studies, and the possibilities that these so-called specific depleting agents were having other than the predicted effect on central monoamines, the question of

adrenergic involvement in morphine anti-nociception remains unanswered. Using the technique of i.c.v. injection to increase the local concentration of amines in certain regions of the brain, the involvement of adrenergic processes has been re-examined both in normal animals and in those whose stores of amines have been depleted with reserpine. The results of this study are presented in this chapter.

2. THE TAIL-FLICK TEST

(a) Reserpine

Reserpine, 2.0 mg/kg i.p., at various pretreatment times, produced a complete abolition of morphine anti-nociception, and in fact reduced the tail-flick reaction time of morphine-treated mice to below that of non-reserpinised, saline-treated mice. A slight potentiation of morphine was noted one hour after the reserpine injection, but thereafter the effect of morphine was reduced by about half (at two hours after reserpine) and completely abolished at three hours (Fig. 41). The period of maximal reduction in the anti-nociceptive effect of morphine lasted from 5 to 24 hours after reserpine; thereafter, the antagonism declined and morphine's activity was fully restored nine days after the single reserpine injection.

(b) Tetrabenazine

Tetrabenazine, 40.0 mg/kg i.p., produced a significant reduction (60.0%) in the anti-nociceptive effect of morphine when given three hours prior to morphine (5.0 mg/kg, s.c.) (Fig. 42). This dose of tetrabenazine was unable to abolish entirely the anti-nociceptive effect of morphine, the maximal effect being a reduction of 60%.

(c) Noradrenaline

Noradrenaline, 5.0 µg, injected i.c.v. 15 min after s.c. morphine,

5.0 mg/kg, produced a 30% attenuation of the activity of morphine in the tail-flick test; this effect was short-lived, however, the morphine activity re-establishing itself 45 min after the noradrenaline injection. Higher doses of noradrenaline produced a proportionately greater attenuation. Assuming the re-establishment of morphine activity to be a result of short duration of action on the part of the noradrenaline, further experiments were carried out in which the noradrenaline was injected 30 min after the morphine (Fig. 44).

In this case, the noradrenaline was injected later in the time-course of morphine activity, with the result that the attenuation was more marked.

Noradrenaline injected i.c.v. after reserpine pretreatment was without effect on the nociceptive threshold, and also had no effect on the threshold of animals which had received reserpine and morphine.

(d) Dopamine

Initially, relatively high doses of dopamine were employed because previous workers had shown that high doses were necessary to alter body temperature, if given *via* the i.c.v. route (Brittain & Handley, 1967). Thus 50.0 µg dopamine injected i.c.v. 15 min after morphine (5.0 mg/kg s.c.) produced a short-lived attenuation of morphine's effect, giving a time-effect curve very similar to that produced by i.c.v. noradrenaline with s.c. morphine.

Subsequently, further experiments were carried out, whereby the dopamine (50.0 µg i.c.v.) was injected 30 min (as opposed to 15 min as in the earlier experiments) after morphine (5.0 mg/kg s.c.), whereupon there was marked attenuation of morphine's anti-nociceptive activity, rather greater than that produced by 5.0 µg of noradrenaline injected at a similar time (Fig. 45). The maximal effect noted at two hours after the morphine injection was

a 100% attenuation.

In later experiments, low doses of dopamine were shown to potentiate morphine's anti-nociceptive effect, and this is demonstrated in Fig. 46. Dopamine, 5.0 µg i.c.v. was injected 15 min after morphine, 2.5 mg/kg s.c., resulting in a significant potentiation of morphine's effect which was detectable five minutes after the i.c.v. injection. The effect was potentiated by about 120%.

Dopamine at low and high doses (5.0-50.0 µg i.c.v.) had no effect on the nociceptive threshold when it was injected after reserpine or after reserpine-plus-morphine.

3. THE RAT FOOT-PRESSURE TEST

(a) Reserpine

Reserpine, 5.0 mg/kg i.p., completely abolished the anti-nociceptive effect of morphine, 5.0 mg/kg s.c. in the rat, when given 18 h before the morphine (Fig. 47), as measured by the foot-pressure test.

(b) Noradrenaline

Noradrenaline, 20.0 µg i.c.v., produced an immediate and complete attenuation of morphine's anti-nociceptive effect (Fig. 48) which lasted throughout the normal period of morphine anti-nociceptive activity. Smaller doses of noradrenaline produced a block which was proportionately less marked than that produced by the larger doses.

(c) Dopamine

Relatively large doses of dopamine (200.0 µg i.c.v.) had no effect on morphine's anti-nociceptive effect, as determined by the foot-pressure test

in rats. However, smaller doses (of the order of 20.0 µg i.c.v.) were capable of producing some potentiation of morphine activity (Fig. 49) although the effect was not prolonged. The potentiation noted was (at its maximum) of the order of 100%, but at this point a 't'-test could not be carried out because too many animals in the test group exceeded the cut-off stimulus.

4. DISCUSSION

Our results, using the tail-flick and foot-pressure nociceptive tests, have confirmed the findings of other workers, that reserpine and tetrabenazine antagonise the anti-nociceptive activity of morphine (for previous references, see this chapter, Introduction). This aspect of the work will be discussed at greater length at the conclusion of the next chapter (Chapter Seven), when the possible relationships of morphine with both adrenergic and 5-HT systems will be further considered.

Noradrenaline injected i.c.v. causes a dramatic and immediate reversal of morphine's anti-nociceptive effect, a phenomenon demonstrated both in the rat (Sparkes & Spencer, 1969, 1971; this work) and in the mouse. In the mouse, the effect is similar, though less complete than in the rat. Noradrenaline injected into the cerebral ventricles comes into contact with nerve fibres which normally contain NA, and, therefore, presumably, with nerves which normally have NA released on to them from these adrenergic fibres. Fuxe and Ungerstedt (1966) have shown that NA injected i.c.v. is taken up by neurones lying close to the ventricles, and that uptake occurs not just at the nerve terminals but also at the axons and the cell-bodies. Their study showed that catecholamines injected i.c.v. penetrate only a short distance from the ventricular spaces. In order to carry out their experiments, Fuxe and Ungerstedt (1966) first had to deplete stores of endogenous amines.

Even then, little uptake could be demonstrated (using the fluorescence histochemical method) unless monoamine oxidase (MAO) had been inhibited. Thus in intact animals, which were mainly used in our study, it is probable that the effects we have observed were due to actions on post-synaptic sites close to the ventricles, since the injected amine would be subject to rapid metabolism by MAO once it had entered the nerve-cell bodies or axons. Further evidence on amine distribution has been provided by Fuxe and Ungerstedt (1968a) using the fluorescence histochemical method, and by Chalmers and Wurtman (1971) and Glowinski and Iverson (1966) using tritiated amines. The work of Steinman *et al.* (1969) suggests that the majority of NA injected i.c.v. does not, in fact, enter the neurones, since the principal route of metabolism following this route of injection was conjugation. If much of the injected NA was entering the neurones, the expected major route of metabolism would be deamination *via* MAO. Also, the rapid onset of effect noted in our experiments would favour the hypothesis of a direct post-synaptic effect on neurones lying close to the ventricular spaces.

A possibility exists that NA might be reducing the activity of morphine, by causing a redistribution of the drug away from the CNS. Thus cerebral vasoconstriction might be expected to reduce cerebral blood flow and thereby reduce the concentration of morphine in the brain. This explanation is unlikely, however, for a number of reasons. Lowered cerebral blood-flow would decrease the rate of ingress of morphine into brain, but would also decrease the rate at which morphine was removed from the brain, presumably to an approximately similar extent. We have shown that NA injected i.c.v. is capable of reducing the anti-nociceptive effect of morphine, even when this is already established, suggesting that effective brain concentrations of morphine had already been reached before the i.c.v. injection of NA. Also, there is evidence to suggest that cerebral arterioles are particularly

insensitive to exogenous catecholamines. Fog (1939) applied adrenaline both intravenously and directly to the pial arteries, and by both routes of administration it had little effect on the diameter of the vessels (the systemic blood pressure was held constant by artificial means). Conversely, a rise in systemic blood pressure produced a constriction of the pial arteries, leading Fog to suggest that this was a reflex constriction aimed at maintaining constant cerebral blood-flow. Further evidence was added by Handley (1970), who detected no change in cerebral blood-flow following i.c.v. NA (using a 20.0 µl injection volume), whilst a measurable cerebral vasoconstriction did occur following an increase in the oxygen content of the inhaled air (thus proving the sensitivity of the method, employing a dye-distribution technique).

Large doses of DA injected i.c.v. in the mouse had a similar effect to NA injected i.c.v. It is tempting to relate this effect of DA to the conversion of the exogenous DA to NA intraneuronally, following i.c.v. injection. However, Fuxe and Ungerstedt (1968a) reported that no uptake or accumulation of DA or NA was detectable in central neurones following i.c.v. injection unless MAO had been inhibited. However, they did add that in some areas where the concentrations of exogenous amines were high (which would be the case in our experiments involving high doses of DA) some accumulation was evident even in the presence of normal MAO activity. Also, Glowinski and Iverson (1966), using the rat, have shown that 27% of the radioactivity in the medulla following a dose of tritiated DA injected i.c.v was accounted for by tritiated NA, 15 min after the DA injection. Similarly, 17% of the DA taken up by the hypothalamus was converted to NA during the 30 min following the i.c.v. injection of DA. Thus it is possible that the effects seen following the i.c.v. injection of high doses of DA were the result of NA formed *in vivo* from the injected DA.

Small doses of DA injected i.c.v. had a potentiating effect on the anti-nociceptive activity of morphine in both the mouse and the rat, an effect opposite to that of NA. This suggest that the two amines have quite separate roles in the CNS, and that DA is not present merely as the precursor of NA. Doggett *et al.* (1970) have shown that small doses of ouabain injected i.c.v. produce a 100% increase in whole-brain levels of DA. These doses of ouabain are also capable of potentiating the anti-nociceptive activity of morphine (Calcutt *et al.*, 1971), suggesting further the involvement of DA in morphine's anti-nociceptive effect. Saarnivaara (1969b), using the rabbit, also obtained evidence implicating DA in the action of morphine.

Thus the results reported in this chapter suggest that increased noradrenaline concentrations at central synapses reduce the efficacy of morphine as an anti-nociceptive agent, whereas increased dopamine levels potentiate the anti-nociceptive activity of morphine. It is also probable that the sites of action of these amines in bringing about the effects described lie close to the ventricular spaces.

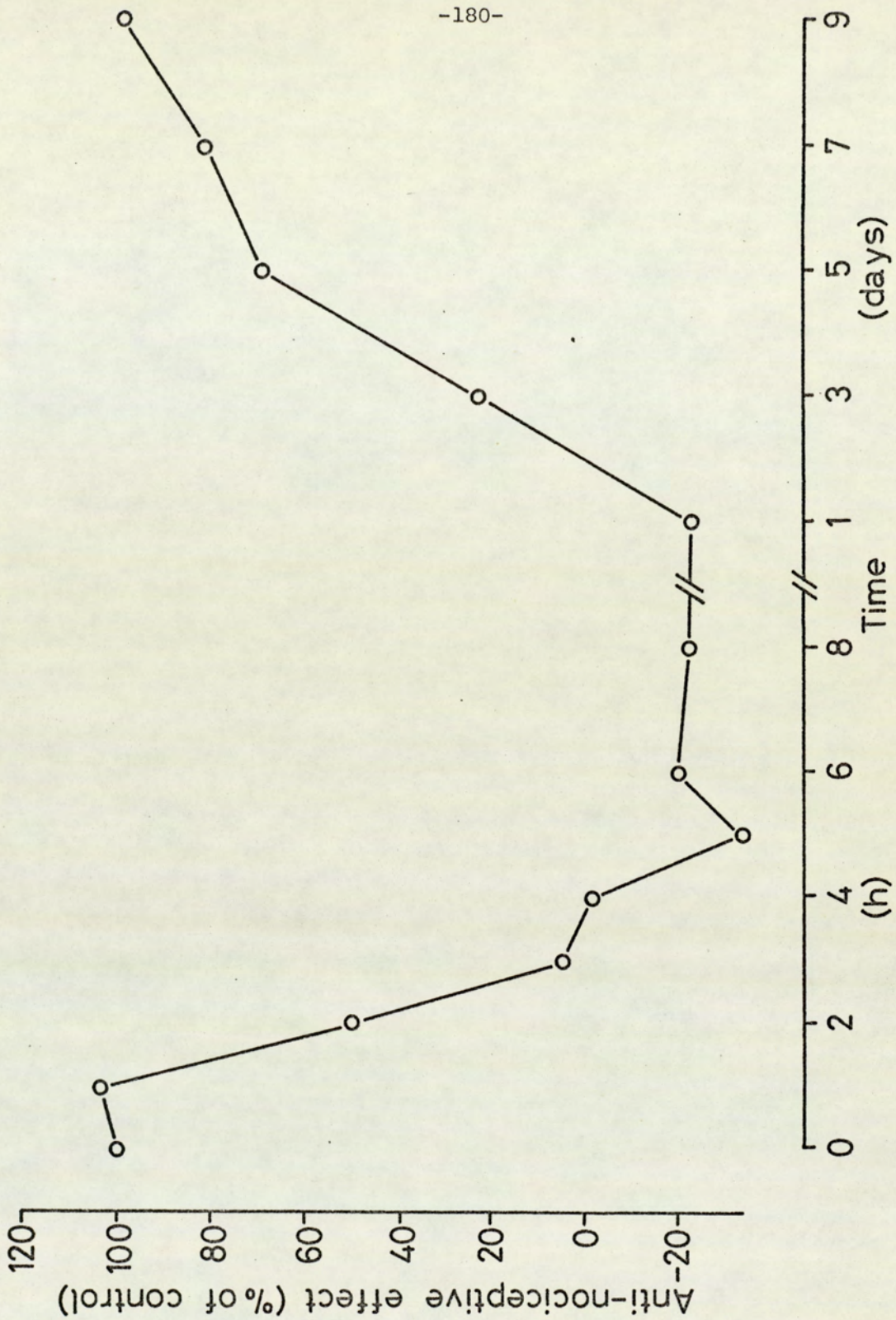

FIGURE 41: THE EFFECT OF RESERPINE ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received reserpine, 2.0 mg/kg i.p. at 0. Subsequently, each received a challenge dose of morphine, 5.0 mg/kg s.c. at the times indicated on the abscissa. The tail-flick test was then carried out 20 min after the morphine injection. Results are plotted as a percentage of the increase in reaction time produced by a challenge dose of morphine, 5.0 mg/kg s.c., in the absence of reserpine.

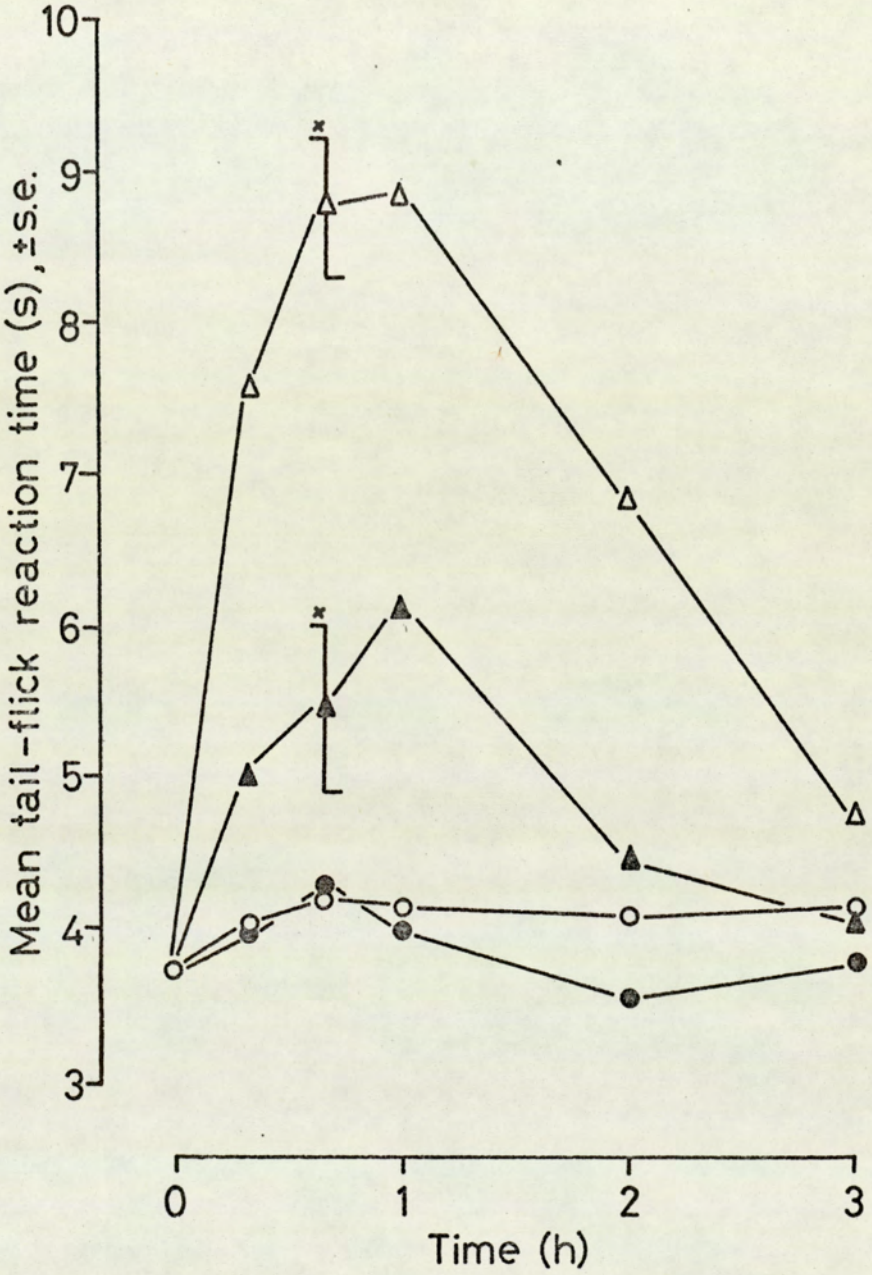

FIGURE 42: THE EFFECT OF TETRABENAZINE ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

○—○: Saline, 10.0 ml/kg i.p. at -3 h + saline, 10.0 ml/kg s.c. at 0.
●—●: Tetrabenazine, 40.0 mg/kg i.p. at -3 h + saline, 10.0 ml/kg s.c. at 0.
△—△: Saline, 10.0 ml/kg i.p. at -3 h + morphine, 5.0 mg/kg s.c. at 0.
▲—▲: Tetrabenazine, 40.0 mg/kg i.p. at -3 h + morphine, 5.0 mg/kg s.c. at 0.

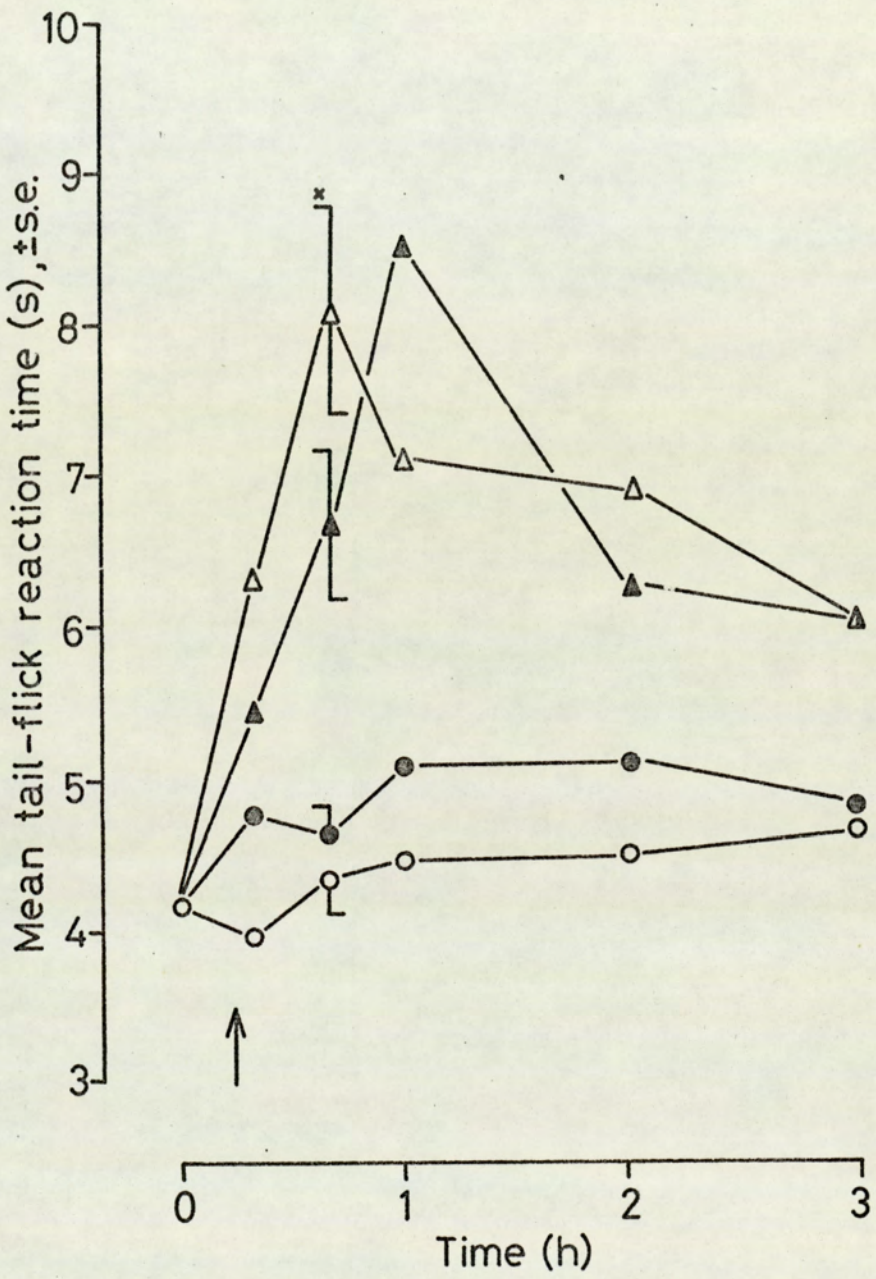

FIGURE 43: THE EFFECT OF NORADRENALINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

(i) NA injected 15 min after morphine

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + NA, 5.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 5.0 mg/kg s.c. at 0 + NA, 5.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

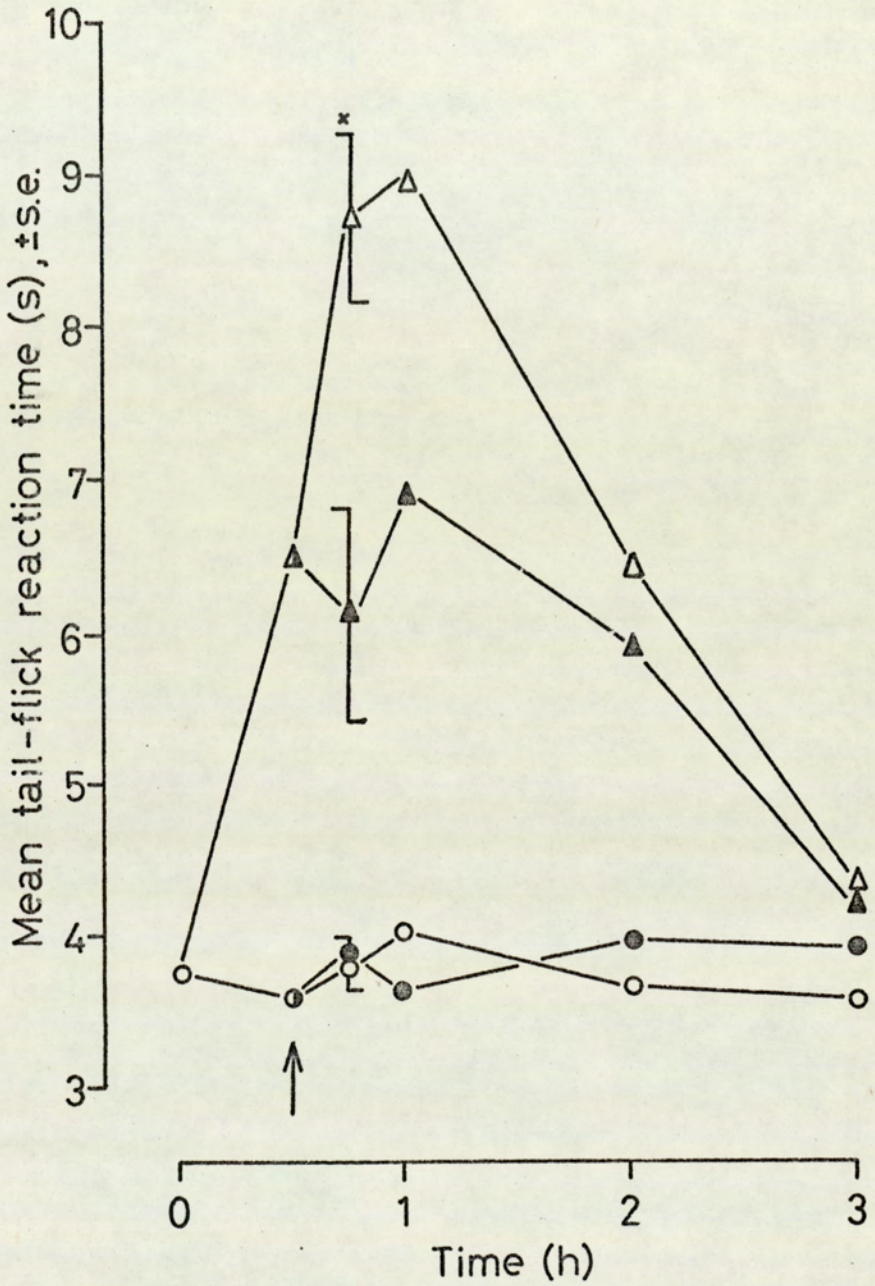

FIGURE 44: THE EFFECT OF NORADRENALINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

(ii) NA injected 30 min after morphine
Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + NA, 5.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 5.0 mg/kg s.c. at 0 + NA, 5.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

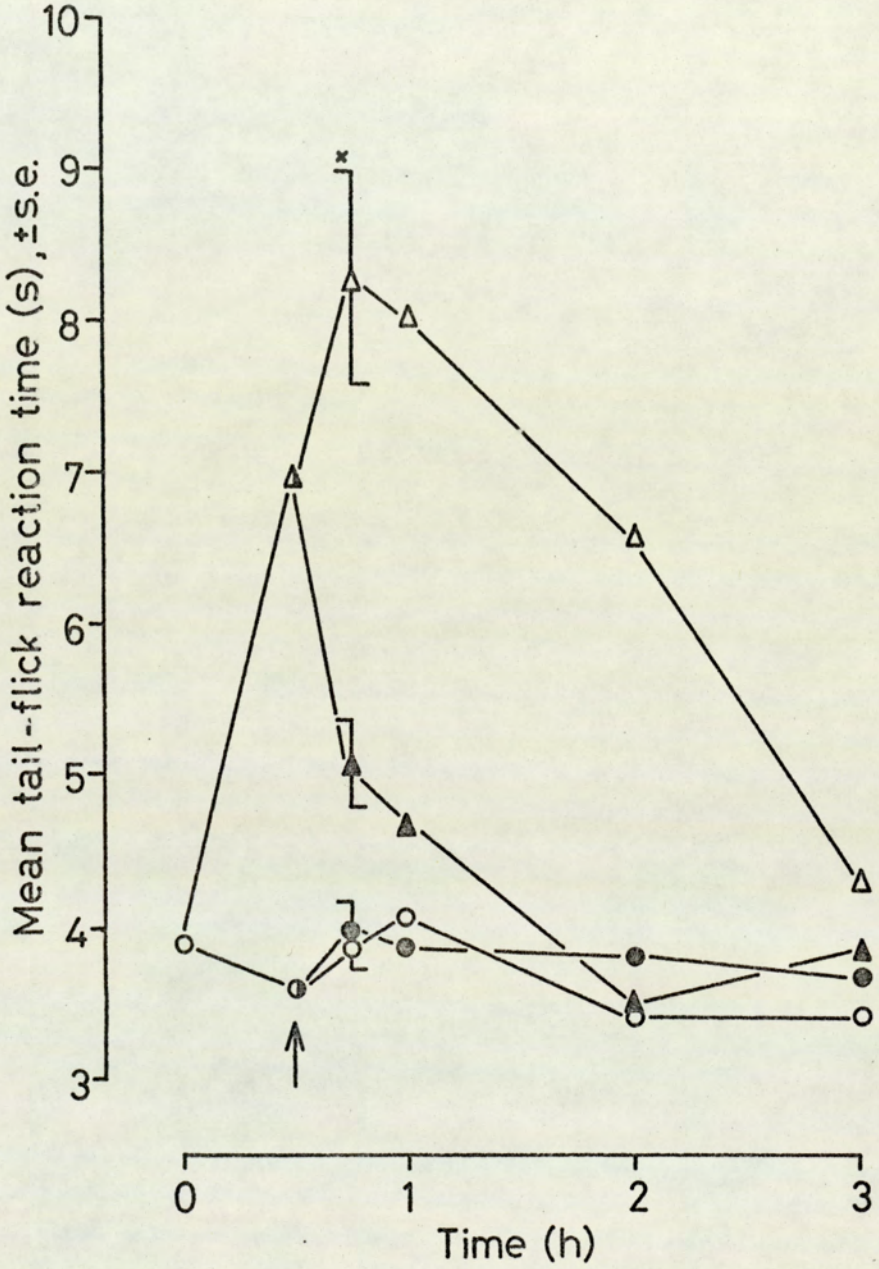

FIGURE 45: THE EFFECT OF DOPAMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

(i) High dose of DA

Groups of ten mice were injected as follows:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + DA, 50.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 5.0 mg/kg s.c. at 0 + DA, 50.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

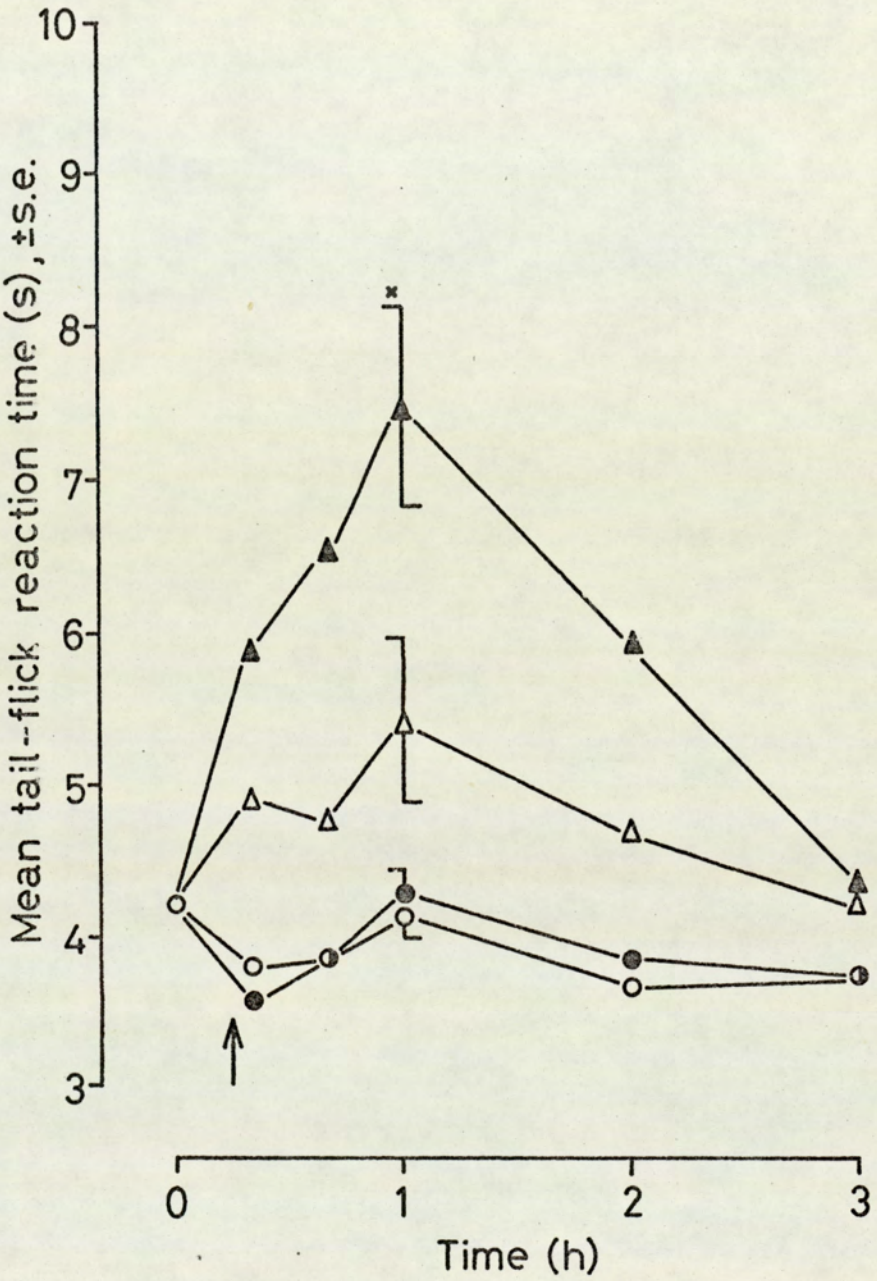

FIGURE 46: THE EFFECT OF DOPAMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

(ii) Low dose of DA

Groups of ten animals were injected as follows:

○—○: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

◉—◉: Saline, 10.0 ml/kg s.c. at 0 + DA, 5.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 2.5 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 2.5 mg/kg s.c. at 0 + DA, 5.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

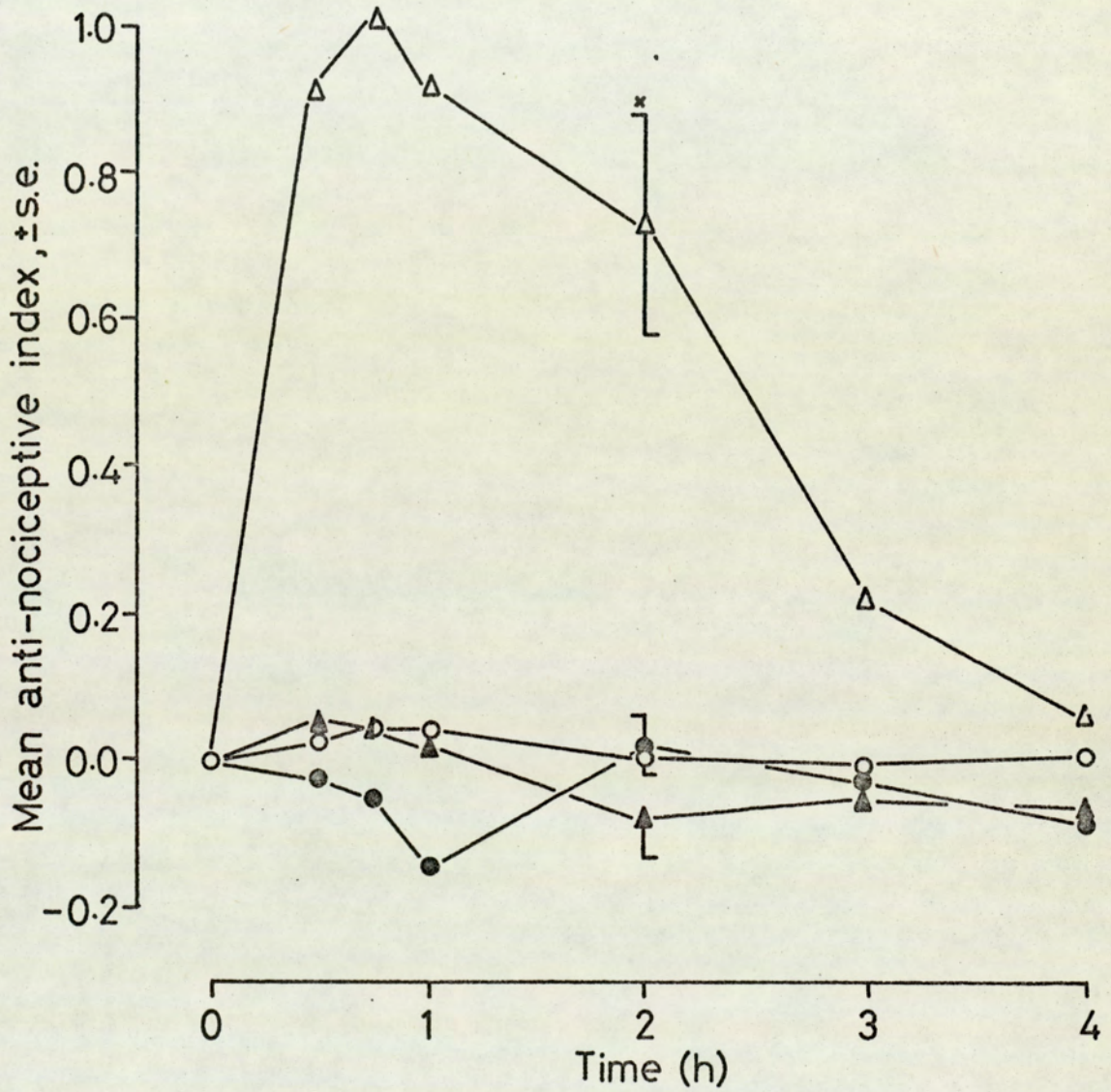

FIGURE 47: THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AFTER RESERPINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

○—○: Saline, 1.0 ml/kg s.c. at 0.
●—●: Reserpine, 5.0 mg/kg i.p. at -18 h + saline, 1.0 ml/kg s.c. at 0.
△—△: Morphine, 5.0 mg/kg s.c. at 0.
▲—▲: Reserpine, 5.0 mg/kg i.p. at -18 h + morphine, 8.0 mg/kg s.c. at 0.

