

"A COMPARISON BETWEEN THE PHARMACOLOGICAL  
RESPONSIVENESS OF SENSORY NERVE ENDINGS  
AND SYMPATHETIC GANGLION CELLS"

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SUMMARY

The effects of a number of agonists, antagonists and local anaesthetics on electrical activity in fibres of the rabbit saphenous nerve in situ, and on the surface potential of the isolated superior cervical ganglion of the rat have been studied.

Acetylcholine, carbachol, methacholine, pilocarpine, McN A343, nicotine, tetramethylammonium, dimethylphenylpiperazinium, histamine, 5-hydroxytryptamine, bradykinin and angiotensin induced an afferent discharge in fibres of the rabbit saphenous nerve on intra-arterial injection into the skin. The same compounds depolarized the isolated ganglion, although the rank orders of potency differed somewhat in the two preparations. The use of the appropriate agonists and antagonists showed that the cholinceptive sites could be subdivided into muscarinic and nicotinic receptors, the latter predominating in both tissues.

Adrenaline and noradrenaline first augmented and then depressed the acetylcholine-induced discharge in the saphenous nerve; these effects were mediated via  $\alpha$ -adrenoreceptors. Isoprenaline produced a prolonged small increase in the acetylcholine-induced discharge and this effect was mediated via  $\beta$ -adrenoreceptors. In the isolated ganglion, adrenaline and noradrenaline produced a weak,  $\alpha$ -receptor-mediated hyperpolarization.

Drugs possessing a nicotinic action gave rise to a secondary hyperpolarization of the ganglion cells which followed the initial depolarization on washing the tissue. Some evidence was obtained suggesting that this hyperpolarization was not a rebound phenomenon consequent upon the initial depolarization, but was due to the release of catecholamine within the ganglion. Drugs with a

(ii)

muscarinic action gave rise to a late post-washing depolarization which appeared to be mediated via muscarinic receptors.

Guanethidine blocked the action of acetylcholine on the saphenous nerve and on the ganglion, but only in the former preparation did the effect resemble the adrenergic neurone blocking action of the drug in being reversed by dexamphetamine.

No evidence was forthcoming to support the concept of the existence of a synaptic gap associated with sensory endings: natural touch responses were still present at a time when drug-induced discharges had been extinguished by antagonistic agents.

A brief study was made of the effect of local anaesthetics and drugs with local anaesthetic properties upon both preparations. It is suggested that the rabbit saphenous nerve preparation may provide an additional means of assessing the potency and durability of local anaesthetic agents.

The results are discussed in relation to the effects of the drugs on other non-myelinated neuronal membranes.

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INTRODUCTION

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The theory that transmission across peripheral synapses and neuro-effector junctions in mammals is mediated by the liberation of chemical substances from the nerve endings is now firmly established. For reviews of the evidence supporting this theory see Minz (1955); Perry (1956, 1957); McLennan (1963); Eccles (1964b) and Volle (1966a). The chemical transmitters involved in the peripheral nervous system are acetylcholine, released from cholinergic nerve endings, and noradrenaline or adrenaline, released from adrenergic nerve endings, the terms 'cholinergic' and 'adrenergic' having been coined by Dale (1914) to denote the nerve fibres liberating one or other of these transmitters.

The nerve endings in autonomic ganglia are cholinergic. Acetylcholine, released by nerve impulses from the presynaptic nerve endings, diffuses across the synaptic gap and reacts with receptor sites on the post-synaptic cell membrane. The combination of acetylcholine with these receptor sites produces a localized increase in the permeability of the post-synaptic membrane to small ions, including sodium, potassium and calcium. The post-synaptic membrane is consequently depolarized, and the synaptic potential thereby generated initiates propagated nerve impulses in the post-ganglionic neurone.

The nerve endings at neuro-effector junctions may be cholinergic or adrenergic. Acetylcholine, adrenaline or noradrenaline released from the nerve endings may depolarize, and therefore excite the post-junctional effector cell, or, by producing a selective increase in the permeability to some ions (probably potassium and chloride) they may hyperpolarize, and therefore inhibit the post-junctional effector cell. Most

physiologists believe that synaptic transmission in the central nervous system, at least in mammals, is also brought about mainly chemically, although evidence as convincing as that for peripheral junctions is lacking, and, with few exceptions, the identity of the central transmitters is unknown (see review by Crossland, 1967).

In the classical view of the mechanism of chemical transmission, the chemical agents act specifically on the post-junctional membrane. However, evidence, in many cases of an indirect nature, is accumulating to suggest that chemical transmitters, as well as other substances, are also capable of acting on the nerve endings, and some workers, notably Burn & Rand (1958), Koelle (1962a) and Riker and his co-workers (Riker, Roberts, Standaert, Fujimori, 1957; Riker, Werner, Roberts & Kuperman, 1959a & b) have attempted to formulate modified theories of junctional transmission which incorporate pre- as well as post-junctional transmitter function. In the central nervous system, the mechanism of presynaptic inhibition (Eccles, 1964 a & b) implies an action of a chemical transmitter on nerve endings. In the peripheral nervous system, a similar presynaptic mechanism of inhibition may be present in parts of the intestine, at least in some species. Evidence from electron microscope studies (Norberg, 1964; Jacobowitz, 1965; Norberg & Sjöqvist, 1966) shows that the adrenergic fibres in the gut embrace the myenteric plexus without directly innervating the smooth muscle, and Paton & Vizi (1969) have shown that noradrenaline reduces the output of acetylcholine from the isolated guinea pig ileum. Numerous biologically active substances are present in the central



nervous system (for reviews see Salmoiraghi, Costa & Bloom, 1965; McLennan, 1963; Eccles, 1964b and Crossland, 1967). It is unlikely that most of them fulfil a true transmitter function, although some of them may act as local hormones to modulate synaptic transmission, and their site of action may be pre- or post-synaptic.

It may be that all nerve membranes are sensitive to the same chemical agents, and differ only quantitatively in their responses. The experiments described in this thesis were made to compare the pharmacological reactivity of two examples of nervous tissue - ganglion cells and sensory nerve endings - in order to see whether any generalizations might be made with regard to their sensitivity to chemical agents. A brief review of published work on the actions of chemical agents on the relevant nervous structures is given below.

#### SYMPATHETIC GANGLIA

##### 1. Acetylcholine and related drugs

The stimulant and depressant effects of nicotine were first described by Langley and his co-workers (1891, 1893, 1898, Langley & Dickinson, 1889; Langley & Sherrington, 1891), who demonstrated that surface application of the drug to sympathetic ganglia initially imitated, and later suppressed, the response of various end-organs to electrical stimulation of preganglionic nerves. These effects of nicotine were limited to synaptic regions. Tetramethylammonium (Paton & Perry, 1953; Pascoe, 1956; Ginsborg & Guerrero, 1964) has been shown to resemble nicotine in its action on ganglion cells.

The nicotine-like actions of choline and several of its esters were described by Dale (1914), who at this time also made

the distinction between 'nicotinic' and 'muscarinic' actions of acetylcholine. Nicotinic actions were produced at ganglion cells, whereas muscarinic effects were believed to be confined to autonomic neuro-effector junctions. Feldberg & Gaddum (1934) and J.C. Eccles (1935) demonstrated that acetylcholine, injected into the fluid perfusing the superior cervical ganglion of the cat, caused stimulation of the ganglion, and that curare blocked the response both to injected acetylcholine and to nerve stimulation. Atropine was without blocking effect on ganglionic transmission. These results suggested that the cholinceptive sites present in ganglion cells are of the 'nicotinic' type, and that acetylcholine released by nerve impulses from the presynaptic fibres effects transmission only by acting upon these nicotinic receptor sites. Later work, including the use of ganglion blocking drugs such as hexamethonium (Perry, 1957; Paton & Zaimis, 1952; Perry & Reinert, 1954), has confirmed that the physiological transmission process in autonomic ganglia mainly involves nicotinic receptor sites. However, nicotinic sites are not the only cholinceptive receptors present as became apparent as early as 1932 when Koppányi demonstrated that pilocarpine stimulates the superior cervical ganglion of the cat, and that this effect is blocked by atropine. Muscarine has also been shown to stimulate the cat superior cervical ganglion (Ambache, Perry & Robertson, 1956; Jones, 1963; Jones & Trendelenburg, 1963; Sanghvi, Murayama, Smith & Unna, 1963; Sanghvi & Unna, 1963) and its effect is blocked by atropine, whereas hexamethonium is ineffective. A similar atropine-sensitive effect is produced by other agents that are believed to be specifically muscarinic in their type of action (Roszkowski,

1961; Sanghvi, Murayama, Smith & Unna, 1963; Franko, Ward & Alphin, 1963; Pappano & Volle, 1963; Takeshige, Pappano, De Groat & Volle, 1963). Acetylcholine itself and many related esters stimulate ganglion cells both muscarinically and nicotinicly (Herr & Gyermek, 1960).

Although the muscarinic receptor sites do not appear to play an important role in synaptic transmission, their stimulation by transmitter acetylcholine can be demonstrated, especially after treatment with anticholinesterase agents. Volle and his co-workers (Volle & Koelle, 1961; Takeshige & Volle, 1962, 1963) have shown that, in the presence of an anticholinesterase, repetitive stimulation of the preganglionic cervical sympathetic trunk produces a double response from the ganglion cells composed of an initial tubocurarine-sensitive burst of potentials and a secondary atropine-sensitive one. It may be that the muscarinic receptor sites are protected by a cholinesterase barrier, and that activation of them is severely limited in extent unless stable stimulant drugs are used, or cholinesterase is inhibited. Nevertheless, R.M. Eccles (1952) and R.M. Eccles & Libet (1961), who studied the isolated superior cervical ganglion of the rabbit using electrophysiological recording techniques, were able to demonstrate the activation of both types of cholinceptive receptor by the transmitter released by preganglionic nerve volleys. In their experiments, preganglionic volleys gave rise to a complex waveform from the ganglion cells. This took the form of an initial negative potential (N), a positive potential (P), and a late negative potential (LN). That all three potentials were evoked by acetylcholine was indicated by the

fact that all of them were absent after treatment with Botulinum toxin. Curare blocked the initial N potential but not the P or LN potentials, whereas atropine blocked the P and LN potentials but was without effect on the N potential. The P potential was also blocked by the  $\alpha$ -adreno-receptor blocking drug, dibenamine, and the significance of this result is referred to below, under the sub-heading 'catecholamines'.