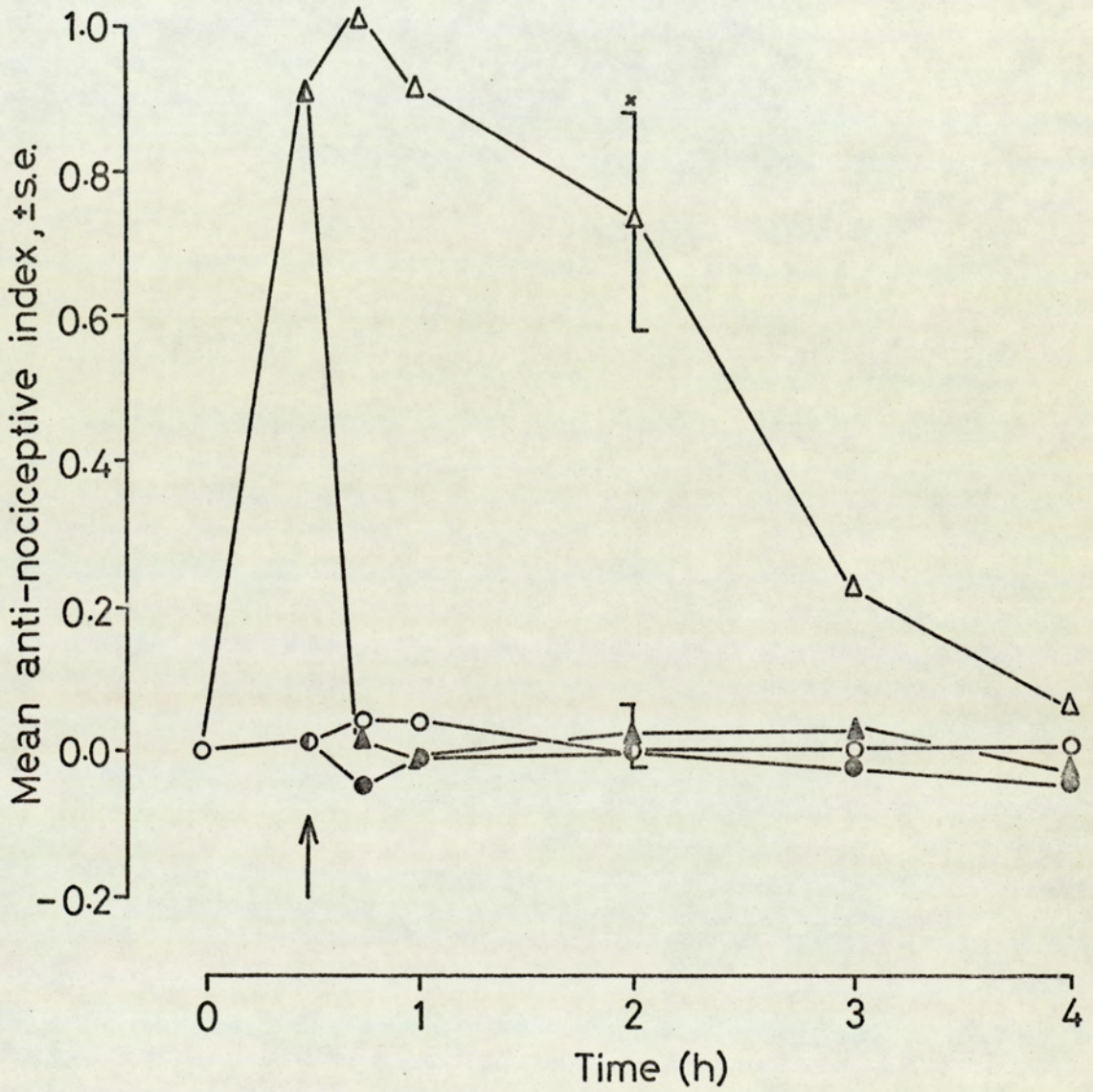

FIGURE 48: THE EFFECT OF NORADRENALINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

○—○: Saline, 1.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 1.0 ml/kg s.c. at 0 + NA, 20.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 5.0 mg/kg s.c. at 0 + NA, 20.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

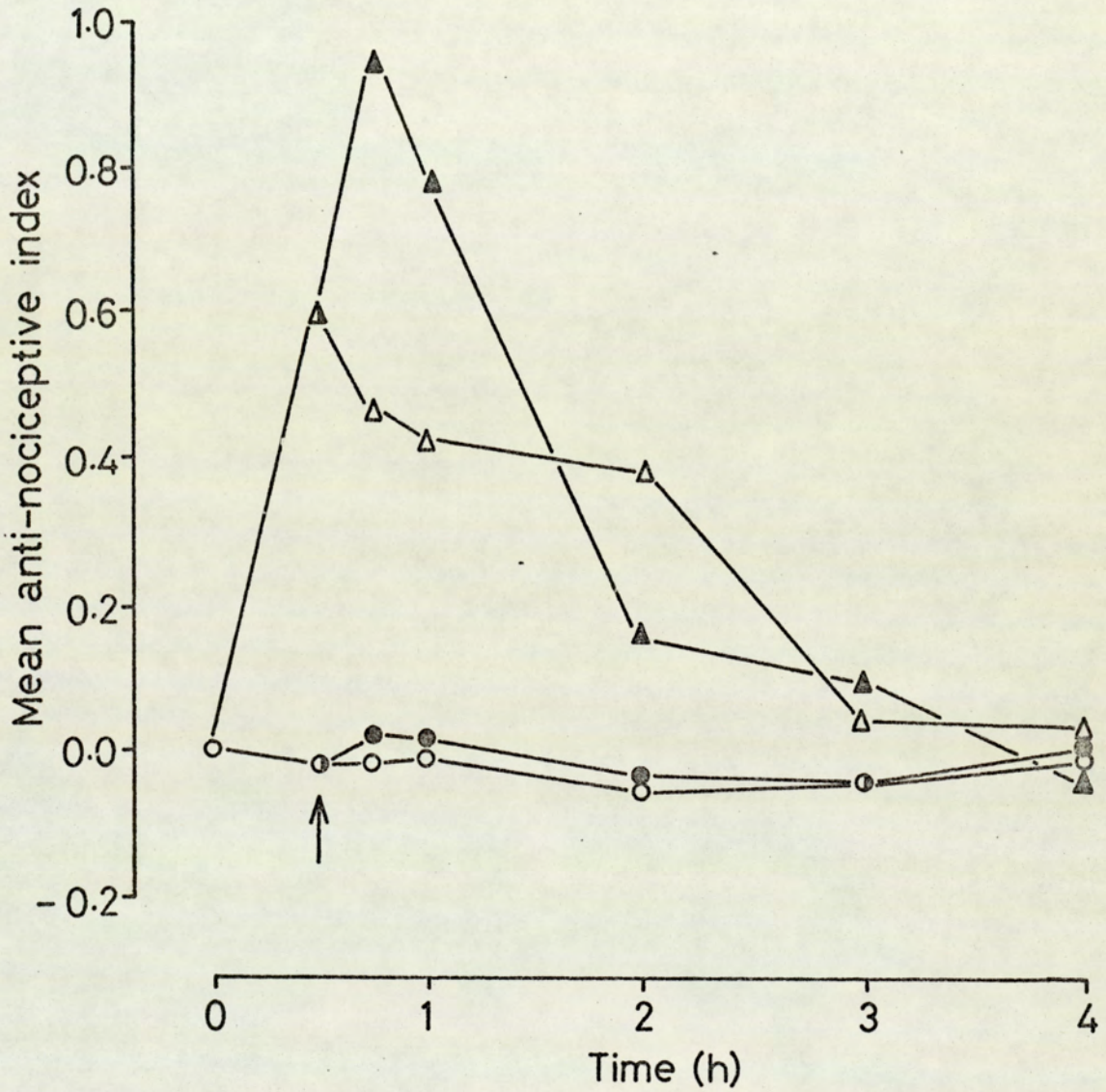

FIGURE 49: THE EFFECT OF DOPAMINE (LOW DOSE) INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

○—○: Saline, 1.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 1.0 ml/kg s.c. at 0 + DA, 20.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 2.5 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 2.5 mg/kg s.c. at 0 + DA 20.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

CHAPTER SEVEN

THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE AS MODIFIED BY 5-HT AND 5-HT ANTAGONISTS

1. INTRODUCTION

The occurrence of 5-hydroxytryptamine (5-HT; serotonin) in brain was first reported by Twarog and Page (1953), and its distribution found to be similar to that of NA by Amin *et al.* (1954). The possibility that 5-HT might have a role to play in brain function was put forward by Woolley and Shaw (1954), possibly as a neurohumoral agent (Brodie *et al.*, 1955). In contrast to the effect of morphine on NA (see Introduction; Results: Chapter Six), early experiments suggested that neither single injections (Brodie, Shore & Pletscher, 1956) nor chronic administration (Cochin & Axelrod, 1959) of morphine would influence brain levels of 5-HT. However, more recently, Fennessy and Lee (1970) reported that single doses of morphine reduced brain 5-HT levels in mice, concommitant with the development of the anti-nociceptive effect, and Yarbrough *et al.* (1971) suggested that single doses of morphine resulted in a small but significant rise in brain 5-HT turnover in rats.

The earlier results led some workers to suggest that although brain levels of 5-HT remained unaltered by morphine, changes in turnover rate may occur without necessarily being reflected in the overall tissue concentration of the amine. Loh *et al.* (1968; 1969) detected an increase in 5-HT turnover on chronic morphine administration, which was prevented by concurrent administration of actinomycin-D, which also prevented the development of tolerance. This led to the conclusion that an alteration in 5-HT turnover was associated with, and was possibly responsible for, the development of tolerance. However, in a later paper, Loh *et al.* (1971) have suggested that at least part of the action of actinomycin-D is a result of an increase in brain permeability to morphine. Cheney *et al.* (1971) attempted to repeat the earlier observations of Loh *et al.* (1968; 1969) with regard to the reported increase in brain 5-HT turnover, and were unable to produce any measurable increase in turnover rate with the development of tolerance. Cheney and

Goldstein (1971) have also attempted to prevent tolerance development by concurrently administering *p*-chlorophenylalanine (*p*-CPA), a specific inhibitor of 5-HT biosynthesis (Koe & Weissman, 1966), without success, as have Marshall and Grahame-Smith (1970). Hence the effects of acute and chronic morphine administration on the metabolism of 5-HT in brain remain to be determined.

A number of workers have tried to alter the levels of 5-HT in the brain in an attempt to modify the activity of morphine. The results obtained using reserpine for this purpose have been summarised in the preceding chapter; since reserpine depletes the catecholamines as well as 5-HT, no conclusions regarding the specific role of 5-HT may be drawn from these results. A number of studies have involved the use of *p*-CPA on the acute effects of morphine. Thus Tenen (1968) using the "flinch-jump" test of Evans (1961; 1962) found the effect of morphine to be reduced by prior treatment with *p*-CPA. Major and Pleuvry (1971) detected an antagonism, using the hot-plate test. However, Fennessy and Lee (1970) found no detectable antagonism between *p*-CPA and morphine, also using the hot-plate test, although they reported an antagonism when the phenylquinone test was used as an index of morphine's activity. Medaković and Banić (1969) could detect no reduction in the anti-nociceptive activity of morphine following pretreatment with *p*-CPA, but did observe that *p*-CPA pretreatment prevented reserpine from antagonising morphine (using the rat tail-flick test). This result suggested that reserpine antagonised morphine by bringing about a slow release of 5-HT from central neurones.

The action of a number of 5-HT antagonists has been investigated with respect to the anti-nociceptive activity of morphine, in particular the agents possessing psychotomimetic activity (Gaddum, 1953; Shore, Silver &

Brodie, 1955). Thus Dhawan and Gupta (1959) demonstrated an antagonism between LSD-25 and morphine, an observation which has been confirmed by Jacob *et al.* (1964) and by Jacob and Barthélémy (1967), who were also able to report a similar effect with a number of other psychotomimetic agents.

Thus the role of 5-HT in the anti-nociceptive action of morphine is by no means clear, and the experiments reported in this chapter have been aimed at clarifying some of the contradictory data. This has been attempted by studying the effects of directly altering the levels of 5-HT in the central nervous system by the injection of 5-HT in to the cerebral ventricles of mice and rats.

2. THE HOT-PLATE TEST

(a) 5-HT

The injection of 5-HT i.c.v., in doses up to 10.0 µg, produced no alteration of the hot-plate reaction time of otherwise untreated mice (see Results: Chapter Three) and did not modify the anti-nociceptive action of morphine when injected 45 min after the morphine. (The hot-plate test was carried out 15 min after the i.c.v. injection, *i.e.* 60 min after the morphine injection.)

3. THE TAIL-FLICK TEST

(a) Reserpine

As detailed in the preceding chapter, reserpine (2.0 mg/kg i.p.) completely abolished the anti-nociceptive activity of morphine in this test.

(b) 5-HT in intact animals

5-HT, 10.0 µg i.c.v., produced a significant potentiation of the anti-nociceptive activity of morphine when injected 15 min after the morphine

2.5 mg/kg s.c. (Fig. 50). The effect was evident 5 min after the i.c.v. injection, at a time when the effect of 5-HT itself on the nociceptive threshold was minimal (Results: Chapter Three). It was necessary to use doses of 5-HT of this magnitude in order to produce a consistent potentiation of the effect of morphine in mice (*i.e.* doses of 5-HT which alone have a significant effect on the nociceptive threshold, as measured by this test).

(c) 5-HT in reserpinised animals

Doses of 5-HT up to 20.0 µg i.c.v. produced no alteration of the nociceptive threshold in mice pretreated with reserpine, 2.0 mg/kg i.p., 18 h previously. Also, there was no alteration of the nociceptive threshold in mice receiving morphine, 5.0 mg/kg s.c., plus 5-HT i.c.v., up to 20.0 µg, 18 h after reserpine, 2.0 mg/kg i.p. (This result should be contrasted with the result obtained in a similar experiment using the rat: This chapter, para. 4(c).)

(d) LSD-25

LSD-25, 0.05 mg/kg s.c., given 20 min prior to morphine, 5.0 mg/kg s.c., produced a marked attenuation of the anti-nociceptive activity of morphine (Fig. 51), ammounting to a 70 to 75% reduction in anti-nociceptive effect 40 min after the morphine injection, being slightly less than this figure at 60 min after morphine (the peak of morphine's activity).

Similarly, LSD-25, 0.05 µg i.c.v., given 10 min before morphine, 5.0 mg/kg s.c., produced a significant attenuation of the anti-nociceptive effect of morphine. The reduction seen was about 50%, at its maximal, *i.e.* rather less than that seen for the s.c. route (at the particular doses used).

(e) LSD-25 and 5-HT

As a control experiment, the effect of LSD-25 on i.c.v. 5-HT was examined. LSD-25, 0.05 mg/kg s.c., injected 15 min before 5-HT, 20.0 µg i.c.v., completely abolished the anti-nociceptive effect of 5-HT (for the effect of 5-HT alone, see Results: Chapter Three).

4. THE RAT FOOT-PRESSURE TEST

(a) Reserpine

As detailed in the preceding chapter, reserpine, 5.0 mg/kg i.p. completely abolished the anti-nociceptive activity of morphine in this test in the rat.

(b) 5-HT in intact animals

5-HT, 20.0 µg i.c.v., injected 30 min after morphine, 2.5 mg/kg s.c., produced a significant potentiation of the anti-nociceptive activity of morphine (Fig. 52). This was seen both as a potentiation of the peak activity and a prolongation of the anti-nociceptive effect. In this species, the potentiating effect of 5-HT is seen at doses which alone do not consistently produce a significant elevation of the nociceptive threshold (although in some experiments 20.0 µg 5-HT i.c.v. does produce a significant elevation of the threshold — Results: Chapter Three).

(c) 5-HT in reserpinised animals

In rats which had received reserpine, 5.0 mg/kg i.p. 18 h previously, 5-HT, 5.0 µg i.c.v., had no effect on the nociceptive threshold. However, the anti-nociceptive effect of morphine, 5.0 mg/kg s.c., which had been abolished by reserpine, was restored to approximately the degree seen in a non-reserpinised animal (Fig. 53) by the injection of 5-HT, 5.0 µg i.c.v..

The peak effect was noted 15 min after the 5-HT injection, a time corresponding to 45 min after the morphine injection. The duration of the anti-nociceptive effect was rather shorter than that seen with a similar dose of morphine in a non-reserpinised animal (Results: Chapter Two).

(d) LSD-25

LSD-25, 1.0 µg i.c.v., injected 30 min after morphine, 3.0 mg/kg s.c., produced a rapid and marked attenuation of the already-established anti-nociceptive activity of morphine, whilst having no effect itself on the nociceptive threshold (Fig. 54).

5. DISCUSSION

In general, the results reported in this chapter suggest that a decrease in central 5-HT antagonises the anti-nociceptive effect of morphine, and an increase in central 5-HT potentiates it (although in the hot-plate test these theories could not be substantiated).

The results obtained using reserpine may now be discussed more fully (see Chapter Six), in the light of the possible involvement of central 5-HT in the effect of reserpine. It has long been suggested that the behavioural depression brought about by reserpine is due to its effects on 5-HT rather than on the catecholamines (Brodie, Pletscher & Shore, 1956; Brodie *et al.*, 1966, 1960; Shore *et al.*, 1957b). However, the effects mediated by a disturbance of 5-HT may be due to either a lack of 5-HT at postsynaptic sites, or to a steady release of 5-HT on to postsynaptic receptor sites (Shore & Brodie, 1957) following disruption by reserpine of the 5-HT storage mechanisms.

The fact that reserpine abolishes the anti-nociceptive activity of

morphine in the mouse tail-flick and the rat foot-pressure tests suggests that in both species morphine is bringing about its effects *via* either the catecholamines or 5-HT, (or both), or some other system which is disrupted by reserpine treatment. In the rat, we have observed, as have Sparkes and Spencer (1969; 1971) that an injection of 5-HT i.c.v. restores the anti-nociceptive activity of morphine in reserpinised rats. However, in the mouse, we have been unable to obtain a similar result — *i.e.* 5-HT injected i.c.v. does not restore the anti-nociceptive activity of morphine in reserpinised mice, despite examining a wide range of doses of 5-HT and various times of administration with respect to the morphine injection.

This inability to restore the anti-nociceptive activity of morphine in reserpinised mice by the injection of 5-HT i.c.v., as we had been able to do in reserpinised rats, is perplexing, and remains unexplained. One possible reason may be that the doses of 5-HT which we used were too rapidly taken up by depleted neurones in the mouse, thus preventing the appearance of the (presumably) post-synaptic effects. However, this seems unlikely, since we were able to restore the anti-nociceptive activity of morphine fully in reserpinised rats with a dose of 5-HT, given i.c.v., which was 1/4 of that required to produce a significant potentiation of morphine in intact rats. Of course, it may be unwise to draw too close a comparison between the two species, although such a comparison has been possible with respect to other experiments described in this study.

5-HT injected i.c.v. in both species is capable of raising the nociceptive threshold (Results: Chapter Three) and of potentiating the anti-nociceptive activity of morphine in intact animals (this chapter). These results, taken together with the reserpine results, suggest that morphine may be acting, at least in part, either by the release of endogenous 5-HT or by

reducing the effect of endogenous 5-HT (for example, by preventing its release, or by blocking its activity at receptor sites). The latter hypothesis is possible if one considers that an excess of 5-HT (such as might occur following i.c.v. injection) could block 5-HT receptors (Shore & Brodie, 1957). This might correlate with the hypothesis of Herold and Cahn (1968), that 5-HT normally subserves the function of a transmitter involved in the affective component of pain — *i.e.* that raised activity in a neurone system involving release of 5-HT would antagonise morphine. However, it seems more likely that an increase in central 5-HT activity results in an anti-nociceptive effect (or potentiation of that of a narcotic analgesic), when the results using LSD-25 are considered also, despite the fact that LSD-25 has been shown to be capable of stimulating some central 5-HT receptors (Andén, Corrodi, Fuxe & Hökfelt, 1968). Further evidence is supplied by the fact that in the early hours after reserpine, the anti-nociceptive activity of morphine is potentiated, at a time when reserpine is presumed to be releasing central amines prior to their depletion (Everett *et al.*, 1957).

More direct evidence has been obtained with respect to central 5-HT neurones and morphine's anti-nociceptive effect. Thus Samanin *et al.* (1970) placed lesions in the mid-brain raphé system of rats. Such lesions reduced brain levels of both 5-HT and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and decreased the anti-nociceptive activity of morphine in the hot-plate, tail-pressure and electric shock tests. The mid-brain raphé system has been shown to contain 5-HT neurones (Andén *et al.*, 1966; Ungerstedt, 1971), and stimulation of this system in the mid-brain increases turnover rate of 5-HT at the rostral terminations of the pathway in the forebrain (Sheard & Aghajanian, 1968).

The effects of administering 5-HT i.c.v. have been studied by a number

of workers, and the majority suggest that 5-HT administered in such a way is taken up by neurones, lying close to the ventricles, which normally contain 5-HT. Similar results have been obtained using both labelled 5-HT (Aghajanian & Bloom, 1967a; Schildkraut *et al.*, 1969) and fluorescence histochemical methods (Fuxe & Ungerstedt, 1967, 1968a, 1968b). However, as was pointed out in the preceding chapter (Chapter Six), it is probable that the effects seen following the injection of biogenic amines i.c.v. are due more to direct actions of the amines on postsynaptic sites rather than to any effect which might be secondary to uptake by the amine-containing neurones lying close to the ventricles. The relatively short duration of action of 5-HT injected i.c.v. in the test situations described in the present study may well reflect deactivation of the 5-HT by uptake into the neurones. Palaić *et al.* (1967) have also suggested that some removal from the ventricular spaces may be brought about by the choroid plexus.

Other studies have shown that 5-HT injected i.c.v. may have anti-nociceptive activity (Tsou & Jang, 1964) and that it is capable of prolonging the effect of morphine (Sparkes & Spencer, 1969, 1971). Another approach has involved the administration of the precursor of 5-HT, 5-hydroxytryptophan (5-HTP) in an attempt to elevate the levels of endogenous 5-HT. Thus 5-HTP was reported to possess anti-nociceptive activity alone (Contreras & Tamayo, 1967; Contreras *et al.*, 1970; Radouco-Thomas *et al.*, 1967) and to be synergistic with morphine (Contreras & Tamayo, 1967; Contreras *et al.*, 1970; Dewey *et al.*, 1968, 1970; Radouco-Thomas *et al.*, 1967).

The results reported in this chapter (and that preceding it) indicate that in both rats and mice, the anti-nociceptive effect of morphine is antagonised by reserpine, and that this effect is probably brought about as a result of depletion of neuronal 5-HT in the central nervous system. This

suggests that 5-HT plays an important part in the anti-nociceptive activity of morphine in the rat, and perhaps to a lesser extent in the mouse. This conclusion is supported by the evidence obtained with LSD-25 and with centrally-administered 5-HT itself.

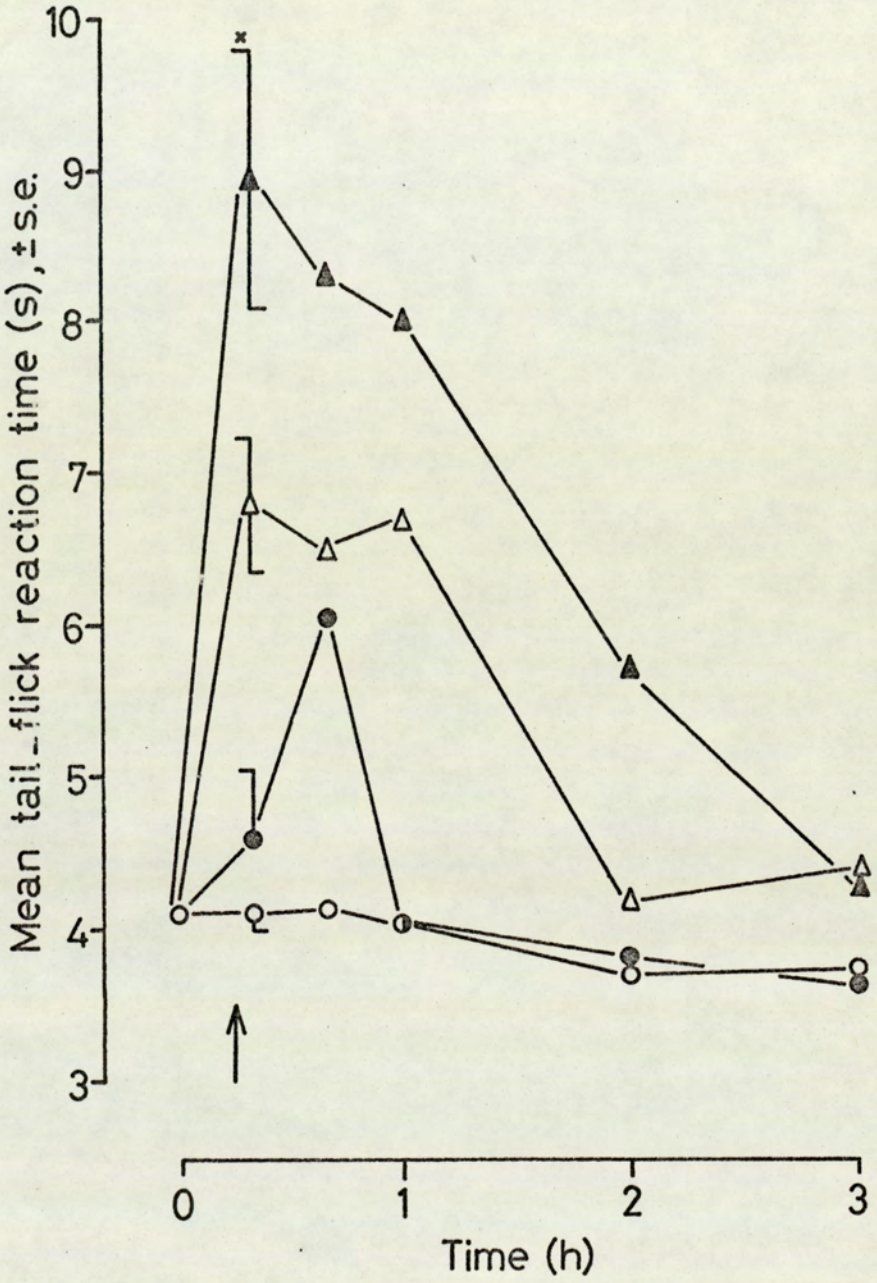

FIGURE 50: THE EFFECT OF 5-HT INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of 5 (O—O; ●—●) or 10 (Δ—Δ; ▲—▲) mice were injected as follows:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + 5-HT, 10.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 2.5 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 2.5 mg/kg s.c. at 0 + 5-HT, 10.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

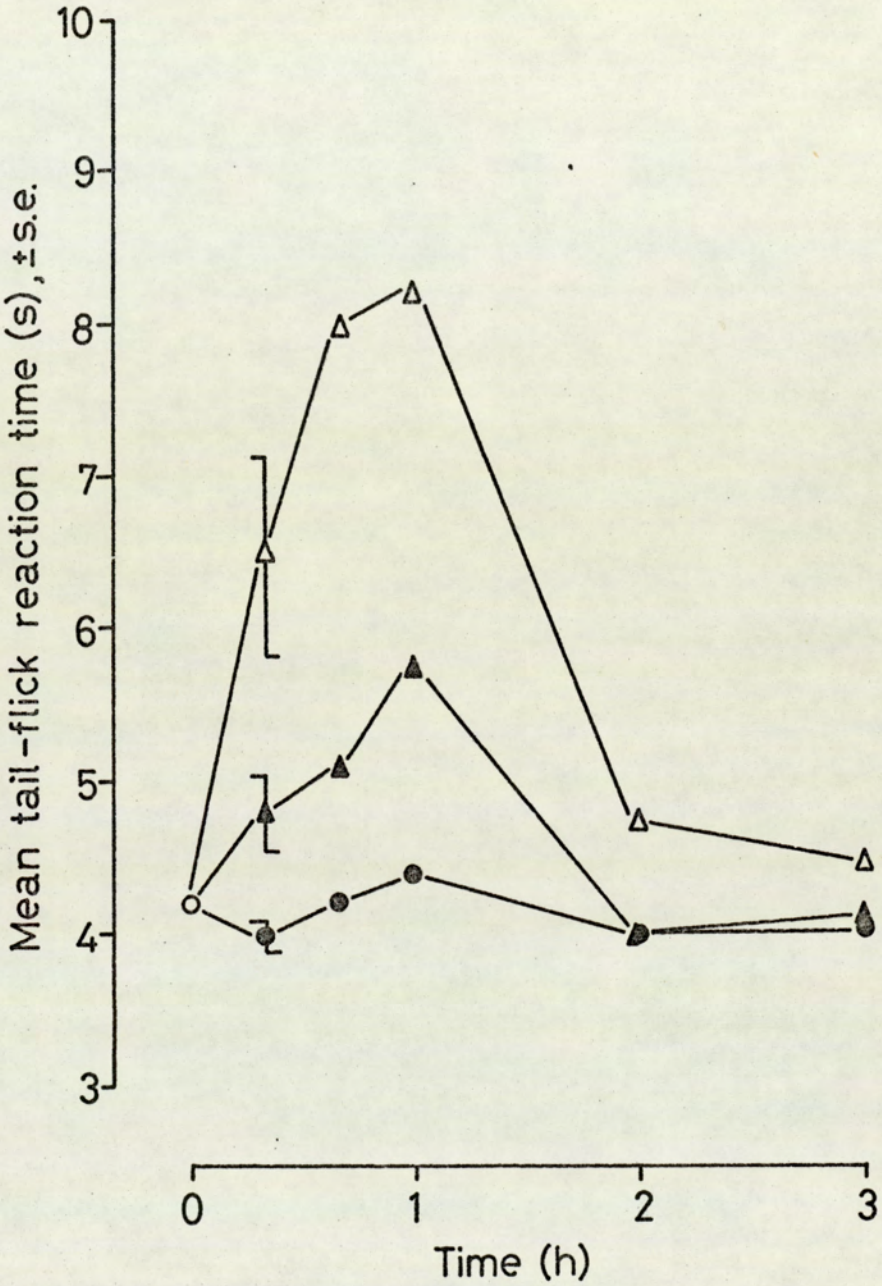

FIGURE 51: THE EFFECT OF LSD-25 ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: LSD-25, 0.05 mg/kg s.c. at -20 min + saline, 10.0 ml/kg s.c. at 0.

Δ—Δ: Saline, 10.0 ml/kg s.c. at -20 min + morphine, 5.0 mg/kg s.c. at 0.

▲—▲: LSD-25, 0.05 mg/kg s.c. at -20 min + morphine, 5.0 mg/kg s.c. at 0.

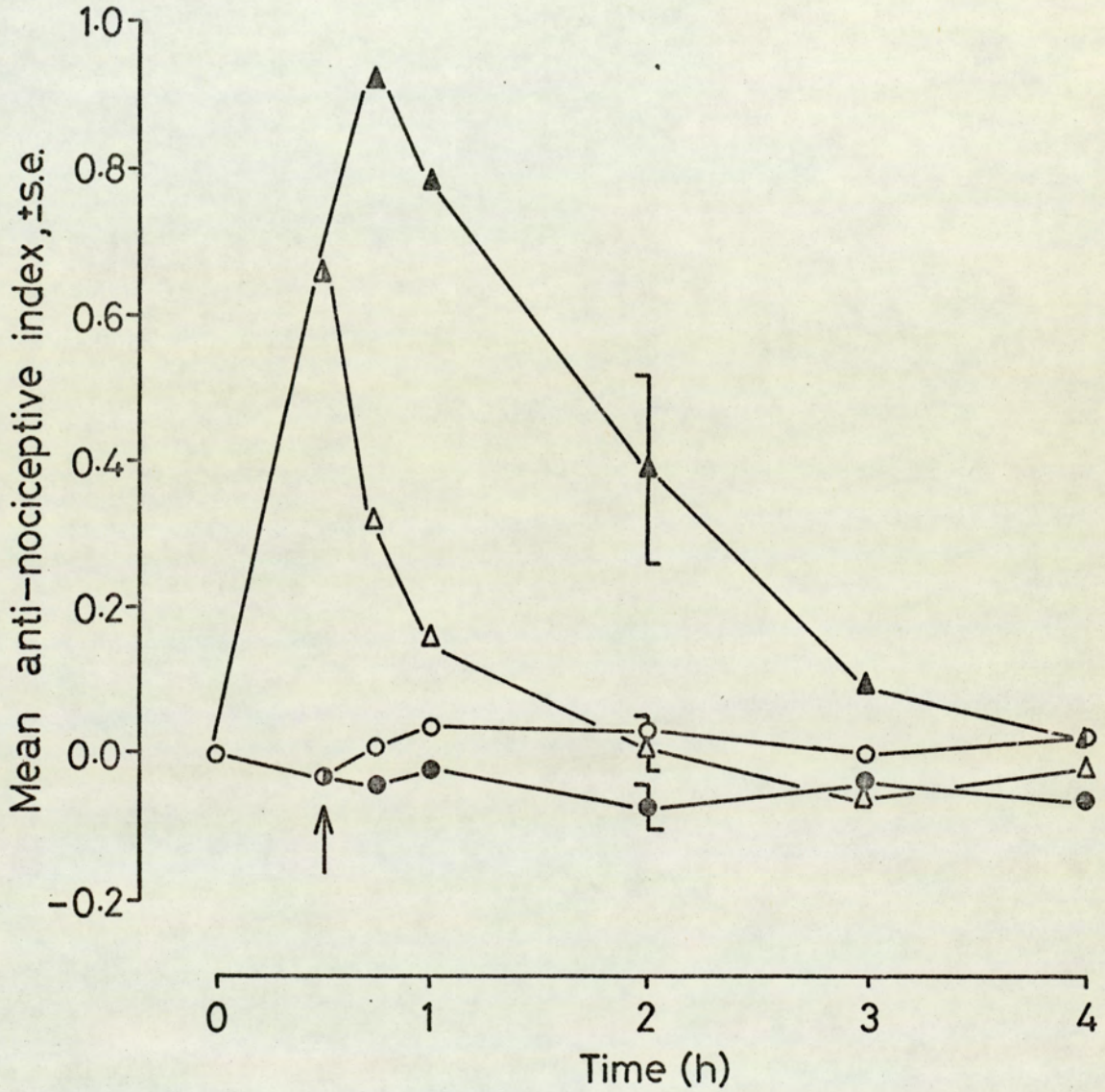

FIGURE 52: THE EFFECT OF 5-HT INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of 5 rats were injected as follows:

O—O: Saline, 1.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 1.0 ml/kg s.c. at 0 + 5-HT, 20.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 2.5 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 2.5 mg/kg s.c. at 0 + 5-HT, 20.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

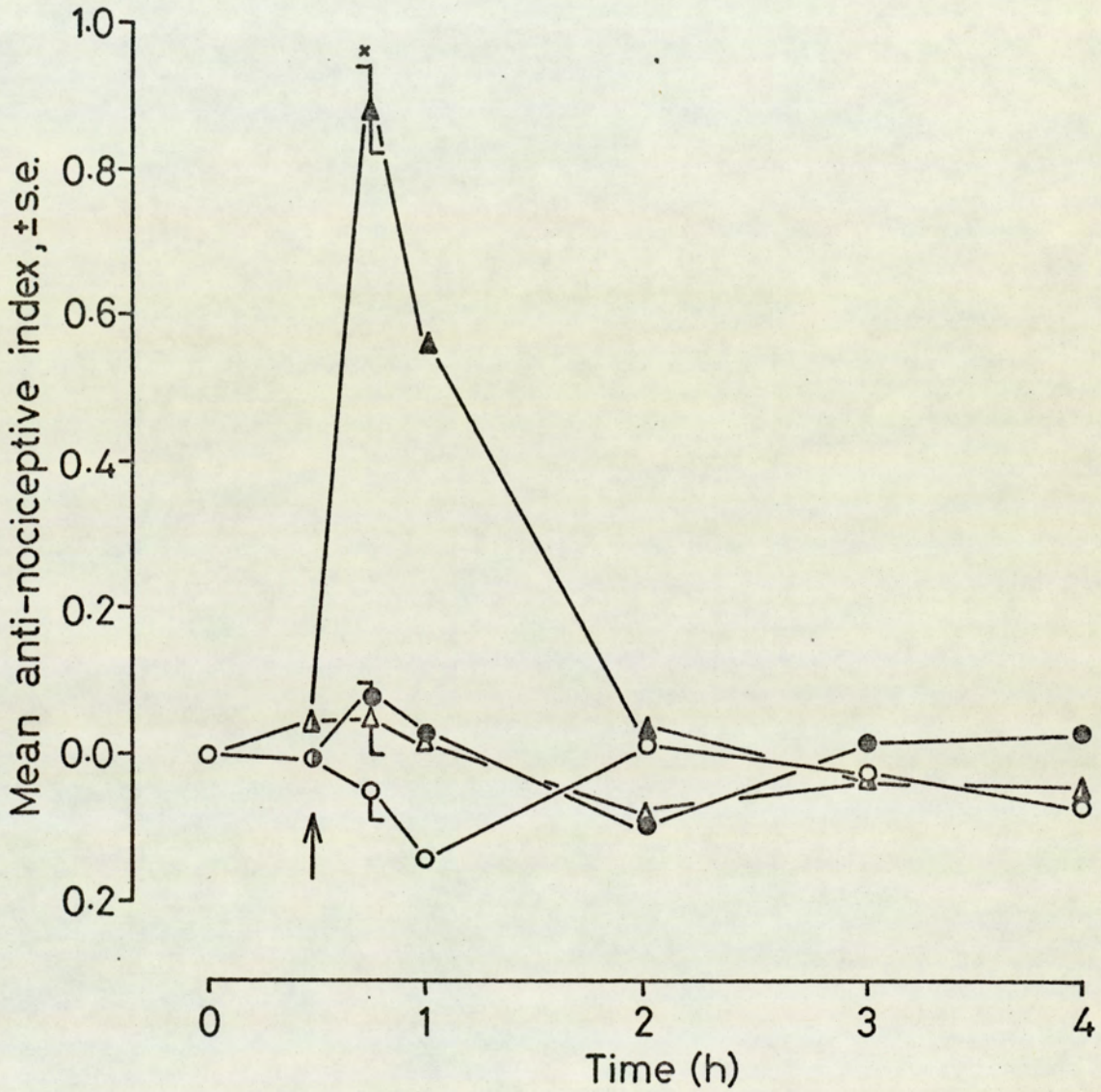

FIGURE 53: THE EFFECT OF 5-HT INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN RATS WHICH HAD PREVIOUSLY RECEIVED RESERPINE

Groups of five rats received reserpine, 5.0 mg/kg i.p. at -18 h, followed by:

○—○: Saline, 1.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 1.0 ml/kg s.c. at 0 + 5-HT, 5.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 5.0 mg/kg s.c. at 0 + 5-HT, 5.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

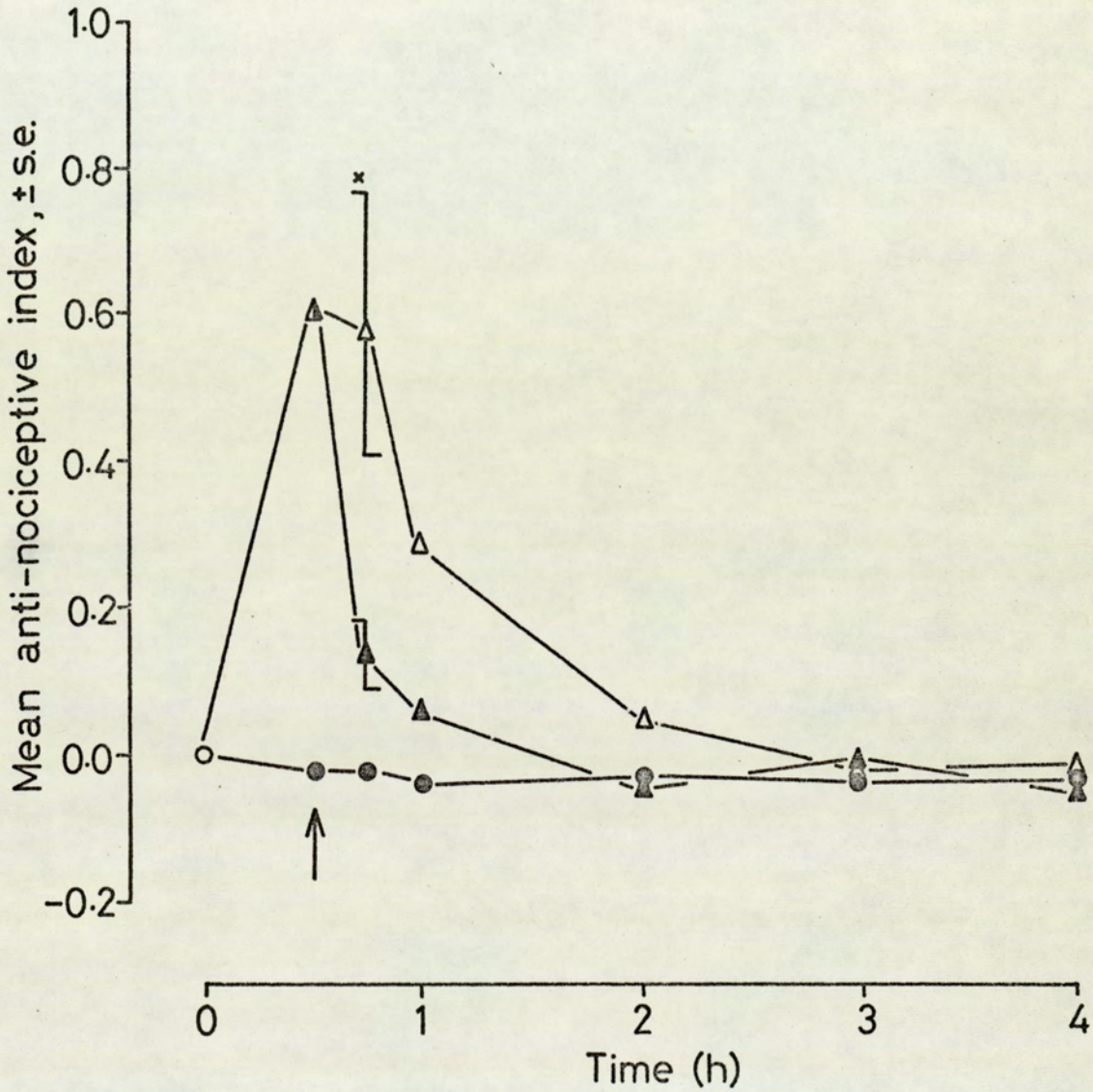

FIGURE 54: THE EFFECT OF LSD-25 INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of 5 rats were injected as follows:

●—●: Saline, 1.0 ml/kg s.c. at 0 + LSD-25, 1.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 3.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 3.0 mg/kg s.c. at 0 + LSD-25, 1.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

PART D

ANTI-NOCICEPTIVE ACTIVITY OF NARCOTIC-ANTAGONIST ANALGESICS AS MODIFIED BY OTHER AGENTS

CHAPTER EIGHT

ANTI-NOCICEPTIVE ACTIVITY OF NARCOTIC-ANTAGONIST ANALGESICS AS MODIFIED BY NALOXONE, CHOLINERGIC AND ANTI-CHOLINERGIC COMPOUNDS

1. INTRODUCTION

Because of the obvious differences in the activities of morphine and the narcotic-antagonist analgesics, especially with regard to their effects in the various nociceptive tests, we decided to observe the interactions of the narcotic antagonists with various agents, whose interactions with morphine we had already studied. Comparison was made difficult by the lack of effect of the narcotic-antagonists in three of the four tests used, but nevertheless some interesting results have been obtained, which are reported in this and in the following chapter.