## 2. Histamine

The stimulant action of histamine on the superior cervical ganglion of the cat was demonstrated by Trendelenburg (1954). Its effect was not blocked by hexamethonium or small doses of atropine, but was antagonised by cocaine, morphine or mepyramine. Ganglia which have been subjected to a brief period of preganglionic stimulation react more strongly to histamine (Trendelenburg & Jones, 1965). Lewis & Reit (1966) confirmed the stimulant action of histamine on the superior cervical ganglion of the cat, and showed that tachyphylaxis develops with repeated dosage.

## 3. Angiotensin and bradykinin

These two polypeptides have been shown to stimulate the superior cervical ganglion of the cat, angiotensin being by far the more potent. Similar effects are produced after the preganglionic nerve has been sectioned and allowed to degenerate, showing that the drugs act on the post-synaptic membrane (Lewis & Reit, 1965, 1966), though not necessarily exclusively so. Angiotensin has also been shown to facilitate the transmission of nerve impulses through the ganglion (Machova & Boska, 1967). The activities of bradykinin and angiotensin on the superior cervical ganglion of the cat are unaffected by hexamethonium or atropine, indicating

that the known ganglionic cholinceptive sites are not involved in their action. Tachyphylaxis to repeated addition of either polypeptide occurs, but there is no cross-tachyphylaxis between them, suggesting that the two polypeptides do not act on the same receptor sites. Recent work has shown that the action of angiotensin is blocked by the antihistamine drug mepyramine, and that cross-tachyphylaxis between histamine and angiotensin can occur, suggesting that the action of angiotensin is in some way mediated through histamine or through receptors activated by histamine (Lewis & Reit, 1966).

Species differences in sensitivity to the ganglionic actions of the two polypeptides exist, bradykinin exerting only a very weak stimulant action on the superior cervical ganglia of dogs and rabbits. In the rabbit, angiotensin produces a similar weak effect, but it is inactive on the superior cervical ganglion of the dog (Lewis & Reit, 1966).

#### 4. 5-Hydroxytryptamine

Very little 5-HT is detectable in most sympathetic ganglia, despite the fact that in the cat these ganglia contain more 5-hydroxytryptophan decarboxylase than does any other nervous tissue (Amin, Crawford & Gaddum, 1954; Gaddum & Giarman, 1956). However, histological investigation of the rat superior cervical ganglion by Eranko & Harkonen (1965) demonstrated the presence of non-chromaffin amine-storing granules, and these authors suggested that the granules contain high concentrations of monoamine, possibly 5-HT.

Robertson (1954) showed that small doses of 5-HT stimulate the perfused superior cervical ganglion. This was confirmed by

Trendelenburg (1956a) who went on to show that the effect is prevented by cocaine, but not by hexamethonium, atropine or mepyramine. A similar stimulant effect is produced in the inferior mesenteric ganglion of the cat, this effect being blocked by morphine, cocaine, lysergic acid diethylamide and phenoxybenzamine (Gyermek & Bindler, 1962). 5-Hydroxytryptamine also stimulates the cells of the rat stellate ganglion, and in sub-stimulant doses facilitates transmission through the ganglion. These effects can be blocked by 5-HT antagonists (Hertzler, 1961).

These results indicate that specific tryptamine receptors are present on some ganglion cells.

#### 5. Catecholamines

That catecholamines are capable of both facilitating and inhibiting sympathetic ganglionic transmission has been known for many years. Bulbring & Burn (1942) reported a facilitatory effect on transmission through the superior cervical ganglion of the cat with small doses of adrenaline, and Bulbring (1944) noted that the response of the perfused ganglion to acetylcholine was also augmented. Birks & MacIntosh (1961) found that the facilitatory effect of adrenaline was accompanied by an increased release of acetylcholine from the presynaptic nerve endings, so that the effect appears to involve both pre- and post-synaptic components. Facilitation of transmission by small amounts of noradrenaline was reported by Trendelenburg (1956b), this amine being more effective than adrenaline.

Marrazzi (1939) first showed that larger amounts of adrenaline inhibited ganglionic transmission and this has been confirmed for both adrenaline and noradrenaline by other workers (Bulbring, 1944;

Trendelenburg, 1956b; Matthews, 1956; Costa, Revzin, Kuntzman, Spector & Brodie, 1961; Pardo, Cato, Gijón & Alonso-de Florida, 1963; Weir & McLennan, 1963; McIsaac, 1966; Volle, 1966a).

Bülbring (1944) showed that in contrast to smaller doses, larger doses of adrenaline depressed the response of the ganglion cells to acetylcholine.

De Groat & Volle (1965) and Volle (1966a) examined the ganglionic response to catecholamines using electrophysiological techniques. They showed that inhibition of ganglionic transmission produced by adrenaline or noradrenaline is accompanied by an increase in the demarcation potential of the ganglion cells. This effect was not reproducible with isoprenaline. The hyperpolarization produced by adrenaline and noradrenaline was antagonised by  $\alpha$ -adrenoreceptor blocking drugs, such as dihydroergotamine, but was enhanced by  $\beta$ -adrenoreceptor blocking drugs, such as dichloroisoprenaline and pronethalol. After dihydroergotamine, administration of adrenaline and noradrenaline produced ganglionic depolarization which accompanied an enhancement of transmission. De Groat & Volle (1966a, b) concluded that there are two pharmacologically distinct adrenoceptive elements; the  $\alpha$ -site produces hyperpolarization and hence depression of ganglionic transmission and of applied acetylcholine, whereas the  $\beta$ -site induces depolarization thereby facilitating transmission. However, there is evidence that adrenaline may also inhibit transmission by a presynaptic action which results in a decrease in acetylcholine release (Paton & Thompson, 1953; Birks & McIntosh, 1961).

Eccles & Libet (1961) claimed that the P potential which

they recorded is mediated by catecholamines. According to them, nerve stimulation releases acetylcholine which acts on catecholamine stores to liberate an adrenaline-like substance, and this then reacts with specific (P) receptors on the post-synaptic surface; this effect presumably corresponds with the hyperpolarizing effect later recorded by Volle. The P potential is blocked by atropine-like antagonists of acetylcholine acting prior to the catecholamine store, or by  $\alpha$ -adrenoreceptor blocking drugs acting beyond it. The catecholamine cannot be liberated independently of presynaptic acetylcholine release, as evidenced by the total block induced by Botulinum toxin.

Reinert (1963) demonstrated the presence of noradrenaline in the isolated perfused superior cervical ganglion of the cat both at rest and following stimulation of the post-ganglionic nerve. He concluded that this noradrenaline arises from the post-ganglionic adrenergic elements, and he did not consider that under physiological conditions it affects the release of acetylcholine or its action on the post-synaptic membrane. This view differed from an earlier one of Dempsher, Tokumaru & Zabara (1959) who used the sympathetic ganglion of the cat infected with pseudorabies virus to induce hyper-irritability of the nerve endings. They claimed that, following electrical stimulation, acetylcholine is liberated preganglionically and acts on preganglionic excitatory or inhibitory fibres: these then release either excitatory or inhibitory transmitters which affect the post-synaptic membrane. They concluded that, while acetylcholine is responsible for excitation, inhibition may be due to adrenaline, noradrenaline, or gamma-aminobutyric acid. Additional evidence of the existence of



catecholamines in sympathetic ganglia includes the findings of Eränkö & Härkönen (1964) who suggested that some sympathetic cells could simultaneously be both adrenergic and cholinergic in the rat, and they claimed to have shown the presence of both noradrenaline and acetylcholine in the same nerve fibres innervating the rat iris. Histochemical investigations have confirmed the presence of adrenergic synaptic terminals in various autonomic ganglia in both cat and rabbit (Hamberger, Norberg & Sjöqvist, 1964; Hamberger & Norberg, 1965; Hamberger, Norberg & Ungerstedt, 1965). In the rabbit such terminals were not present in all autonomic ganglia. In the cat the presence of catecholamines has been associated with dense-core granules situated in the presynaptic endings (Clementi, Mantegazza & Botturi, 1966). It has been suggested that the catecholamine present in the ganglion acts to modulate ganglionic transmission by exerting an inhibitory effect on the basically cholinergic transmission (Hamberger, Norberg & Ungerstedt, 1965). Monoamineoxidase inhibitors block ganglionic transmission (Costa et al., 1961; Gertner, 1961) and the mechanism underlying this effect may be that they cause the accumulation of catecholamine in the ganglion and this inhibits cholinergic transmission.

Thus it is clear that sympathetic ganglia may contain a variety of chemosensitive receptor sites but, at the present time, only those involved in the nicotinic action of acetylcholine play a clear physiological role in the transmission of impulses.

#### SENSORY RECEPTORS

##### 1. Acetylcholine and related drugs

Brown & Gray (1948) showed that close arterial injection of

acetylcholine or nicotine into the skin and mesentery evoked an impulse discharge in small sensory nerve fibres. By means of their antidromic extinction technique, Douglas & Ritchie (1957b,d, 1959) showed that the afferent discharge produced by acetylcholine in the cat saphenous nerve was carried mainly in mechano-sensitive C fibres, and Fjällbrant & Iggo (1961) confirmed this finding. The last-named authors also showed that the sensory endings of myelinated fibres - hair receptors, touch receptors and high threshold mechanoreceptors - were not affected by acetylcholine. Acetylcholine and related drugs have also been shown to sensitise or excite slowly-adapting pressure receptors (Fjällbrant & Iggo, 1961), chemoreceptors in the carotid body (Dripps & Comroe, 1944; Diamond, 1955; Anichkov & Belenkii, 1963; Eyzaguirre & Koyano, 1965b) and the primary endings of muscle spindles (Granit, Skoglund & Thesleff, 1953; Smith & Eldred, 1961). Intracutaneous injection of acetylcholine in man, or application by iontophoresis, was found not to elicit any sensation (Buchtal, 1954), but a painful sensation is elicited when acetylcholine is injected intra-arterially (Harvey, Lilienthal & Talbot, 1941) or when it is applied to an exposed blister base (Armstrong, Dry, Keele & Markham, 1953). Paintal (1964) has summarized the evidence suggesting that the site of action of these drugs on sensory endings is not on the most peripheral generator region of the ending itself, but on the adjacent regenerative region which is considered to be less protected by diffusion barriers of the nerve sheath than are more central portions of the axons. Nevertheless, after desheathing, acetylcholine can be shown to affect C fibres along their length, causing a fall in resting potential, a fall in

spike height, an enhancement of the positive after-potential and a reduction in conduction velocity. These effects can be reversed by hyperpolarizing electric currents (Armett & Ritchie, 1960).

The ability of acetylcholine to affect sensory nerve fibres is shared by other drugs possessing nicotinic actions (nicotine, T.M.A., suxamethonium, carbachol) but, according to published work, not by drugs possessing only muscarinic actions (methacholine, bethanecol, pilocarpine, arecoline). The effects of the former group of drugs are blocked by hexamethonium and tubocurarine but not by atropine (Douglas & Ritchie, 1962; Ritchie, 1963). As at other sites of the nicotinic action of acetylcholine, large amounts of acetylcholine itself and of related drugs block conduction, presumably by depolarizing the membrane.

The possibility that acetylcholine plays a physiological role in the initiation of sensory impulses in the exteroceptive system has been put forward (Duner & Pernow, 1952; Liljestrang, 1954; Davis, 1961; Koelle, 1962a & b), but most authors reject this view (Harvey et al., 1941; Douglas, 1954; Diamond, 1955; Hebb & Hill, 1955; Paintal, 1956; Gray & Diamond, 1957; Gray, 1959; Douglas & Ritchie, 1962; Paintal, 1964), at least in so far as skin receptors are concerned. Histochemical evidence, too, has so far failed to confirm the existence of such a process (Loewenstein & Molins, 1958; Gerebtzoff, 1959; Giacobini, 1959; Koelle, 1962b). Part of the evidence against a physiological role for acetylcholine is that when the effects of acetylcholine are blocked by, for example, hexamethonium, the nerve is still capable of responding to its normal sensory stimulus. In the interoceptive sensory system, there may be exceptions. The sensory endings of

carotid nerve fibres form synaptic-like terminations with carotid body cells (de Castro, 1951; Biscoe & Stebhens, 1966; Hess, 1967). The presynaptic element of the junction is the carotid glomus cell which is sensitive to agents such as low  $P_{O_2}$ , high  $P_{CO_2}$  or acidity (Eyzaguirre & Koyano, 1965a & b; Eyzaguirre & Zapata, 1967, 1968a). Activation of the glomus cell subsequently excites the closely associated sensory nerve endings probably through the release of a chemical transmitter (Landgren, Liljestr and & Zotterman, 1952; Eyzaguirre, Koyano & Taylor, 1965; Eyzaguirre & Zapata, 1967). Recent experiments have provided direct evidence that the chemical transmitter involved is acetylcholine (Eyzaguirre & Zapata, 1968b).

## 2. Histamine

Histamine, along with acetylcholine, is present in the stinging nettle (Emmelin & Feldberg, 1947) and it elicits pain when applied to an exposed blister base (Armstrong et al., 1953). It has been suggested that histamine is a chemical mediator of cutaneous pain (Rosenthal, 1964) but others have rejected this view (Gilfoil & Klavins, 1965). Histamine has been found to produce a discharge in fibres from slowly-adapting cutaneous pressure receptors (Fj allbrant & Iggo, 1961) and it increases the resting discharge during expiration in some pulmonary stretch receptors (Widdicombe, 1954), but these effects may be secondary to the contractile action of the drug on smooth muscle, rather than to a direct action on the sensory nerve endings.

## 3. Angiotensin and bradykinin

Angiotensin does not appear to have been tested on sensory receptors. Bradykinin produces pain when applied to an exposed blister base (Keele, 1962), and an impure preparation of bradykinin

has been shown to excite the slowly-adapting cutaneous pressure receptors (Fjällbrant & Iggo, 1961). However, this latter effect, which had a prolonged latency, may again be secondary to some motor effect of bradykinin.

#### 4. 5-Hydroxytryptamine

5-Hydroxytryptamine is more potent than acetylcholine in producing pain when applied to an exposed blister base. It differs from acetylcholine in that its effect follows a latent period of 10-45 sec whereas that of acetylcholine is abrupt. Repeated administration of 5-HT produces diminishing responses (Ginzel & Kottegoda, 1954). Keele (1962) considered that 5-HT acts on receptors more deeply situated than those affected by acetylcholine and he suggested that this accounts for its relatively slow onset of action. Fjällbrant & Iggo (1961) showed that 5-hydroxytryptamine stimulates slowly-adapting cutaneous pressure receptors. Large doses have been shown to stimulate pulmonary stretch receptors in dogs and cats but not in rabbits (Schneider & Yonkman, 1953, 1954), and there is some evidence that it stimulates carotid baroreceptors (Ginzel & Kottegoda, 1954). It also affects gastric stretch receptors (Paintal, 1954) and mucosal mechanoreceptors (Paintal, 1957), and these effects may be important in the initiation of intestinal reflexes (Bülbring & Lin, 1957). The actions of 5-HT on spinal reflexes have been attributed to excitation of afferent nerve fibres (Curtis, Eccles & Eccles, 1955; Douglas & Ritchie, 1957a; Van Gelder, 1962).

#### 5. Catecholamines

Sympathetic stimulation or small doses of adrenaline cause sensitization and stimulation of cutaneous, bronchial and cardiovascular

sensory receptors, but in some cases at least these effects are secondary to alterations in the tone of smooth muscles in the vicinity of the receptors (for a review of the relevant literature see Paintal, 1964).

Loewenstein (1956) found that tactile receptors of frog skin are stimulated and their threshold is lowered by sympathetic stimulation or small doses of adrenaline, and Loewenstein & Altamirano-Orrego (1956) found that the threshold of the Pacinian corpuscle is lowered and adaptation is reduced. The endings of muscle spindles are first excited and subsequently depressed by adrenaline and sympathetic stimulation. The secondary depression appears to be the result of vasoconstriction and is prevented by  $\alpha$ -adrenoreceptor blocking drugs. The initial excitatory effect is not blocked by these drugs and appears to be the result of a direct action of the catecholamine on the nerve ending (Paintal, 1964).

Bülbring & Whitteridge (1941) and Goffart & Holmes (1962) found that adrenaline reduces the threshold of mammalian myelinated and non-myelinated nerve fibres, possibly as a result of changes in the membrane constants. In isolated fibres this effect is associated with an increase in the demarcation potential, particularly in C fibres (Goffart & Holmes, 1962). There is also indirect evidence that adrenaline may hyperpolarize nerve membranes in vivo. Thus Krnjević & Miledi (1958) found that adrenaline relieved the presynaptic failure of transmission which occurs in rapidly stimulated isolated nerve muscle preparations, and in a later paper (Krnjević & Miledi, 1959) they found that this effect of adrenaline on the motor nerve endings was mimicked by hyperpolarizing electric currents.

From the results described in this survey it appears that there are at least superficial similarities between the pharmacological responsiveness of ganglion cells and sensory nerve endings, and possibly other nerve membranes also. The experiments described in this thesis were designed to examine this similarity in more detail, and for this purpose the sensitivity to various pharmacological agents of the isolated superior cervical ganglion of the rat has been compared with that of sensory nerve endings in the saphenous nerve of the rabbit.





RABBIT SAPHENOUS NERVE

Most of the experiments were performed upon Californian albino rabbits of either sex, and weighing about 2.5 kg. They were anaesthetised with 6 ml/kg of 25% urethane intravenously. Tracheal and jugular cannulae were inserted, the latter being used for the systemic administration of drugs. The method of stimulating and recording from the saphenous nerve was similar to that used by Douglas & Ritchie (1960) in the cat (Fig. 1). The nerve was exposed high in the thigh and crushed by ligation. In some experiments the nerve was cut, but ligation proved satisfactory, and was preferred as the nerve could be more easily placed on the stimulating electrodes when they were required.

The knee was immobilised by a drill through the lower end of the femur (the drill shank being used as an earth link for the recording electrode input). The ankle joint was secured with a clamp in the later experiments as drills were apt to slip and produce haemorrhage.

The nerve branches near the knee joint were exposed, and bipolar platinum recording electrodes were placed beneath the branch innervating the skin around and below the front of the knee. When stimulation was employed the nerve was earthed between the stimulating and recording electrodes. The saphenous artery was traced below the knee and separated from its two laterally-associated veins. It was then cannulated with a fine polythene cannula, containing heparin (200 IU/ml) in 0.9% w/v NaCl solution, and attached to a hypodermic needle head. A loose ligature was placed under the artery a short distance below the knee. By pulling on this to occlude the artery, retrograde injections of

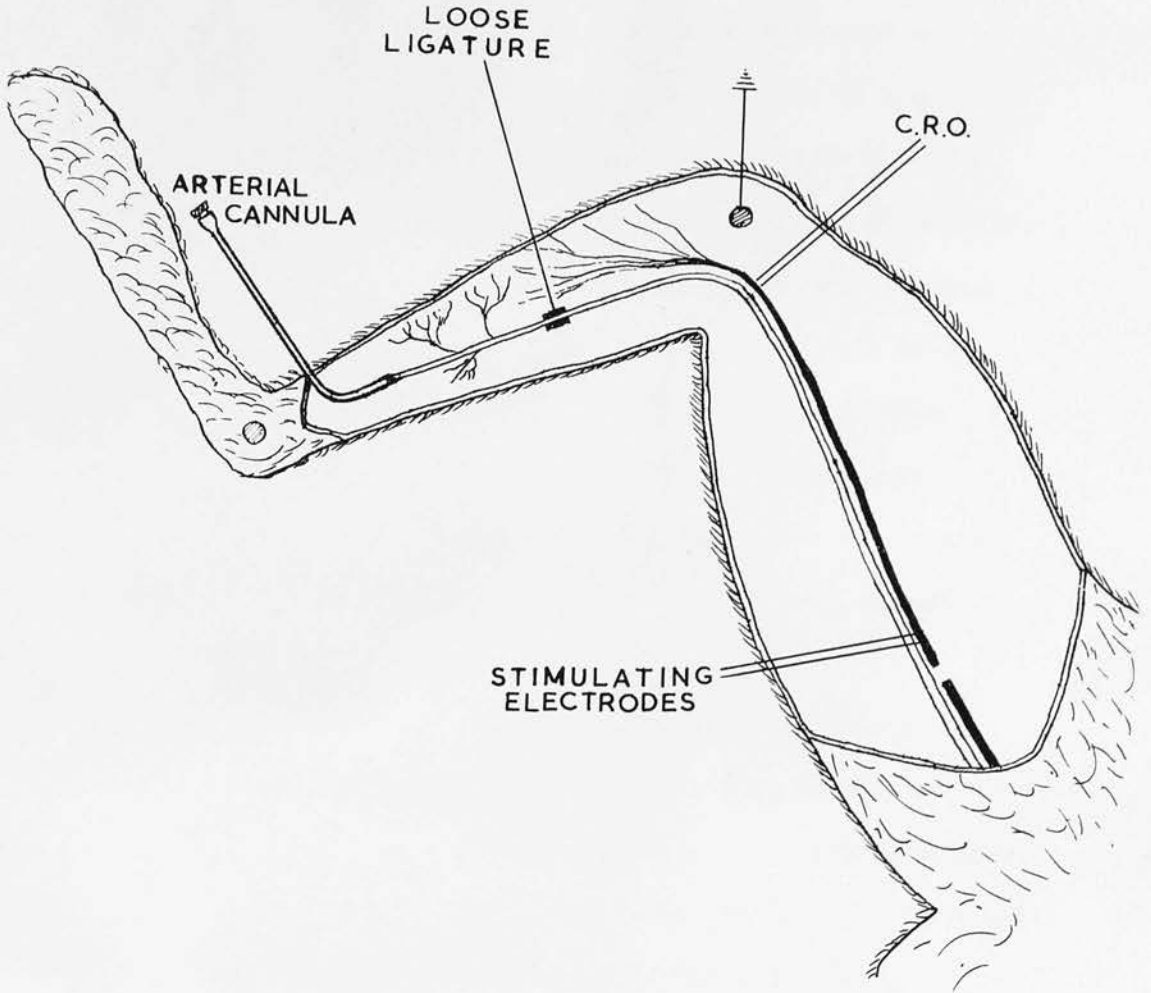


Fig. 1. Diagrammatic representation of the method used for stimulating and recording from the rabbit saphenous nerve. At the moment of injection, the loose ligature around the artery was pulled, thus diverting the injected fluid into the skin branches. The nerve was sectioned or tightly ligated centrally to the stimulating electrodes. The skin area physically stimulated lay above the branches of the saphenous nerve shown to the right of the loose ligature.