2. THE HOT-PLATE TEST

Physostigmine, 0.5 µg i.c.v., did not modify the effects of either nalorphine or pentazocine in the hot-plate test. This dose of physostigmine did, in fact, produce a significant anti-nociceptive effect alone, and prior treatment with nalorphine, 15.0 mg/kg s.c., or pentazocine, 20.0 mg/kg s.c., both of which were without effect alone in this test (Results: Chapter Two), did not significantly alter the effect produced by the physostigmine itself.

3. THE TAIL-FLICK TEST

(a) Nalorphine plus physostigmine

Nalorphine, 20.0 mg/kg s.c., had only a slight anti-nociceptive effect in the mouse tail-flick test, as reported in Chapter Two. This amounted to a 20% elevation of the reaction time. This effect was considerably potentiated by the injection of physostigmine, 1.0 µg i.c.v. 15 min after the nalorphine, the combined effect giving a 50% elevation of the reaction time (Fig. 55). This dose of physostigmine was inactive alone in this test. The peak effect of the combination (nalorphine + physostigmine) occurred rather sooner after injection than that seen with nalorphine alone, and the

time-course of the combined effect seemed to be related more to that of physostigmine (see Chapter Three, Fig. 26) than to that of nalorphine itself (Fig. 55).

(b) Pentazocine plus physostigmine

Pentazocine, 40.0 mg/kg s.c., produced only a small anti-nociceptive effect as determined by the tail-flick test, giving an elevation of some 15% in the reaction time. In Figure 56, this is compared with the effect of pentazocine, 20.0 mg/kg s.c. plus physostigmine, 1.0 µg i.c.v. It can be seen that the physostigmine (which was without effect alone) produced a considerable potentiation of the anti-nociceptive effect of pentazocine, elevating the reaction time by 50%. This potentiation was statistically significant ($P<0.05$).

(c) Nalorphine plus carbachol

Carbachol, 0.025 µg i.c.v., produced a small potentiation of the anti-nociceptive activity of nalorphine, 20.0 mg/kg s.c. With the combination, the reaction time was elevated some 35 to 40%, the potentiation being statistically significant ($P<0.05$) only at 5 min after the i.c.v. injection.

(d) Pentazocine plus carbachol

Carbachol, 0.025 µg i.c.v., produced a small (10%) elevation in the anti-nociceptive activity of pentazocine, 20.0 mg/kg s.c. However, this effect was not statistically significant.

4. THE PHENYLQUINONE TEST

(a) Nalorphine plus naloxone

Naloxone, 10.0 mg/kg s.c., injected 20 min prior to nalorphine, 50.0

mg/kg s.c., only slightly (and not significantly) reduced the anti-nociceptive activity of nalorphine (Table 2) as determined by the phenylquinone test.

(b) Pentazocine plus naloxone

Naloxone, 10.0 mg/kg s.c., injected 20 min prior to pentazocine, 5.0 mg/kg s.c., produced an almost total reversal of the anti-nociceptive activity of pentazocine (Table 2) as determined by the phenylquinone test.

(c) Nalorphine plus hyoscine

Hyoscine, 1.0 mg/kg s.c., produced a 25% reduction of the anti-nociceptive activity of nalorphine, 50.0 mg/kg s.c. (Table 3). This effect was, however, statistically significant ($P<0.05$).

(d) Pentazocine plus hyoscine

Hyoscine, 1.0 mg/kg s.c., failed to inhibit the anti-nociceptive activity of pentazocine, 5.0 mg/kg s.c. (Table 3). A slight inhibition (about 15%) was apparent, but this effect was not statistically significant.

5. THE RAT FOOT-PRESSURE TEST

(a) Nalorphine plus physostigmine

When given alone, neither physostigmine, 2.0 µg i.c.v., nor nalorphine, 15.0 mg/kg s.c., exhibited any anti-nociceptive effect in this test. However, when physostigmine was injected i.c.v. 30 min after nalorphine s.c., a marked anti-nociceptive effect was apparent (Fig. 57). Three of the five animals so treated exhibited an anti-nociceptive index of 1.0, which precluded a statistical analysis of this marked effect of the drug combination.

It will be noted that the time-course of the anti-nociceptive effect resembles that of higher doses of physostigmine injected alone, i.c.v., in

this test (Chapter Three, Fig. 30). Thus this property is similar in both the rat and the mouse (this chapter, para. 3(a)).

(b) Pentazocine plus physostigmine

Physostigmine, 2.0 µg i.c.v., produced a significant potentiation of the anti-nociceptive activity of pentazocine, 20.0 mg/kg s.c. (Fig. 58). In this case, the time-course of the combined effects of pentazocine plus physostigmine appears to be rather longer than that recorded for physostigmine alone in this test (Fig. 30).

6. DISCUSSION

The lack of activity of the narcotic-antagonist analgesics in animal tests has been discussed elsewhere (Chapter Two). This has made comparison of the narcotic-antagonists with morphine particularly difficult, especially so since the injection of saline i.c.v. has such a profound effect in the only test situation in which the antagonists show a dose-dependent effect (the phenylquinone test — Chapter Two). For this reason, none of the interaction experiments involving the injection of agents *via* the i.c.v. route has been attempted in the phenylquinone test. Similarly, since the antagonist-analgesics have virtually no activity in the other tests used, it was necessary to restrict our experiments to those in which a potentiation of a slight existing effect might be seen.

In the mouse tail-flick test, pentazocine and nalorphine were both potentiated markedly by physostigmine injected i.c.v. The dose of physostigmine was below that necessary to produce an effect of its own on the nociceptive threshold. This suggests that an increase in cholinergic activity in the CNS augments the activity of the narcotic-antagonist analgesics, as has been suggested also by Harris *et al.* (1969) who injected physostigmine

peripherally in mice. In fact, by reducing the stimulus intensity in the tail-flick test, Gray *et al.* (1970) were able to demonstrate the anti-nociceptive activity of pentazocine in a dose-dependent manner. However, naloxone also produced an increase in reaction time when the test parameters were thus altered (Gray *et al.*, 1970). Gray *et al.* (1970) observed some difficulty, however, in detecting the anti-nociceptive activity of nalorphine in this modified version of the test, and attributed this to the greater potency of nalorphine as compared with pentazocine. The same authors suggested that pentazocine might possess more morphine-like activity than had at first been suspected by Harris and Pierson (1964). The ability (or otherwise) of naloxone to inhibit this measurable effect of pentazocine in the modified tail-flick test would appear not to have been investigated by Gray *et al.* (1970). Such an experiment would presumably give some indication as to how much of pentazocine's anti-nociceptive activity was due to morphine-like properties.

Our own results with naloxone and pentazocine in the phenylquinone test suggest that a large proportion of the activity of pentazocine is due to its morphine-like effect, since naloxone brought about an almost total reversal of pentazocine's anti-nociceptive effect. It is interesting, also, to note that Gray *et al.* (1970) had difficulty in detecting nalorphine's activity in their modified tail-flick test, since we were unable to block the effect of nalorphine in the phenylquinone test with a dose of naloxone which almost totally abolished the activity of pentazocine in this test. The doses of pentazocine and nalorphine used in this experiment produced similar degrees of anti-nociceptive activity in the absence of naloxone.

The results discussed above are, however, at variance with those of Blumberg, Dayton and Wolf (1966), who were able to block both pentazocine

and nalorphine in the phenylquinone test, using naloxone. The discrepancy may be a result of the way in which the test was evaluated, since Blumberg *et al.* (1966) used a quantal method which required the treated animals to writhe less than 50% of the number of times of the control animals in a given period (Blumberg, Wolf & Dayton, 1965). This resulted in nalorphine being much more potent as evaluated in their method (1.0 mg/kg producing 87% anti-nociceptive effect) as compared with the method used in the present study (ED_{50}: 52.0 mg/kg s.c.); the figures for pentazocine are of the same order (Blumberg *et al.*: 10.0 mg/kg gave an 87% anti-nociceptive effect; the present study: ED_{50}: 4.8 mg/kg s.c.) in the two studies.

A further difference between nalorphine and pentazocine was revealed by the use of hyoscine in the phenylquinone test. Hyoscine reduced the anti-nociceptive effect of nalorphine by about 25%, whereas it failed to produce a significant reduction of the effect of pentazocine in this test, at a dose (1.0 mg/kg s.c.) which significantly reduced the anti-nociceptive effect of morphine in the tail-flick test (Results: Chapter Five). These results suggest that it is difficult to make valid comparisons between the same drug combinations in different test situations. Thus although an increase in central cholinergic activity potentiates nalorphine and pentazocine in the tail-flick test (also shown by Harris *et al.*, 1969) blockade of central cholinergic mechanisms antagonises nalorphine but not pentazocine in the phenylquinone test. Ireson (1969) was unable to block morphine or nalorphine with atropine in a number of nociceptive test situations, and in a later paper (Ireson, 1970) suggested that narcotic and cholinergic anti-nociceptive effects were brought about by separate but complementary mechanisms. However, it is possible that inability to block morphine with atropine was the result of a phenomenon which has been observed in the present study; large doses of atropine do not block the anti-nociceptive activity of morphine whereas

smaller doses do (Chapter Five). This explanation is unlikely in the case of Ireson's results, however, since he used 0.5 mg/kg s.c. atropine, which was of the same order as our "smaller" doses which did antagonise morphine.

The potentiation of both nalorphine and pentazocine by physostigmine injected i.c.v. has been observed both in the mouse tail-flick and in the rat foot-pressure tests. It is unfortunate that the narcotic antagonist analgesics are not sufficiently active in either of these tests to be able to demonstrate an antagonism (with other agents) of their individual anti-nociceptive effects. The results reported in Chapter Five, whereby morphine was partially antagonised by hyoscine, suggest that part of the anti-nociceptive activity of morphine may be cholinergic with a second component acting *via* an independent mechanism.. This could also be true of nalorphine as judged by the experiment reported in this chapter where hyoscine was able to partially antagonise the effect of nalorphine; however, pentazocine, despite possessing morphine-like activity (as revealed by its antagonism by naloxone) has little cholinergic-mediated activity (as revealed by its lack of antagonism by hyoscine).

This latter hypothesis is supported by the results using physostigmine injected i.c.v. in the tail-flick and foot-pressure tests. In these tests, the anti-nociceptive effects of the nalorphine/physostigmine and pentazocine/physostigmine combinations are similar, the doses being similar. However, when the ED_{50}'s of nalorphine and pentazocine alone are compared in the phenylquinone test, then pentazocine is ten times as active as nalorphine. Although direct comparisons of potency from one test to another may be inadvisable, this does suggest that nalorphine is relatively more easily potentiated by i.c.v. physostigmine than is pentazocine. This in turn indicates that nalorphine may possess a greater cholinergic component than

does pentazocine.

The results presented in this chapter, together with those reported in Chapter Five, may, therefore, be conveniently summarised: morphine and the narcotic-antagonist analgesics pentazocine and nalorphine, possess two separate but interrelated modes of anti-nociceptive action. One component involves a specific morphine-like type of activity, which is blocked by the narcotic antagonist naloxone; the other involves a cholinergic system, the ability of anti-cholinergic agents to block any individual anti-nociceptive agent depending upon the proportion of cholinergic component possessed by that anti-nociceptive agent. This statement is, however, an obvious over-simplification, for a number of reasons. Firstly, it does not take into account the fact that the anti-nociceptive effects of morphine and pentazocine are capable of complete blockade by naloxone, yet that of morphine may be at least partially blocked by anti-cholinergic agents. Secondly, this statement takes no account of the possible involvement of 5-HT, which would appear to be important in both the narcotic (see Chapter Seven) and cholinergic (see below, Chapter Twelve) anti-nociceptive effects. However, it is a useful basis for the later discussion of results, and will be expanded in the General Discussion to include results from succeding chapters, particularly those dealing with the anti-nociceptive activity of cholinergic compounds and the modification thereof by various agents (Chapters Ten, Eleven & Twelve).

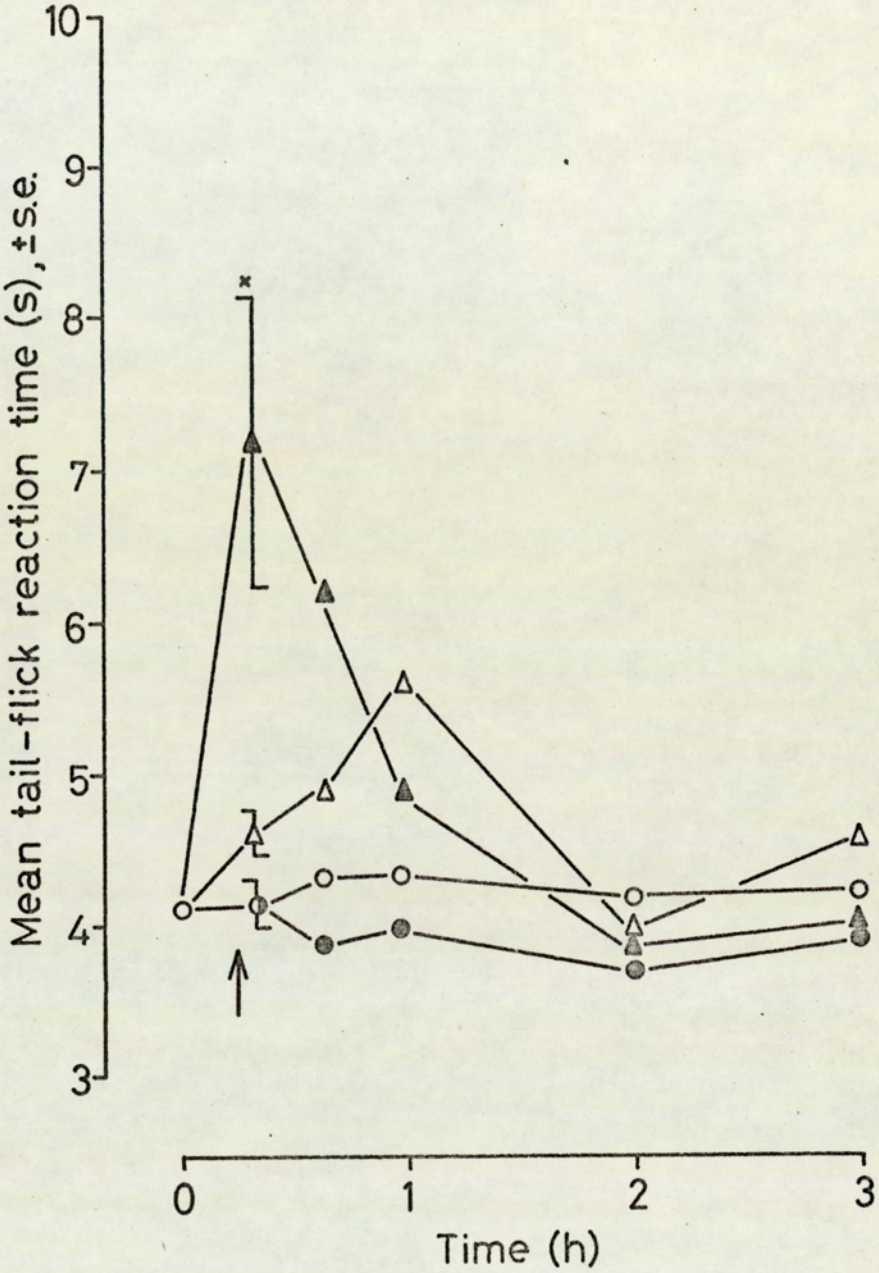

FIGURE 55: POTENTIATION BY PHYSOSTIGMINE INJECTED I.C.V. OF THE ANTI-NOCICEPTIVE ACTIVITY OF NALORPHINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

Δ—Δ: Nalorphine, 20.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Nalorphine, 20.0 mg/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

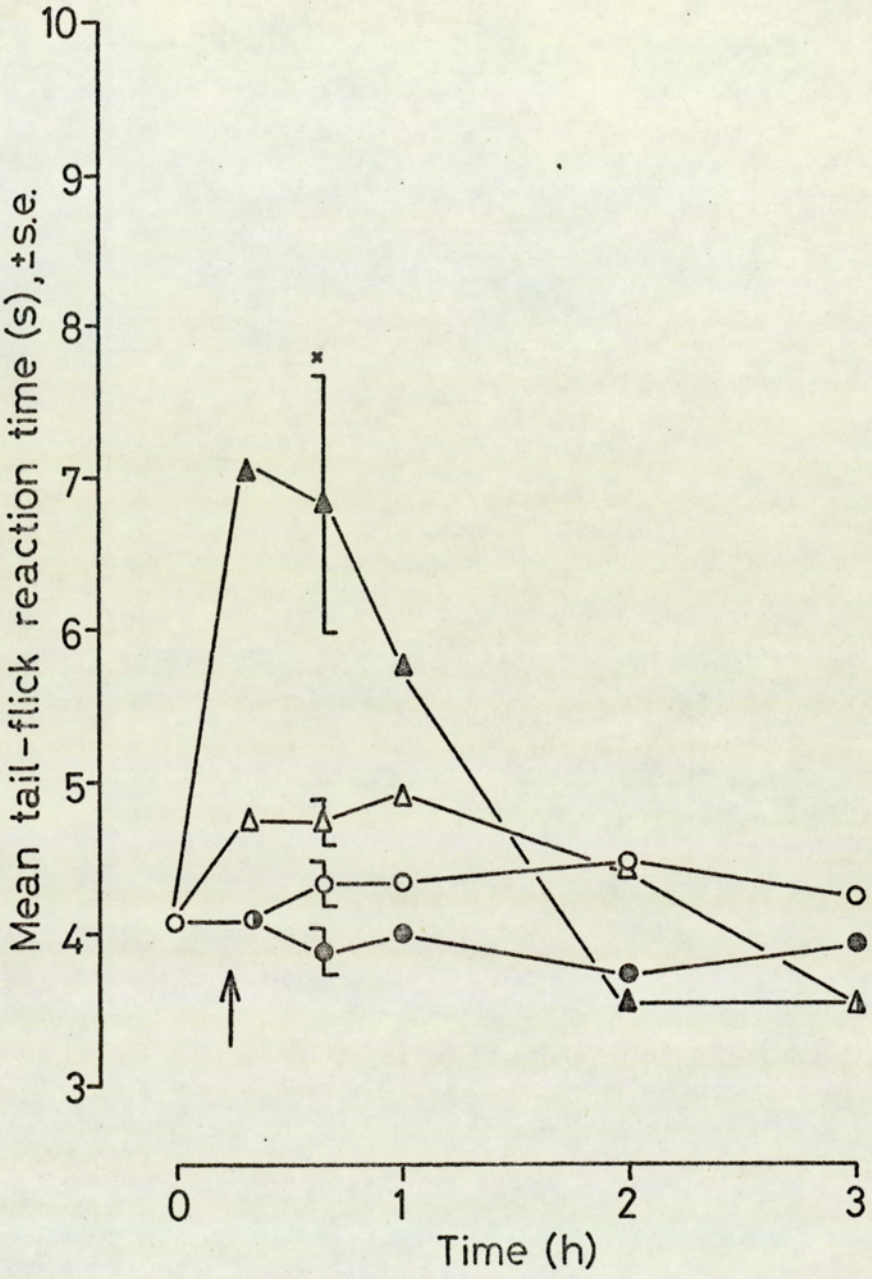

FIGURE 56: POTENTIATION BY PHYSOSTIGMINE INJECTED I.C.V. OF THE ANTI-NOCICEPTIVE ACTIVITY OF PENTAZOCINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

Δ—Δ: Pentazocine, 40.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Pentazocine, 20.0 mg/kg s.c. at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

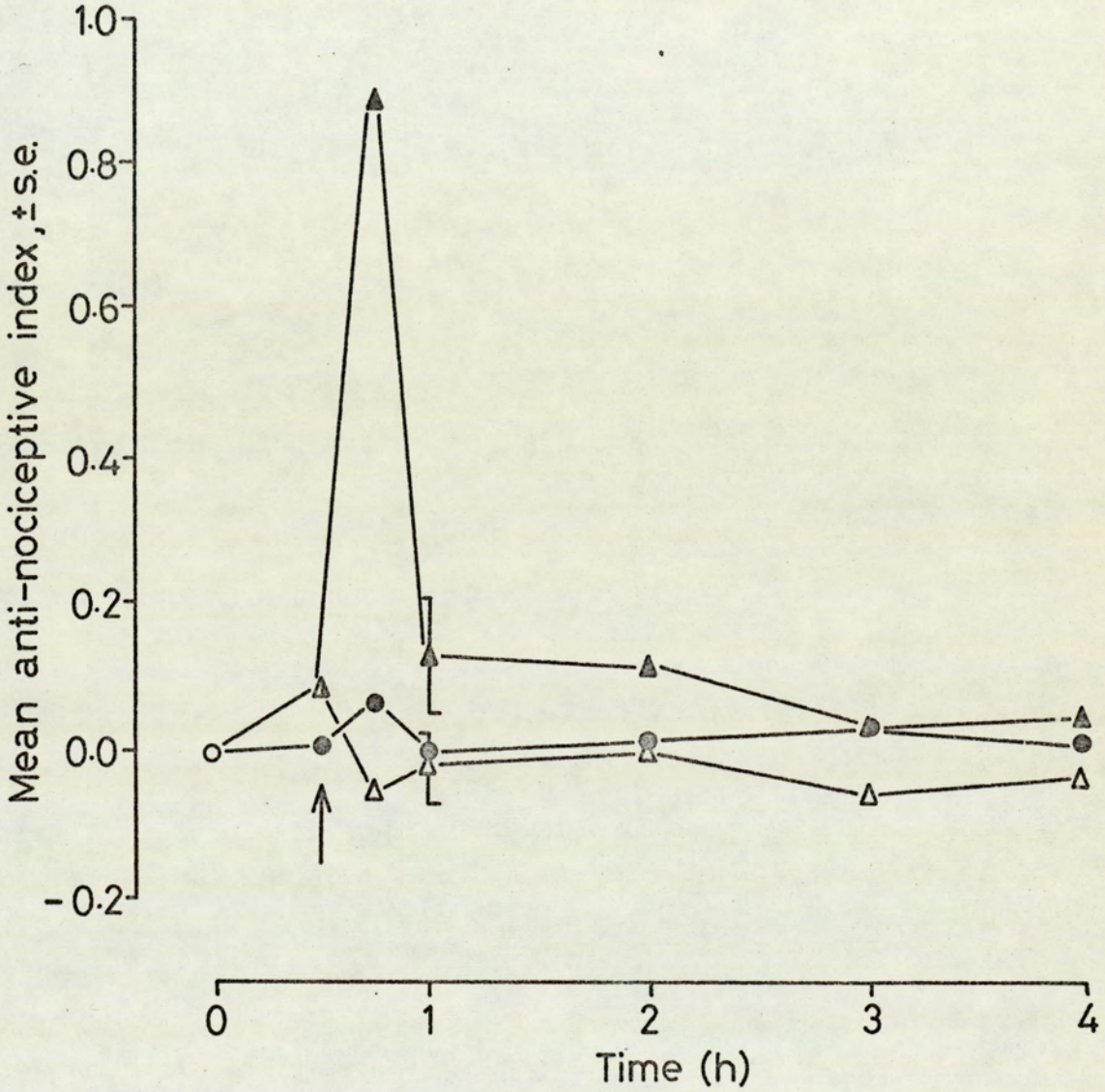

FIGURE 57: EFFECT OF PHYSOSTIGMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF NALORPHINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

●—●: Saline, 1.0 ml/kg s.c. at 0 + physostigmine, 2.0 µg i.c.v. at the arrow.

Δ—Δ: Nalorphine, 15.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Nalorphine, 15.0 mg/kg s.c. at 0 + physostigmine, 2.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

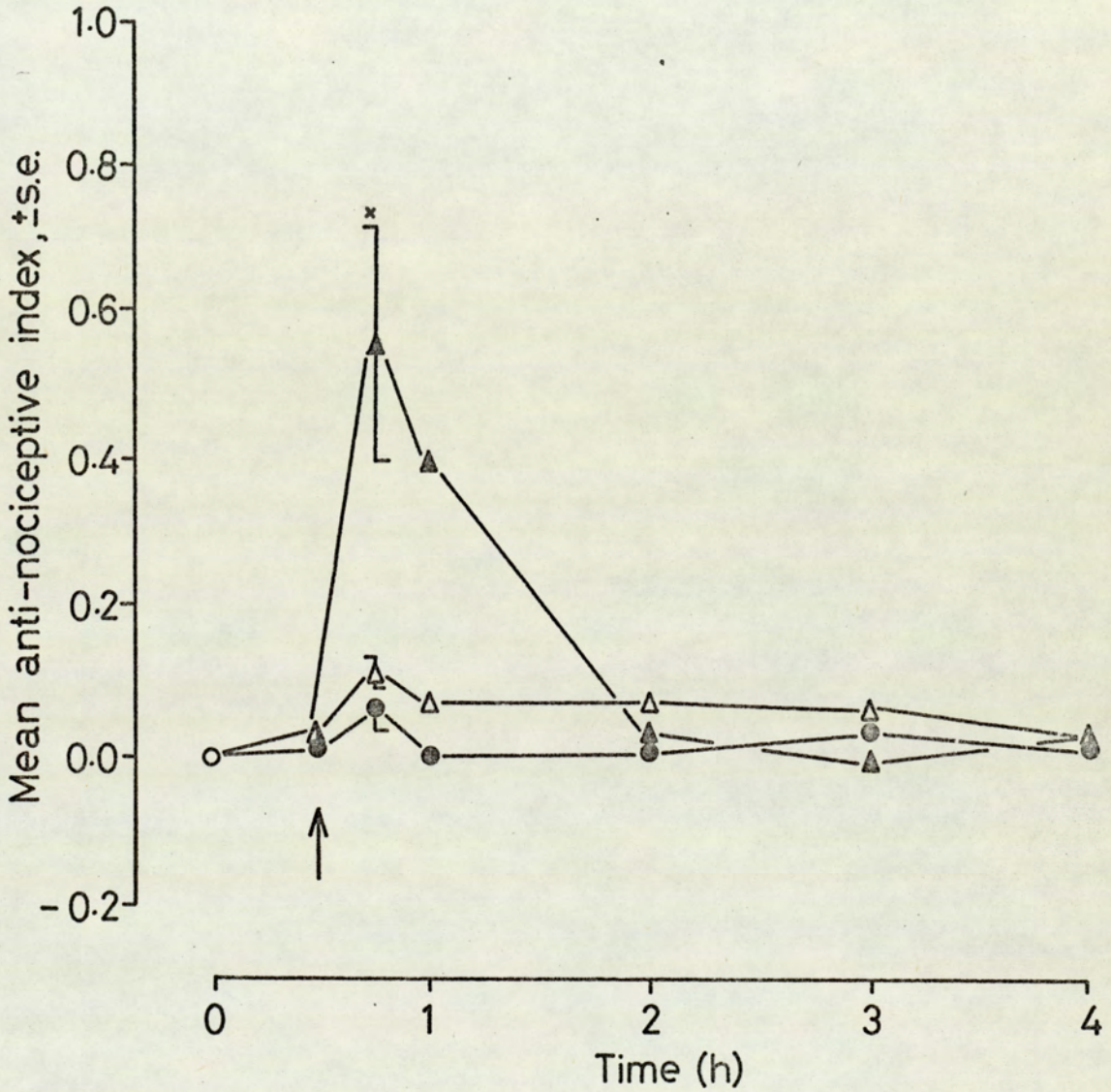

FIGURE 58: EFFECT OF PHYSOSTIGMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF PENTAZOCINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

●—●: Saline, 1.0 ml/kg s.c. at 0 + physostigmine, 2.0 µg i.c.v. at the arrow.

Δ—Δ: Pentazocine, 20.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Pentazocine, 20.0 mg/kg s.c. at 0 + physostigmine, 2.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

Treatment prior to phenylquinone 2.0 mg/kg i.p.	Mean No of writhes, ±s.e.
Saline 10.0 ml/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	34.9 ±3.3
Naloxone 10.0 mg/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	33.2 ±3.7
Saline 10.0 ml/kg s.c. at -40 min + nalorphine 50.0 mg/kg s.c. at -20 min	2.2 ±1.2
Naloxone 10.0 mg/kg s.c. at -40 min + nalorphine 50.0 mg/kg s.c. at -20 min	5.2 ±1.3
Saline 10.0 ml/kg s.c. at -40 min + pentazocine 5.0 mg/kg s.c. at -20 min	3.3 ±0.9
Naloxone 10.0 mg/kg s.c. at -40 min + pentazocine 5.0 mg/kg s.c. at -20 min	25.1 ±4.5

TABLE 2: EFFECT OF NALOXONE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITIES OF NALORPHINE AND PENTAZOCINE IN THE PHENYLQUINONE TEST

Groups of ten mice were injected as detailed in the table.

Treatment prior to phenylquinone 2.0 mg/kg i.p.	Mean No of writhes, ±s.e.
Saline 10.0 ml/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	29.4 ±3.2
Hyoscine 1.0 mg/kg s.c. at -40 min + saline 10.0 ml/kg s.c. at -20 min	30.2 ±4.1
Saline 10.0 ml/kg s.c. at -40 min + nalorphine 50.0 mg/kg s.c. at -20 min	4.2 ±1.4
Hyoscine 1.0 mg/kg s.c. at -40 min + nalorphine 50.0 mg/kg s.c. at -20 min	10.8 ±2.7
Saline 10.0 ml/kg s.c. at -40 min + pentazocine 5.0 mg/kg s.c. at -20 min	1.8 ±1.0
Hyoscine 1.0 mg/kg s.c. at -40 min + pentazocine 5.0 mg/kg s.c. at -20 min	5.8 ±4.4

TABLE 3: EFFECT OF HYOSCINE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITIES OF NALORPHINE AND PENTAZOCINE IN THE PHENYLQUINONE TEST

Groups of ten mice were injected as detailed in the table.

CHAPTER NINE

ANTI-NOCICEPTIVE ACTIVITY OF NARCOTIC ANTAGONIST ANALGESICS AS MODIFIED BY ADRENERGIC COMPOUNDS AND BY 5-HT

1. INTRODUCTION

Having examined the effects of noradrenaline, dopamine and 5-HT on the anti-nociceptive activity of morphine (Results: Chapters Six & Seven), it was necessary to determine their action on the narcotic-antagonist analgesics. However, as detailed elsewhere, the narcotic-antagonists were more than marginally active only in the phenylquinone test (Results: Chapter Two). We were unable to utilise the i.c.v. route of injection with the phenylquinone test, because the injection technique resulted in a significant reduction in the number of writhes elicited during the period in which any centrally-administered compound would have its peak effect (Results: Chapter One). Also, LSD-25 could not be used in this test because this compound itself produced a significant anti-nociceptive action in the phenylquinone test (Results: Chapter Three).

For these reasons, the number of experiments which could usefully be carried out in this part of the investigation was limited. However, those results which were obtained are reported below.

2. ADRENERGIC COMPOUNDS

All experiments involving adrenergic compounds and the narcotic-antagonist analgesics were carried out using the tail-flick test.

(a) Nalorphine

Neither NA, 0.5 µg i.c.v., nor DA, 5.0 µg i.c.v. modified in any way the marginal anti-nociceptive activity exhibited by nalorphine in the tail-flick test (doses of 5.0 to 20.0 mg/kg nalorphine s.c. were employed).

(b) Pentazocine

Similarly, neither NA, 0.5 µg i.c.v., nor DA, 5.0 µg i.c.v. modified

the marginal anti-nociceptive effect produced by pentazocine (10.0 to 40.0 mg/kg s.c.) in the tail-flick test.

(c) Naloxone

Naloxone, 5.0 to 20.0 mg/kg s.c., had no effect on the reaction time of mice tested by the tail-flick procedure (Results: Chapter Two), and neither NA, 0.5 µg i.c.v., nor DA, 5.0 µg i.c.v., modified this response.

3. 5-HT

Experiments were carried out with 5-HT in the tail-flick test as follows:

(a) Nalorphine

5-HT, 10.0 µg i.c.v., did not potentiate the activity of nalorphine, 20.0 mg/kg s.c. in the tail-flick test. In fact, the elevation in reaction-time noted with 5-HT alone (Results: Chapter Three) was somewhat reduced by the nalorphine, although this result was not statistically significant.

(b) Pentazocine

5-HT, 10.0 µg i.c.v., did not potentiate the anti-nociceptive effect of pentazocine, 10.0 to 40.0 mg/kg s.c., in the tail-flick test. Again, there appeared to be some antagonism of the 5-HT effect, but this was not statistically significant.

(c) Naloxone

The small elevation in reaction time produced by 5-HT, 10.0 µg i.c.v., was reduced by pretreatment with naloxone, 5.0 mg/kg s.c., although this effect was not statistically significant. The results of a typical experiment are depicted in Fig. 59, where the typical small effect of 5-HT

alone (and the wide scatter of reaction times within this group) is apparent.

Further experiments with 5-HT were carried out in the rat foot-pressure test, as follows:

(a) Nalorphine

The characteristic anti-nociceptive effect of 5-HT, 20.0 µg i.c.v., in the rat (Results: Chapter Three) was not modified by pretreatment with nalorphine, 15.0 mg/kg s.c., which itself was without effect on the nociceptive threshold in rats (Results: Chapter Two).

(b) Pentazocine

The raised nociceptive threshold of rats produced by 5-HT, 20.0 µg i.c.v., was not modified by pretreatment with pentazocine, 20.0 mg/kg s.c., which itself was without effect on the threshold (Results: Chapter Two).

(c) Naloxone

The anti-nociceptive effect of 5-HT, 20.0 µg i.c.v., in the rat, was not modified by pretreatment with naloxone, 20.0 mg/kg s.c., which was itself without effect on the nociceptive threshold of the rat (Fig. 60). This figure is typical of those seen with 5-HT plus nalorphine, pentazocine or naloxone in the rat.

4. DISCUSSION

As outlined in the introduction to this chapter, it was not possible, for a number of reasons, to carry out a full investigation of the interactions of adrenergic agents and 5-HT with the narcotic antagonists. The results which have been obtained are not entirely conclusive. However, in both the

rat and the mouse it is possible to say that the injection of 5-HT i.c.v. does not potentiate the anti-nociceptive activity of the narcotic-antagonist analgesics. In the mouse, some evidence has been obtained that, conversely, the antagonists will antagonise the effect of 5-HT (but this is not supported by the experiments using the rat).

In the mouse, there was no evidence of any interaction between the narcotic-antagonist analgesics and the catecholamines. Particularly, a dose of DA injected i.c.v. which potentiated the anti-nociceptive activity of morphine had no effect on the antagonist analgesics.

Thus it would appear that the antagonist analgesics differ from morphine in not being potentiated by 5-HT injected i.c.v. or by DA injected i.c.v. These results further lend support to the hypothesis that the antagonist analgesics exert their anti-nociceptive effect (at least in part) *via* a different mechanism to that of morphine itself (Eddy & Martin, 1970; Smits & Takemori, 1970).