drugs could be made: on release of the ligature they were carried into the skin receptor area served by the saphenous nerve branch referred to above. The skin flaps of the upper leg were raised to form a pool around the electrodes. This pool was filled with warm mineral oil (Heavy Liquid Paraffin B.P.). After differential amplification by either a Tektronix Type 122 or Grass P8 pre-amplifier the nerve action potentials were displayed on a Tektronix 502 or 502A oscilloscope, and photographed on 35 mm film. In the early experiments the photographic record was made with a moving film and a stationary spot, but it was later found to be practicable to use a moving spot, and to change the film frame at the moment of flyback. This procedure economised considerably on both film and time taken for analysis. The time of injection, or stroking, was signalled by means of a deflection of the second (free) beam of the oscilloscope.

In some experiments bipolar platinum stimulating electrodes were placed on the trunk of the nerve, as described above, and stimulation was effected by rectangular pulses of varying strength and duration from a Tektronix 161 stimulator. The action potentials were recorded antidromically from the recording electrodes. Mechanical stimulation was produced by lightly touching the hair with a wisp of cotton wool, or with the back of the first two fingers. Though seemingly crude, the latter method yielded consistent and reproducible results. Trials with automatic mechanical strokers proved unsatisfactory due to the unwieldiness of the apparatus required and the prohibition of electrical devices within the screened experimental area. The drugs were dissolved in 0.9% w/v NaCl, and the doses quoted refer to the salts. When given

intra-arterially the volume of solution did not exceed 0.3 ml in case a massive flow over and past the receptors should occur.

#### RAT SUPERIOR CERVICAL GANGLION

The method used was that described by Pascoe (1956), Mason (1962) and Brown (1966a).

Wistar albino rats of either sex, and usually weighing 250-300 g were used. The rats were anaesthetised with 6 ml/kg of 25% urethane injected intra-peritoneally. The left superior cervical ganglion was removed in most cases: occasionally the right one was used, and there was no obvious difference in the results obtained. The ganglion was exposed, after tracheal cannulation, by forward reflection of the tracheal-oesophageal complex together with their encircling muscles. All small blood vessels hindering approach to the ganglion were cauterised. The external lateral branch of the ganglion was cut, and the ganglion, together with a few mm. of post-ganglionic nerve and a longer length of preganglionic nerve, was secured with gauge 00 thread and then transferred to Krebs' solution (previously equilibrated with 5% CO<sub>2</sub> in O<sub>2</sub>) at room temperature. Desheathing was performed with the aid of a binocular dissecting microscope, using watchmakers No.5 forceps and fine-pointed spring-loaded scissors. It was essential to remove all obvious connective tissue and at the same time the preparation was trimmed of all superficial emergent fibres. The ganglion was mounted vertically, with the post-ganglionic trunk uppermost, in a bath containing 25 ml of equilibrated Krebs' solution at room temperature (19-20°C) which was situated inside an earthed metal cabinet (see Fig. 2). The Krebs' solution used had the following

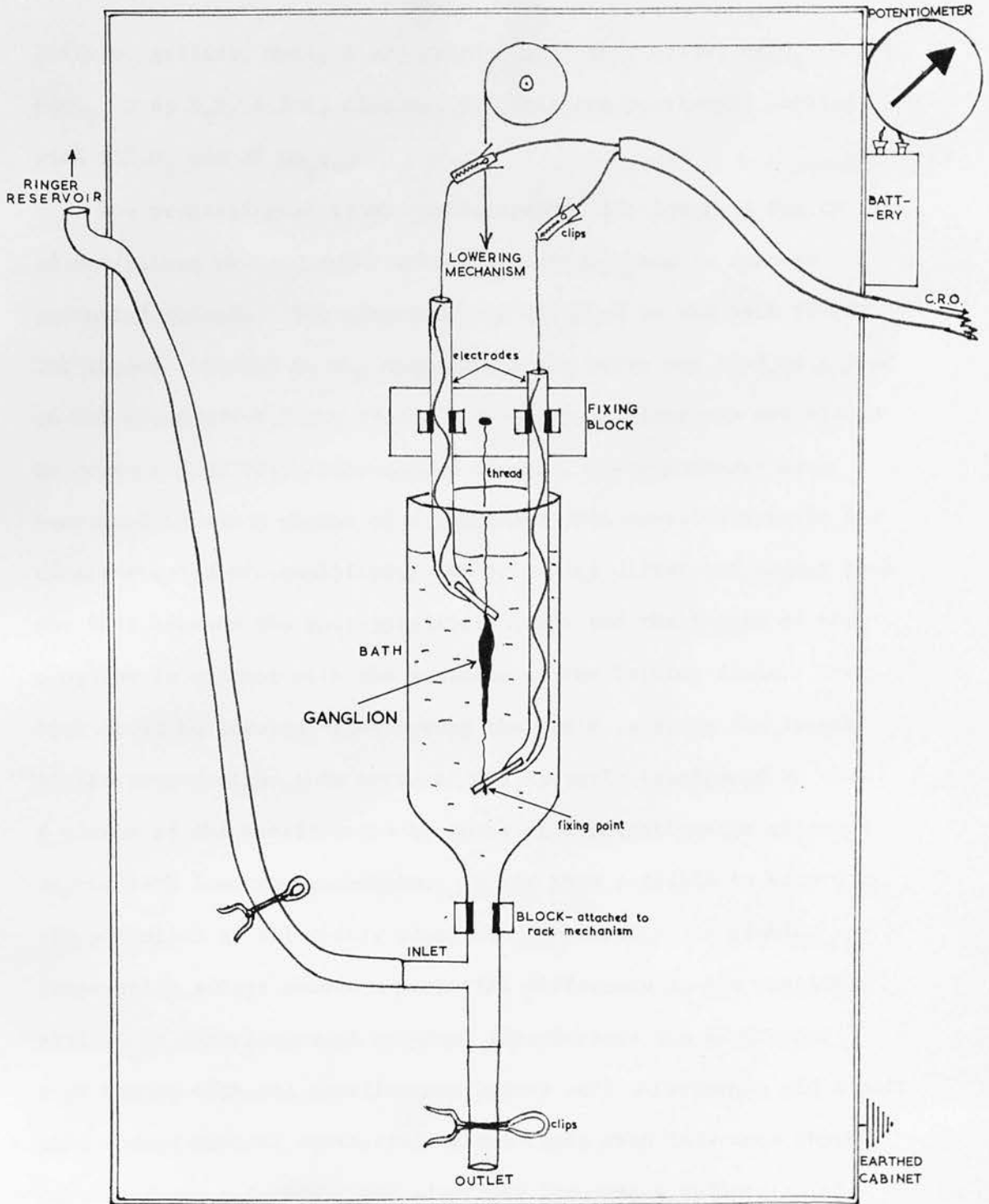


Fig. 2. Diagram of the arrangement for recording surface potentials from the rat superior cervical ganglion. When the bath was lowered the meniscus swept over the ganglion, acting as a moving electrode. The potential difference in air between the meniscus and the fixed upper electrode was recorded on the oscilloscope. The movement of the fluid was coupled to the X plates of the oscilloscope by means of the potentiometer, allowing the potential at all parts along the preparation to be recorded.

formula: g/litre, NaCl, 6.92; NaHCO<sub>3</sub>, 2.1; KCl, 0.324; MgSO<sub>4</sub>, 0.294; CaCl<sub>2</sub>, 0.6; H<sub>2</sub>O, 0.306; Glucose, 2. This was previously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

The preganglionic trunk was secured to the lower of the two silver/silver chloride/agar saline electrodes used to measure potential changes. The electrode was situated in the bath fluid. The thread attached to the post-ganglionic nerve was tied to a post on the electrode-holding block, and the upper electrode was placed in contact with this Krebs-soaked thread. The electrodes were connected to the Y plates of a Tektronix 502A oscilloscope via its direct-coupled pre-amplifier. The potential difference across them was that between the post-ganglionic trunk and the region of the ganglion in contact with the meniscus of the bathing fluid. The bath could be lowered, thus moving the meniscus along the length of the preparation: this movement was linearly transposed to the X plates of the oscilloscope by means of a potentiometer attached to the bath lowering mechanism. It was thus possible to record the potential at all points along the preparation. A viable preparation always showed a potential difference in the resting state. To ensure minimal external interference the electrodes were tested with the oscilloscope before each experiment, and a pair that showed minimal deflection of the trace when they were short-circuited was selected. Any electrode inducing a deflection of 500  $\mu$ V or more was rejected. There was often considerable variation between a given pair of electrodes from one day to another, but by retaining a stock of 5 or 6 an optimal pairing selection could always be made. (See Appendix for preparation of electrodes).

The preparation was kept at room temperature throughout, since Pascoe (1956) showed that the depolarizing effect of acetylcholine is not materially affected by temperature changes in the range of 10-30°C. Despite quite large variations in environmental temperature, the preparation never displayed discernible variability or irregularity.

Drugs were diluted to required concentrations with oxygenated Krebs' solution, and were added to the bath from a 1 ml syringe. To minimise changes in the bathing fluid the added volume was kept small, the syringe being used to mix the added drug with the bath contents by rapidly filling and discharging it twice. An amount of fluid equal to that added was finally withdrawn, and measurements then commenced.

Prior to any drug administration a control tracing was taken. This was important as the shape of the spontaneous potential difference between the post-ganglionic fibres and the body of the ganglion changes (albeit slowly) during the course of an experiment, although the drug-evoked responses remain remarkably consistent.

Carbachol was used throughout as the reference drug, since, unlike acetylcholine, it is not significantly affected by cholinesterases in the ganglion. The standard concentration adopted throughout was 12 µg/ml. This did not produce maximal depolarization, but was sufficient to ensure penetration and depolarization within the exposure time.

Fresh preparations were made for each experiment, and every experiment was usually confined to the study of one drug and its action and interactions with carbachol or with a specific antagonist, since it was felt that otherwise there might have been residual

effects from a previously added substance. The test method was initially based on a 47 min cycle. This comprised 2 min exposure to the drug, with readings being taken at 30 sec, 60 sec and 2 minutes. During the first 30 sec the depolarization was rapid, but it became slower thereafter: it was not fully complete at 2 min, and this effectively ensured that the control response was large and measurable, but not maximal, as could be proved clearly when larger doses of carbachol, tetramethylammonium or acetylthiocholine were employed. The bath was next drained and filled twice (effectively producing a double wash). Further readings were taken 3, 5, 10 and 15 min after these washes. The disproportionate time before the first reading was necessary to allow the bath level to be reset accurately so that the fluid volume remained constant. This was achieved by means of reference points on the resting potential trace. In most experiments the progress of the recovery phase was observed without taking records other than those quoted above: any drug which showed a transient effect after washing could thus be detected, and additional records made if the effect was considered relevant. At 15 min after washing the bath was again emptied and refilled, and the preparation rested for 30 min, at which time a control trace was recorded and the next drug addition made without further washing.

After the first 15 experiments it was found practicable to shorten the recovery phase to 15 min. This was more satisfactory because it meant that the bath fluid was changed at roughly equal time intervals, and it also allowed more tests to be made during an experiment. This 32 min cycle had to be varied occasionally for some drugs which exhibited sustained effects so preventing a return



to the control resting level in the normal time.

The Krebs' solution was kept in a reservoir outside the metal cabinet, and continuously bubbled with the 95% O<sub>2</sub>/5% CO<sub>2</sub> mixture. Random checks with a Pye Dynocap pH meter indicated that the pH was well maintained at an average value of 7.2. No more than 3 litres was prepared at any one time, thus ensuring freshness throughout.

The potential was displayed simultaneously on the oscilloscope screen and on a slave tube. A film record was made of the former, while the latter was used to make an immediate estimate (with reasonable accuracy, as confirmed by the film record) of changes in potential. The analysis of the film records was performed with a Durst enlarger, superimposing the traces in each series of drug applications (i.e. during the 32 min cycle). The concentrations of drugs used in these experiments refer to the salts.

#### Histological Examination

The ganglia, suspended by a linen thread and with a light weight (of plasticine) on the opposite end to keep them extended, were fixed in 5% formol saline for 48-72 hours. After fixation they were washed two or three times in distilled water and then placed in saturated acid fuchsin for 24 hours; at the end of this time they were again washed in distilled water. They were then differentiated in 0.1% alcoholic potassium hydroxide, and finally washed in a dilute solution of hydrochloric acid (1 part HCl to 5 parts distilled water). Following this, the stained intact tissue was processed through 50, 60, 70, 80, 95% and absolute alcohol (74 O.P. Spirit). It was then cleared in chloroform (2 changes), followed by three changes of wax, and finally

embedded in polyester resin wax. Sections were cut at 5 microns thickness using an M.S.E. base sledge microtome.

#### GUINEA PIG WHEEL TEST FOR LOCAL ANAESTHETIC ACTIVITY

A modification of the method described by Bülbring & Wajda (1945) was used. This method has the advantage that several drugs may be tested on the same animal and that the course of local anaesthesia, both in terms of efficacy and duration, can be followed.

The method, as originally described, depends on a fixed end point of 30 minutes at which time the number of negative responses in a series of 36 test stimulations is calculated. In the present experiments the tests were continued until full recovery had occurred. To do this with high dose levels the time interval had to be extended between tests, in order to avoid excessive damage to the skin caused by the repeated pin-pricking. Full recovery was admitted when the animal responded 6 times out of 6, and continued to do so thereafter.

For convenience, in the small animals available, the dose volume was reduced to 0.1 ml, instead of the 0.25 ml originally specified. It was thus possible to test four drugs plus a saline control each time. On several occasions the same strength of solution was used on more than one animal: as there was variability in sensitivity between animals, it appeared advantageous to pool all the results of a single dose for the final analysis. The positions of the drugs in the skin were altered in every experiment, to minimise any differences in the sensitivity of skin areas.

Albino guinea pigs were used throughout, and both shaving or depilatory creams were used on the day previous to testing.

The latter appeared to be rather more satisfactory. No sex differences were apparent. In some experiments 'masked' administration was employed - the tester had no knowledge of the contents of the five skin blebs: these results did not differ from the others.

The results were expressed in terms of a) comparative potency of the drug as judged at a set time of 20 min (to include some of the weaker solutions), b) duration.

#### DRUGS AND SOLUTIONS

Acetylcholine chloride (Roche), acetyl- $\beta$ -methylcholine chloride (methacholine) (Sigma), acetylthiocholine iodide (British Drug Houses), adrenaline acid tartrate (British Drug Houses), ambenonium chloride (Sterling Winthrop), angiotensin amide (Ciba), aspirin (calcium) (Boots), atropine sulphate (British Drug Houses), bradykinin (gift from Sandoz), carbachol chloride (British Drug Houses), cocaine hydrochloride (British Drug Houses), dexamphetamine sulphate (British Drug Houses), dibenamine hydrochloride (Koch-Light), dichloroisoprenaline chloride (Lilly), 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) (K & K Laboratories), guanethidine sulphate (gift from Ciba), hexamethonium bromide (May & Baker), histamine acid phosphate (British Drug Houses), 5-hydroxytryptamine creatinine sulphate (British Drug Houses), hyoscine hydrobromide (British Drug Houses), isoprenaline sulphate (Burroughs Wellcome), lignocaine hydrochloride (Astra-Hewlett), McNeil A 343 chloride (gift from McNeil Laboratories), mecamlamine hydrochloride (gift from Merck, Sharp & Dohme), mepyramine maleate (May & Baker), methysergide maleate (Sandoz), neostigmine methyl sulphate (Roche), nicotine hydrogen tartrate (British Drug Houses), (-)noradrenaline

bitartrate (Koch Light), phentolamine methane-sulphonate (Ciba), phenylbutazone (Geigy), physostigmine salicylate (British Drug Houses), pilocarpine nitrate (British Drug Houses), procaine hydrochloride (British Drug Houses), pronethalol hydrochloride (Imperial Chemical Industries), propranadid (gift from Bayer), propranolol hydrochloride (Imperial Chemical Industries), sodium salicylate (British Drug Houses), tetraethylammonium chloride (British Drug Houses), tetramethylammonium chloride (British Drug Houses), tubocurarine chloride (Burroughs Wellcome).

Stock solutions were made up in distilled water, and diluted before use in 0.9% w/v sodium chloride for experiments on the rabbit, and in the Krebs' solution for the experiments on the rat ganglion.

Doses used refer to the weight of the salt.

SECTION 1

Rabbit Saphenous Nerve

RESULTS

RABBIT SAPHENOUS NERVECompound action potential

Electrical stimulation of the whole saphenous nerve trunk with rectangular pulses evoked a compound action potential which was recorded antidromically as illustrated in Fig. 3. Weak stimulation selectively evoked a group of potentials from two types of A fibres, conducting at about 44 and 8 m/sec (Fig. 3a, b). Increasing the strength of the stimulus evoked a large C fibre component, conducting at about 0.9 m/sec (Fig. 3c). Both A and C fibre components were also visible when the recording electrodes were placed on the saphenous nerve below the knee, but when they were on the small nerve branch innervating the skin around the knee, the A fibre component was very small, the main deflection being due to C fibres. In all the experiments involving drugs or stroking, the recording electrodes were placed on this small nerve.

Response to stroking

Lightly stroking the skin over the knee and the leading aspect of the lower leg produced a discharge of nerve impulses (see Fig. 16a). This discharge largely extinguished the antidromic C fibre potential evoked by electrical stimulation, showing that the discharge was partly carried by C fibres. Douglas & Ritchie (1960) described similar results using the cat saphenous nerve. They had previously found (Douglas & Ritchie, 1957b, c) that C fibres are chiefly concerned with sensations of touch and light pressure.

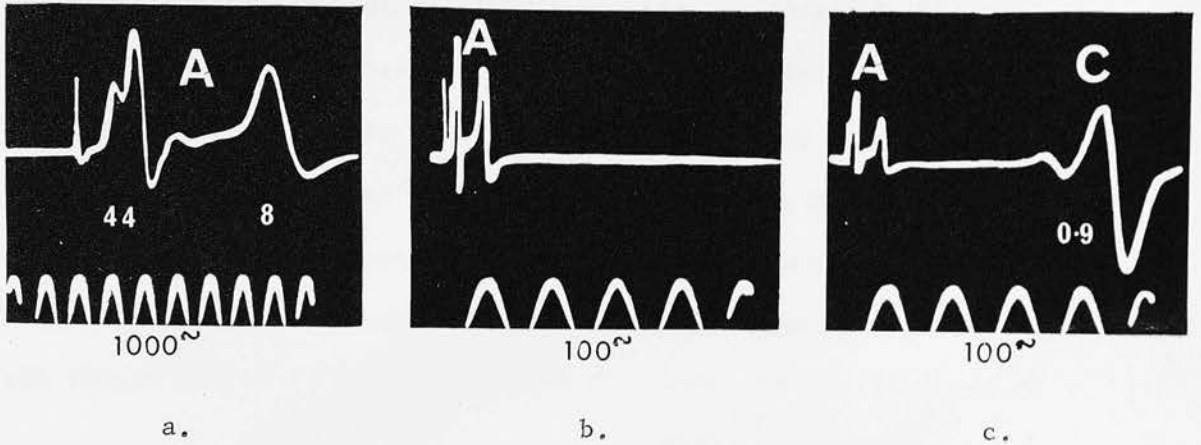


Fig. 3. Compound action potential: rabbit saphenous nerve. The nerve was stimulated with rectangular shocks and the compound action potential was recorded antidromically at a distance of 4 cm. from the stimulating electrodes. a. and b. show the A fibre potentials evoked by a rectangular pulse of 100  $\mu$  sec duration. b. is the same as a. but with a slower time base to show the absence of a C fibre component with these stimulus parameters. For c. the strength of the stimulus was doubled and the duration of the pulse was increased to 0.5 msec. With these stimulus parameters a large C fibre component was excited. The gain for c. was half that for a. and b. The numbers below the potentials denote the approximate conduction velocities in m/sec.