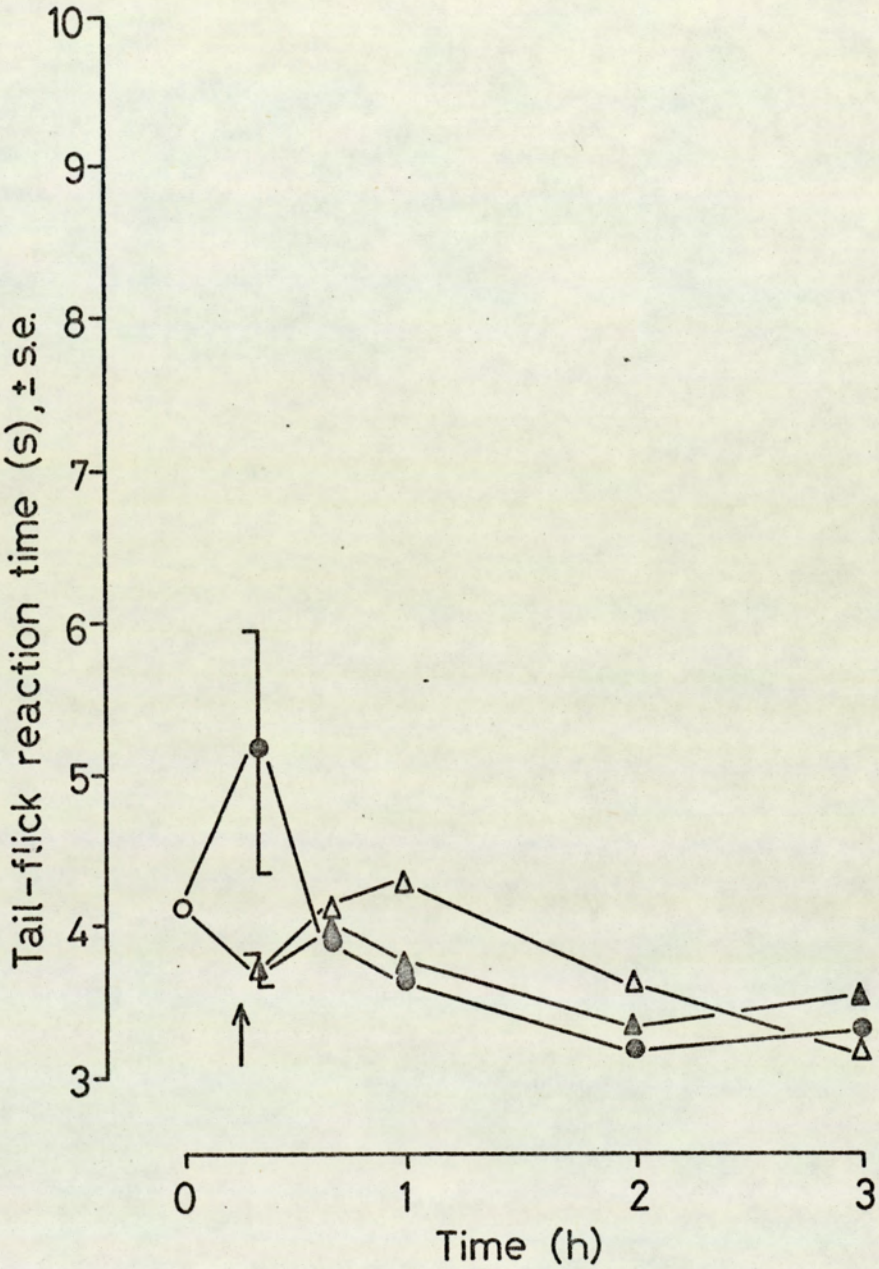

FIGURE 59: EFFECT OF NALOXONE ON THE ANTI-NOCICEPTIVE ACTIVITY OF 5-HT IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: Saline, 10.0 ml/kg s.c. at 0 + 5-HT, 10.0 µg i.c.v. at the arrow.

Δ—Δ: Naloxone, 5.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Naloxone, 5.0 mg/kg s.c. at 0 + 5-HT, 10.0 µg i.c.v. at the arrow.

The arrow is at +15 min.

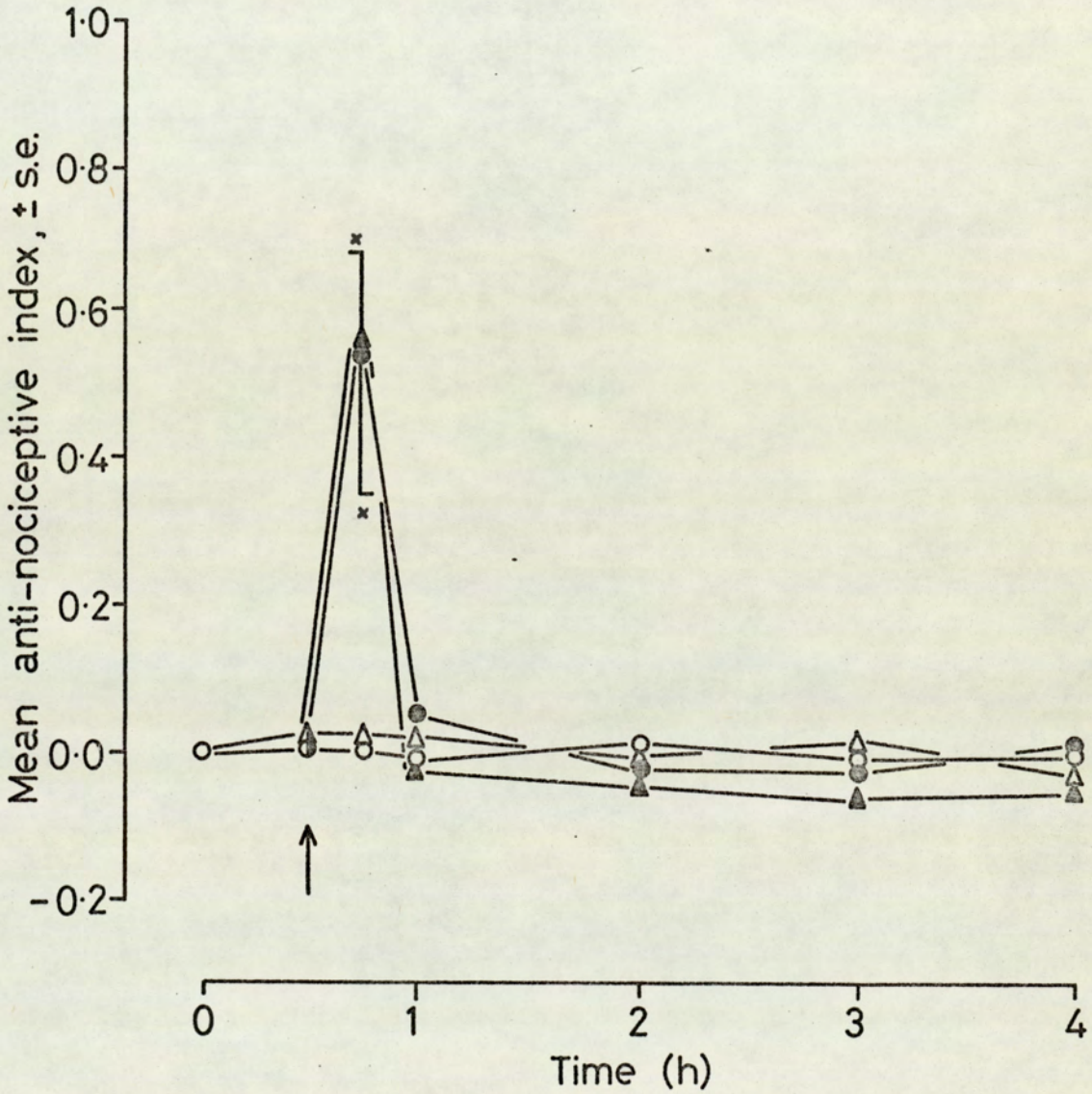

FIGURE 60: EFFECT OF NALOXONE ON THE ANTI-NOCICEPTIVE ACTIVITY OF 5-HT IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

O—O: Saline, 2.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 2.0 ml/kg s.c. at 0 + 5-HT, 20.0 µg i.c.v. at the arrow.

Δ—Δ: Naloxone, 20.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Naloxone, 20.0 mg/kg s.c. at 0 + 5-HT, 20.0 µg i.c.v. at the arrow.

The arrow is at +30 min.

PART E

ANTI-NOCICEPTIVE ACTIVITY OF CHOLINERGIC COMPOUNDS AS MODIFIED BY OTHER AGENTS

CHAPTER TEN

ANTI-NOCICEPTIVE ACTIVITY OF CHOLINERGIC COMPOUNDS AS MODIFIED BY NALOXONE, CHOLINERGIC AND ANTI-CHOLINERGIC COMPOUNDS

1. INTRODUCTION

In Chapter Three of the Experimental Results, experiments were described wherein the anti-nociceptive activity of a number of cholinomimetic agents was investigated. The activity of these agents was discussed with respect to that of morphine, and in Chapter Five, the effects of combinations of morphine with cholinomimetic agents were described. In the present chapter, and in Chapters Eleven and Twelve, the anti-nociceptive activity of cholinomimetic agents as modified by other drugs will be discussed. Oxotremorine is the cholinomimetic which we have used most widely in this study.

Because of the similarity of the anti-nociceptive activities of oxotremorine and morphine, we have attempted to modify the action of oxotremorine with the same agents as were used to modify the anti-nociceptive action of morphine, in order to confirm this similarity. In the present chapter, we describe the results obtained using the narcotic antagonist naloxone, combinations of cholinergic agents, and various anti-cholinergic agents. As noted in Chapter Three, except in those experiments specifically designed to examine the activity of anti-cholinergic agents, it was normal to pretreat all animals (in experiments involving oxotremorine) with atropine methonitrate, 0.5 mg/kg s.c., 20 to 30 min before the oxotremorine injection, in order to prevent the peripheral effects of the latter agent. Such a dose of atropine methonitrate had no effect on the anti-nociceptive activity of oxotremorine, 0.05 mg/kg s.c., in the tail-flick test (this Chapter, para. 4(c)), and had been used for a similar purpose by Ireson (1969, 1970).

2. EXPERIMENTS WITH NALOXONE

(a) Physostigmine with naloxone

In the hot-plate test, naloxone, 20.0 mg/kg s.c., had no significant

effect on the anti-nociceptive activity of physostigmine, 0.5 µg i.c.v., when injected 20 min before the physostigmine.

Similarly, in the tail-flick test, naloxone, 20.0 mg/kg s.c., had no significant effect on the anti-nociceptive activity of physostigmine, 3.0 µg i.c.v., when injected 20 min before the physostigmine.

(b) Oxotremorine with naloxone

In the hot-plate test, oxotremorine, 0.1 mg/kg s.c., was not antagonised by the injection of naloxone, 1.0 µg i.c.v., a dose which previously had antagonised completely the effect of morphine, 10.0 mg/kg s.c., in this test (Results: Chapter Four).

Using the tail-flick test, naloxone, 10.0 mg/kg s.c., produced some degree of antagonism towards the anti-nociceptive activity of oxotremorine, 0.05 µg i.c.v. Again, however, this effect was not statistically significant, and raising the dose of naloxone to 20.0 mg/kg s.c. did not increase the inhibitory effect (Fig. 61).

In the phenylquinone test, naloxone, 10.0 mg/kg s.c., had no effect on the anti-nociceptive activity of oxotremorine, 0.05 mg/kg s.c. This dose of naloxone completely abolished the anti-nociceptive effect of morphine, 1.0 mg/kg s.c., in this test (Results: Chapter Four).

3. EXPERIMENTS WITH OTHER CHOLINERGIC AGENTS

(a) Physostigmine and oxotremorine in the hot-plate test

Physostigmine, 1.0 µg i.c.v., did not potentiate the anti-nociceptive activity of oxotremorine, 0.1 mg/kg s.c. in this test. In fact physostigmine was active itself in this test at the dose used in this experiment, producing a 30% elevation in the mean reaction time, but there was no apparent additive

effect with oxotremorine.

(b) Physostigmine and oxotremorine in the tail-flick test

Physostigmine, 1.0 µg i.c.v., produced a significant elevation of the anti-nociceptive effect of oxotremorine, 0.025 mg/kg s.c., as shown in Fig. 62. The combination produced an elevation in reaction time about 3 × that of either agent alone. However, since both oxotremorine and physostigmine were active alone at these dose-levels, the effect seen may be an addition of the effects of the two drugs, rather than true potentiation of one by the other.

4. EXPERIMENTS WITH ANTI-CHOLINERGIC AGENTS

(a) Atropine injected s.c. in the tail-flick test

Atropine sulphate, 2.0 mg/kg s.c., injected 15 min before oxotremorine, 0.05 mg/kg s.c., completely abolished the anti-nociceptive effect of the oxotremorine (Fig. 63). Similarly, a high dose of atropine sulphate, 20.0 mg/kg s.c., also blocked the effect of oxotremorine, 0.05 mg/kg s.c. This result contrasts with that of the antagonism of morphine by atropine, when small doses of atropine antagonised morphine but large doses did not (Results: Chapter Five).

(b) Atropine injected i.c.v. in the tail-flick test

Atropine sulphate, 0.5 µg i.c.v., injected 5 min before oxotremorine, 0.05 mg/kg s.c., produced a 90% reduction in the anti-nociceptive activity of oxotremorine ($P<0.005$), although the anti-nociceptive activity was not completely abolished (Fig. 64). This dose of atropine injected i.c.v. significantly reduced the anti-nociceptive activity of morphine, 5.0 mg/kg s.c., by about 65% (Results: Chapter Five).

(c) Methylatropine injected s.c. in the tail-flick test

Methylatropine, 2.0 mg/kg s.c., injected 15 min before oxotremorine, 0.05 mg/kg s.c., did not attenuate the anti-nociceptive activity of the oxotremorine (Fig. 65).

(d) Methylatropine injected i.c.v. in the tail-flick test

Methylatropine, 0.5 µg i.c.v., injected 5 min before oxotremorine, 0.05 mg/kg s.c., produced a highly significant attenuation (by about 90%) of the anti-nociceptive effect of oxotremorine ($P<0.005$; Fig. 66).

(e) Hyoscine injected s.c. in the tail-flick test

Hyoscine, 1.0 mg/kg s.c., completely abolished the anti-nociceptive effect of oxotremorine, 0.05 mg/kg s.c., when injected 20 min before the oxotremorine ($P<0.001$). Figure 67 shows this effect, the control group having received 20.0 mg/kg s.c. of hyoscine, which had no effect alone on the nociceptive threshold.

(f) Hyoscine injected s.c. in the phenylquinone test

Hyoscine, 1.0 mg/kg s.c., injected 20 min before oxotremorine, 0.05 mg/kg s.c., completely abolished the anti-nociceptive activity of oxotremorine as measured by the phenylquinone test ($P<0.001$; Table 4).

5. DISCUSSION

The results reported in this chapter show that i.c.v. physostigmine, (although capable of potentiating the weak anti-nociceptive activity of nalorphine and pentazocine, agents which possess some degree of agonist activity: Results: Chapter Eight), does not reveal any activity in the agent naloxone, which is devoid of agonist activity (Kosterlitz & Watt,

1968). This suggests that the potentiation of morphine (Results: Chapter Five) and that of nalorphine and pentazocine (Results: Chapter Eight) by physostigmine injected i.c.v. is an effect concerned with the narcotic agonist component of these agents. We have been unable to demonstrate any antagonism of the cholinergic agent with naloxone.

As might be expected, we have shown that physostigmine injected i.c.v. is capable of potentiating (or, at least, exerting an additive effect upon) the anti-nociceptive activity of oxotremorine in the tail-flick test. However, in the hot-plate test, physostigmine did not potentiate oxotremorine, although it had potentiated the anti-nociceptive action of morphine in this test (Results: Chapter Five). The mechanism by which physostigmine might potentiate oxotremorine is not clear, since the way in which these two individual agents exert their anti-nociceptive activities is also unclear.

In the case of oxotremorine, it may be acting *via* release of endogenous ACh (Holmstedt & Lundgren, 1966), or by a direct effect on muscarinic sites (Cox & Potkonjak, 1969). This latter effect could then easily explain the increase in whole-brain levels of ACh following oxotremorine injection: Polak (1971) has postulated a feed back mechanism controlling ACh release, whereby released ACh stimulates presynaptic receptors, which then results in a reduced release of ACh. If oxotremorine were to stimulate these pre-synaptic receptors, then the release of endogenous ACh would be reduced and hence brain levels would rise.

In the case of physostigmine, several studies have suggested that its anti-nociceptive activity is a result of a direct effect on muscarinic receptors (Burgen & Chipman, 1952) rather than its ability to inhibit cholinesterase. Thus Oelssner and Andreas (1969) demonstrated the anti-nociceptive activity of physostigmine, but could detect no effect with

certain of the irreversible organophosphorus cholinesterase inhibitors. This led them to suggest that a direct effect of physostigmine was responsible for the observed effects. However, it is possible that neuronally-released ACh may be involved in the anti-nociceptive effect, because physostigmine is more efficient at protecting ACh from cholinesterase than are the organophosphorus inhibitors. The reason is that physostigmine is equally antagonistic towards acetylcholinesterase and pseudocholinesterase (Augustinsson, 1948), whilst the organophosphorus compound dyflos is more potent against pseudocholinesterase (Aldridge, 1953). Thus measurement of total cholinesterase inhibition in the brain would not give a true picture of the extent of inhibition of acetylcholinesterase, which is presumably more important with regard to the possible involvement of neuronally-released ACh in anti-nociceptive activity (Tha, 1970). However, whatever the exact mechanisms of action of oxotremorine and physostigmine, it seems clear that stimulation of central cholinergic receptors, probably muscarinic in nature, is responsible for the anti-nociceptive activity of these agents. It is difficult, at this stage, to attempt to suggest to what extent endogenous ACh is involved, until a definitive explanation of the pharmacological properties of oxotremorine has been achieved.

The evidence obtained with the cholinergic blocking drugs atropine, methylatropine and hyoscine, further supports the hypothesis that stimulation of muscarinic sites in the central nervous system is responsible for the anti-nociceptive activity of oxotremorine. Methylatropine, which is a quaternary amine and therefore not readily capable of crossing the blood-brain barrier, had no effect on the anti-nociceptive activity of oxotremorine. As mentioned previously, 0.5 mg/kg s.c. methylatropine has been used to prevent the peripheral effects of oxotremorine in all experiments involving

oxotremorine except when the experiment specifically involved the investigation of cholinolytic agents.

Both low and high doses of atropine sulphate antagonised the anti-nociceptive activity of oxotremorine. Other workers have demonstrated this effect, using low doses (Chen, 1958; Cho *et al.*, 1964; Handley & Spencer, 1969; Ireson, 1969). This result is interesting in view of our earlier findings that relatively high doses of atropine failed to block the anti-nociceptive action of morphine whereas low doses did block morphine's effect (Results: Chapter Five).

Both atropine sulphate and methylatropine were effective in low doses at blocking the anti-nociceptive activity of oxotremorine when they were injected *via* the i.c.v. route: further evidence in support of a central site of action for oxotremorine.

The similarity between the anti-nociceptive activities of morphine and oxotremorine is thus demonstrated by the properties of both drugs in the presence of cholinergic and cholinolytic agents.

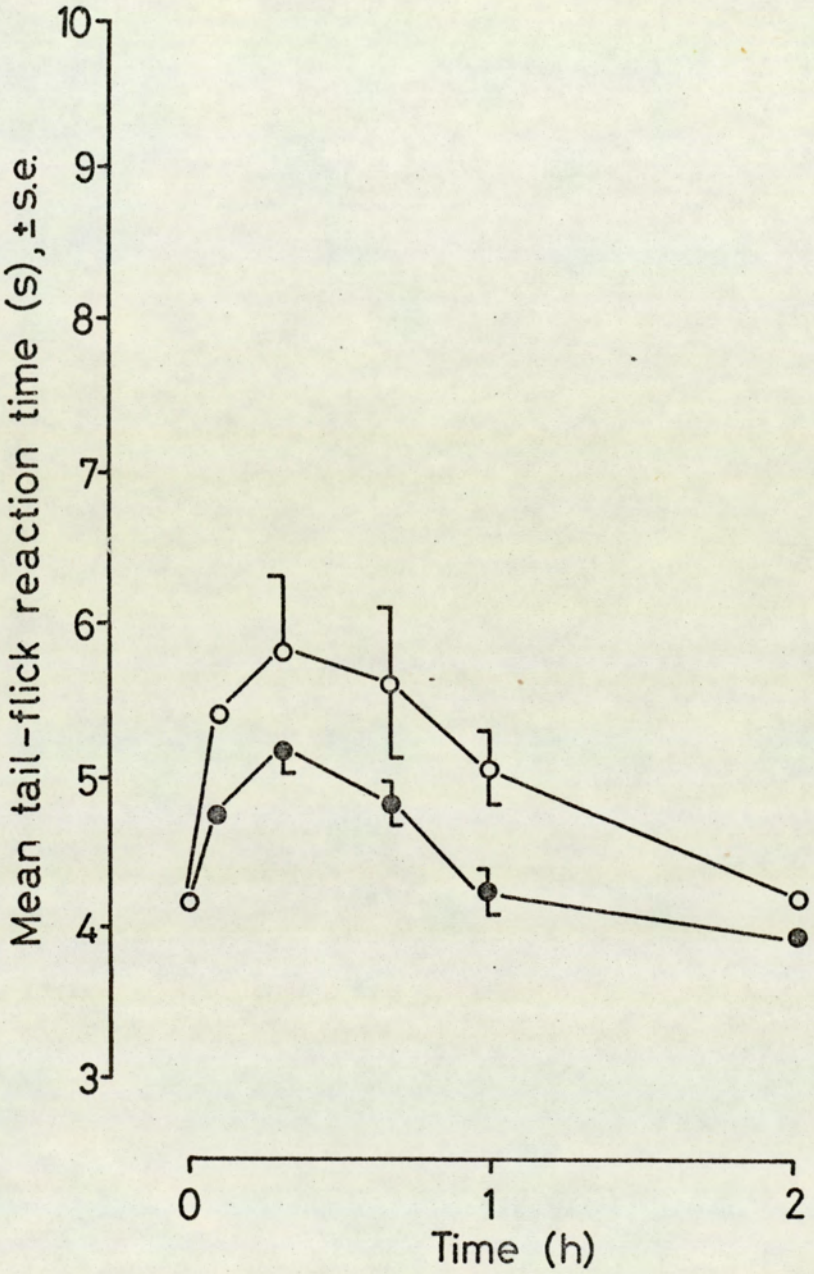

FIGURE 61: ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE AS MODIFIED BY NALOXONE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

O—O: Saline, 10.0 ml/kg s.c. at -20 min + oxotremorine, 0.05 µg i.c.v. at 0.

●—●: Naloxone, 20.0 mg/kg s.c. at -20 min + oxotremorine, 0.05 µg i.c.v. at 0.

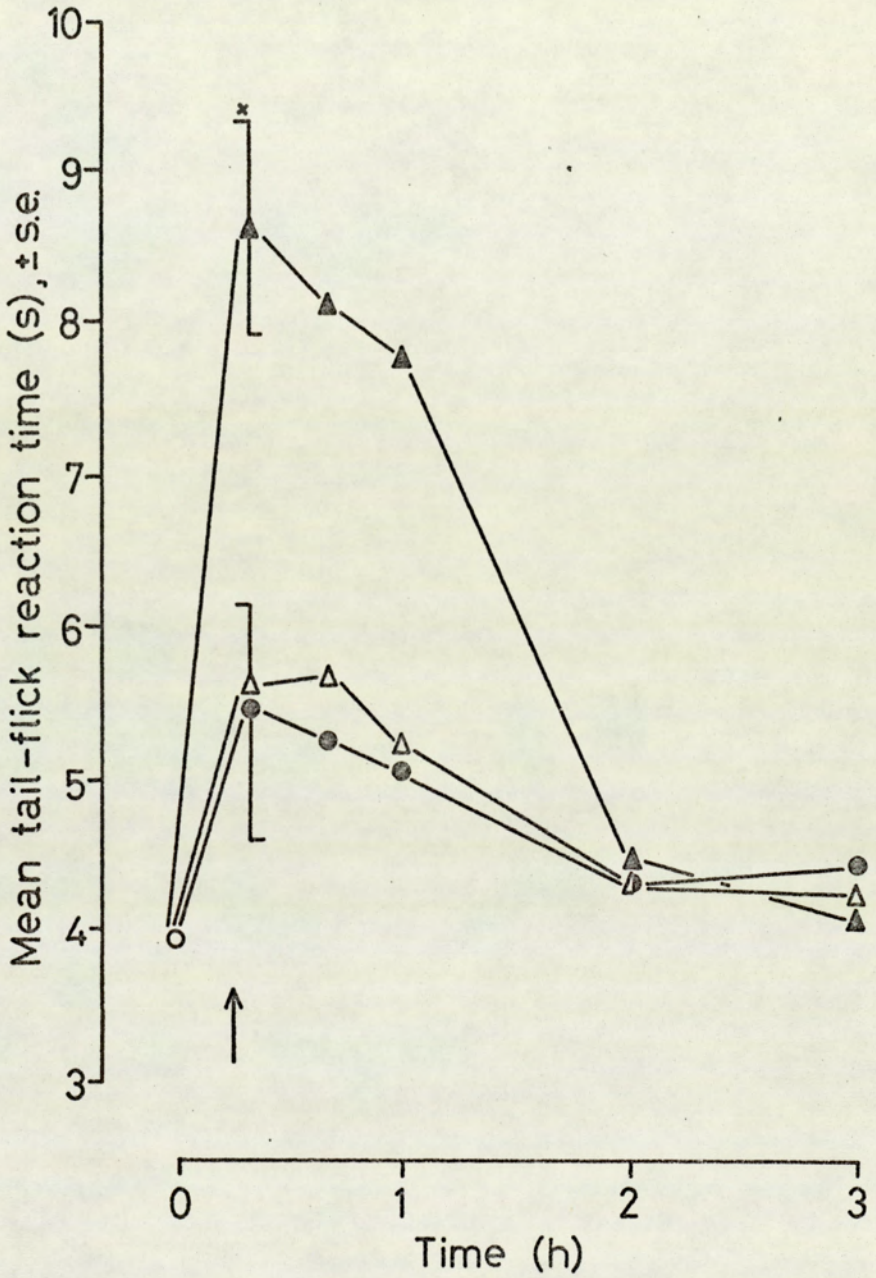

FIGURE 62: ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE WITH I.C.V. PHYSOSTIGMINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: Saline, 10.0 ml/kg s.c., at 0 + physostigmine, 1.0 µg i.c.v. at the arrow.

Δ—Δ: Oxotremorine, 0.025 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Oxotremorine, 0.025 mg/kg s.c., at 0 + physostigmine, 1.0 µg i.c.v., at the arrow.

The arrow is at +15 min.

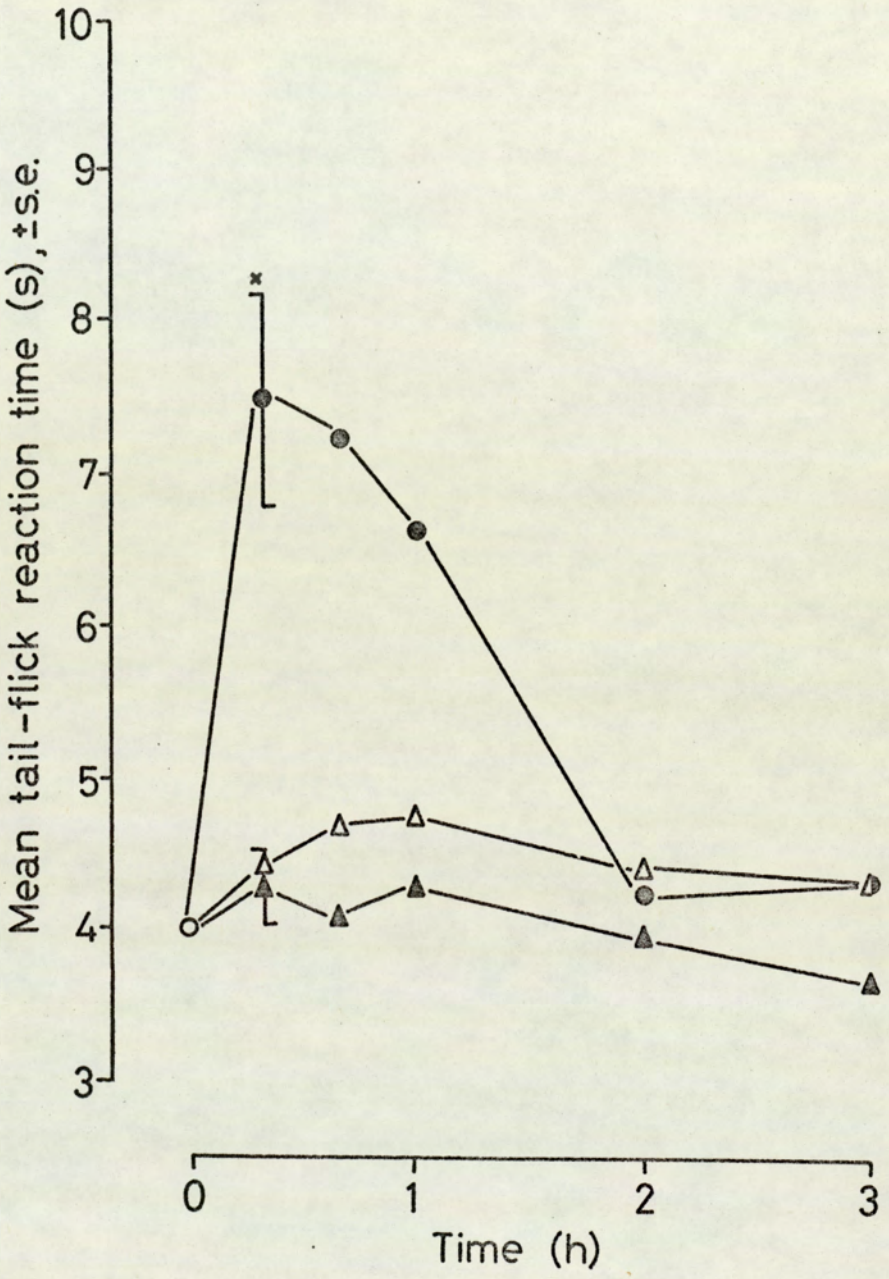

FIGURE 63: EFFECT OF ATROPINE SULPHATE ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN MICE

Groups of ten mice were injected as follows:

●—●: Saline, 10.0 ml/kg s.c., at -15 min + oxotremorine, 0.05 mg/kg s.c., at 0.

Δ—Δ: Atropine, 2.0 mg/kg s.c., at -15 min + saline, 10.0 ml/kg s.c., at 0.

▲—▲: Atropine, 2.0 mg/kg s.c., at -15 min + oxotremorine, 0.05 mg/kg s.c., at 0.

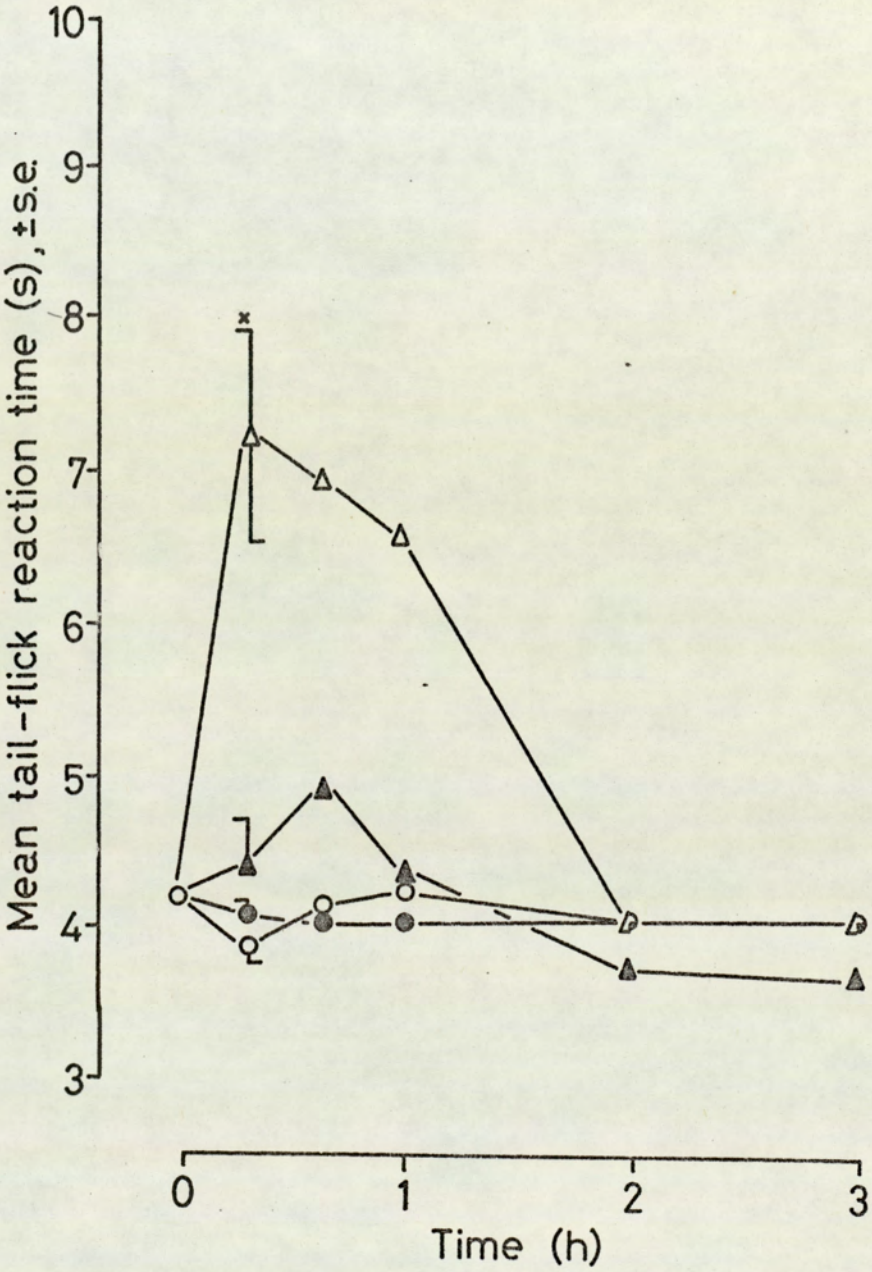

FIGURE 64: INHIBITION OF THE ANTI-NOCICEPTIVE EFFECT OF OXOTREMORINE BY ATROPINE SULPHATE INJECTED I.C.V. IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

O—O: Saline, 10.0 µl i.c.v., at -5 min + saline, 10.0 ml/kg s.c. at 0.

●—●: Atropine, 0.5 µg i.c.v., at -5 min + saline, 10.0 ml/kg s.c. at 0.

Δ—Δ: Saline, 10.0 µl i.c.v., at -5 min + oxotremorine, 0.05 mg/kg s.c. at 0.

▲—▲: Atropine, 0.5 µg i.c.v., at -5 min + oxotremorine, 0.05 mg/kg s.c. at 0.

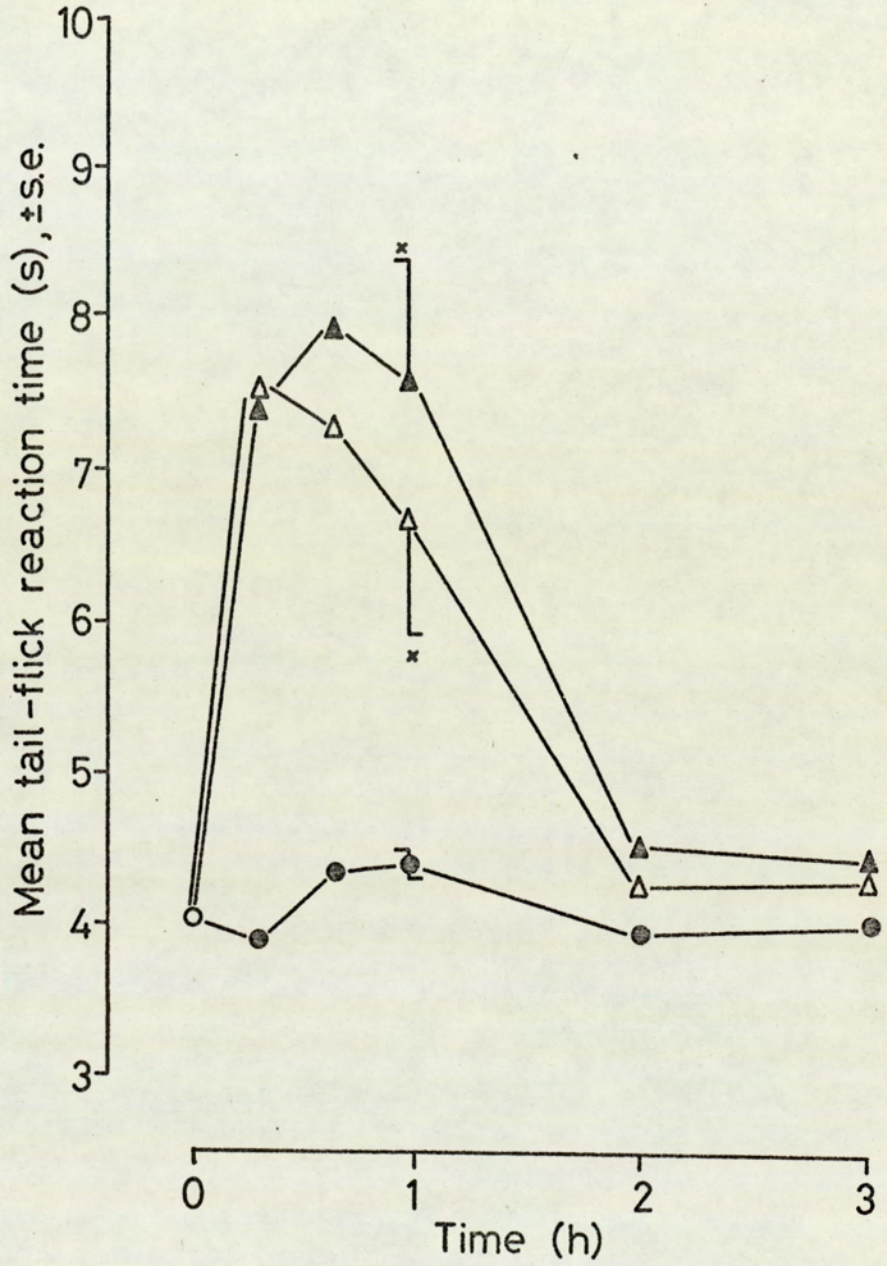

FIGURE 65: EFFECT OF METHYLATROPINE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: Atropine methonitrate, 2.0 mg/kg s.c., at -15 min + saline, 10.0 ml/kg s.c., at 0.

Δ—Δ: Saline, 10.0 ml/kg s.c., at -15 min + oxotremorine, 0.05 mg/kg s.c., at 0.

▲—▲: Atropine methonitrate, 2.0 mg/kg s.c., at -15 min + oxotremorine, 0.05 mg/kg s.c., at 0.

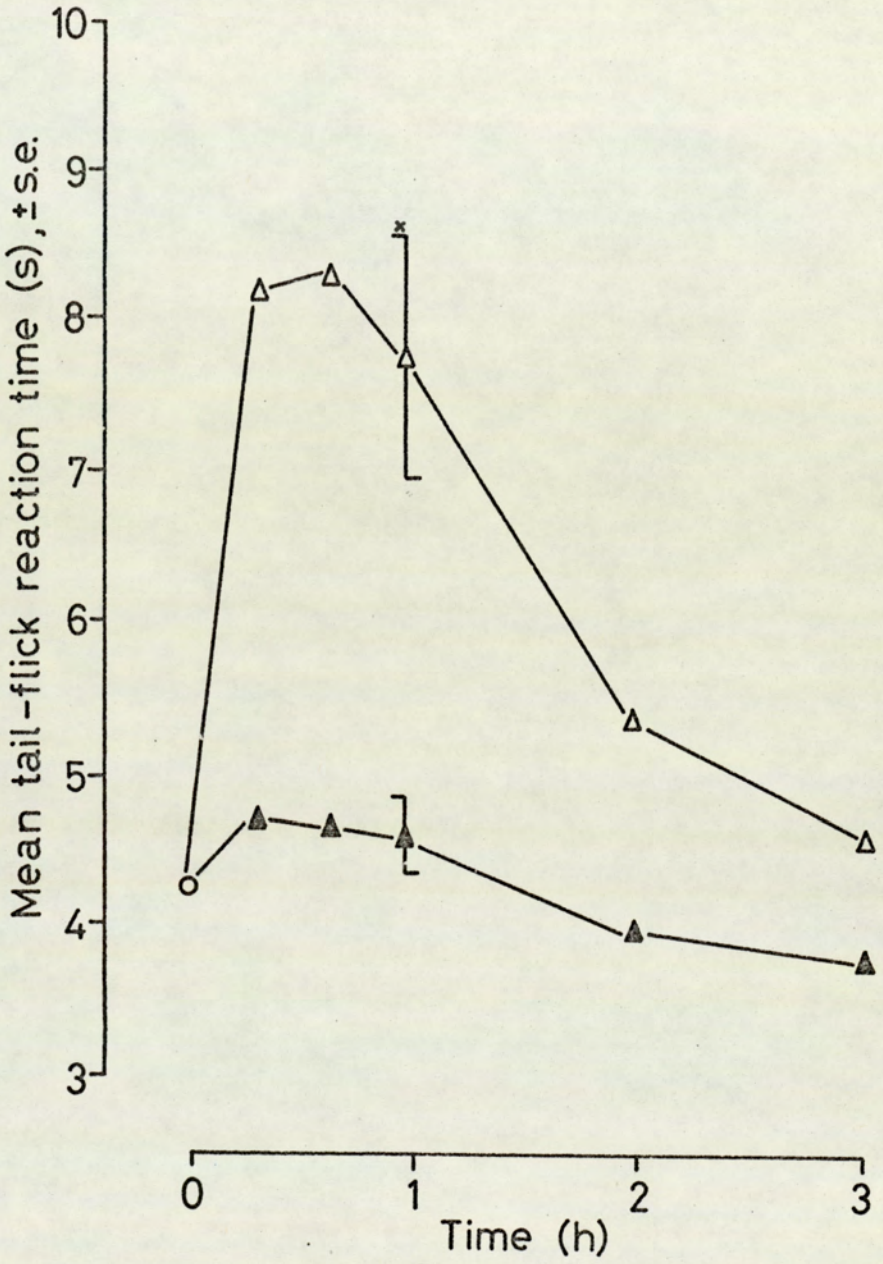

FIGURE 66: EFFECT OF METHYLATROPINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

Δ—Δ: Saline, 10.0 µl i.c.v., at -5 min + oxotremorine, 0.05 mg/kg s.c., at 0.

▲—▲: Atropine methonitrate, 0.5 µg i.c.v., at -5 min + oxotremorine, 0.05 mg/kg s.c., at 0.

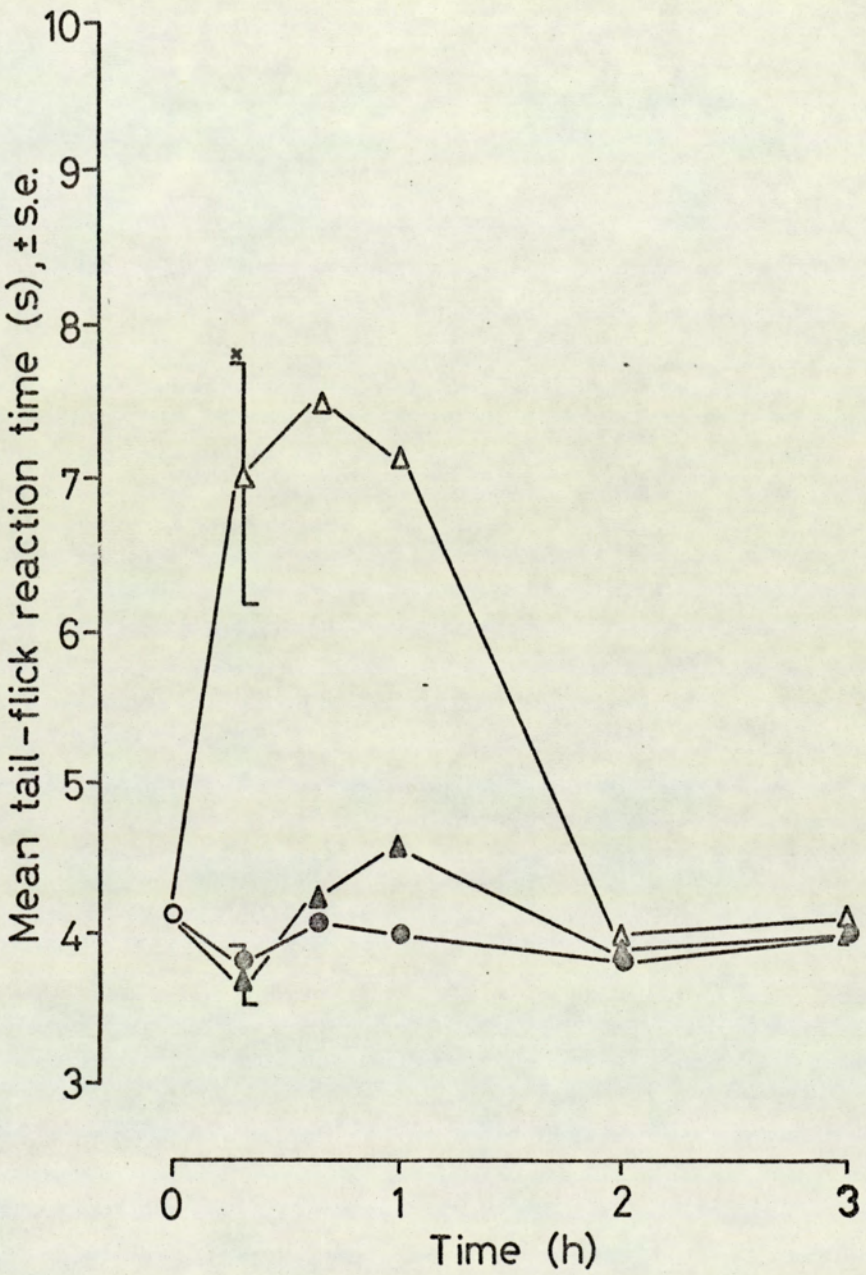

FIGURE 67: EFFECT OF HYOSCINE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: Hyoscine, 20.0 mg/kg s.c., at -20 min + saline, 10.0 ml/kg s.c., at 0.

Δ—Δ: Saline, 10.0 ml/kg s.c., at -20 min + oxotremorine, 0.05 mg/kg s.c., at 0.

▲—▲: Hyoscine, 1.0 mg/kg s.c., at -20 min + oxotremorine, 0.05 mg/kg s.c., at 0.

Treatment prior to phenylquinone 2.0 mg/kg i.p. at 0	Mean No of writhes, ±s.e.
Saline, 10.0 ml/kg s.c. at -40 min + saline, 10.0 ml/kg s.c. at -20 min	32.3 ±3.3
Hyoscine, 1.0 mg/kg s.c. at -40 min + saline, 10.0 ml/kg s.c. at -20 min	43.2 ±4.3
Saline, 10.0 ml/kg s.c. at -40 min + oxotremorine, 0.05 mg/kg s.c. at -20 min	3.7 ±1.8
Hyoscine, 1.0 mg/kg s.c. at -40 min + oxotremorine, 0.05 mg/kg s.c. at -20 min	42.1 ±3.7

TABLE 4: EFFECT OF HYOSCINE INJECTED S.C. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE PHENYLQUINONE TEST

Groups of ten mice were injected as detailed in the table.

CHAPTER ELEVEN

ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE AS MODIFIED BY ADRENERGIC AND ANTI-ADRENERGIC COMPOUNDS

1. INTRODUCTION

In order to demonstrate further the similarities between the anti-nociceptive actions of oxotremorine and morphine, we have studied the interactions between oxotremorine and some adrenergic and anti-adrenergic compounds. The results reported in this chapter can, therefore, be compared with analagous experiments using morphine and reported previously in Chapter Six.

2. THE MOUSE TAIL-FLICK TEST

(a) Reserpine

Reserpine, 2.0 mg/kg i.p., was injected at various time intervals before oxotremorine, 0.05 mg/kg s.c., the mice then being subjected to the tail-flick test 20 min after the dose of oxotremorine. Figure 68 shows, initially, that at one and two hours after the reserpine injection there was a potentiation of the oxotremorine, such that the total anti-nociceptive effect was some 30% greater than that achieved using oxotremorine alone.

Thereafter, the anti-nociceptive activity of oxotremorine was abolished progressively with increasing duration of reserpine pretreatment, so that with 8 h of pretreatment, a slight hyperalgesic effect was observed. After 24 h pretreatment, the anti-nociceptive action of oxotremorine was again detectable, although still only 20% of the activity of oxotremorine in intact animals. The effect of oxotremorine was fully restored 5 days after reserpinisation (this time-course may be compared with that of morphine at various times after reserpine: Chapter Six, para. 2(a) and Fig. 41).

(b) Noradrenaline

Noradrenaline, 5.0 µg i.c.v., injected 15 min after oxotremorine, 0.05 mg/kg s.c., produced a highly significant attenuation of the anti-nociceptive

activity of oxotremorine ($P<0.01$; Fig. 69). The attenuation was most marked 5 min after the NA injection (*i.e.* 20 min after the oxotremorine injection), ammounting to a reduction of approximately 75% in the anti-nociceptive effect of oxotremorine, as measured by this test. Subsequently, however, the oxotremorine effect became re-established, until at 45 min after the NA injection (*i.e.* 60 min after the oxotremorine injection) the NA-injected group was no longer significantly more sensitive to the noxious stimulus than the saline-injected group. (An analagous situation occurred when i.c.v. NA was used to modify the anti-nociceptive activity of morphine: Chapter Six, para. 2(c); Figs. 43 & 44.)

(c) Dopamine

Dopamine, 50.0 µg i.c.v., injected 15 min after oxotremorine, 0.05 mg/kg s.c., produced a large reduction in the anti-nociceptive activity of the oxotremorine (Fig. 70). This effect was maximal 5 min after the DA injection (*i.e.* 20 min after the oxotremorine injection), when the reduction in anti-nociceptive activity was about 80%. Subsequently, the anti-nociceptive effect became re-established, but only to about 50% of that of the oxotremorine/saline group. In this particular experiment, no estimate of statistical significance could be made because of the high proportion of animals in the oxotremorine/saline group which exceeded the cut-off time. However, the attenuation of the anti-nociceptive effect of oxotremorine may be clearly seen. (The effect of DA injected i.c.v. on the anti-nociceptive activity of oxotremorine may be compared with that of DA injected i.c.v. on the anti-nociceptive activity of morphine: Chapter Six, para. 2(d); Fig. 45.)

On injection of a much smaller dose of DA, 5.0 µg i.c.v., a dose which potentiated the anti-nociceptive activity of morphine in this test (Chapter Six, para. 2(d); Fig. 46) there was a significant potentiation ($P<0.05$) of

the anti-nociceptive activity of oxotremorine, 0.025 mg/kg s.c., at 20, 40 and 60 min after the oxotremorine injection. This effect was noted in some experiments when the dose of oxotremorine alone had been ineffective, whereupon the addition of DA, 5.0 µg i.c.v. (also ineffective alone) elevated the reaction time to about 7.0 s.

3. THE RAT FOOT-PRESSURE TEST

(a) Reserpine

Reserpine, 5.0 mg/kg i.p., 18 h before oxotremorine, 0.1 mg/kg s.c., produced a significant prolongation of the anti-nociceptive effect of the oxotremorine (Fig. 71). The anti-nociceptive activity was still significant 2 h after the injection of oxotremorine, at a time when that of the oxotremorine in the non-reserpinised rats was no longer apparent. This result is in direct contrast to that observed with morphine in reserpinised rats: Chapter Six, para. 3(a); Fig. 47.

(b) Noradrenaline

Noradrenaline, 20.0 µg i.c.v., produced a marked and highly significant ($P<0.001$) attenuation of the anti-nociceptive effect of oxotremorine, 0.1 mg/kg s.c., in the rat (Fig. 72). The anti-nociceptive effect of the oxotremorine did not become re-established at any time after the NA injection: the effect of the oxotremorine itself being very short-lived (see Chapter Three, para. 12 and discussion).

4. DISCUSSION

The antagonistic effect of reserpine against physostigmine and oxotremorine at a time when brain amine depletion was maximal has been previously observed in the mouse tail-flick test (Dewey *et al.*, 1970).

These authors also noted that a potentiation of oxotremorine (and, indeed, morphine) occurred at a time when reserpine was bringing about a release of brain amines (Everett *et al.*, 1957). Our results using the mouse tail-flick test show that the time-course of the reserpine effect is similar, though not identical, for both morphine and oxotremorine (for data on morphine/reserpine, see: Chapter Six, para. 2(a), and Fig. 41). This suggests that whichever of the brain amines is involved in the anti-nociceptive effect of morphine (in that its depletion abolishes morphine's activity), that amine is involved also in the anti-nociceptive effect of oxotremorine.

The injection of NA i.c.v. attenuated the anti-nociceptive effect of oxotremorine in the mouse tail-flick test, in a manner similar to that in which it reduced the activity of morphine in this test (Results: Chapter Six, para. 2(c); Figs. 43 & 44). Similarly, a relatively large dose of DA (50.0 µg) i.c.v. produced an attenuation of the anti-nociceptive activity of oxotremorine, which was remarkably similar to that seen with DA i.c.v. and morphine (Chapter Six, para. 2(d); Fig. 45). Also, a small dose of DA i.c.v. (5.0 µg) produced a significant potentiation of the anti-nociceptive activity of oxotremorine as determined by the tail-flick test. Therefore, the results reported in this chapter for the modification of the anti-nociceptive effect of oxotremorine by catecholamines in the mouse bear a striking resemblance to those reported in Chapter Six for the modification of the anti-nociceptive activity of morphine by catecholamines.

In the rat, however, using the foot-pressure test, some discrepancy has appeared between the results for morphine and those involving oxotremorine. Thus in the rat, reserpine failed to attenuate the anti-nociceptive activity of oxotremorine — in fact the action of oxotremorine was prolonged somewhat by reserpine pretreatment 18 h previously; at this time after reserpine, the

antagonism of the anti-nociceptive activity of morphine was maximal in the rat (Sparkes & Spencer, 1971). This result suggests that, in the rat, there may be a different mechanism involved in the anti-nociceptive activities of morphine and oxotremorine, at least as far as the involvement of amines in the CNS is concerned.

The prolongation of the activity of oxotremorine by reserpine in the rat may possibly be due to a reduced rate of metabolism of oxotremorine in the presence of reserpine. The normal rate of metabolism of oxotremorine in this species is high (Hammer *et al.*, 1968), and reserpine may interfere with its oxidative metabolism in the liver by means of its hypothermic effect. Indeed, Hammer *et al.* (1968) showed that, in the mouse, the rate of oxotremorine metabolism fell with increasing hypothermia, approaching zero as the hypothermia became maximal. However, this seems to offer only a partial explanation, since the degree of hypothermia produced by reserpine in the rat (about 1°C 18 h after 5.0 mg/kg reserpine i.p.) is considerably less marked than that produced in the mouse in the situation described by Hammer *et al.* (1968).

A further explanation may be afforded by the recent work of Karlén *et al.* (1971), who have shown that by blocking the peripherally-mediated vasodilatation produced by oxotremorine, less of the drug was able to penetrate to the brain. Conversely, therefore, since reserpine itself produces vasodilatation, it would be expected to have the opposite effect to that noted in the study of Karlén *et al.* (1971), and thereby *increase* the amount of oxotremorine in the brain. The observed effect in the present study may, therefore, be a result of a combination of the two suggested effects.

The possibility that altered adrenergic activity within the central

nervous system might be capable of influencing function in a cholinergic system has been supported by some recent evidence both in rats and in rabbits. In the rabbit, lowered brain amine levels (using reserpine) resulted in the blockade of the attenuation by physostigmine of the thalamo-cortical recruiting response (van Meter & Karczmar, 1971). In the rat, i.c.v. injections of NA were shown to have no effect on whole-brain choline acetylase activity on acute administration, except in the brain-stem. However, chronic injections of NA i.c.v. resulted in increased choline acetylase activity in all regions of the CNS (Ho *et al.*, 1971).

Thus it seems that aminergic systems in the brain are capable of influencing the activity of cholinergic systems. The results reported in this chapter would seem to support the possibility of a cholinergic system involved in anti-nociceptive activity. For the time being, the species difference observed must remain unexplained.

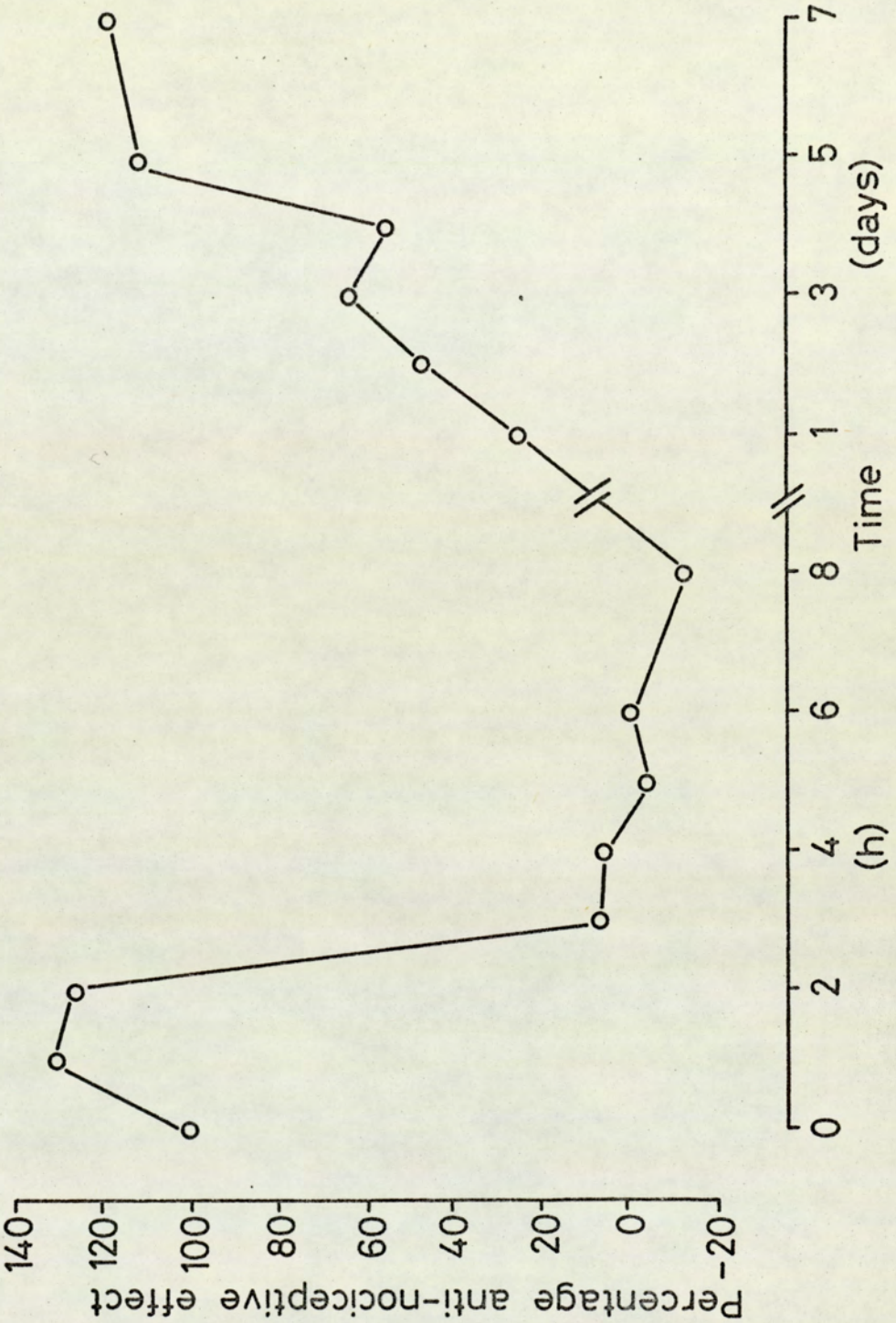

FIGURE 68: EFFECT OF RESERPINE ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received a single injection of reserpine, 2.0 mg/kg i.p., at 0, then oxotremorine, 0.05 mg/kg s.c., at the intervals stated on the graph after the reserpine injection. Each point represents the anti-nociceptive effect of the oxotremorine as a percentage of the non-reserpinised controls. Each point represents a different group of ten animals.

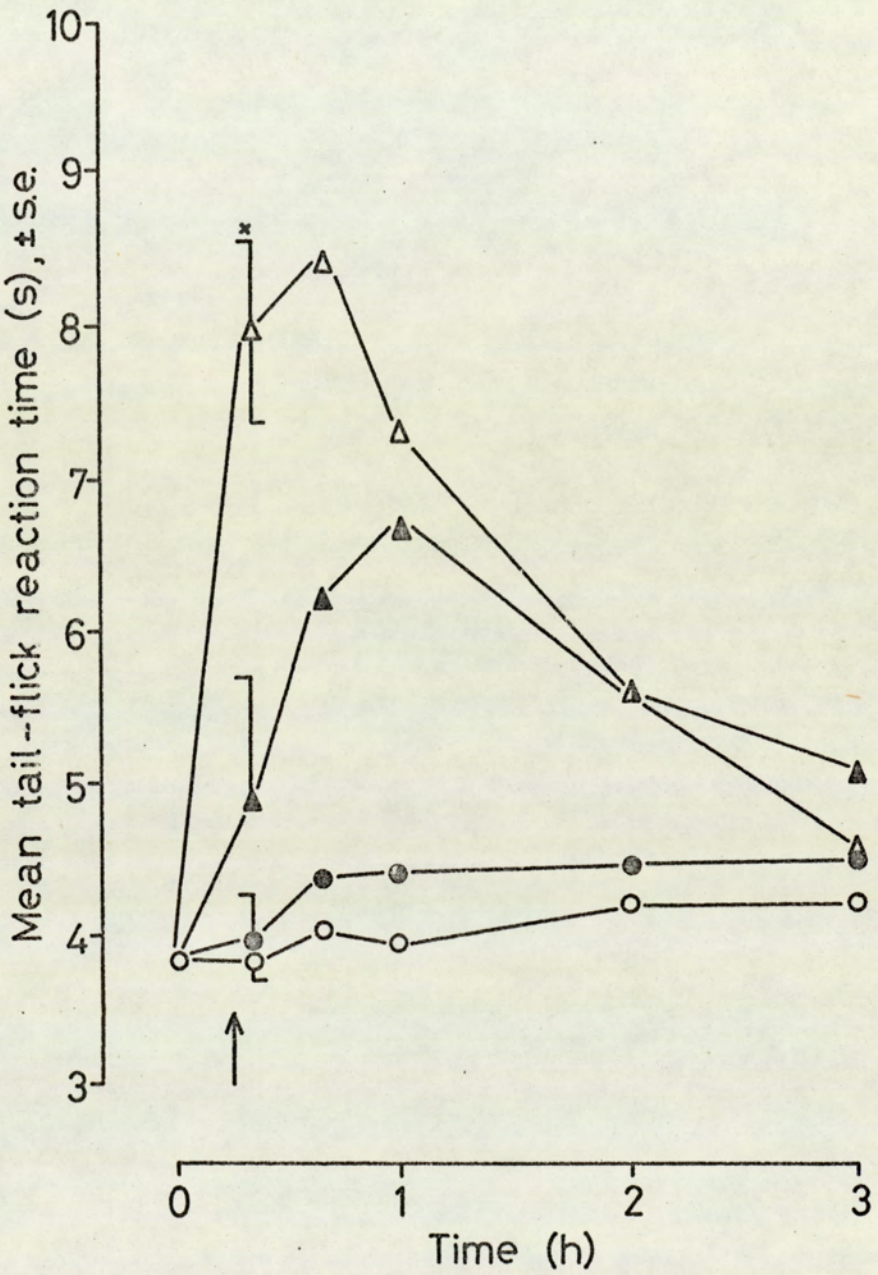

FIGURE 69: EFFECT OF NORADRENALINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

○—○: Saline, 10.0 ml/kg s.c., at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c., at 0 + NA, 5.0 µg i.c.v., at the arrow.

Δ—Δ: Oxotremorine, 0.05 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Oxotremorine, 0.05 mg/kg s.c., at 0 + NA, 5.0 µg i.c.v., at the arrow.

The arrow is at +15 min.

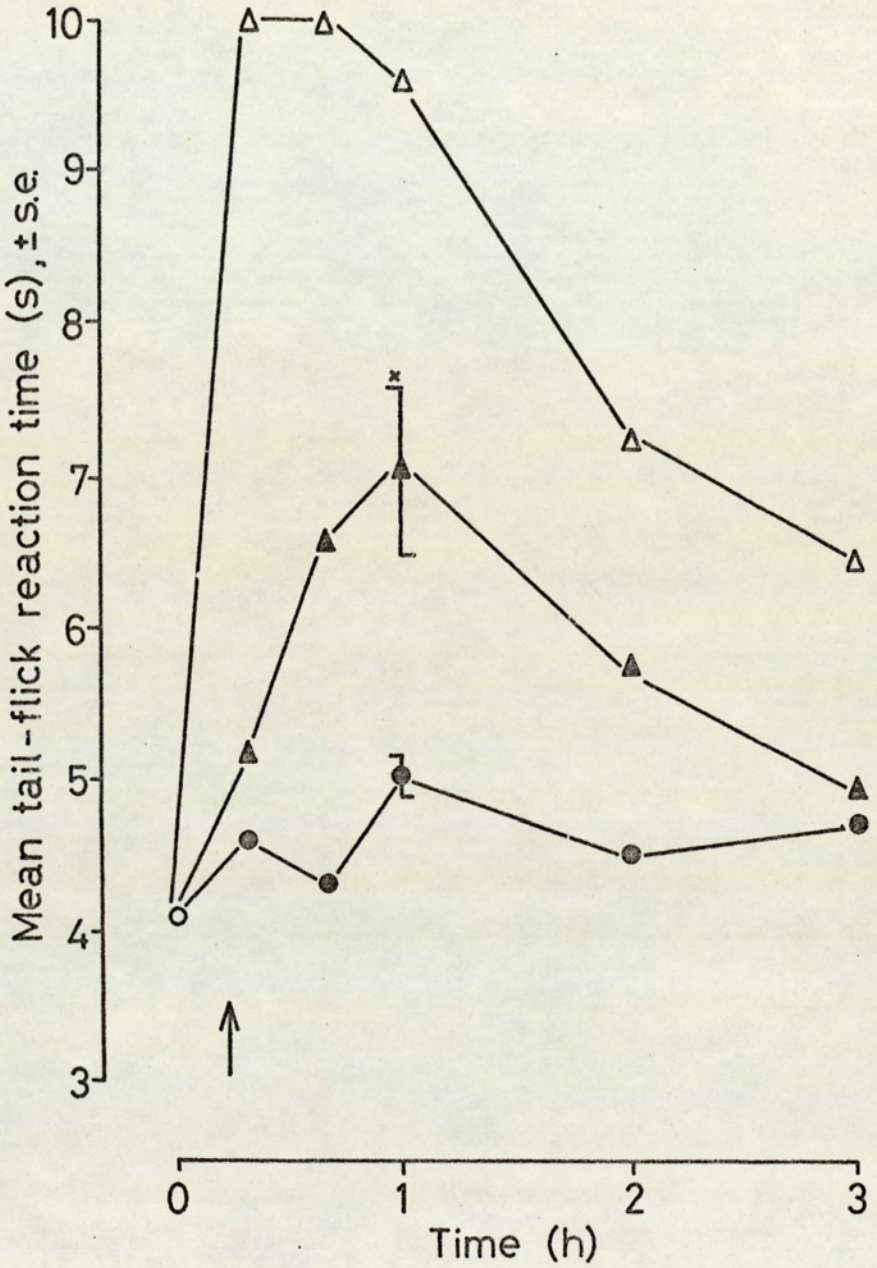

FIGURE 70: EFFECT OF DOPAMINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice were injected as follows:

●—●: Saline, 10.0 ml/kg s.c., at 0 + DA, 50.0 µg i.c.v., at the arrow.

Δ—Δ: Oxotremorine, 0.05 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Oxotremorine, 0.05 mg/kg s.c., at 0 + DA, 50.0 µg i.c.v., at the arrow.

The arrow is at +15 min.

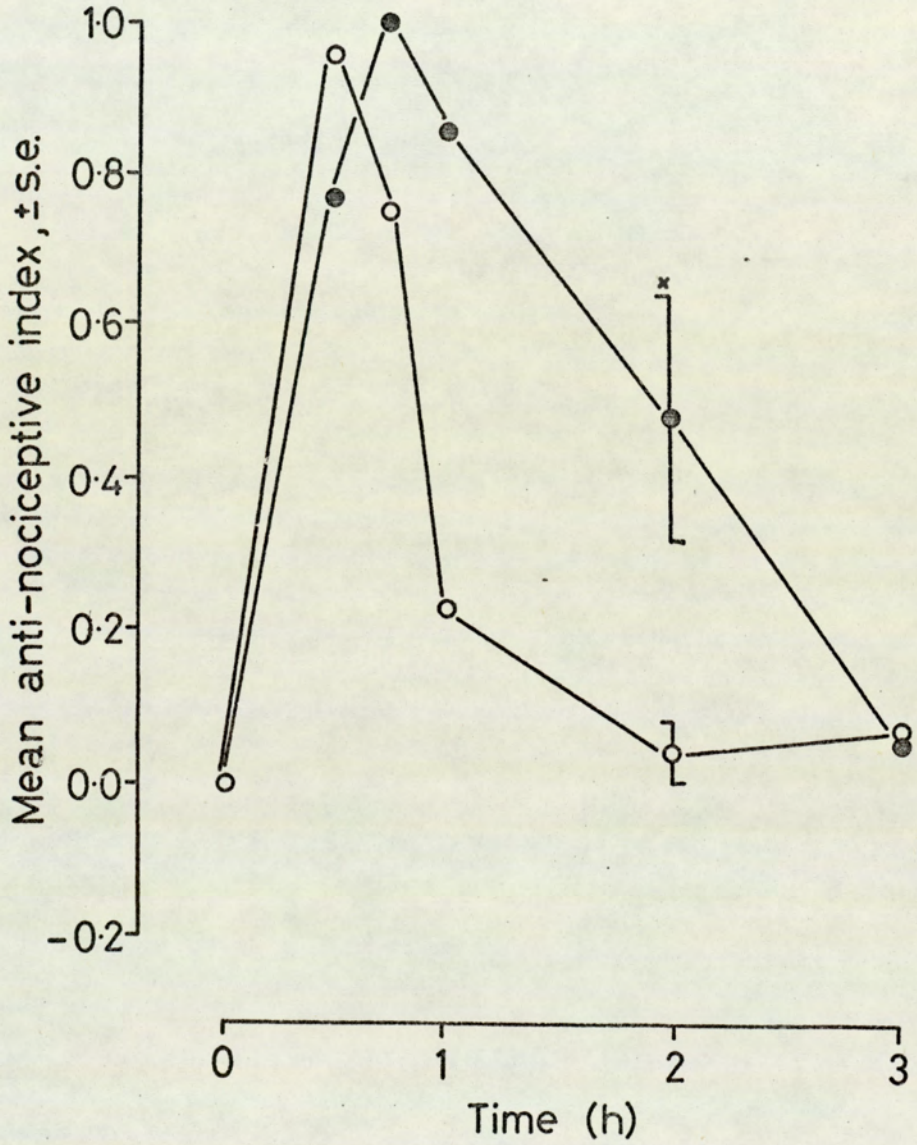

FIGURE 71: ANTI-NOCICEPTIVE EFFECT OF OXOTREMORINE AFTER RESERPINE IN THE RAT FOOT-PRESSURE TEST

Two groups of five rats were used:

O—O: received only oxotremorine, 0.1 mg/kg s.c., at 0.
●—●: received oxotremorine, 0.1 mg/kg s.c., 18 h after reserpine, 5.0 mg/kg i.p.

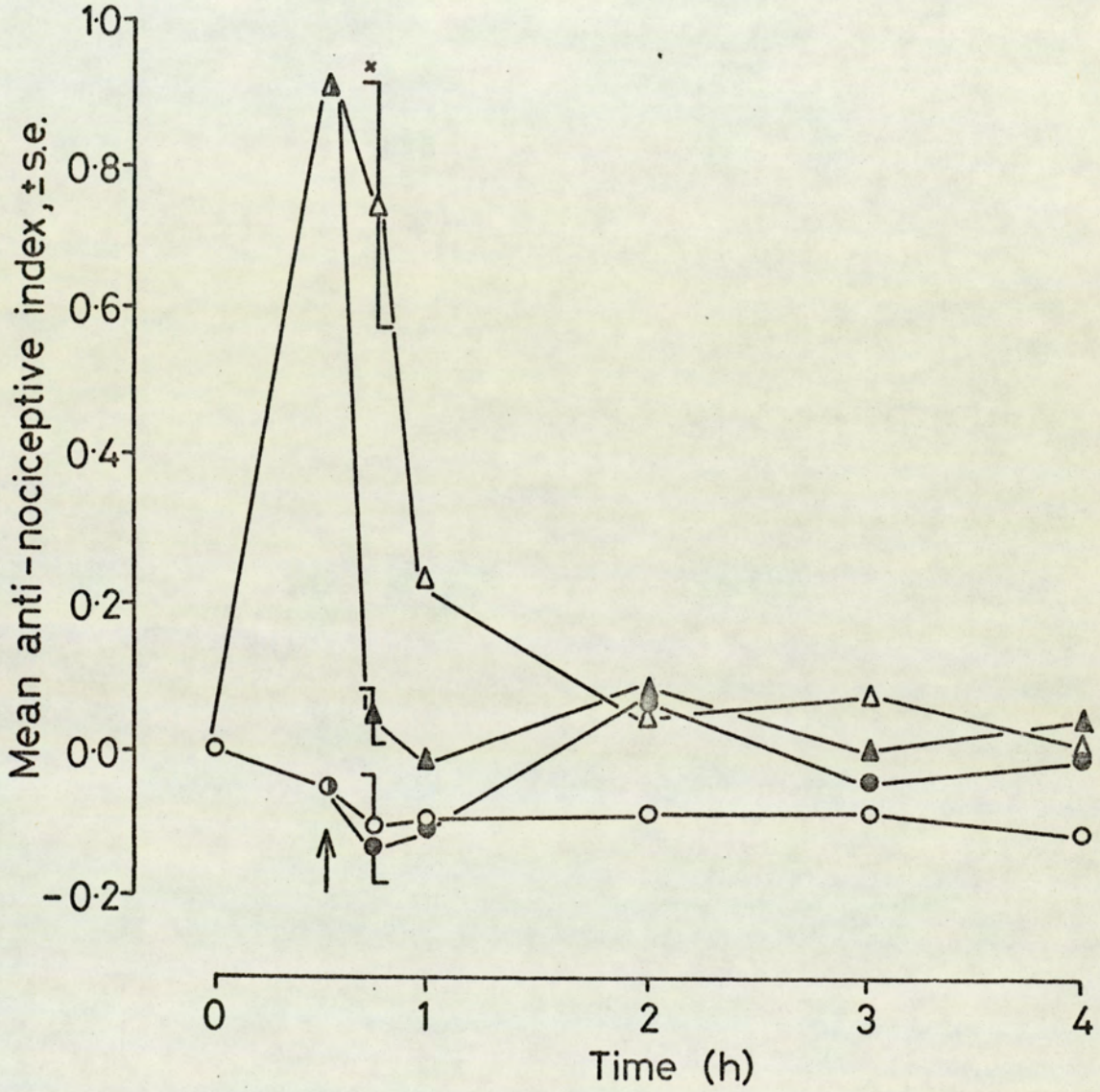

FIGURE 72: EFFECT OF NORADRENALINE INJECTED I.C.V. ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats received the following:

○—○: Saline, 1.0 ml/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

●—●: Saline, 1.0 ml/kg s.c., at 0 + NA, 20.0 µg i.c.v., at the arrow.

Δ—Δ: Oxotremorine, 0.1 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Oxotremorine, 0.1 mg/kg s.c., at 0 + NA, 20.0 µg i.c.v., at the arrow.

The arrow is at +30 min.

CHAPTER TWELVE

ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE AS MODIFIED BY 5-HT AND 5-HT ANTAGONISTS

1. INTRODUCTION

The experiments reported in this chapter were carried out to compare the interactions of oxotremorine and 5-HT with those of morphine and 5-HT.

2. THE MOUSE HOT-PLATE TEST

(a) 5-HT

5-HT, 10.0 µg i.c.v., injected 20 min after oxotremorine, 0.1 mg/kg s.c., did not alter the anti-nociceptive effect of oxotremorine in the hot-plate test. The test was carried out 30 min after the oxotremorine injection (*i.e.* 10 min after the 5-HT injection).

3. THE MOUSE TAIL-FLICK TEST

(a) Reserpine

As detailed in the previous chapter, reserpine, 2.0 mg/kg i.p., completely abolished the anti-nociceptive activity of oxotremorine, 0.05 mg/kg s.c. (Chapter Eleven, para. 2(a), and Fig. 68).

(b) 5-HT

5-HT, 10.0 µg i.c.v., enhanced the anti-nociceptive effect of oxotremorine, 0.025 mg/kg s.c., as determined by the tail-flick test. (Despite carrying out this experiment several times, in all cases the scatter of results within each group was sufficiently great to prevent the differences between groups being statistically significant.)

(c) 5-HT after reserpine

5-HT, 10.0 µg i.c.v., failed to restore the anti-nociceptive effect of oxotremorine, 0.1 mg/kg s.c., injected 18 h after reserpine, 2.0 mg/kg i.p., a dose of reserpine which completely abolished the anti-nociceptive activity

of oxotremorine (Chapter Eleven, para. 2(a), and Fig. 68).

(d) LSD-25

LSD-25, 0.05 mg/kg s.c., injected 20 min before oxotremorine, 0.05 mg/kg s.c., produced a partial (65%) but statistically significant reduction in the anti-nociceptive effect of the oxotremorine (Fig. 73). The LSD-25 alone was without effect on the nociceptive threshold as determined by the tail-flick test (*cf.* Chapter Three).

4. THE RAT FOOT-PRESSURE TEST

(a) Reserpine

As detailed in the preceding chapter, reserpine, 5.0 mg/kg i.p., produced a prolongation of the anti-nociceptive effect of oxotremorine, 0.1 mg/kg s.c. (Chapter Eleven, para. 3(a), and Fig. 71).

(b) 5-HT

5-HT, 20.0 µg i.c.v., injected 30 min after oxotremorine, 0.025 mg/kg s.c., produced a marked potentiation of the anti-nociceptive effect of the oxotremorine (Fig. 74). The mean anti-nociceptive index of the oxotremorine plus 5-HT group was almost maximal 15 min after the i.c.v. injection of 5-HT, whereas that of the group receiving oxotremorine plus saline was not significantly different from the saline plus saline control group at this time.

(c) 5-HT after reserpine

5-HT, 5.0 µg i.c.v., produced no significant alteration in the anti-nociceptive effect of oxotremorine, 0.1 mg/kg s.c., injected 18 h after reserpine, 5.0 mg/kg i.p.

(d) LSD-25

LSD-25, 1.0 µg i.c.v., injected 30 min after oxotremorine, 0.05 mg/kg s.c., failed to attenuate the anti-nociceptive activity of oxotremorine. This dose of LSD-25 had effectively antagonised the anti-nociceptive effect of morphine, 3.0 mg/kg s.c., when injected *via* the i.c.v. route (Chapter Seven, para. 4(d), and Fig. 54).