In the course of over 100 experiments the intensity of the recorded reaction to stroking varied considerably. It was usually present to some degree, but occasionally it was difficult to distinguish from the basal spontaneous firing which always occurred as long as the animal survived. However, on most occasions the reaction to stroking was very brisk. Variations in sensitivity probably exist between different rabbits, just as there is threshold variability of sensitivity in man, as judged by the radiant heat and other test methods (Hardy, 1962; Watson, unpublished observations). If liquid paraffin accidentally came into contact with the fur, it produced a cohesion of hairs and a marked depression of response. This suggests that the mechanoreceptors involved were activated by movements of the hairs rather than by touching the skin.

#### Responses to other stimuli

Unlike the same nerve in the cat (Douglas & Ritchie, 1960), the saphenous nerve of the rabbit was found to be insensitive to thermal changes, and even intense heat which damaged the skin failed to generate impulses in a preparation which responded well to stroking. Pinching the skin or pulling on the hairs likewise failed to produce an obvious afferent discharge.

#### Responses to injected volume changes

Brown & Gray (1948) reported reactions to control injections which they attributed to volume effects. However, in the rabbit, volumes of 0.9% NaCl solution up to 2 ml (10 times the standard drug injection volume) never produced any reaction, thus indicating that volume effects did not complicate the results of the present



experiments. Distilled water, in volumes up to 0.3 ml was also without effect.

Heparin solution (100 units/ml) was used in all experiments from time to time to wash out the cannula. In a few preparations volumes of 1 ml of this solution evoked a weak afferent discharge.

#### Responses to stimulant drugs

Responses to acetylcholine and to stroking were tested in all rabbits. Responses to all other drugs were tested in between 3 and 8 preparations. The effects produced by the drugs described under this heading are summarised in Table 1. (See page 54).

#### Acetylcholine

Intra-arterial injections of acetylcholine, in doses of 5-10  $\mu\text{g}$  and above, usually produced a discharge of impulses in the knee branch of the saphenous nerve (Fig. 4). Douglas & Ritchie (1960) obtained similar results in the cat, and found that the threshold dose was usually about 2  $\mu\text{g}$ . In most experiments a definite and reproducible response in the rabbit was produced by 20  $\mu\text{g}$  acetylcholine, and this was the standard dose adopted in most cases. Occasional preparations did not respond even to very large doses (200  $\mu\text{g}$ ) although they responded well to stroking. Throughout the experiments in which the effects of both acetylcholine and stroking were studied, there appeared to be an inverse relationship between the sensitivities to the two. When stroking produced a vigorous discharge, the effects of acetylcholine were relatively weak. Conversely, preparations which responded powerfully to small amounts of acetylcholine responded relatively weakly to stroking. No obvious explanation of this inverse relationship can be offered,

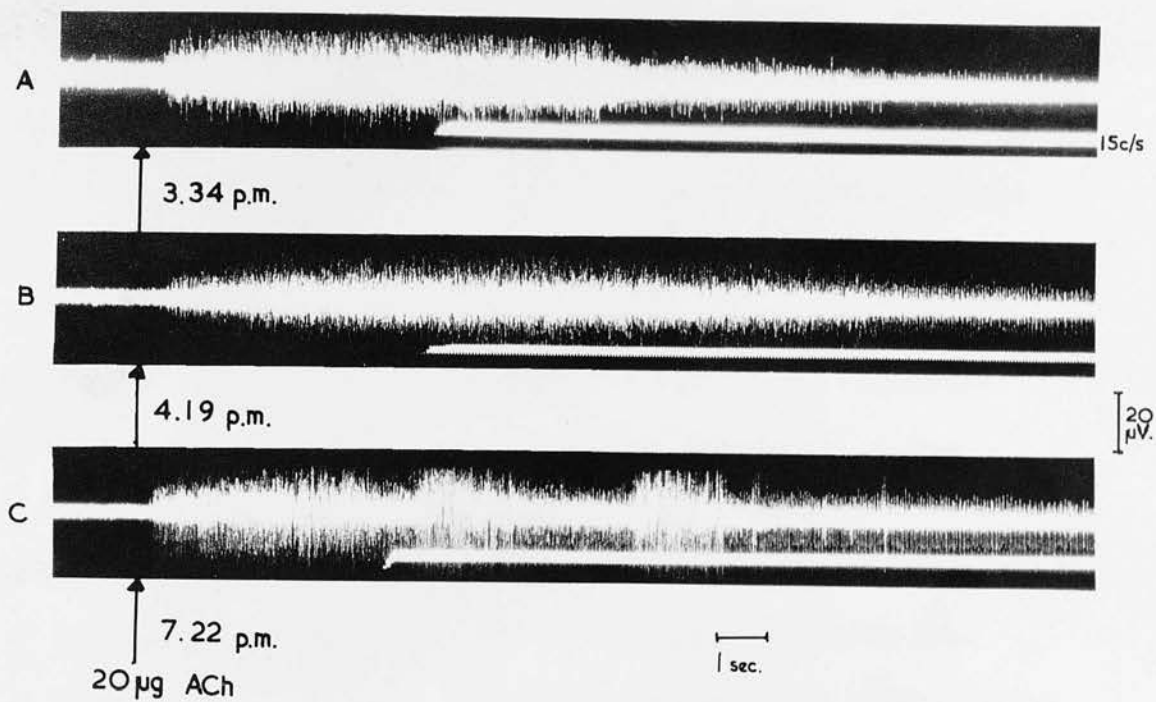


Fig. 4. Afferent discharges in the knee branch of the rabbit saphenous nerve evoked by three of a series of injections of 20  $\mu\text{g}$  of acetylcholine (indicated by the arrows). The duration of the injection was between the arrow and the reappearance of the time trace.

but it occurred too frequently for it to be likely that it was simply coincidence. The most suitable preparations were therefore those which gave an intermediate response both to stroking and to acetylcholine.

In early experiments, doses of acetylcholine were injected at intervals of half an hour or more, but this long interval proved to be unnecessary as demonstrated in several control experiments. In one of the control experiments, six doses of acetylcholine (20  $\mu$ g) were injected at intervals of only 5 min; there was some reduction in response to the second dose, an improvement in response to the third, and a return to responses equal to that of the second dose with the remaining three doses. In another experiment, a dose of 20  $\mu$ g was repeated after two minutes, and was followed one minute later by a third dose; the responses, though diminished, continued to be produced. A feature of every preparation, even with dose intervals of 30-40 min, was that the first response to acetylcholine was always greater than that to subsequent injections. However, after this initial loss of effectiveness, repetition of the same dose produced responses which were constant over several hours providing that an adequate dose interval was allowed (Fig. 4). All the control experiments showed that a 15 min dose interval was ample to ensure constancy of responses, and this interval was subsequently adopted in most experiments. At no time, or dose level, did acetylcholine interfere with the response to stroking. However, it was regularly observed that, in those animals which displayed vigorous spontaneous background firing, there was always a reduction in the amplitude and frequency of the larger spikes for a short time (5-10 min) after the firing caused by the injection had worn off.

The collision technique of Douglas & Ritchie (1960) was again used to check that the recorded discharge was travelling in C fibres. Antidromic C fibre potentials were evoked every 2 sec by stimulating the central end of the whole saphenous nerve trunk, and were recorded with the recording electrodes in the usual position on the knee branch of the nerve. The discharge evoked by intra-arterial injection of 20  $\mu$ g acetylcholine rendered the C fibres refractory and so partially extinguished the stimulus-evoked potential (Fig. 5). In these experiments the gain of the recording was too low to observe the acetylcholine-induced discharge clearly.

#### Carbachol

Carbachol on a weight basis was slightly less potent than acetylcholine in producing an afferent discharge, 30  $\mu$ g of carbachol being required to elicit a response comparable to that produced by 20  $\mu$ g of acetylcholine. When acetylcholine was injected less than 15 min after carbachol, the response to it was always depressed when compared with control injections given before carbachol. Successive doses of carbachol given at 15 min intervals produced progressively lessening responses, presumably because of the stability of the drug to cholinesterase and its consequent persistent depolarizing action. Even large doses of acetylcholine (200  $\mu$ g) when repeated, failed to show a similar tachyphylaxis. With carbachol (30  $\mu$ g) an interval of 45 min was necessary between doses in order to ensure constancy of responses. Responses to stroking were not depressed by carbachol in doses of 30  $\mu$ g no matter how soon after the injection the test was made.

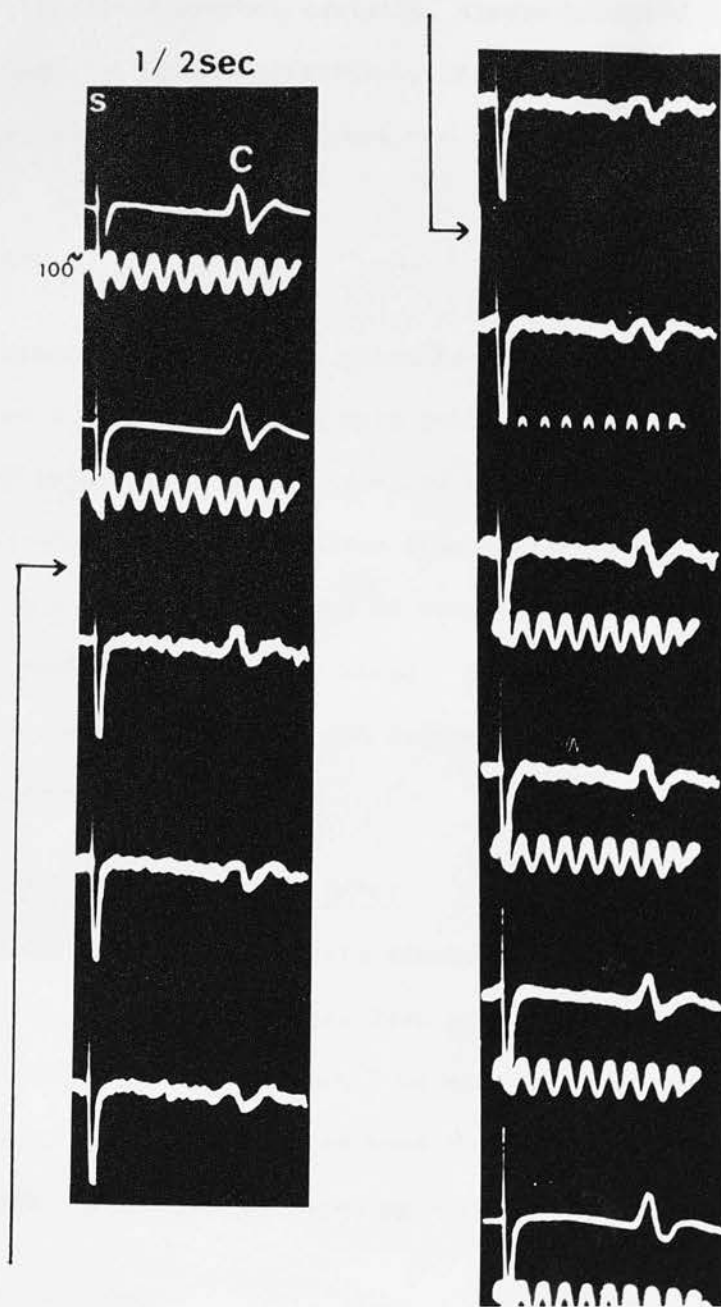


Fig. 5. Rabbit saphenous nerve stimulated once every 2 sec with rectangular pulses of 0.5 msec duration and of a strength greater than that necessary to evoke a maximal C fibre potential. The action potential was recorded antidromically from the knee branch of the saphenous nerve. This branch contains C fibres. Acetylcholine ( $20 \mu\text{g}$ ) was injected intra-arterially for the period marked by the absence of the time trace and indicated by the arrows. Note that the C fibre potential was partially extinguished by the acetylcholine injection. S is the stimulus artifact.