5. DISCUSSION

As reported in the previous chapter, reserpine pretreatment in the mouse abolishes the anti-nociceptive effect of oxotremorine at a time when amine depletion would be maximal. This effect could be due to depletion of NA, DA, 5-HT, or to an action on some other agent or system in the CNS. Since NA injected i.c.v. antagonises oxotremorine, then it seems unlikely that depletion of NA would result in the observed attenuation of oxotremorine by reserpine. Low doses of DA and 5-HT given *via* the i.c.v. route both potentiate the anti-nociceptive activity of oxotremorine, and therefore depletion of either of these amines could be responsible for the observed reduction of the oxotremorine activity. However, the activity of oxotremorine was not restored by the injection of 5-HT i.c.v. after reserpine treatment. We have also been unable to restore the activity of morphine with 5-HT injected i.c.v. after reserpine in the mouse, although this was possible in the rat (Results: Chapter Seven). The fact that oxotremorine was antagonised by pretreatment with LSD-25 in the mouse does suggest, however, that a central neurone system involving 5-HT as the transmitter substance is concerned in the production of the anti-nociceptive effect of oxotremorine in this species.

In the foot-pressure test, using the rat, the picture is a little

different from that reported in the mouse. Also, the results using oxotremorine in the rat differ somewhat from those using morphine in the rat (Chapter Seven). Thus, although i.c.v. 5-HT potentiates and NA attenuates the anti-nociceptive activity of oxotremorine in the rat, reserpine pretreatment does not abolish the anti-nociceptive effect. Also, LSD-25 injected i.c.v. does not antagonise the anti-nociceptive effect of oxotremorine. Thus in the rat, although increasing 5-HT activity in the central nervous system potentiates the activity of a cholinergic anti-nociceptive agent, lowering 5-HT activity in the CNS does not attenuate this apparently cholinergic effect. Thus there would appear to be a disparity between the rat and the mouse with regard to the anti-nociceptive activities of the cholinergic agent oxotremorine, and also in the rat a disparity between the activities of narcotic and cholinergic anti-nociceptive effects. In the mouse, using a number of tests of nociceptive sensitivity, we have been able to demonstrate several points of similarity between the anti-nociceptive properties of the narcotic and cholinergic agents.

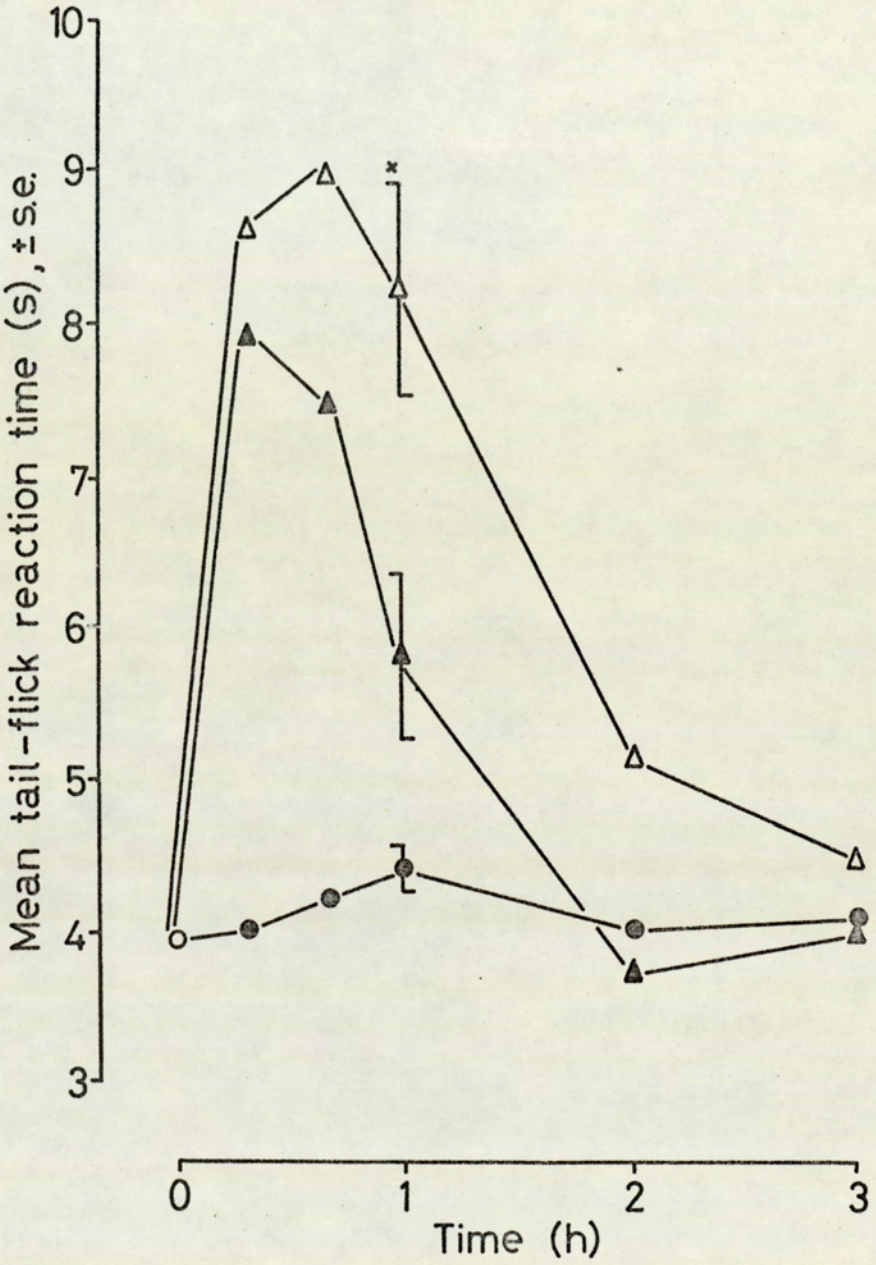

FIGURE 73: EFFECT OF LSD-25 ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE MOUSE TAIL-FLICK TEST

Groups of ten mice received the following:

●—●: LSD-25, 0.05 mg/kg s.c., at -20 min + saline, 10.0 ml/kg s.c., at 0.

Δ—Δ: Saline, 10.0 ml/kg s.c., at -20 min + oxotremorine, 0.05 mg/kg s.c., at 0.

▲—▲: LSD-25, 0.05 mg/kg s.c., at -20 min + oxotremorine, 0.05 mg/kg s.c., at 0.

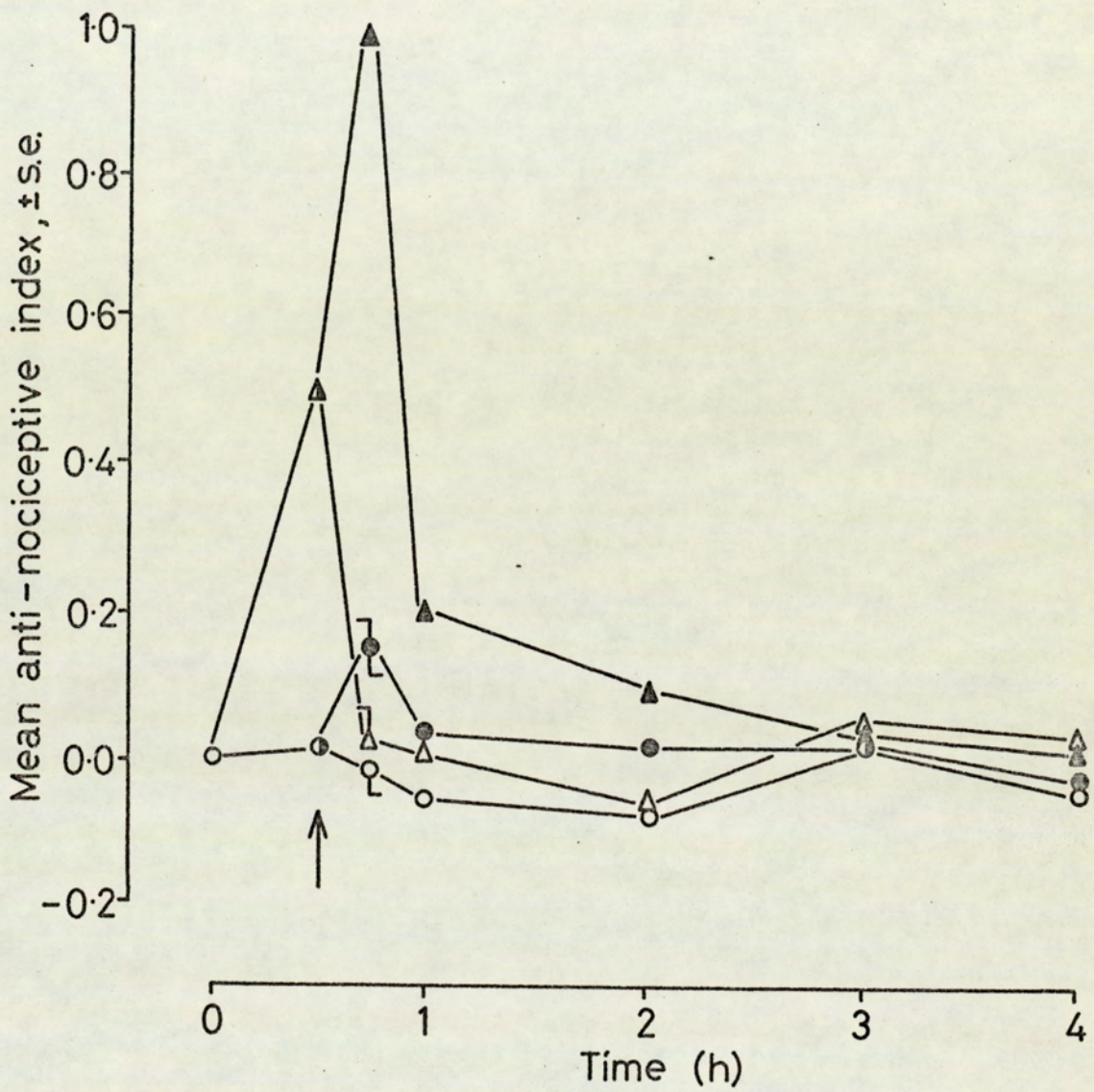

FIGURE 74: EFFECT OF I.C.V. 5-HT ON THE ANTI-NOCICEPTIVE ACTIVITY OF OXOTREMORINE IN THE RAT FOOT-PRESSURE TEST

Groups of five rats were injected as follows:

O—O: Saline, 1.0 ml/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

●—●: Saline, 1.0 ml/kg s.c., at 0 + 5-HT, 20.0 µg i.c.v., at the arrow.

Δ—Δ: Oxotremorine, 0.025 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Oxotremorine, 0.025 mg/kg s.c., at 0 + 5-HT, 20.0 µg i.c.v., at the arrow.

The arrow is at +30 min.

PART F

EXPERIMENTS ON BODY TEMPERATURE

CHAPTER THIRTEEN

THE MODIFICATION OF BODY TEMPERATURE BY ANTI-NOCICEPTIVE AGENTS AND ITS POSSIBLE RELEVANCE TO THEIR ANTI-NOCICEPTIVE EFFECTS

1. INTRODUCTION

In a number of animal species, morphine produced a rise in body temperature, the maximal effect occurring 3.5 to 4.5 h after giving the drug to mice (Helfrich, 1934). Since these early observations, many studies have been directed at elucidating the mechanism by which morphine alters the body temperature of animals. However, subsequent work had shown that the effect of morphine on rodents depends upon the dose employed, hypothermia occurring with larger doses, hyperthermia with smaller ones (Gunne, 1960; Herrmann, 1942), and some workers have reported a fall followed by a rise, the latter being accompanied by a period of increased locomotor activity (Sloan *et al.*, 1962) — possibly the rise detected by Helfrich (1934).

Subsequently, Lotti and co-workers have more fully investigated the hypothermia produced in rats following injection into the hypothalamus (Lotti, George & Lomax, 1965; Lotti, Lomax & George, 1965a). Further, they have demonstrated that this effect is blocked by nalorphine administration (Lotti, Lomax & George, 1965b, 1965c). Also, rats developed tolerance to this effect of morphine (Lotti, Lomax & George, 1966), an effect which was again blocked by concommitant administration of nalorphine (Lomax & Kirkpatrick, 1967). Hence a number of similarities are apparent between the hypothermic effect of morphine in rodents and the anti-nociceptive effects in the same animals (nalorphine has been shown to block the anti-nociceptive activity of morphine (Unna, 1943) and its concommitant injection prevents the appearance of tolerance to the anti-nociceptive effect of morphine (Orahovats *et al.*, 1953)).

With the demonstration of the presence of biogenic amines in the central nervous system (von Euler, 1946; Twarog & Page, 1953) and the ability of those amines to modify body temperature when injected into the cerebral

ventricles (Feldberg & Myers, 1963, 1964), attempts were made to relate the effects of morphine on body temperature to an interaction with these amines (see, for instance, Banerjee *et al.*, 1968). Similarly, the peripheral, i.c.v. or intracerebral injection of cholinergic agents produced a profound fall in body temperature (Cho *et al.*, 1964; Lomax & Jenden, 1966, Meeter, 1971), *i.e.* a change similar to that produced by morphine on body temperature under some circumstances (Sharkawi, 1972, has recently suggested that morphine hyperthermia occurs as a result of inhibition of ACh release in the CNS). These results have led to a number of theories of body temperature control, involving interactions between cholinergic and monoaminergic influences in the hypothalamus (Beckman & Carlisle, 1969; Feldberg & Myers, 1963, 1964; Lomax *et al.*, 1969; Myers & Yaksh, 1969) and it is suggested that morphine may alter body temperature by interfering with this hypothalamic regulating system (Lomax, 1967; Lotti, George & Lomax, 1965), for example by altering the hypothalamic set-point (of Hammel *et al.*, 1963).

The results reported by previous workers leads to two important questions: firstly, will a study of the effects of morphine and the narcotic-antagonist analgesics on body temperature yield further information about their possible interactions with putative central transmitter substances?; secondly, since many of the agents used in this study do alter body temperature, is it possible that some of the observed changes in nociceptive threshold are a result of these alterations of body temperature — particularly when the nociceptive stimulus has been a thermal one? Thus the experiments carried out and reported in this chapter were performed mainly to exclude the latter of the above possibilities and where possible to add to the information already obtained on the interactions between the anti-nociceptive agents used and the postulated CNS transmitter-substances.

2. EFFECT OF MORPHINE

(a) Injected subcutaneously

Morphine, 10.0 mg/kg s.c., produced a fall in mouse body temperature of between 2.0° and 4.0°C, the peak effect occurring 30 to 45 min after injection (Fig. 75). Thereafter, the temperature rose steadily until it was no longer significantly different from that of the control animals at 2 h after the injection. This compares with the time-course of anti-nociceptive effect in the mouse tail-flick test of a peak effect at 40-60 min and a termination of effect which varied between 2 and 3 h after injection (Results: Chapter Two).

(b) Injected intracerebroventricularly

Morphine, 5.0 µg i.c.v., produced a similar degree of hypothermia to that produced by 10.0 mg/kg morphine s.c. in the mouse (Fig. 76). However, the effect was maximal 15 min after injection, and temperature tended to remain depressed for about 90 min after the injection, after which time recovery occurred so rapidly that at 120 min after injection, the temperature of the morphine-treated animals was no longer significantly different from that of the saline-treated mice. It should be pointed out at this stage that an injection of saline i.c.v. tended itself to produce a hypothermia of about 1.0°C, measured 15 min after the injection, but this effect was always rapidly reversed during the following 15 to 30 min.

This effect of i.c.v. morphine compares with the time-course of its anti-nociceptive effect in the tail-flick test, in that the peak of anti-nociceptive activity was reached more rapidly after i.c.v. than after s.c. injection, and the effect also tended to be more prolonged after i.c.v. than after s.c. injection (Results: Chapter Two).

3. EFFECT OF NALORPHINE, ALONE AND WITH MORPHINE

In the experiment shown in Fig. 76, the effect of an i.c.v. injection of saline, 10.0 µl, is clearly shown, as is the initial rise in temperature of the group receiving saline s.c., which is presumably a result of disturbing the animals after taking the initial reading at time 0 before giving the injections.

Nalorphine, 2.0 µg i.c.v., produced a slightly greater fall in oesophageal temperature than did 10.0 µl saline i.c.v., but this was not statistically significant. However, the hypothermic effect of morphine was significantly antagonised by the injection of nalorphine i.c.v. ($P<0.05$) at 1 and 2 h after the morphine injection, from some 3.0°C below controls to about 1.0° to 1.5°C below controls.

4. EFFECT OF NORADRENALINE, ALONE AND WITH MORPHINE

Noradrenaline, 10.0 µg i.c.v., produced a slight rise in temperature, which was significant when compared with the fall produced by 10.0 µl saline i.c.v. (Fig. 77). Also, this dose of NA injected i.c.v. produced, initially, a marked attenuation of the morphine-induced hypothermia, which again was a statistically significant effect ($P<0.05$). However, the effect of the NA rapidly disappeared, such that the group receiving NA alone attained a temperature similar to that of the saline-treated group, whereas that of the morphine plus NA group fell so markedly that at 2 h after the morphine injection, a degree of hypothermia was present considerably in excess of that seen in the morphine-only group at its peak. Thus, despite the fact that i.c.v. NA initially antagonised the morphine hypothermia, an apparent potentiation of morphine's effect then followed (Fig. 77). Having reached this peak level of hypothermia, the temperature recovered to the control level within a further 2 hours.

5. EFFECT OF DOPAMINE, ALONE AND WITH MORPHINE

Dopamine, 50.0 µg i.c.v., produced a significant degree of hypothermia in the mouse (Fig. 78), the temperature of the animals receiving DA i.c.v. being 2.5°C below that of the animals receiving saline i.c.v., at the time of peak effect — 20 min after injection. Dopamine injected in this way produced an increase in the hypothermic effect of morphine (injected s.c.), which was statistically significant at 45 min after the morphine injection — *i.e.* at a time when the DA hypothermia itself was maximal.

6. EFFECT OF 5-HT, ALONE AND WITH MORPHINE

5-HT, 20.0 µg i.c.v., did not produce any change in the body temperature of mice which was statistically significantly different from that produced by saline, 10.0 µl i.c.v. Also, there was no alteration by 5-HT injected i.c.v. of the hypothermia induced by morphine.

7. EFFECT OF OXOTREMORINE

(a) Injected subcutaneously

The hypothermia produced by oxotremorine in mice is demonstrated in Fig. 79, where 0.1 mg/kg produced a fall in temperature of 6.0°C, the peak effect being 45 to 60 min after injection. This compares with a peak anti-nociceptive effect occurring at about 40 min after s.c. injection in the tail-flick test (Results: Chapter Three). The hypothermia was no longer significant at 3 h after injection.

(b) Injected intracerebroventricularly

Oxotremorine, 0.1 µg i.c.v., produced a fall in the body temperature of mice of about 3.0°C, which was significantly greater than the (transient) fall produced by saline, 10.0 µl i.c.v. The peak hypothermic effect

occurred 20 min after the i.c.v. injection, temperatures re-attaining control levels 120 min after the injection.

These results compare with a peak anti-nociceptive effect occurring in the tail-flick test at 20 min after injection of 0.05 µg oxotremorine i.c.v. The anti-nociceptive effect was no longer detectable at 120 min after the injection (Results: Chapter Three).

8. EFFECT OF NALORPHINE ON OXOTREMORINE HYPOTHERMIA

The injection of nalorphine, 2.0 µg i.c.v., did not significantly modify the hypothermia induced by the injection of oxotremorine, 0.1 mg/kg s.c. (Fig. 79).

9. EFFECT OF DOPAMINE ON OXOTREMORINE HYPOTHERMIA

The hypothermic effect of DA, 50.0 µg i.c.v., was additive with that of oxotremorine, 0.1 mg/kg s.c., as it was with that of morphine (this chapter, para. 5). A significantly greater degree of hypothermia was observed with the combination than with either of the two agents alone.

10. EFFECT OF RESERPINE

The hypothermic effect of reserpine, 2.0 mg/kg i.p., is shown in Fig. 80. The figure also includes the data from Fig. 41, (Results: Chapter Six), to show the time-course of the effect of reserpine on the anti-nociceptive activity of morphine in the mouse tail-flick test. It will be noted that recovery of body temperature takes place rather more rapidly than recovery of the anti-nociceptive effect of morphine.

11. DISCUSSION

The results reported in this chapter show that morphine is capable of

producing a fall in the body temperature of the mouse, both when injected peripherally (s.c.) and centrally (i.c.v.). Winter and Flataker (1953) were able to show that, in the dog, a fall in skin temperature produced by the injection of morphine was responsible for much of the noted rise in threshold to a thermal stimulus. Subsequently, Cox and Weinstock (1964), using mice on the hot-plate, demonstrated a linear relationship between skin temperature and reaction-time — with a fall in skin temperature the reaction-time became larger. This led to the possibility that part of the increase in reaction-time produced by morphine in nociceptive tests depending on a thermal stimulus, as used in the present study, might be a result of changes induced in body and/or skin temperature.

Lotti, Lomax and George (1965) suggested that, in the rat, the fall in body temperature produced by morphine was a result of depression of the hypothalamic set-point of Hammel *et al.* (1963). This would result in the initiation of heat loss mechanisms, which would initially cause a rise in skin temperature as the "excess" heat was dissipated (Hardy, 1961). Handley (1970) has shown that such a rise in skin temperature does occur initially before the oesophageal temperature falls, but this is short-lived, rapidly leading to a fall in skin temperature also. Thus it seems unlikely that the anti-nociceptive effect of morphine as determined by the tail-flick test is modified to any great extent by the production of hypothermia. (Our data from the hot-plate test do not include time-course studies for reasons given in Methods, para. 9(b).)

Further evidence against a significant effect of body temperature changes on the nociceptive threshold as determined by the tail-flick test is provided by the experiments with reserpine, which drug produced a profound degree of hypothermia and yet antagonised the anti-nociceptive activity of

morphine. Also, the reserpine treatment resulted in an increased sensitivity to the noxious stimulus in animals which did not receive morphine. The results using NA and DA also suggest a lack of involvement of temperature effects in the changes of nociceptive threshold. Intracerebroventricular NA resulted in an attenuation of the anti-nociceptive effect of morphine in the tail-flick test (Results: Chapter Six) and indeed it initially attenuated the hypothermic effect of morphine (Results: this chapter). However, i.c.v. DA, which again had attenuated the anti-nociceptive effect of morphine at this dose (50.0 µg i.c.v.: Results, Chapter Six) in fact had an additive hypothermic effect with morphine (Results: this chapter). Similarly, i.c.v. DA antagonised the anti-nociceptive activity of oxotremorine (Results: Chapter Eleven) whereas it produced an additive hypothermic effect with oxotremorine (Results: this chapter).

The reason for the fall in body temperature noted following the injection of saline i.c.v. is presumably related to the effects reported in Chapter One of the Results, concerning the effects of i.c.v. injections in the phenylquinone test and on behaviour generally in mice. The conclusion reached was that since the effects on general behaviour seemed related to the volume of solution injected, the effects were probably due to the increase in csf pressure following the introduction of the injection fluid into the ventricles. The fall in body temperature was typically short-lived (Fig. 76), and has been noted previously in mice (Brittain & Handley, 1967).

A difference was noted in the time-course of morphine's hypothermic effect depending on the route of injection employed. Thus the hypothermia following i.c.v. injection tended to be more rapid in onset and of longer duration than that following s.c. injection. These effects parallel those

noted for the anti-nociceptive effect of morphine (in the tail-flick test) when given *via* the two routes (Results: Chapter Two). This suggests a site of action more readily accessible from the csf rather than from the plasma, and also suggests a site of metabolism less readily accessible from the csf than from the plasma. Presumably, transport in either direction between plasma and csf involves the passage of the drug across the choroid plexus (Craig *et al.*, 1971; Miller & Elliott, 1955; Wang & Takemori, 1972).

Similar comments may be made with respect to oxotremorine injected *via* the different routes, the hypothermia reaching a peak earlier following i.c.v. injection than following s.c. injection. The recovery times may not strictly be compared in the case of oxotremorine with the particular doses used, because the hypothermic effect seen following s.c. injection was some 6.0°C, whereas that following i.c.v. injection was 3.0°C. Presumably the time taken to restore normal body temperature is dependent upon the rate of heat production and the depth of hypothermia present when the hypothermogenic effect of the drug is removed.

A point of great interest is that morphine produces a hypothermic response in some species (*e.g.* the mouse: this study; the rat: Sloan *et al.*, 1962; the dog: Fichtel *et al.*, 1969, but after an initial rise) whilst in others a rise in temperature is seen, depending on the dose used (*e.g.* the rabbit: Banerjee, Burks, Feldberg & Goodrich, 1968; the cat: Banerjee, Feldberg & Lotti, 1968). Also, Paolino and Bernard (1968) have shown that, in the rat, a rise in body temperature may be elicited at high ambient temperatures by a dose of morphine which would produce a fall at normal room temperature. This argues against a lowering of the set-point (Lotti, Lomax & George, 1965) and suggests rather a disruption of temperature control. This is particularly interesting since tremorine

(the precursor of oxotremorine: Cho *et al.*, 1961) has also been shown to raise body temperature of rats at high ambient temperatures (Cox & Potkonjak, 1966). Thus, in the rat, there is noted a further similarity between morphine and oxotremorine.

Our results using nalorphine (nalorphine was used in these experiments because naloxone was not available at the time they were carried out) have confirmed those of Lotti, Lomax and George (1965b, 1965c), in that morphine-induced hypothermia is reversed by nalorphine. Also, we have supported our own results with respect to oxotremorine, in that naloxone was unable to antagonise the anti-nociceptive effect (Results: Chapter Ten), and nalorphine was unable to reverse the hypothermic effect of this compound.

The results reported in this chapter support the view that the hypothermic effect of morphine in rodents is brought about by the action of morphine upon a specific receptor, which is presumably similar to that which is responsible for the anti-nociceptive effect of morphine. Also, the similarities to morphine of the cholinergic agent oxotremorine are again apparent. Further, our results suggest that the hypothermia produced by morphine and by oxotremorine is not directly responsible for the anti-nociceptive actions of these agents as determined by a test which uses a thermal noxious stimulus (the tail-flick test).

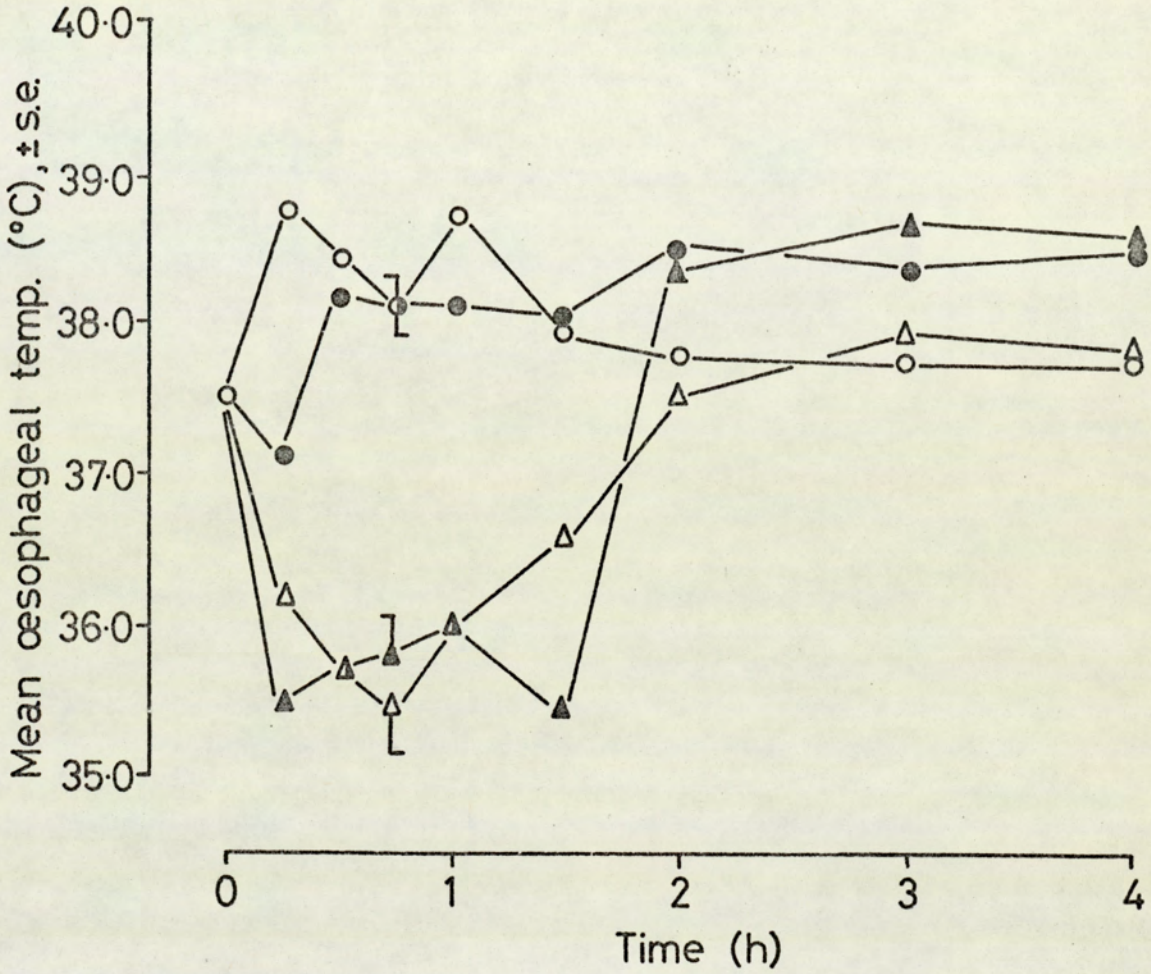

FIGURE 75: EFFECT OF MORPHINE ON THE OESOPHAGEAL TEMPERATURE OF MICE

Groups of eight mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0.
●—●: Saline, 10.0 µl i.c.v at 0.
Δ—Δ: Morphine, 10.0 mg/kg s.c. at 0.
▲—▲: Morphine, 5.0 µg i.c.v. at 0.

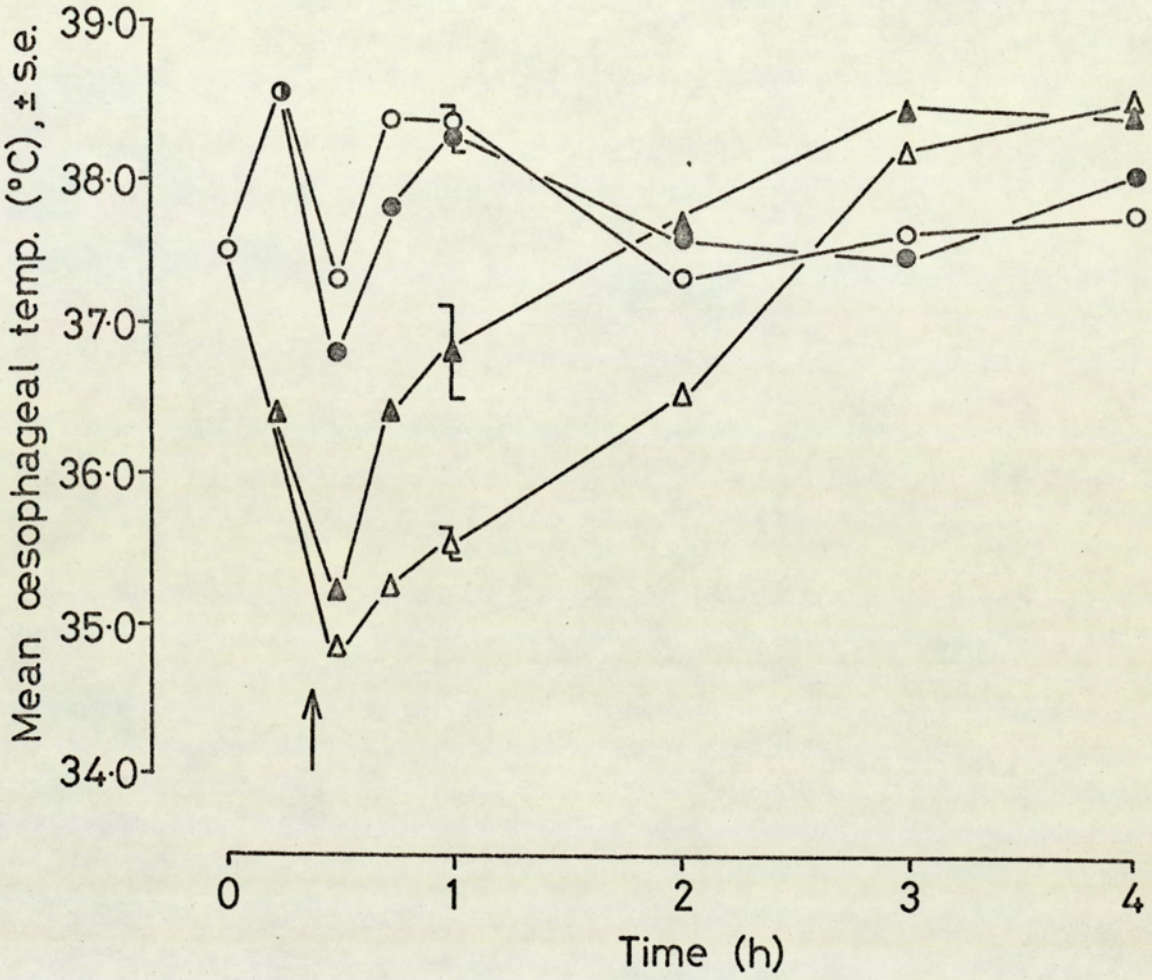

FIGURE 76: EFFECT OF NALORPHINE INJECTED I.C.V. ON THE HYPOTHERMIA INDUCED BY MORPHINE IN MICE

Groups of five mice received the following:

O—O: Saline, 10.0 ml/kg s.c., at 0 + saline 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c., at 0 + nalorphine, 2.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 10.0 mg/kg s.c., at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 10.0 mg/kg s.c., at 0 + nalorphine, 2.0 µg i.c.v. at the arrow.

The arrow is at +25 min.

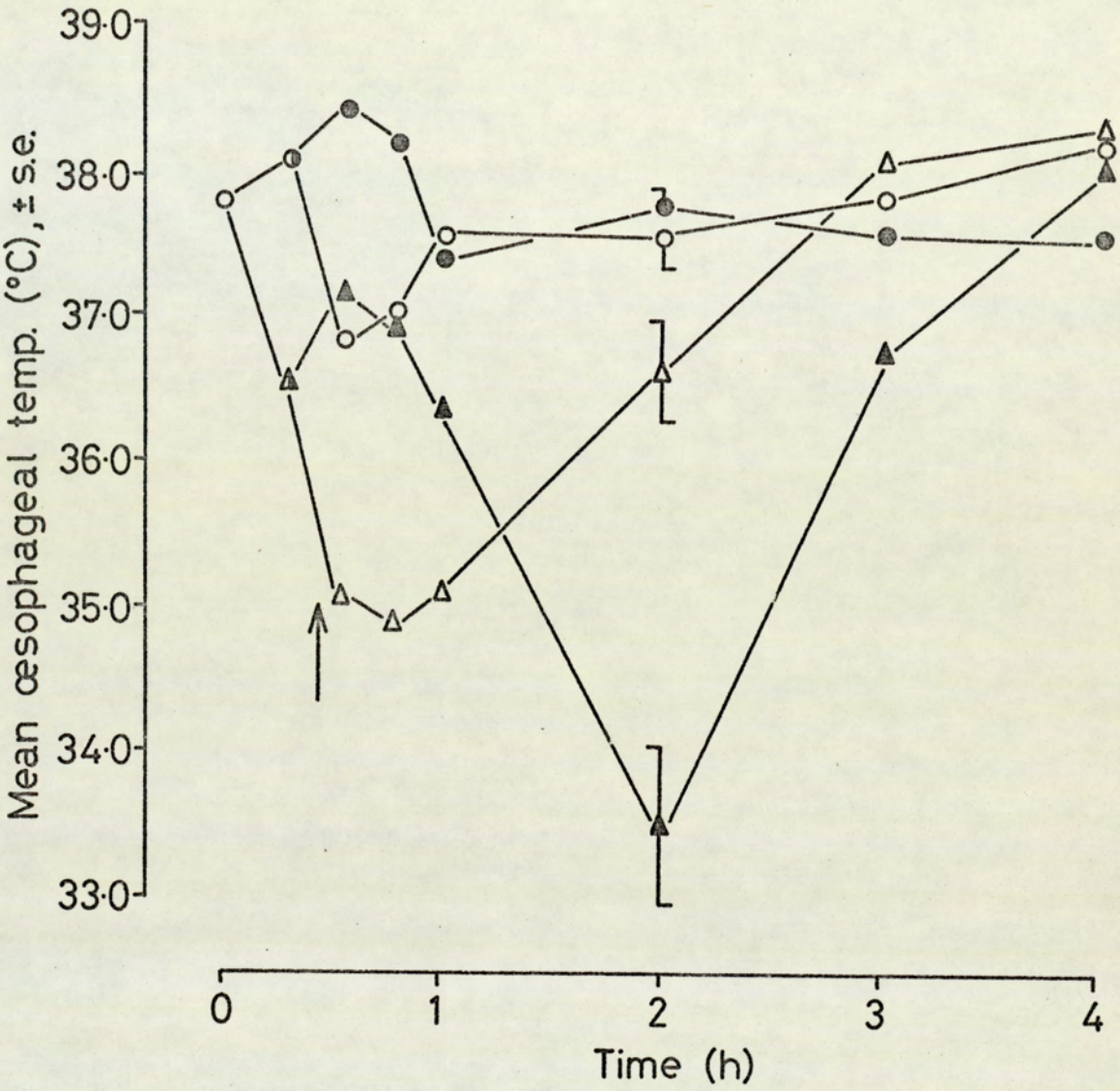

FIGURE 77: EFFECT OF NA INJECTED I.C.V. ON THE HYPOTHERMIA INDUCED BY MORPHINE IN MICE

Groups of five mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v., at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + NA, 10.0 µg i.c.v., at the arrow.

Δ—Δ: Morphine, 10.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Morphine, 10.0 mg/kg s.c. at 0 + NA, 10.0 µg i.c.v., at the arrow.

The arrow is at +25 min.

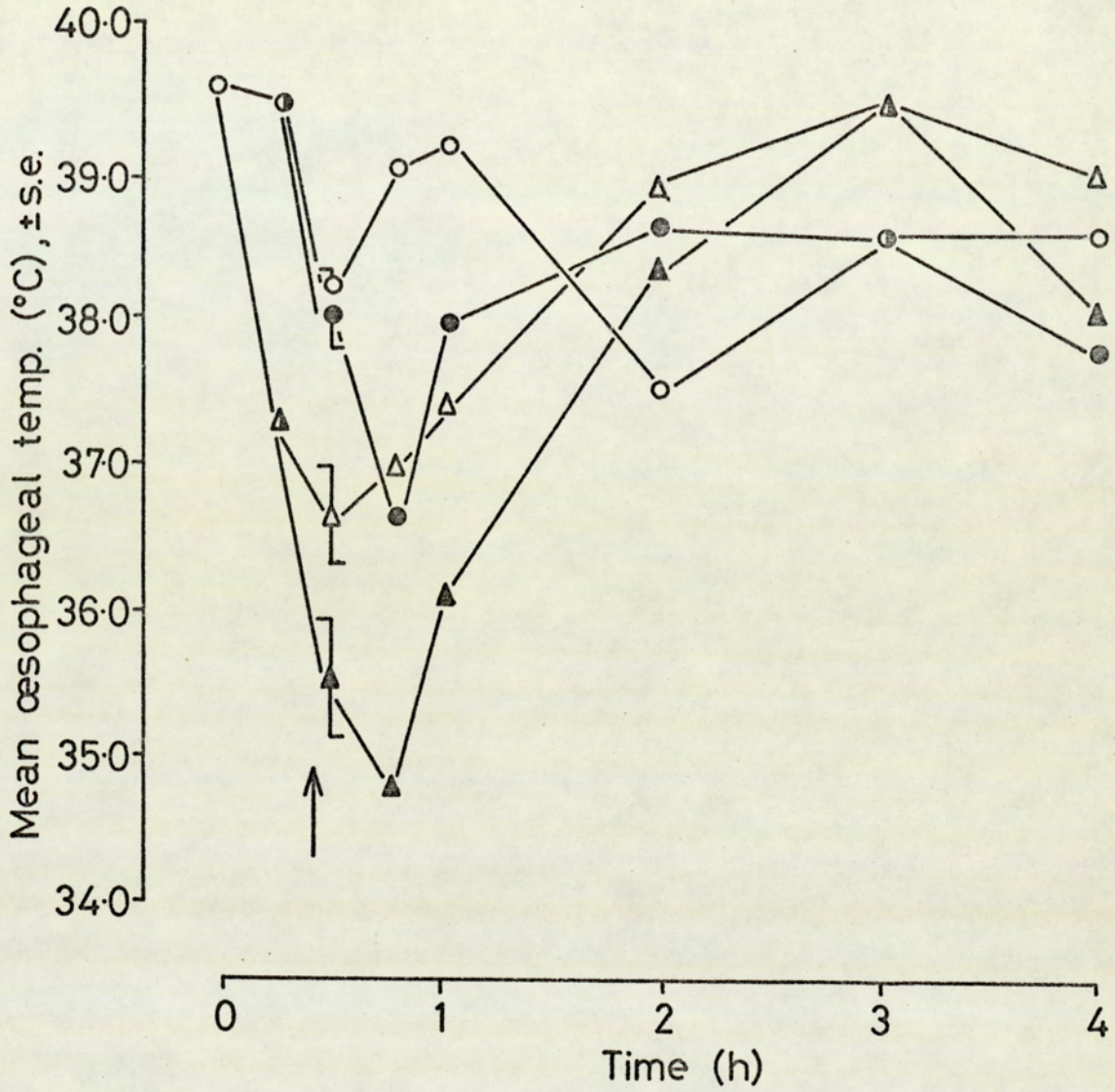

FIGURE 78: EFFECT OF DA INJECTED I.C.V. ON THE HYPOTHERMIA INDUCED BY MORPHINE IN MICE

Groups of eight mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + DA, 50.0 µg i.c.v. at the arrow.

Δ—Δ: Morphine, 10.0 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v. at the arrow.

▲—▲: Morphine, 10.0 mg/kg s.c. at 0 + DA, 50.0 µg i.c.v. at the arrow.

The arrow is at +25 min.

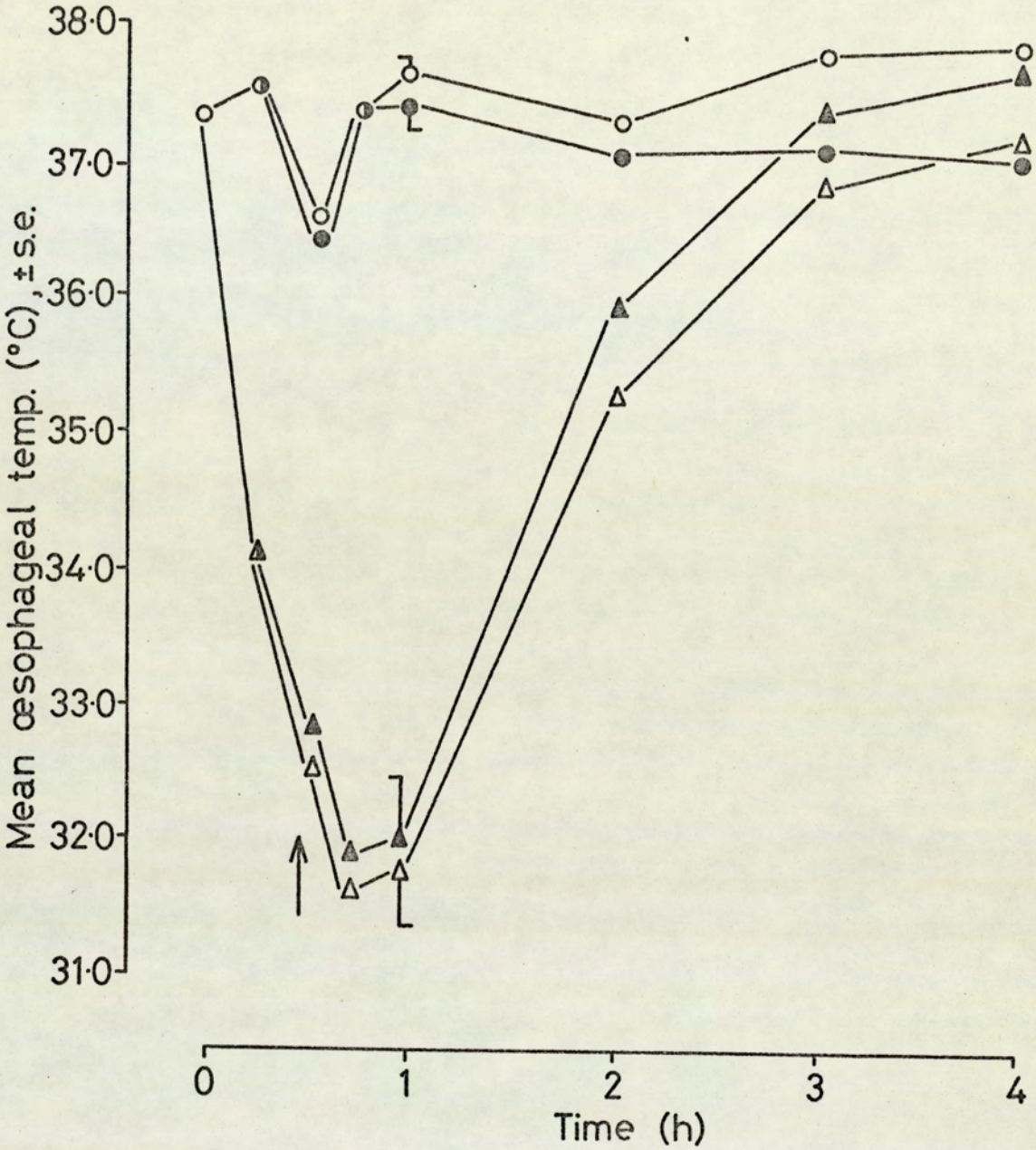

FIGURE 79: EFFECT OF NALORPHINE INJECTED I.C.V. ON THE HYPOTHERMIA INDUCED BY OXOTREMORINE IN MICE

Groups of ten mice received the following:

O—O: Saline, 10.0 ml/kg s.c. at 0 + saline 10.0 µl i.c.v., at the arrow.

●—●: Saline, 10.0 ml/kg s.c. at 0 + nalorphine, 2.0 µg i.c.v., at the arrow.

Δ—Δ: Oxotremorine, 0.1 mg/kg s.c. at 0 + saline, 10.0 µl i.c.v., at the arrow.

▲—▲: Oxotremorine, 0.1 mg/kg s.c. at 0 + nalorphine, 2.0 µg i.c.v., at the arrow.

The arrow is at +25 min.

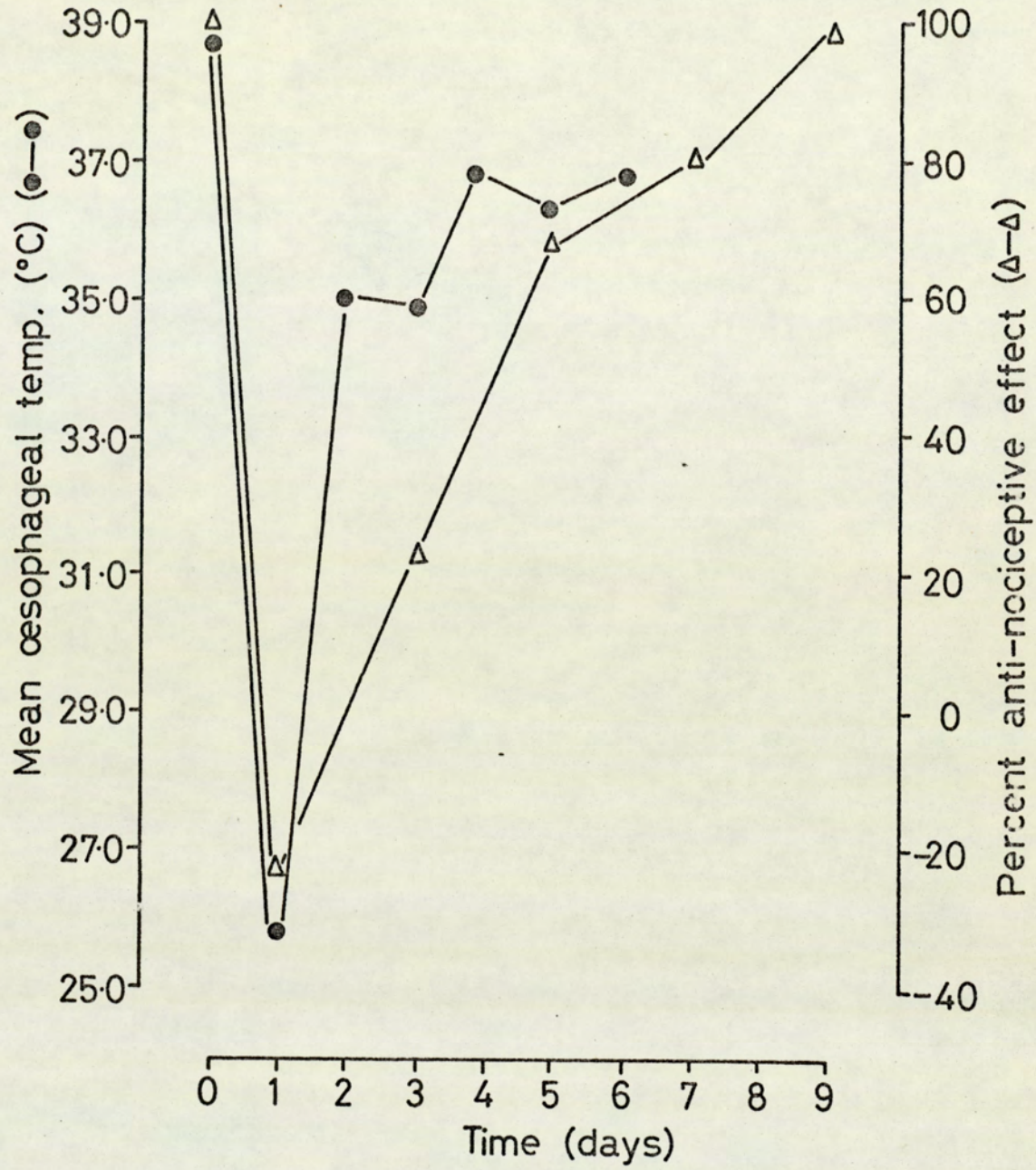

FIGURE 80: TO SHOW RECOVERY OF BODY TEMPERATURE AND RECOVERY OF THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE IN MICE FOLLOWING A SINGLE DOSE OF RESERPINE

Groups of ten mice received a single injection of reserpine, 2.0 mg/kg i.p. at 0, and their body temperature was determined at intervals thereafter. (Data on morphine from Fig. 41, Results: Chapter Six.)

SECTION FOUR

GENERAL DISCUSSION

GENERAL DISCUSSION

1. REASONS FOR CHOICE OF EXPERIMENTAL TECHNIQUES

(a) Use of the i.c.v. injection technique

The existence of a so-called 'blood-brain barrier' is well-documented; however, the structures (or properties of those structures) responsible for the observed phenomena have yet to be fully elucidated (*c.f.* reviews by Dobbing, 1961; Levin & Scicli, 1969). With particular relevance to the present study, it has been shown that monoamines injected into the systemic circulation do not penetrate the brain substance (Bertler *et al.*, 1966; Bertler, Falck & Rosengren, 1963; Weil-Malherbe *et al.*, 1961), and indeed that a barrier mechanism exists within the capillary walls of the cerebral circulation which impedes the passage of the amino-acid precursors of these monoamines (Bertler *et al.*, 1966).

Thus the i.c.v. injection technique has been employed in order to circumvent such barrier phenomena, and also to achieve some localisation of effect of the agents thus administered. Hence it has been shown that monoamines injected *via* this route tend to be located in neuronal tissue which is close to the ventricular spaces, although the pattern of uptake does not necessarily follow the distribution of the endogenous monoamines (Aghajanian & Bloom, 1967a, 1967b; Chalmers & Wurtman, 1971; Fuxe & Ungerstedt, 1966, 1967, 1968a, 1968b). However, as mentioned earlier in this thesis, it seems likely that many of the effects seen following the injection of agents i.c.v. were the result of an effect on post-synaptic sites directly accessible to the injected agent rather than an effect secondary to neuronal uptake of that agent (Chapter Six).

Many of the other agents used in this study have been injected using the i.c.v. route, despite the fact that they gain access to the brain easily from the blood. In these cases the method has been used purely

in an attempt to localise their effect to the CNS. Many studies of this nature have demonstrated that the site of action (or, one of the sites of action) of morphine in bringing about its anti-nociceptive effect is close to the ventricular spaces (Albus *et al.*, 1970; Herz *et al.*, 1970; Metyš *et al.*, 1969a, 1969b; Tsou & Jang, 1964). This demonstration is supported by the work of Cube *et al.* (1970), who related the effectiveness of morphine and other narcotic analgesics to their lipid solubilities, showing morphine to be poorly lipid-soluble. This correlates with the work of Lomax (1966) who showed that morphine penetrated only a small distance into the surrounding brain tissue following intracerebral microinjection.

The results reported in this thesis using the i.c.v. injection technique are consistent with the view that the effects of agents seen following their injection *via* this route were due to an action on the CNS rather than to any direct peripheral action.

No attempt has been made in this study to further localise the area to which injected agents have been applied; instead, attention has been paid more to the interactions between the agents used. The main reason for this was that it was considered sufficient at this stage to attempt to implicate a particular putative transmitter (or transmitters) in the anti-nociceptive action of morphine and the narcotic-antagonist analgesics, without attempting to define the locus of action of the transmitter. Further studies employing microinjection into specific areas of the brain substance could now be usefully carried out, especially following the mapping of the monoaminergic neurone systems in the brain (Ungerstedt, 1971), such that we might conclude in what areas of the CNS the putative transmitter might be released in the normal animal.

(b) Choice of nociceptive testing methods

A careful study of the literature on nociceptive testing methods has suggested that possibly one of the most useful techniques is the electric shock test of Carroll and Lim (1960), in that three different thresholds are detectable, each of which is denoted by a different reaction to the noxious stimulus. The reactions are integrated at various levels of the CNS, and Herold and Cahn (1968) have suggested that 5-HT is involved in the vocalisation after-discharge reaction, which is integrated at the higher centres. However, there are many problems involved in the use of electrical testing methods, notably concerning reproduceability of results, because of changes in electrode resistance (*c.f.* Beecher, 1957). Also, because one is attempting to define a threshold, it is necessary to carry out a number of trials, increasing the stimulus strength between each. This is, of necessity, time-consuming.

Because of these criticisms, the simpler methods of nociceptive testing were examined. The tail-clip test (Bianchi & Franceschini, 1954) is rapid in use, and gives reasonably reproduceable results when used as a quantal test. The response elicited by the stimulus is a complex, co-ordinated movement, requiring the integrity of the higher centres (Jacob, 1966). However, this last factor does mean that a drug interfering with muscle tone or motor co-ordination would reduce the ability of the animal to carry out the necessary movements, and hence could produce a false anti-nociceptive result. Collier (1964) has compared the anti-nociceptive ED_{50}'s in the tail-clip test with those in a rotating drum test for locomotor co-ordination, and only one known analgesic (in man), propoxyphene, had an anti-nociceptive/disco-ordination ratio of less than 2.0. Other apparently-anti-nociceptive agents (*e.g.* mephenesin, phenobarbitone) had ratios of 1.0 or less, indicating that their 'anti-nociceptive' activity was a result

of their disturbance of co-ordinated motor activity.

The hot-plate test is reasonably rapid in use, gives reproduceable results, and once again, the end-point requires a highly co-ordinated movement, whether it is licking of the fore- or hind-paws, or jumping out of the restraining cylinder (Janssen & Jageneau, 1957). Again, however, it is open to criticism because of this latter property. Thus agents interfering with locomotor activity can produce apparent anti-nociceptive effects (*e.g.* chlorpromazine: Weinstock, 1961; reserpine: this study, and others). Also any agent producing excitation renders judgement of the end-point impossible (Nott, 1968; this study). A considerable degree of learning seems to be acquired by animals which are exposed to the hot-plate more than once (Jacob, 1966), and Mitchell and co-workers have implicated a drug-test interaction in the development of apparent tolerance to narcotic analgesics when using this test situation in the rat (Adams *et al.*, 1969; Gebhardt & Mitchell, 1971; Gebhardt *et al.*, 1971; Kayan & Mitchell, 1969; Kayan *et al.*, 1969, 1971; Mitchell *et al.*, 1968). Thus when used in the present study, each animal was exposed only once to the hot-plate environment.

The tail-flick test, in the mouse, involves a simple spinal reflex as the end-point (Dewey *et al.*, 1969b) and therefore agents depressing co-ordinated locomotor activity would presumably not produce an anti-nociceptive effect in this test. In other species, it has been shown that the narcotic analgesics are active in depressing the reflex in spinal animals only at higher doses than those required in intact animals (Cook & Bonnycastle, 1951; Irwin *et al.*, 1951; Winter & Flataker, 1951). Also, Dewey *et al.* (1969b) reported morphine to be far less effective in suppressing the reflex in spinal mice, and a low ceiling effect was

produced. On the basis of this evidence, they suggested that, in the intact animal, morphine owes its anti-nociceptive activity to a stimulation of supraspinal inhibitory influences.

The main criticism of the phenylquinone test is its lack of specificity, a large variety of drugs suppressing the syndrome. These include the narcotic analgesics (Siegmund *et al.*, 1957), narcotic-antagonist analgesics (Blumberg *et al.*, 1965; Taber *et al.*, 1964) and also: parasympathomimetics, sympathomimetics, anticholinesterases, adrenergic blocking drugs, antihistamines, local anaesthetics and CNS stimulants (Brittain *et al.*, 1963; Hendershot & Forsaith, 1959). Also, the injection of saline i.c.v. significantly reduces the number of writhes elicited by a challenge dose of phenylquinone (Results: Chapter One).

In the present study, the main test used has been the mouse tail-flick test. Its useful features are its rapidity in use, the fact that reproduceable results are obtained, that time-course experiments may be carried out, and that the effects which are observed are probably the result of actions on supraspinal mechanisms. The hot-plate test has been used to a limited extent, mainly for comparison with the tail-flick test. The phenylquinone test was used because the narcotic-antagonist analgesics are active in this test, although its use was, in the event, restricted by the inability to combine with it the i.c.v. route of administration. The foot-pressure test was chosen for use in the rat because of its simplicity, in that the response was, again, a simple withdrawal reflex (as far as could be ascertained: there has been little material published on this test) and therefore comparable to the tail-flick reflex in the mouse. Earlier studies in these laboratories have shown that rats of sufficient age and size to be fitted with i.c.v. cannula guides had tails which were

too scaly to respond to the radiant heat stimulus before irreversible damage became apparent, and therefore a direct comparison using the tail-flick test could not be carried out between the rat and the mouse. The phenylquinone test was used in the present study because the narcotic-antagonist analgesics are active in this test, this being the only simple test available for which this is true.

2. DISCUSSION OF EXPERIMENTAL RESULTS

(a) *The involvement of putative CNS transmitter-substances in the anti-nociceptive effects of morphine*

The results reported in this thesis suggest that the anti-nociceptive action of morphine is due, at least in part, to an increase in cholinergic activity in the CNS. Thus agents which are known to have a central cholinomimetic action potentiate morphine's anti-nociceptive effect, whilst those which are known to inhibit central cholinergic transmission antagonise it.

The similarity between the anti-nociceptive activities of morphine and oxotremorine (in our *acute* experiments) has been well demonstrated. Thus both are potentiated by the administration of physostigmine i.c.v., both are antagonised by peripheral and central administration of atropine or hyoscine, whereas neither are significantly antagonised by the peripheral injection of quaternary atropine. Thus further evidence is added to suggest the involvement of a central cholinergic system in the anti-nociceptive action of morphine. Future experiments might profitably be carried out in an attempt to localise the precise site of the cholinergic system or systems which are involved (Metyš *et al.*, 1969b). Good correlation was obtained between the mouse and the rat with respect to cholinergic interactions of morphine, which suggests that the involvement

of cholinergic systems is common to both species.

The possible clinical significance of these findings has to an extent been pointed out by some earlier work in man. Thus both atropine and hyoscine have been shown to increase the sensitivity of a patient to pain at normal premedication doses (Dundee *et al.*, 1961) and atropine also to diminish the analgesic action of pethidine and pethidine/phenothiazine combinations (Moore & Dundee, 1962).

We have shown that an increase in 5-HT in the CNS produces a transient anti-nociceptive effect in the rat and in the mouse. Further, we have demonstrated that 5-HT in the CNS is probably involved in bringing about the anti-nociceptive effects of morphine in these species. Injecting 5-HT i.c.v. potentiates morphine; depletion of 5-HT with reserpine antagonises morphine; and in the rat, the activity of morphine is restored in reserpinised animals following an i.c.v. injection of 5-HT. We were unable to duplicate this last result in the mouse, and this might offer a suitable area for further research, considering the high degree of correlation between the two species up to this point.

The interactions of oxotremorine with 5-HT are in many instances closely comparable to those of morphine with 5-HT, further linking narcotic and cholinomimetic anti-nociceptive effects, but again there have been some differences between the rat and the mouse, and until further work is carried out in this field, the resultant confusion must remain.

The possibility that 5-HT metabolism might be altered in the genesis of tolerance has been discussed earlier (Results: Chapter Seven), in some studies an increase in 5-HT turnover being detected (Loh *et al.*, 1968, 1969) whilst other workers were unable to confirm these findings

(Cheney *et al.*, 1971). Sparkes (1971) was able to prevent the appearance of acute tolerance to morphine (infused i.v.) by infusing 5-HT i.c.v. at the same time in rats. Recent work (Spencer, 1972, Personal communication) has shown that the anti-nociceptive effect of morphine is restored in tolerant mice by an injection of 5-HT i.c.v. Hence there is strong evidence that 5-HT in the CNS is involved in the production of the anti-nociceptive action of morphine.

Correlation with a specific neuronal system containing 5-HT has produced remarkable results. Thus the mid-brain raphé system has been shown to contain 5-HT neurones (Andén *et al.*, 1966; Ungerstedt, 1971), and stimulation of this system increases turnover of 5-HT at its rostral terminations in the forebrain (Sheard & Aghajanan, 1968). Lesions in the mid-brain raphé reduce forebrain levels of 5-HT and 5-HIAA, and decrease the anti-nociceptive activity of morphine in the hot-plate, tail-pressure and electric shock tests (Samanin *et al.*, 1970). Thus there is a considerable likelihood that 5-HT in the CNS is involved in the anti-nociceptive action of morphine.

As far as the catecholamines are concerned, it would appear that i.c.v. DA potentiates both morphine and oxotremorine, whilst NA antagonises both of these agents. Any further work with these putative CNS transmitter substances could usefully involve intracerebral microinjection into specific areas of the brain, in an attempt to correlate these effects with a particular neuronal system (Ungerstedt, 1971) as has been attempted with 5-HT and the mid-brain raphé system. It is possible that an increase in activity on the part of a neurone system containing NA might be responsible, at least in part, for the development of tolerance to morphine and other narcotic analgesics, although as stated in the Introduction,

there is no clear-cut evidence for such a suggestion based on alterations of NA levels or turnover rates in brain during the development of tolerance. This is a further line of investigation which might be pursued, however.

It should be emphasised at this point that all the experiments carried out in the present study were acute experiments, and therefore we have been examining only the anti-nociceptive action of a single dose of morphine. Corroborative evidence may well be obtained (such as that regarding the possible rôle of 5-HT in the development of tolerance, mentioned above) by carrying out experiments involving the chronic administration of morphine and related substances.

(b) The involvement of putative CNS-transmitter substances in the anti-nociceptive effects of the narcotic-antagonist analgesics

In view of the differences which have become apparent between the two narcotic-antagonist analgesics used in this study (nalorphine and pentazocine) it is perhaps unwise to consider them together under a single heading (see Results: Chapter Eight). Nevertheless, for the sake of clarity, this has been attempted.

The anti-nociceptive effects of both agents were potentiated by the anticholinesterase physostigmine (injected i.c.v.), suggesting a similarity to morphine. However, only nalorphine was antagonised by hyoscine (as morphine had been). This suggests that nalorphine shares with morphine an action *via* a cholinergic system, whilst it seems that pentazocine does not act *via* such a system.

However, the narcotic antagonist naloxone antagonised morphine and pentazocine, but did not antagonise nalorphine. This evidence suggests that the agents morphine and pentazocine both act, at least in part, *via*

a mechanism which involves stimulation of a 'narcotic agonist' receptor, which is blocked by the antagonist naloxone. On the other hand, morphine and nalorphine act (or can act) in part, *via* a cholinergic mechanism (which is blocked by hyoscine).

Thus, from our results, morphine would appear to have a dual action on 'narcotic' and cholinergic systems, nalorphine acting almost entirely *via* a cholinergic system, whilst pentazocine acts almost entirely *via* a 'narcotic' system. However, as mentioned in Chapter Eight, this is almost certainly a gross oversimplification, because the anti-nociceptive activity of morphine is capable of complete blockade by naloxone, suggesting that only a 'narcotic' action is involved in its effect. The suggestion can be made, however, based upon these results, that the narcotic-antagonist analgesics should not be considered as a group, because it would appear that their properties may differ considerably from one agent to another within the heading 'narcotic-antagonist analgesic'.

There was considerable difficulty when attempting to study the interactions of the narcotic-antagonist analgesics with 5-HT and the catecholamines, because of the inadequacies of the nociceptive tests available. Therefore, a large amount of research remains to be carried out in this field following the adoption of a testing method which is sufficiently sensitive to detect the antagonist analgesics but yet not prone to false positive results such as is the phenylquinone test following i.c.v. injection (Results: Chapter One).

In summary: it may be concluded that morphine depends upon both ACh and 5-HT in the brain in order to produce its anti-nociceptive effect, and that DA may be involved to a lesser extent. 5-HT would itself appear to be

necessary for the production of the anti-nociceptive activity exhibited by the cholinergic agent oxotremorine, thus suggesting a close link between the narcotic and cholinergic anti-nociceptive effects.

The narcotic-antagonist analgesics nalorphine and pentazocine differ considerably in their spectra of activity, in that pentazocine appears to act to a large degree *via* stimulation of morphine-type receptors, but does not involve a cholinergic system to any great extent. Nalorphine, in contrast, has little or no action *via* the morphine-type receptor but its anti-nociceptive effect does appear to involve a cholinergic system. The differences between these drugs as analgesics/anti-nociceptive agents require further study in experimental animals and at the clinical level.

SECTION FIVE

APPENDICES

APPENDIX A

TABULATED DATA CORRESPONDING TO FIGURES APPEARING IN THE TEXT

The tables are annotated with the figure number of the appropriate figure as it is numbered in the text.

FIG. 11

Treatment prior to phenylquinone, 2.0 mg/kg i.p.	Mean Nº of writhes ± s.e.
No pretreatment (control)	36.5 ± 4.4
10.0 µl saline i.c.v. concurrent with phenylquinone	10.9 ± 2.6*
10.0 µl saline i.c.v. 10 minutes prior to phenylquinone	18.3 ± 4.1*
10.0 µl saline i.c.v. 20 " " " "	21.9 ± 4.2*
10.0 µl saline i.c.v. 40 " " " "	28.0 ± 4.6
10.0 µl saline i.c.v. 60 " " " "	33.6 ± 3.7

* = Significantly different from control value ($P<0.05$; $n = 10$).

FIG. 12

Dose of morphine and route of injection	Anti-nociceptive effect (%)
1.25 µg/kg i.c.v.	12.5
2.5 µg/kg i.c.v.	25.0
5.0 µg/kg i.c.v.	37.5
10.0 µg/kg i.c.v.	75.0
1.25 mg/kg s.c.	5.0
2.5 mg/kg s.c.	50.0
5.0 mg/kg s.c.	50.0
10.0 mg/kg s.c.	70.0
20.0 mg/kg s.c.	95.0

n = 8 for i.c.v. doses.

n = 10 for s.c. doses.

FIG. 13

	Tail-flick reaction time (mean ± s.e.)(s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c.	4.18 ±0.05	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Morphine 5.0 mg/kg s.c.	—	6.48 ±0.64	7.96 ±0.69	8.18 ±0.62	4.76 ±0.29	4.48 ±0.11

n = 10.

FIG. 14

Dose of morphine and route of injection	Anti-nociceptive effect (%)
15.0 µg/kg i.c.v.	10.0
30.0 µg/kg i.c.v.	40.0
60.0 µg/kg i.c.v.	50.0
5.0 mg/kg s.c.	10.0
7.5 mg/kg s.c.	50.0
10.0 mg/kg s.c.	70.0

n = 10 for each dose plotted.

FIG. 15

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v.	3.99 ±0.06	3.80 ±0.13	4.20 ±0.12	4.42 ±0.13	4.04 ±0.14	4.14 ±0.20
Morphine 1.5 ug i.c.v.	—	7.78 -	7.98 -	7.84 ±0.79	6.62 ±0.88	4.08 ±0.26

n = 10.

FIG. 16

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c.	4.08 ±0.05	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Nalorphine 5.0 mg/kg s.c.	—	4.72 ±0.15	5.22 ±0.24	5.08 ±0.15	4.34 ±0.12	4.24 ±0.13
Nalorphine 20.0 mg/kg s.c.	—	4.58 ±0.12	4.86 ±0.17	5.26 ±0.23	4.00 ±0.12	4.00 ±0.31

n = 10.

FIG. 17

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c.	4.08 ±0.05	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Pentazocine 10.0 mg/kg s.c.	—	5.38 ±0.27	4.93 ±0.15	5.62 ±0.22	4.92 ±0.22	4.56 ±0.25
Pentazocine 40.0 mg/kg s.c.	—	4.50 ±0.15	4.96 ±0.09	5.74 ±0.30	4.58 ±0.27	3.88 ±0.10

n = 10.

FIG. 18

Dose of morphine and route of injection	Anti-nociceptive effect (%)
3.125 µg/kg i.c.v.	30.0
6.25 µg/kg i.c.v.	Not tested
12.5 µg/kg i.c.v.	60.0
25.0 µg/kg i.c.v.	80.0
50.0 µg/kg i.c.v.	90.0
0.375 mg/kg s.c.	8.0
0.75 mg/kg s.c.	40.0
1.5 mg/kg s.c.	80.0
3.0 mg/kg s.c.	95.0

n = 10 for each dose plotted.

FIG. 19

Dose of nalorphine by s.c. injection	Anti-nociceptive effect (%)
9.4 mg/kg	5.0
18.8 mg/kg	30.0
37.5 mg/kg	50.0
75.0 mg/kg	50.0
150.0 mg/kg	80.0
Dose of pentazocine by s.c. injection	**Anti-nociceptive effect (%)**
0.625 mg/kg	10.0
1.25 mg/kg	20.0
2.5 mg/kg	20.0
5.0 mg/kg	50.0
10.0 mg/kg	70.0

n = 10 for each dose plotted.

FIG. 20

	Anti-nociceptive index (mean ± s.e.)						
Time after injection (min)	0	30	45	60	120	180	240
Saline 1.0 ml/kg s.c.	0.0	0.02 ±0.04	0.04 ±0.02	0.04 ±0.02	0.00 ±0.02	0.00 ±0.02	0.01 ±0.01
Morphine 5.0 mg/kg s.c.	0.0	0.90 -	1.00 -	0.91 -	0.72 ±0.15	0.22 ±0.07	0.06 ±0.04

n = 5.

FIG. 21

Dose of morphine by s.c. injection	Anti-nociceptive effect (%)
1.25 mg/kg	0.75
2.5 mg/kg	40.0
5.0 mg/kg	80.0

n = 5 for each dose plotted.

FIG. 22

Dose of oxotremorine and route of injection	Anti-nociceptive effect (%)
1.25 µg/kg i.c.v.	37.5
2.5 µg/kg i.c.v.	50.0
5.0 µg/kg i.c.v.	62.5
10.0 µg/kg i.c.v.	92.5
0.025 mg/kg s.c.	10.0
0.05 mg/kg s.c.	50.0
0.10 mg/kg s.c.	70.0

n = 8 for each i.c.v. dose plotted.

n = 10 for each s.c. dose plotted.

FIG. 23

Dose of NA given by i.c.v. injection	Anti-nociceptive effect (%)
12.5 µg/kg	12.5
25.0 µg/kg	25.0
37.5 µg/kg	62.5
50.0 µg/kg	62.5

n = 8 for each dose plotted.

FIG. 24

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v.	3.99 ±0.06	3.80 ±0.13	4.20 ±0.12	4.42 ±0.13	4.04 ±0.14	4.14 ±0.20
Oxotremorine 0.05 µg i.c.v.	—	5.82 ±0.48	5.58 ±0.50	5.06 ±0.25	4.22 ±0.21	4.20 ±0.18
Oxotremorine 0.05 mg/kg s.c.	—	8.68 ±0.66	8.96 ±0.46	8.22 ±0.70	5.12 ±0.56	4.46 ±0.18

n = 10.

FIG. 25

Dose of oxotremorine and route of injection	Anti-nociceptive effect (%)
1.25 µg/kg i.c.v.	25.0
2.5 µg/kg i.c.v.	37.5
5.0 µg/kg i.c.v.	94.3
0.0125 mg/kg s.c.	10.0
0.025 mg/kg s.c.	20.0
0.05 mg/kg s.c.	50.0
0.10 mg/kg s.c.	80.0

n = 8 for each i.c.v. dose plotted.

n = 10 for each s.c. dose plotted.

FIG. 26

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v.	4.08 ±0.15	4.08 ±0.19	4.16 ±0.28	4.04 ±0.12	3.68 ±0.10	3.76 ±0.23
5-HT 10.0 µg i.c.v.	4.04 ±0.16	4.60 ±0.45	6.04 ±0.79	4.04 ±0.12	3.80 ±0.36	3.64 ±0.16
Carbachol 0.2 µg i.c.v.	3.98 ±0.08	5.10 ±0.67	4.66 ±0.52	4.60 ±0.28	4.12 ±0.34	3.68 ±0.12
Physostigmine 3.0 µg i.c.v.	4.14 ±0.08	8.52 ±0.63	7.66 ±0.63	7.52 ±0.82	4.60 ±0.13	—

n = 5 for saline and 5-HT.

n = 10 for carbachol and physostigmine.

FIG. 27

Dose of oxotremorine by s.c. injection	Anti-nociceptive effect (%)
0.01 mg/kg	10.0
0.02 mg/kg	30.0
0.04 mg/kg	50.0
0.08 mg/kg	90.0

n = 10.

FIG. 28

	Anti-nociceptive index (mean ± s.e.)						
Time after injection (min)	0	30	45	60	120	180	240
Saline 1.0 ml/kg s.c.	0	-0.08 ±0.06	-0.10 ±0.07	-0.10 ±0.06	-0.09 ±0.06	-0.09 ±0.02	-0.12 ±0.04
Oxotremorine 0.1 mg/kg s.c.	0	0.95 -	0.75 ±0.17	0.23 ±0.17	0.04 ±0.04	0.07 ±0.06	0.00 ±0.04

n = 5.

FIG. 29

Dose of oxotremorine by s.c. injection	Anti-nociceptive effect (%)
0.0125 mg/kg	3.2
0.025 mg/kg	6.2
0.05 mg/kg	40.0
0.10 mg/kg	60.0

n = 5.

FIG. 30

	Anti-nociceptive index (mean ± s.e.)						
Time of experiment (min)	0	30	45	60	120	180	240
Saline 10.0 µl i.c.v.	0	0.05 ±0.04	0.03 ±0.02	0.04 ±0.02	0.04 ±0.02	-0.01 ±0.04	-0.01 ±0.03
Physostigmine 2.5 µg i.c.v.	0	0.05 ±0.04	0.56 ±0.15	0.15 ±0.08	-0.01 ±0.04	-0.03 ±0.03	0.01 ±0.02
5-HT 20.0 µg i.c.v.	0	0.05 ±0.04	0.55 ±0.19	0.05 ±0.02	-0.03 ±0.03	-0.02 ±0.02	0.02 ±0.02

n = 5.

FIG. 31

	Tail-flick reaction time (mean ± s.e.) (s)					
Time after s.c. injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v. + saline 10.0 ml/kg s.c.	4.24 ±0.04	4.34 ±0.11	4.26 ±0.16	4.36 ±0.11	3.96 ±0.10	4.02 ±0.09
Naloxone 1.0 µg i.c.v. + saline 10.0 ml/kg s.c.	—	4.10 ±0.10	4.14 ±0.14	4.28 ±0.17	4.34 ±0.16	4.16 ±0.14
Saline 10.0 µl i.c.v. + morphine 5.0 mg/kg s.c.	—	7.00 ±0.83	7.76 ±0.76	7.30 ±0.80	4.58 ±0.37	4.66 ±0.15
Naloxone 1.0 µg i.c.v. + morphine 5.0 mg/kg s.c.	—	4.30 ±0.11	4.42 ±0.14	4.48 ±0.01	4.48 ±0.12	4.28 ±0.13

n = 10.

FIG. 32

Drug treatment	Mean hot-plate reaction time, (s), ± standard error.
Saline (10.0 ml/kg) s.c. + saline (10.0 µl) i.c.v.	8.21 ± 2.03
Morphine (5.0 mg/kg) s.c. + saline (10.0 µl) i.c.v.	15.70 ± 3.04
Saline (10.0 ml/kg) s.c. + physostigmine (1.0 µg) i.c.v.	16.42 ± 3.11
Morphine (5.0 mg/kg) s.c. + physostigmine (1.0 µg) i.c.v.	26.38 ± 2.52

n = 10.

FIG. 33

	Tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µg i.c.v.	4.08 ±0.05	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Saline 10.0 ml/kg s.c. + physostigmine 1.0 µg i.c.v.	—	4.08 ±0.12	3.88 ±0.13	4.02 ±0.13	3.74 ±0.22	3.96 ±0.21
Morphine 2.5 mg/kg s.c. + saline 10.0 µl i.c.v.	—	4.60 ±0.33	5.18 ±0.61	5.38 ±0.62	5.42 ±0.70	3.92 ±0.20
Morphine 2.5 mg/kg s.c. + physostigmine 1.0 µg i.c.v.	—	7.94 ±0.75	8.00 ±0.81	7.94 ±0.90	5.00 ±0.68	4.62 ±0.47

n = 10.