Carbachol, injected intra-arterially, always produced a profuse salivary secretion, which was effectively blocked by intravenous atropine. This salivation response was not seen with any other drug used.

### Nicotine

In doses ranging from 5-25  $\mu\text{g}$ , nicotine produced a definite response, and on a weight basis was more powerful than acetylcholine. After injection of small doses of nicotine (5-10  $\mu\text{g}$ ), the response to acetylcholine was often temporarily slightly augmented, whereas after larger doses of nicotine (15-25  $\mu\text{g}$ ) responses to acetylcholine were depressed. At no time or dose level within the range 5-25  $\mu\text{g}$  was the response to stroking altered by nicotine.

### 1,1-dimethyl-4-phenylpiperazinium (DMPP)

DMPP produced a weak and variable discharge of impulses in doses of 30-100  $\mu\text{g}$ , and was therefore less active on a weight basis than was acetylcholine, carbachol or nicotine. Responses to acetylcholine were slightly augmented when it was injected within 15 min after DMPP. Responses to stroking were unaffected.

### Tetramethylammonium (TMA)

TMA was ineffective in doses below 100  $\mu\text{g}$  but produced a vigorous response in a dose of 200  $\mu\text{g}$ . The response produced by 200  $\mu\text{g}$  was about equivalent to that produced by 20  $\mu\text{g}$  of acetylcholine (Fig. 6). The response to acetylcholine injected 5 min after TMA did not differ from controls. TMA was without effect on responses to stroking.

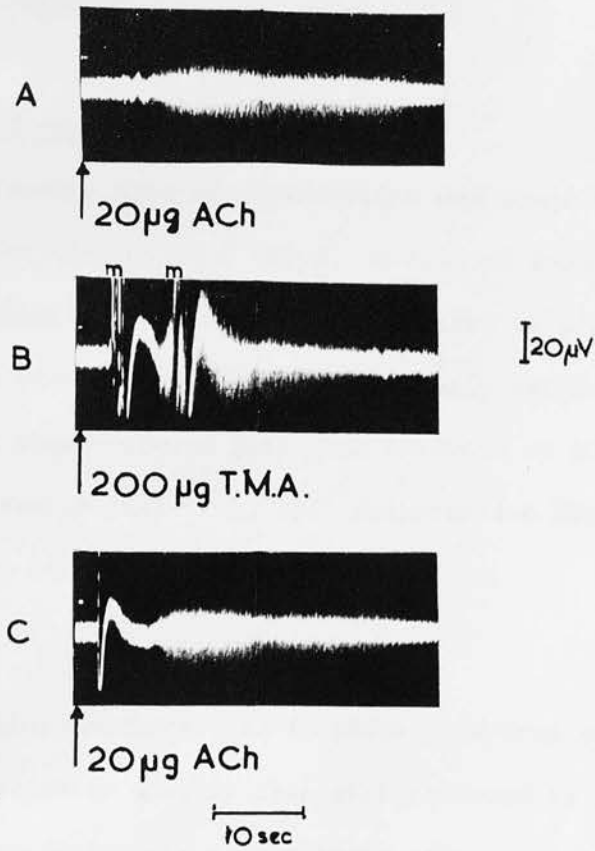


Fig. 6. Afferent discharges in the knee branch of the rabbit saphenous nerve evoked by intra-arterial injection of 20  $\mu\text{g}$  of acetylcholine and 200  $\mu\text{g}$  tetramethylammonium. The response in B was recorded 30 min after A. C was recorded 5 min after B and was quite normal. In this experiment T.M.A. evoked some movement artifacts labelled 'm'.

Acetylthiocholine

The response to acetylthiocholine resembled that to acetylcholine except that, on a weight basis, doses 2-3 times greater were required.

Methacholine (acetyl- $\beta$ -methylcholine)

The smallest effective dose of methacholine was found to be 80  $\mu$ g in two rabbits and 120  $\mu$ g in a third. A dose of 200  $\mu$ g was tested in each of 5 rabbits and found to be effective in every case. The response produced with 200  $\mu$ g was always slightly larger and more delayed in onset when compared with that produced by 20  $\mu$ g of acetylcholine in the same animal (Fig. 7). Methacholine was without effect on responses to stroking.

Pilocarpine

Pilocarpine resembled methacholine in potency, a dose of 200  $\mu$ g producing a response slightly smaller than that produced by 20  $\mu$ g of acetylcholine. Pilocarpine was also without effect on responses to stroking.

Histamine

Histamine caused stimulation in many but not all preparations at a dose level of 20  $\mu$ g (Fig. 8i). When present the discharge produced was about the same as that produced by 20  $\mu$ g of acetylcholine. Histamine did not interfere with the response to stroking. Even when histamine by itself was without obvious effect, it slightly potentiated a subsequent dose of acetylcholine for times varying from 5-15 min after injection (Fig. 8ii). Some tachyphylaxis was evident in the responses to repeated doses of histamine. Massive doses of histamine (1 mg) were used in two animals, both of which



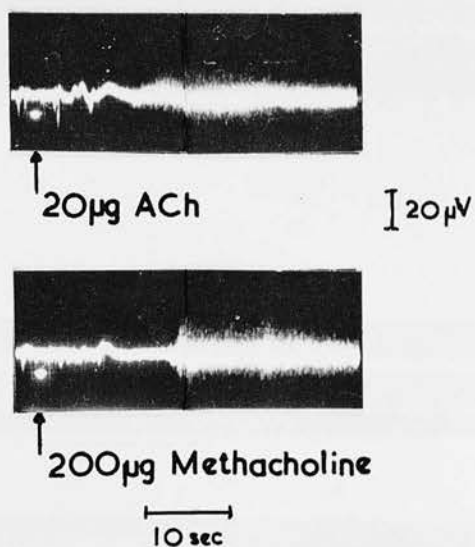


Fig. 7. Afferent discharges evoked in the rabbit saphenous nerve by intra-arterial injection of 20  $\mu$ g acetylcholine (ACh) and 200  $\mu$ g methacholine. 15 min elapsed between injections. Note the greater delay (approximately 5 sec) in the response to methacholine.

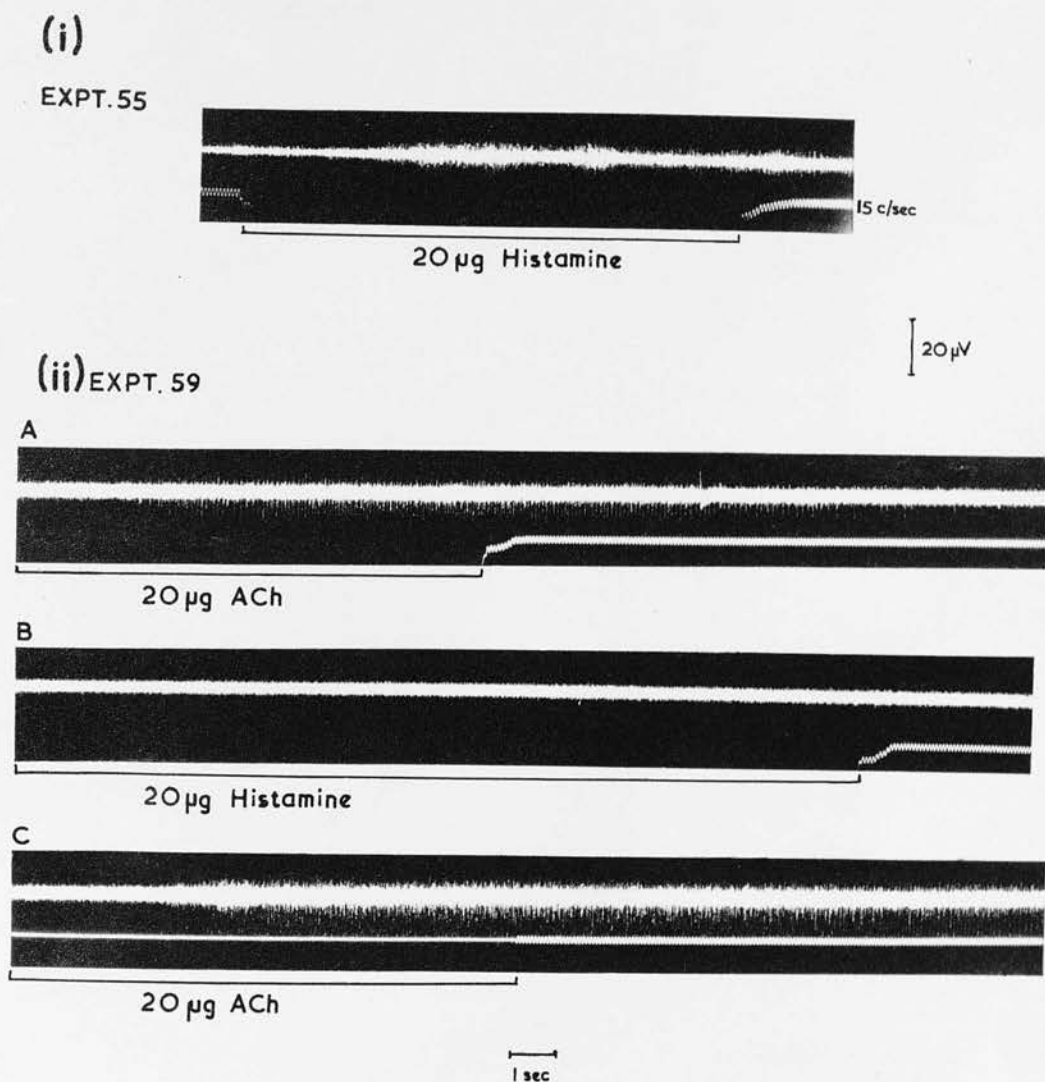


Fig. 8. Afferent discharges evoked in the rabbit saphenous nerve:  
 (i) Response to 20 µg histamine in a sensitive preparation.  
 (ii) Potentiation of acetylcholine (20 µg) by an injection of histamine (20 µg) given 15 min previously. This preparation was insensitive to the same dose of histamine alone (B).

responded well to acetylcholine. An obvious reduction in response to repeated doses of histamine could be seen, but there was no cross tachyphylaxis to acetylcholine which continued to produce its normal response (Fig. 9). Tachyphylaxis to histamine was reversible. In one animal, 4 doses, each of 1 mg, were injected at 15 min intervals. There was a reduction in the response to each dose. A fifth dose was injected after a further interval of 1 hr and by this time some recovery had occurred. Histamine tachyphylaxis has also been reported in cats by Trendelenburg (1956b). Responses to histamine were selectively reduced or abolished by mepyramine in doses of 0.5-1 mg intra-arterially. In doses of 2 mg intra-arterially, mepyramine also depressed the response to acetylcholine.