FIG. 34

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Atropine sulphate 10.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	3.88 ±0.13	3.82 ±0.10	3.74 ±0.12	3.86 ±0.08	3.60 ±0.11	3.36 ±0.05
Saline 10.0 ml/kg s.c. + morphine 5.0 mg/kg s.c.	—	7.82 ±0.73	8.52 ±0.53	8.74 ±0.54	6.34 ±0.90	5.22 ±0.30
Atropine sulphate 2.0 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	4.54 ±0.15	4.68 ±0.18	4.78 ±0.21	3.42 ±0.11	3.46 ±0.15
Atropine sulphate 10.0 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	6.80 ±0.68	8.12 ±0.73	7.30 ±0.84	4.50 ±0.27	4.74 ±0.31

n = 10.

FIG. 35

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	3.99 ±0.06	3.80 ±0.13	4.20 ±0.12	4.42 ±0.13	4.04 ±0.14	4.14 ±0.20
Saline 10.0 ml/kg s.c. + morphine 1.5 µg i.c.v.	—	7.78 -	7.98 -	7.84 ±0.79	6.62 ±0.88	4.08 ±0.26
Atropine sulphate 2.0 mg/kg s.c. + morphine 1.5 µg i.c.v.	—	4.42 ±0.16	5.32 ±0.56	6.28 ±0.70	5.80 ±0.76	3.88 ±0.22
Atropine sulphate 20.0 mg/kg s.c. + morphine 1.5 µg i.c.v.	—	4.58 ±0.13	5.74 ±0.76	6.70 ±0.90	6.30 ±0.85	3.62 ±0.20

n = 10.

FIG. 36

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Atropine sulphate 0.5 µg i.c.v. + saline 10.0 ml/kg s.c.	4.06 ±0.08	3.93 ±0.10	3.98 ±0.08	3.94 ±0.10	3.86 ±0.12	4.04 ±0.10
Saline 10.0 µl i.c.v. + morphine 5.0 mg/kg s.c.	—	6.98 ±0.66	7.70 ±0.62	7.78 ±0.72	6.32 ±0.81	4.34 ±0.30
Atropine sulphate 0.5 µg i.c.v. + morphine 5.0 mg/kg s.c.	—	4.80 ±0.18	5.27 ±0.32	5.33 ±0.28	5.22 ±0.50	3.90 ±0.13

n = 10.

FIG. 37

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Atropine methonitrate 2.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	4.05 ±0.27	3.92 ±0.13	4.34 ±0.14	4.42 ±0.09	3.94 ±0.23	3.98 ±0.17
Saline 10.0 ml/kg s.c. + morphine 5.0 mg/kg s.c.	—	7.82 ±0.73	8.52 ±0.53	8.74 ±0.54	6.34 ±0.90	5.22 ±0.30
Atropine methonitrate 2.0 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	6.56 ±0.82	7.82 ±0.70	8.30 ±0.55	5.56 ±0.75	4.62 ±0.35
Atropine methonitrate 10.0 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	5.78 ±0.70	6.72 ±0.76	6.65 ±0.81	5.86 ±0.94	4.38 ±0.20

n = 10.

FIG. 38

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Atropine methonitrate 2.0 µg i.c.v. + saline 10.0 ml/kg s.c.	4.01 ±0.12	3.76 ±0.10	4.14 ±0.11	4.06 ±0.12	3.86 ±0.13	3.60 ±0.13
Saline 10.0 µg i.c.v. + morphine 5.0 mg/kg s.c.	—	7.38 ±0.76	7.96 ±0.68	7.80 ±0.76	6.16 ±0.80	4.32 ±0.57
Atropine methonitrate 0.5 µg i.c.v. + morphine 5.0 mg/kg s.c.	—	6.70 ±0.73	7.72 ±0.75	7.78 ±0.81	5.16 ±0.72	4.48 ±0.34
Atropine methonitrate 2.0 µg i.c.v. + morphine 5.0 mg/kg s.c.	—	5.48 ±0.77	5.84 ±0.82	5.50 ±0.62	4.32 ±0.53	3.80 ±0.16

n = 10.

FIG. 39

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 ml/kg s.c.	4.21 ±0.05	4.32 ±0.10	4.24 ±0.16	4.06 ±0.11	4.06 ±0.13	3.70 ±0.10
Hyoscine 1.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	—	4.30 ±0.09	4.28 ±0.08	4.08 ±0.09	3.80 ±0.08	3.84 ±0.11
Saline 10.0 ml/kg s.c. + morphine 5.0 mg/kg s.c.	—	7.60 ±0.80	9.00 -	9.70 -	6.54 ±0.82	4.52 ±0.26
Hyoscine 1.0 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	4.42 ±0.17	5.44 ±0.31	5.50 ±0.52	4.12 ±0.10	4.00 ±0.12

n = 10.

FIG. 40

	Anti-nociceptive index (mean ± s.e.)					
Time after s.c. injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	0.01 ±0.03	0.01 ±0.02	-0.03 ±0.02	0.00 ±0.02	0.00 ±0.01	0.01 ±0.01
Saline 1.0 ml/kg s.c. + physostigmine 1.0 µg i.c.v.	0.01 ±0.03	0.19 ±0.07	0.06 ±0.04	-0.01 ±0.02	-0.01 ±0.02	-0.01 ±0.02
Morphine 1.25 mg/kg s.c. + saline 10.0 µl i.c.v.	0.34 ±0.07	0.37 ±0.06	0.31 ±0.14	0.31 ±0.11	0.15 ±0.04	-0.02 ±0.02
Morphine 1.25 mg/kg s.c. + physostigmine 1.0 µg i.c.v.	0.34 ±0.07	0.97 -	0.41 ±0.12	0.18 ±0.04	0.10 ±0.03	-0.02 ±0.04

n = 5.

FIG. 41

Time interval between reserpine and morphine	Anti-nociceptive effect (% of morphine control)
Control group : no reserpine	100.0
1 hour	104.0
2 "	51.0
3 "	4.8
4 "	-0.8
5 "	-29.0
6 "	-20.0
8 "	-22.0
1 day	-22.0
3 "	23.0
5 "	69.0
7 "	81.0
9 "	99.0

n = 10.

FIG. 42

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg i.p. + saline 10.0 ml/kg s.c.	3.73 ±0.07	4.06 ±0.16	4.20 ±0.13	4.14 ±0.09	4.12 ±0.09	4.14 ±0.08
Tetrabenazine 40.0 mg/kg i.p. + saline 10.0 ml/kg s.c.	—	4.02 ±0.16	4.30 ±0.14	4.02 ±0.16	3.56 ±0.12	4.28 ±0.16
Saline 10.0 ml/kg i.p. + morphine 5.0 mg/kg s.c.	—	7.58 ±0.58	8.78 ±0.45	8.82 ±0.54	6.82 ±0.67	4.74 ±0.21
Tetrabenazine 40.0 mg/kg i.p. + morphine 5.0 mg/kg s.c.	—	4.98 ±0.58	5.46 ±0.54	6.10 ±0.58	4.46 ±0.22	4.12 ±0.07

n = 10.

FIG. 43

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.17 ±0.08	3.98 ±0.11	4.36 ±0.20	4.52 ±0.18	4.54 ±0.17	4.70 ±0.18
Saline 10.0 ml/kg s.c. + NA 5.0 µg i.c.v.	—	4.72 ±0.45	4.64 ±0.20	5.08 ±0.21	5.16 ±0.20	4.86 ±0.14
Morphine 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	6.36 ±0.50	8.08 ±0.69	7.12 ±0.82	6.94 ±0.69	6.06 ±0.20
Morphine 5.0 mg/kg s.c. + NA 5.0 µg i.c.v.	—	5.52 ±0.26	6.72 ±0.51	8.50 ±0.53	6.30 ±0.71	6.02 ±0.50

n = 10.

FIG. 44

	Mean tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	30	45	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	3.73 ±0.06	3.59 ±0.08	3.76 ±0.10	4.02 ±0.12	3.66 ±0.22	3.56 ±0.11
Saline 10.0 ml/kg s.c. + NA 5.0 µg i.c.v.	—	—	3.86 ±0.12	3.62 ±0.14	3.96 ±0.15	3.88 ±0.14
Morphine 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	6.50 ±0.49	8.70 ±0.55	8.94 ±0.54	6.40 ±0.94	4.28 ±0.39
Morphine 5.0 mg/kg s.c. + NA 5.0 µg i.c.v.	—	—	6.10 ±0.69	6.90 ±0.67	5.92 ±0.72	4.22 ±0.16

n = 10.

FIG. 45

	Mean tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	30	45	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	3.80 ±0.06	3.58 ±0.06	3.92 ±0.10	3.88 ±0.11	3.42 ±0.08	3.46 ±0.08
Saline 10.0 ml/kg s.c. + DA 50.0 µg i.c.v.	—	—	3.96 ±0.20	4.12 ±0.17	3.80 ±0.17	3.70 ±0.15
Morphine 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	6.90 ±0.70	8.30 ±0.71	8.00 ±0.81	6.54 ±0.78	4.32 ±0.31
Morphine 5.0 mg/kg s.c. + DA 50.0 µg i.c.v.	—	—	5.12 ±0.29	4.64 ±0.31	3.44 ±0.13	3.76 ±0.15

n = 10

FIG. 46

	Mean tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.15 ±0.08	3.80 ±0.07	3.90 ±0.10	4.12 ±0.13	3.60 ±0.12	3.70 ±0.13
Saline 10.0 ml/kg s.c. + DA 5.0 µg i.c.v.	—	3.54 ±0.09	3.90 ±0.10	4.24 ±0.15	3.80 ±0.13	3.90 ±0.10
Morphine 2.5 mg/kg s.c. + saline 10.0 µl i.c.v.	—	4.78 ±0.52	4.73 ±0.60	5.36 ±0.60	4.64 ±0.34	4.21 ±0.14
Morphine 2.5 mg/kg s.c. + DA 5.0 µg i.c.v.	—	5.86 ±0.51	6.52 ±0.59	7.44 ±0.66	5.90 ±0.60	4.31 ±0.20

n = 10.

FIG. 47

	Mean anti-nociceptive index, ±s.e.						
Time after s.c, injection (min)	0	30	45	60	120	180	240
Saline 1.0 ml/kg i.p. + saline 1.0 ml/kg s.c.	0.0 -	0.02 ±0.04	0.04 ±0.02	0.04 ±0.02	0.00 ±0.02	0.00 ±0.02	0.01 ±0.01
Reserpine 5.0 mg/kg i.p. + saline 1.0 ml/kg s.c.	0.0 -	-0.01 ±0.05	-0.05 ±0.04	-0.14 ±0.02	0.02 ±0.04	-0.02 ±0.03	-0.07 ±0.06
Saline 1.0 ml/kg i.p. + morphine 5.0 mg/kg s.c.	0.0 -	0.90 -	1.00 -	0.91 -	0.72 ±0.15	0.22 ±0.07	0.06 ±0.04
Reserpine 5.0 mg/kg i.p. + morphine 8.0 mg/kg s.c.	0.0 -	0.05 ±0.06	0.05 ±0.05	0.02 ±0.11	-0.08 ±0.05	-0.04 ±0.04	-0.06 ±0.06

n = 5.

FIG. 48

	Mean anti-nociceptive index, ±s.e.					
Time after s.c. injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	0.02 ±0.04	0.04 ±0.02	0.04 ±0.02	0.00 ±0.02	0.00 ±0.02	0.01 ±0.01
Saline 1.0 ml/kg s.c. + NA 20.0 µg i.c.v.	—	-0.05 ±0.03	-0.01 ±0.02	0.00 ±0.03	-0.02 ±0.02	-0.04 ±0.02
Morphine 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	0.90 -	1.00 -	0.91 -	0.72 ±0.15	0.22 ±0.07	0.06 ±0.04
Morphine 5.0 mg/kg s.c. + NA 20.0 µg i.c.v.	—	0.01 ±0.06	-0.01 ±0.03	0.02 ±0.04	0.03 ±0.04	-0.02 ±0.04

n = 5.

FIG. 49

	Mean anti-nociceptive index, ±s.e.					
Time after s.c. injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	-0.02 ±0.04	-0.02 ±0.02	-0.01 ±0.03	-0.05 ±0.03	-0.04 ±0.04	-0.01 ±0.03
Saline 1.0 ml/kg s.c. + DA 20.0 µg i.c.v.	—	0.02 ±0.02	0.02 ±0.02	-0.03 ±0.03	-0.04 ±0.03	0.02 ±0.03
Morphine 2.5 mg/kg s.c. + saline 10.0 µl i.c.v.	0.59 ±0.15	0.46 ±0.15	0.42 ±0.13	0.38 ±0.16	0.04 ±0.01	0.04 ±0.02
Morphine 2.5 mg/kg s.c. + DA 20.0 µg i.c.v.	—	0.93 -	0.77 -	0.16 ±0.04	0.09 ±0.03	-0.02 ±0.04

n = 5.

FIG. 50

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.05 ±0.15	4.08 ±0.19	4.16 ±0.28	4.04 ±0.12	3.68 ±0.10	3.76 ±0.23
Saline 10.0 ml/kg s.c. + 5-HT 10.0 µg i.c.v.	—	4.60 ±0.45	6.04 ±0.79	4.04 ±0.12	3.80 ±0.36	3.64 ±0.16
Morphine 2.5 mg/kg s.c. + saline 10.0 µl i.c.v.	—	6.82 ±0.87	6.52 ±0.78	6.68 ±0.66	4.14 ±0.32	4.40 ±0.44
Morphine 2.5 mg/kg s.c. + 5-HT 10.0 µg i.c.v.	—	8.96 ±0.18	8.28 ±0.72	8.02 ±0.65	5.70 ±0.59	4.28 ±0.50

n = 5 (saline/saline; saline/5-HT).

n = 10 (morphine/saline; morphine/5-HT).

FIG. 51

	Mean tail-flick reaction time (s), ±s.e.					
Time after morphine injection (min)	0	20	40	60	120	180
LSD-25 0.05 mg/kg s.c. + saline 10.0 ml/kg s.c.	4.18 ±0.05	4.00 ±0.12	4.20 ±0.15	4.40 ±0.17	3.98 ±0.12	4.04 ±0.08
Saline 10.0 ml/kg s.c. + morphine 5.0 mg/kg s.c.	—	6.48 ±0.04	7.96 ±0.69	8.18 -	4.76 ±0.29	4.48 ±0.11
LSD-25 0.05 mg/kg s.c. + morphine 5.0 mg/kg s.c.	—	4.80 ±0.27	5.09 ±0.27	5.78 ±0.34	4.00 ±0.11	4.11 ±0.16

n = 10.

FIG. 52

	Mean anti-nociceptive index, ±s.e.					
Time after s.c. injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	-0.03 ±0.04	0.01 ±0.01	0.04 ±0.05	0.03 ±0.02	0.00 ±0.04	0.02 ±0.03
Saline 1.0 ml/kg s.c. + 5-HT 20.0 µg i.c.v.	—	-0.04 ±0.03	-0.02 ±0.04	-0.07 ±0.03	-0.04 ±0.02	-0.05 ±0.05
Morphine 2.5 mg/kg s.c. + saline 10.0 µl i.c.v.	0.66 ±0.19	0.32 ±0.06	0.55 ±0.10	0.02 ±0.03	-0.05 ±0.04	-0.01 ±0.04
Morphine 2.5 mg/kg s.c. + 5-HT 20.0 µg i.c.v.	—	0.92 -	0.78 -	0.39 ±0.13	0.09 ±0.05	0.02 ±0.03

n = 5.

FIG. 53

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	-0.01 ±0.06	-0.05 ±0.04	-0.14 ±0.02	0.02 ±0.04	-0.02 ±0.03	-0.07 ±0.01
Saline 1.0 ml/kg s.c. + 5-HT 5.0 µg i.c.v.	—	0.08 ±0.06	0.03 ±0.04	-0.09 ±0.10	0.02 ±0.01	0.03 ±0.01
Morphine 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	0.05 ±0.11	0.05 ±0.05	0.02 ±0.11	-0.08 ±0.05	-0.04 ±0.04	-0.06 ±0.06
Morphine 5.0 mg/kg s.c. + 5-HT 5.0 µg i.c.v.	—	0.88 ±0.06	0.56 ±0.16	0.03 ±0.07	-0.04 ±0.08	-0.05 ±0.04

All groups received reserpine 5.0 mg/kg i.p. at -18 h.

n = 5.

FIG. 54

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + LSD-25 1.0 µg i.c.v.	-0.02 ±0.01	-0.02 ±0.01	-0.04 ±0.01	-0.03 ±0.02	-0.03 ±0.03	-0.03 ±0.01
Morphine 3.0 mg/kg s.c. + saline 10.0 µl i.c.v.	0.61 ±0.19	0.58 ±0.18	0.29 ±0.06	0.05 ±0.02	-0.02 ±0.01	-0.01 ±0.02
Morphine 3.0 mg/kg s.c. + LSD-25 1.0 µg i.c.v.	—	0.14 ±0.05	0.06 ±0.04	-0.04 ±0.06	-0.01 ±0.05	-0.03 ±0.03

n = 5.

FIG. 55

	Mean tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.10 ±0.10	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Saline 10.0 ml/kg s.c. + physostigmine 1.0 µg i.c.v.	—	4.08 ±0.12	3.88 ±0.13	4.02 ±0.13	3.74 ±0.22	3.92 ±0.20
Nalorphine 20.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	4.20 ±0.15	4.89 ±0.19	5.58 ±0.37	4.22 ±0.25	4.58 ±0.21
Nalorphine 20.0 mg/kg s.c. + physostigmine 1.0 µg i.c.v.	—	7.20 ±0.94	6.20 ±0.84	4.90 ±0.61	3.88 ±0.27	4.04 ±0.29

n = 10.

FIG. 56

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.10 ±0.10	4.08 ±0.06	4.37 ±0.07	4.37 ±0.08	4.48 ±0.11	4.27 ±0.28
Saline 10.0 ml/kg s.c. + physostigmine 1.0 µg i.c.v.	—	4.08 ±0.12	3.88 ±0.13	4.02 ±0.13	3.74 ±0.22	3.92 ±0.20
Pentazocine 40.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	4.74 ±0.39	4.73 ±0.15	4.91 ±0.36	4.47 ±0.31	3.60 ±0.31
Pentazocine 20.0 mg/kg s.c. + physostigmine 1.0 µg i.c.v.	—	7.04 ±0.93	6.84 ±0.86	5.76 ±0.77	3.56 ±0.13	3.62 ±0.11

n = 10.

FIG. 57

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + physostigmine 2.0 µg i.c.v.	0.01 ±0.02	0.07 ±0.03	0.00 ±0.01	0.01 ±0.02	0.03 ±0.02	0.01 ±0.01
Nalorphine 15.0 mg/kg s.c. + saline 10.0 µl i.c.v.	0.09 ±0.03	-0.05 ±0.04	-0.02 ±0.05	0.00 ±0.03	-0.06 ±0.05	-0.04 ±0.04
Nalorphine 15.0 mg/kg s.c. + physostigmine 2.0 µg i.c.v.	—	0.89 -	0.13 ±0.08	0.11 ±0.06	0.03 ±0.02	0.04 ±0.01

n = 5.

FIG. 58

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 mg/kg s.c. + physostigmine 2.0 µg i.c.v.	0.01 ±0.02	0.07 ±0.03	0.00 ±0.01	0.01 ±0.02	0.03 ±0.02	0.01 ±0.01
Pentazocine 20.0 mg/kg s.c. + saline 10.0 µl i.c.v.	0.02 ±0.03	0.11 ±0.02	0.07 ±0.02	0.07 ±0.02	0.05 ±0.02	0.02 ±0.02
Pentazocine 20.0 mg/kg s.c. + physostigmine 2.0 µg i.c.v.	—	0.56 ±0.16	0.40 ±0.17	0.02 ±0.02	-0.01 ±0.02	0.02 ±0.02

n = 5.

FIG. 59

	Mean tail-flick reaction time (s), ±s.e.					
Time after s.c. injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + 5-HT 10.0 µg i.c.v.	4.10 ±0.10	5.14 ±0.85	3.88 ±0.23	3.76 ±0.10	3.26 ±0.16	3.42 ±0.13
Naloxone 5.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	3.74 ±0.09	4.10 ±0.13	4.28 ±0.12	3.64 ±0.10	3.28 ±0.12
Naloxone 5.0 mg/kg s.c. + 5-HT 10.0 µg i.c.v.	—	3.76 ±0.09	3.94 ±0.17	3.68 ±0.14	3.40 ±0.14	3.54 ±0.12

n = 10.

FIG. 60

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 2.0 ml/kg s.c. + saline 10.0 µl i.c.v.	0.00 ±0.03	0.00 ±0.04	-0.01 ±0.02	0.01 ±0.02	-0.01 ±0.02	0.01 ±0.02
Saline 2.0 ml/kg s.c. + 5-HT 20.0 µg i.c.v.	0.00 ±0.02	0.55 ±0.19	0.05 ±0.02	-0.03 ±0.03	-0.02 ±0.02	0.02 ±0.02
Naloxone 20.0 mg/kg s.c. + saline, 10.0 µl i.c.v.	0.01 ±0.03	0.02 ±0.03	0.02 ±0.01	-0.01 ±0.04	0.01 ±0.04	-0.02 ±0.04
Naloxone 20.0 mg/kg s.c. + 5-HT 20.0 µg i.c.v.	0.01 ±0.03	0.56 ±0.12	-0.02 ±0.03	-0.04 ±0.03	-0.06 ±0.04	-0.04 ±0.03

n = 5.

FIG. 61

	Mean tail-flick reaction time (s), ±s.e.					
Time after i.c.v. injection (min)	0	5	20	40	60	120
Saline 10.0 ml/kg s.c. + oxotremorine 0.05 µg i.c.v.	4.14 ±0.08	5.40 ±0.53	5.82 ±0.48	5.58 ±0.50	5.06 ±0.25	4.22 ±0.21
Naloxone 20.0 mg/kg s.c. + oxotremorine 0.05 µg i.c.v.	—	4.74 ±0.16	5.14 ±0.13	4.78 ±0.16	4.26 ±0.10	3.96 ±0.18

n = 10.

FIG. 62

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + physostigmine 1.0 µg i.c.v.	3.96 ±0.04	5.46 ±0.85	5.26 ±0.55	5.06 ±0.58	4.28 ±0.11	4.38 ±0.22
Oxotremorine 0.025 mg/kg s.c. + saline 10.0 µl i.c.v.	—	5.60 ±0.52	5.66 ±0.58	5.22 ±0.50	4.30 ±0.19	4.22 ±0.10
Oxotremorine 0.025 mg/kg s.c. + physostigmine 1.0 µg i.c.v.	—	8.58 ±0.71	8.12 ±0.63	7.76 ±0.66	4.44 ±0.62	4.04 ±0.16

n = 10.

FIG. 63

	Mean tail-flick reaction time (s), ±s.e.					
Time after oxotremorine injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + oxotremorine 0.05 mg/kg s.c.	4.05 ±0.27	7.50 ±0.71	7.24 ±0.68	6.66 ±0.74	4.24 ±0.19	4.28 ±0.31
Atropine sulphate 2.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	—	4.46 ±0.10	4.70 ±0.12	4.76 ±0.10	4.42 ±0.12	4.28 ±0.12
Atropine sulphate 2.0 mg/kg s.c. + oxotremorine 0.05 mg/kg s.c.	—	4.32 ±0.27	4.10 ±0.24	4.30 ±0.29	3.96 ±0.44	3.66 ±0.12

n = 10.

FIG. 64

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v. + saline 10.0 ml/kg s.c.	4.22 ±0.55	3.86 ±0.08	4.18 ±0.08	4.20 ±0.10	3.98 ±0.09	4.02 ±0.14
Atropine sulphate 0.5 µg i.c.v. + saline 10.0 ml/kg s.c.	—	4.04 ±0.11	4.00 ±0.08	4.00 ±0.16	3.98 ±0.13	3.90 ±0.14
Saline 10.0 µl i.c.v. + oxotremorine 0.05 mg/kg s.c.	—	7.00 ±0.73	6.90 ±0.77	6.64 ±0.90	4.12 ±0.21	3.90 ±0.15
Atropine sulphate 0.5 µg i.c.v. + oxotremorine 0.05 mg/kg s.c.	—	4.40 ±0.29	4.88 ±0.38	4.36 ±0.25	3.70 ±0.20	3.64 ±0.11

n = 10.

FIG. 65

	Mean tail-flick reaction time (s), ±s.e.					
Time after oxotremorine injection (min)	0	20	40	60	120	180
Atropine methonitrate 2.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	4.05 ±0.27	3.92 ±0.13	4.34 ±0.14	4.42 ±0.09	3.94 ±0.23	3.98 ±0.17
Saline 10.0 ml/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	7.50 ±0.71	7.24 ±0.68	6.66 ±0.74	4.24 ±0.19	4.28 ±0.31
Atropine methonitrate 2.0 mg/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	7.46 ±0.74	7.90 ±0.67	7.54 ±0.79	4.48 ±0.36	4.32 ±0.25

n = 10.

FIG. 66

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 µl i.c.v. + oxotremorine 0.05 mg/kg s.c.	4.26 ±0.12	8.16 ±0.78	8.24 ±0.72	7.72 ±0.79	5.28 ±0.54	4.52 ±0.29
Atropine methonitrate 0.5 µg i.c.v. + oxotremorine 0.05 mg/kg s.c.	——	4.72 ±0.49	4.64 ±0.35	4.56 ±0.23	3.96 ±0.10	3.74 ±0.10

n = 10.

FIG. 67

	Mean tail-flick reaction time (s), ±s.e.					
Time after oxotremorine injection (min)	0	20	40	60	120	180
Hyoscine 20.0 mg/kg s.c. + saline 10.0 ml/kg s.c.	4.08 ±0.05	3.80 ±0.10	4.06 ±0.12	3.96 ±0.15	3.80 ±0.11	3.98 ±0.07
Saline 10.0 ml/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	6.90 ±0.78	7.42 ±0.75	7.10 ±0.78	3.96 ±0.13	4.12 ±0.22
Hyoscine 1.0 mg/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	3.66 ±0.14	4.20 ±0.09	4.54 ±0.16	3.92 ±0.10	4.04 ±0.13

FIG. 68

Timé interval between reserpine and oxotremorine	Anti-nociceptive effect (% of oxotremorine control)
Control group : no reserpine	100.0
1 hour	130.0
2 "	127.0
3 "	5.5
4 "	5.0
5 "	-5.0
6 "	-0.5
8 "	-13.0
1 day	23.0
2 "	44.5
3 "	61.0
4 "	54.0
5 "	110.0
7 "	115.0

n = 10.

FIG. 69

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	4.32 ±0.12	4.30 ±0.15	4.52 ±0.12	4.46 ±0.16	4.72 ±0.20	4.72 ±0.23
Saline 10.0 ml/kg s.c. + noradrenaline 5.0 µg i.c.v.	—	4.44 ±0.28	4.84 ±0.13	4.90 ±0.27	4.94 ±0.22	5.00 ±0.26
Oxotremorine 0.05 mg/kg s.c. + saline 10.0 µl i.c.v.	—	8.44 ±0.60	8.88 -	7.78 ±0.66	6.12 ±0.73	5.06 ±0.22
Oxotremorine 0.05 mg/kg s.c. + noradrenaline 5.0 µg i.c.v.	—	5.34 ±0.88	6.72 ±0.58	7.16 ±0.83	6.10 ±0.83	5.52 ±0.12

n = 10.

FIG. 70

	Mean tail-flick reaction time (s), ±s.e.					
Time after subcutaneous injection (min)	0	20	40	60	120	180
Saline 10.0 ml/kg s.c. + dopamine 50.0 µg i.c.v.	4.10 ±0.14	4.62 ±0.31	4.32 ±0.20	5.02 ±0.15	4.56 ±0.19	4.70 ±0.23
Oxotremorine 0.05 mg/kg s.c. + saline 10.0 µl i.c.v.	—	10.0 -	9.96 -	9.56 -	7.18 ±0.95	6.38 ±0.84
Oxotremorine 0.05 µg/kg s.c. + dopamine 50.0 µg i.c.v.	—	5.14 ±0.60	6.54 ±0.80	7.02 ±0.57	5.72 ±0.49	4.92 ±0.32

n = 10.

FIG. 71

	Mean anti-nociceptive index, ±s.e.				
Time after oxotremorine injection (min)	30	45	60	120	180
Oxotremorine 0.1 mg/kg s.c.	0.95 -	0.75 ±0.17	0.23 ±0.17	0.04 ±0.04	0.07 ±0.06
Reserpine 5.0 mg/kg i.p. + oxotremorine 0.1 mg/kg s.c.	0.77 ±0.19	1.00 -	0.86 -	0.48 ±0.16	0.06 ±0.02

n = 5.

FIG. 72

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	-0.05 ±0.06	-0.10 ±0.07	-0.10 ±0.06	-0.09 ±0.06	-0.09 ±0.02	-0.12 ±0.04
Saline 1.0 ml/kg s.c. + NA 20.0 µg i.c.v.	——	-0.13 ±0.05	-0.11 ±0.04	0.06 ±0.05	-0.05 ±0.05	-0.02 ±0.03
Oxotremorine 0.1 mg/kg s.c. + saline 10.0 µl i.c.v.	0.91 ±0.05	0.75 ±0.17	0.23 ±0.17	0.04 ±0.04	0.07 ±0.06	0.00 ±0.04
Oxotremorine 0.1 mg/kg s.c. + NA 20.0 µg i.c.v.	——	0.05 ±0.04	-0.02 ±0.03	0.08 ±0.05	-0.01 ±0.04	0.03 ±0.04

n = 5.

FIG. 73

	Mean tail-flick reaction time (s), ±s.e.					
Time after oxotremorine injection (min)	0	20	40	60	120	180
LSD-25 0.05 mg/kg s.c. + saline 10.0 ml/kg s.c.	3.96 ±0.04	4.00 ±0.12	4.20 ±0.15	4.40 ±0.17	3.98 ±0.12	4.04 ±0.08
Saline 10.0 ml/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	8.68 ±0.66	8.98 ±0.46	8.22 ±0.70	5.12 ±0.56	4.46 ±0.18
LSD-25 0.05 mg/kg s.c. + oxotremorine 0.05 mg/kg s.c.	——	7.90 ±0.62	7.46 ±0.58	5.80 ±0.53	3.70 ±0.16	4.00 ±0.25

n = 10.

FIG. 74

	Mean anti-nociceptive index, ±s.e.					
Time after subcutaneous injection (min)	30	45	60	120	180	240
Saline 1.0 ml/kg s.c. + saline 10.0 µl i.c.v.	0.02 ±0.04	-0.02 ±0.04	-0.06 ±0.02	-0.08 ±0.06	0.01 ±0.02	-0.04 ±0.03
Saline 1.0 ml/kg s.c. + 5-HT 20.0 µg i.c.v.	—	0.15 ±0.05	0.03 ±0.02	0.02 ±0.01	0.01 ±0.03	-0.02 ±0.17
Oxotremorine 0.025 mg/kg s.c. + saline 10.0 µl i.c.v.	0.51 ±0.06	0.02 ±0.03	0.00 ±0.03	-0.07 ±0.06	0.04 ±0.02	0.04 ±0.01
Oxotremorine 0.025 mg/kg s.c. + 5-HT 20.0 µg i.c.v.	—	0.98 -	0.19 ±0.06	0.08 ±0.06	0.02 ±0.04	0.01 ±0.03

n = 5.

FIG. 75

	Mean oesophageal temperature (°C), ±s.e.								
Time after injection (min)	0	15	30	45	60	90	120	180	240
Saline 10.0 ml/kg s.c.	37.5 ±0.2	38.7 ±0.1	38.4 ±0.2	38.1 ±0.2	38.7 ±0.3	37.9 ±0.2	37.8 ±0.2	37.1 ±0.2	37.6 ±0.2
Saline 10.0 µl i.c.v.	—	37.1 ±0.3	38.1 ±0.2	38.1 ±0.2	38.1 ±0.2	38.0 ±0.1	38.4 ±0.1	38.3 ±0.2	38.4 ±0.1
Morphine 10.0 mg/kg s.c.	—	36.2 ±0.2	35.7 ±0.2	35.5 ±0.3	36.0 ±0.3	36.6 ±0.4	37.5 ±0.3	37.9 ±0.1	37.8 ±0.1
Morphine 5.0 µg i.c.v.	—	35.5 ±0.3	35.7 ±0.3	35.8 ±0.2	36.0 ±0.3	35.4 ±0.6	38.3 ±0.3	38.6 ±0.2	38.5 ±0.2

n = 8.

FIG. 76

	Mean oesophageal temperature (°C), ±s.e.							
Time after s.c. injection (min)	0	15	30	45	60	120	180	240
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	37.4 ±0.2	38.7 ±0.1	37.2 ±0.4	38.3 ±0.1	38.2 ±0.1	37.2 ±0.3	37.4 ±0.2	37.6 ±0.2
Saline 10.0 ml/kg s.c. + nalorphine 2.0 µg i.c.v.	—	—	36.7 ±0.2	37.7 ±0.2	38.2 ±0.1	37.5 ±0.3	37.4 ±0.4	37.9 ±0.2
Morphine 10.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	36.4 ±0.2	34.8 ±0.2	35.3 ±0.1	35.5 ±0.1	36.4 ±0.4	38.1 ±0.3	38.3 ±0.3
Morphine 10.0 mg/kg s.c. + nalorphine 2.0 µg i.c.v.	—	—	35.2 ±0.4	36.4 ±0.5	36.8 ±0.3	37.6 ±0.2	38.6 ±0.2	38.5 ±0.3

n = 5.

FIG. 77

	Mean oesophageal temperature (°C), ±s.e.							
Time after s.c. Injection (min)	0	15	30	45	60	120	180	240
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	37.7 ±0.3	38.0 ±0.4	36.8 ±0.1	37.0 ±0.2	37.5 ±0.1	37.5 ±0.2	37.8 ±0.2	38.2 ±0.1
Saline 10.0 ml/kg s.c. + NA 10.0 µg i.c.v.	—	—	38.4 ±0.6	38.1 ±0.3	37.3 ±0.3	37.7 ±0.1	37.6 ±0.2	37.6 ±0.3
Morphine 10.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	36.5 ±0.2	35.1 ±0.1	34.9 ±0.1	35.1 ±0.1	36.6 ±0.4	38.1 ±0.1	38.2 ±0.1
Morphine 10.0 mg/kg s.c. + NA 10.0 µg i.c.v.	—	—	37.1 ±0.3	37.0 ±0.2	36.4 ±0.1	33.5 ±0.6	36.8 ±0.6	38.0 ±0.3

n = 5.

FIG. 78

	Mean oesophageal temperature (°C), ±s.e.							
Time after s.c. injection (min)	0	15	30	45	60	120	180	240
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	39.5 ±0.2	39.4 ±0.3	38.1 ±0.1	39.0 ±0.2	39.1 ±0.1	37.4 ±0.6	38.5 ±0.2	38.5 ±0.2
Saline 10.0 ml/kg s.c. + DA 50.0 µg i.c.v.	—	—	38.0 ±0.2	36.7 ±0.6	37.9 ±0.5	38.6 ±0.3	38.5 ±0.1	37.7 ±0.4
Morphine 10.0 mg/kg s.c. + saline 10.0 µl i.c.v.	—	37.3 ±0.5	36.6 ±0.3	37.1 ±0.2	37.3 ±0.2	37.8 ±0.2	39.4 ±0.2	38.9 ±0.3
Morphine 10.0 mg/kg s.c. + DA 50.0 µg i.c.v.	—	—	35.5 ±0.4	34.9 ±0.7	36.1 ±0.7	38.2 ±0.3	39.4 ±0.2	37.9 ±0.1

n = 8.

FIG. 79

	Mean oesophageal temperature (°C), ±s.e.							
Time after s.c. injection (min)	0	15	30	45	60	120	180	240
Saline 10.0 ml/kg s.c. + saline 10.0 µl i.c.v.	37.3 ±0.2	37.6 ±0.2	36.5 ±0.1	37.3 ±0.1	37.5 ±0.1	37.2 ±0.2	37.7 ±0.2	37.8 ±0.2
Saline 10.0 ml/kg s.c. + nalorphine 2.0 µg i.c.v.	—	—	36.3 ±0.2	37.3 ±0.2	37.3 ±0.2	37.0 ±0.2	37.0 ±0.2	36.9 ±0.3
Oxotremorine 0.1 mg/kg s.c. + saline 10.0 µl i.c.v.	—	34.2 ±0.2	32.5 ±0.3	31.6 ±0.5	31.8 ±0.4	35.3 ±0.3	36.7 ±0.2	37.1 ±0.2
Oxotremorine 0.1 mg/kg s.c. + nalorphine 2.0 µg i.c.v.	—	—	32.8 ±0.2	31.9 ±0.3	32.0 ±0.5	35.9 ±0.3	37.3 ±0.2	37.6 ±0.1

n = 10.

FIG. 80

Time after reserpine injection (days)	Mean oesophageal temperature (°C), ±s.e.
0	38.7 ± 0.1
1	25.8 ± 0.7
2	35.0 ± 0.4
3	34.9 ± 0.2
4	36.8 ± 0.2
5	36.5 ± 0.3
6	36.8 ± 0.3

n = 10.

APPENDIX B

DRUGS USED

DRUG AND SALT		SUPPLIER
Adrenaline acid tartrate	:	Sigma Ltd., London.
Carbachol chloride	:	B.D.H. Ltd., London.
Creatinine sulphate	:	Sigma Ltd., London.
"Crystamycin Forte"	:	Glaxo Laboratories Ltd., Greenford.
Dopamine hydrochloride	:	Sigma Ltd., London.
5-Hydroxytryptamine creatinine sulphate complex	:	Sigma Ltd., London.
Lysergic acid diethylamide tartrate (LSD-25)	:	Sandoz Ltd., Basle, Switzerland; a gift from Dr. M. Mitchard.
Morphine hydrochloride	:	A gift from May & Baker Ltd., Dagenham.
Nalorphine hydrobromide	:	Burroughs Wellcome & Co., London.
Naloxone hydrochloride	:	A gift from Dr. H. Blumberg, Endo Laboratories Inc., Garden City, New York, to Dr. C.G. Sparkes.
Noradrenaline bitartrate monohydrate	:	Sigma Ltd., London.
Oxotremorine oxalate	:	A gift from Dr. Nils Sterner, A.B. Ferrosan, Malmö, Sweden, to Dr. P.S.J. Spencer.
Pentazocine lactate	:	A gift from Dr. M. Boardman, Sterling-Winthrop Laboratories, Surbiton, Surrey.
Phenyl-*p*-quinone	:	Sigma Ltd., London.
Physostigmine sulphate	:	B.D.H. Ltd., London.
Pontamine sky blue	:	B.D.H. Ltd., London.
Procaine hydrochloride	:	B.D.H. Ltd., London.
Reserpine	:	B.D.H. Ltd., London.
Tetrabenazine	:	A gift from Dr. M.W. Parkes, Roche Products Ltd., Welwyn Garden City, to Dr. M.D. Day.

SECTION SIX

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