#### Acetylcholine and histamine mixtures

Emmelin and Feldberg (1947) showed that nettle stings contain both acetylcholine and histamine, and mixtures of the two were tested in a rabbit which displayed pronounced responses to stroking and to acetylcholine. A mixture containing 10  $\mu$ g of each produced as great an effect as did 20  $\mu$ g of acetylcholine and a much greater effect than did 20  $\mu$ g histamine alone which on this preparation was only very weakly active (Fig. 10). Thus there was evidence of potentiation of the one by the other. A mixture of 20  $\mu$ g of each produced a very pronounced and prolonged response, and repetition of this dose after an interval of 5 min produced an even greater response.

#### 5-Hydroxytryptamine (5-HT)

To obtain a definite response to 5-HT, doses of 40  $\mu$ g were

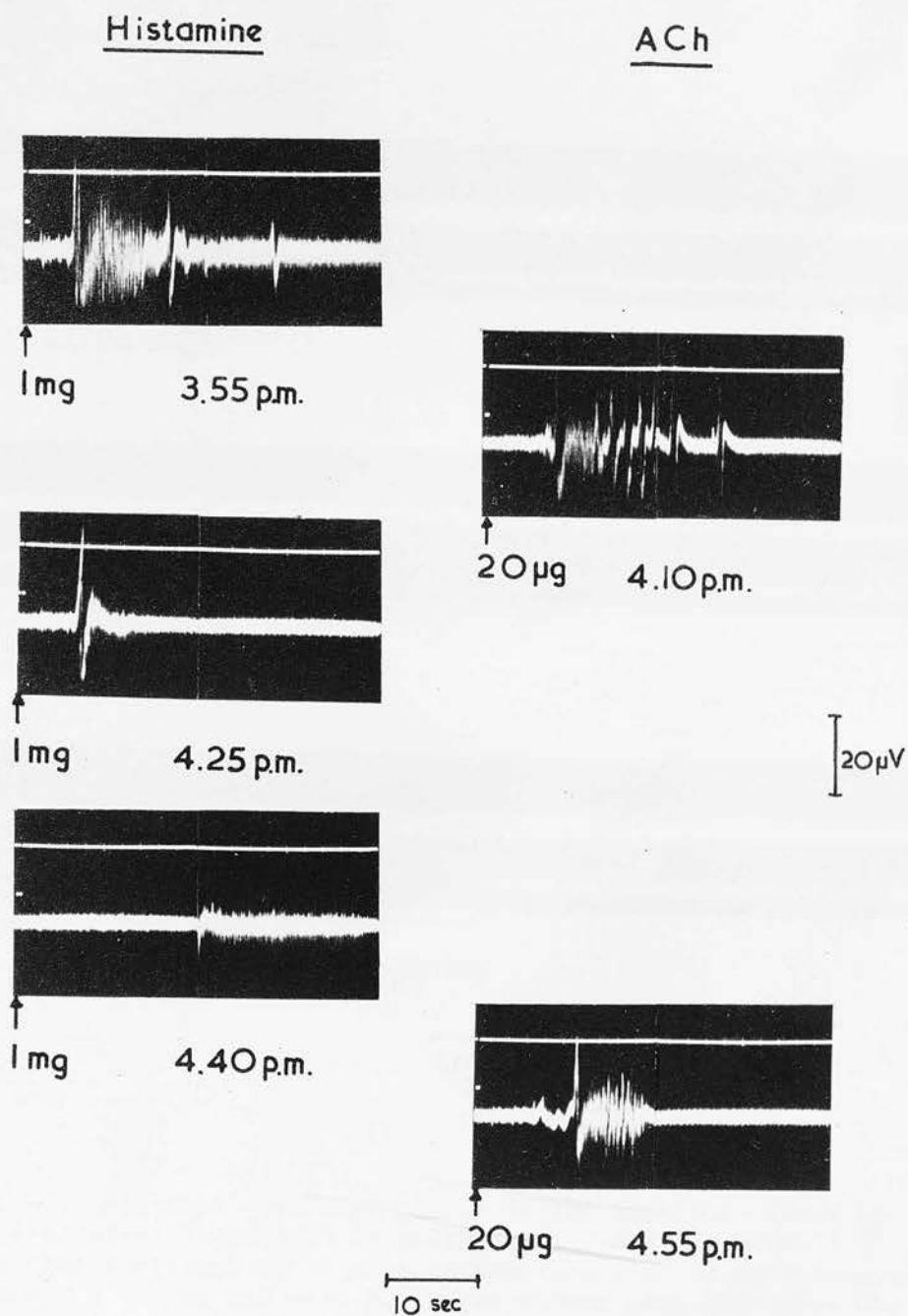


Fig. 9. Afferent discharge evoked in the rabbit saphenous nerve by intra-arterial injection of acetylcholine ( $20 \mu\text{g}$ ) and large doses of histamine ( $1 \text{ mg}$ ). Note the tachyphylaxis to histamine. Acetylcholine responses did not diminish. Large deflections are movement artifacts.

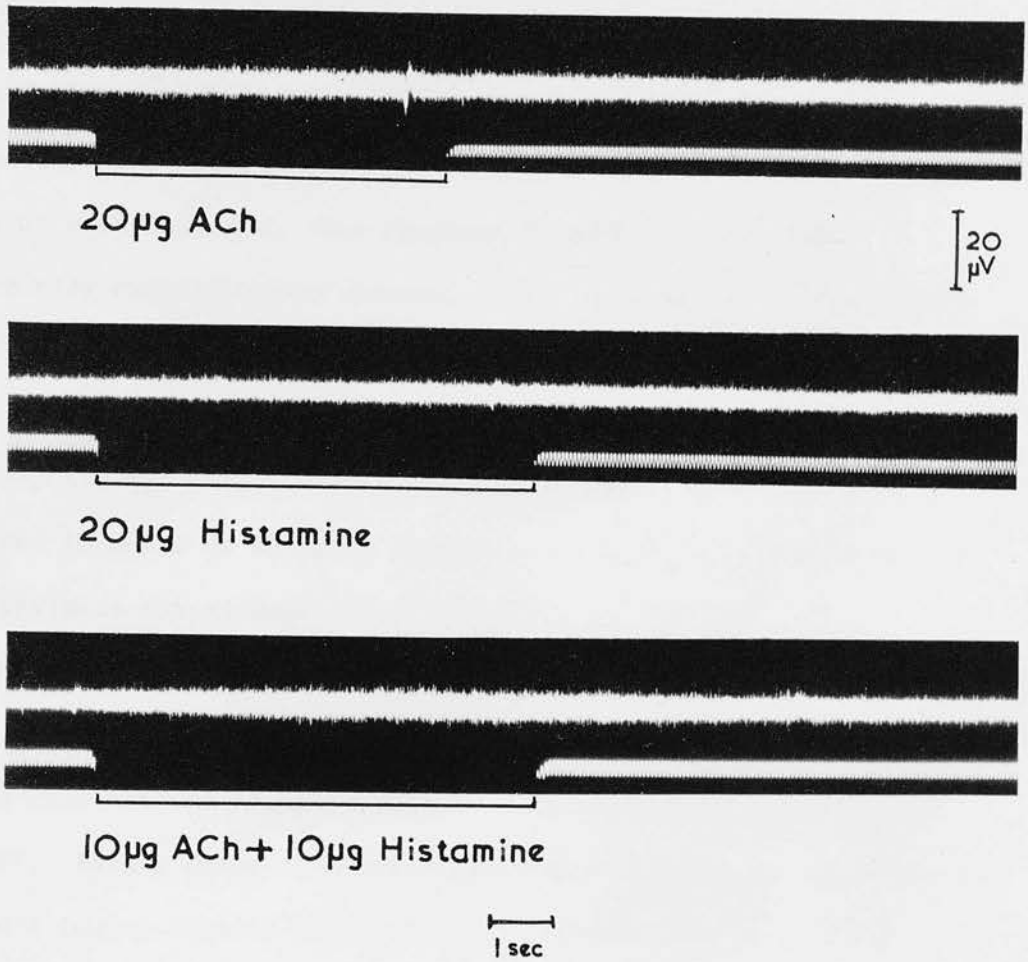


Fig. 10. Afferent discharge evoked in the saphenous nerve by intra-arterial injections of acetylcholine and histamine. Note that a mixture of 10 µg acetylcholine with 10 µg histamine produced a larger and more prolonged effect than did twice the dose of either substance alone. The time interval between each trace was 10 min.

necessary; again no interference with responses to stroking was observed. Repeated dosage with 5-HT at intervals of 15 min resulted in a marked tachyphylaxis which is illustrated in Fig. 11. Similar tachyphylactic effects have been reported for cat aortic arch (Ginzel & Kottegoda, 1956) and superior cervical ganglion (Trendelenburg, 1954). The final response in Fig. 11 is that to 20  $\mu$ g of acetylcholine. The response to acetylcholine was temporarily augmented over control levels despite the tachyphylaxis to 5-HT. In the same experiment, 5-HT was tested after a gap of three hours after the development of tachyphylaxis. By this time, the response was restored to its original size. When 5-HT was injected in doses of 40  $\mu$ g at intervals of 45-60 min, little or no tachyphylaxis was evident.

Methysergide, in doses of 10  $\mu$ g, selectively blocked 5-HT given 2 min later and the block persisted for about 2 hours. During this period responses to acetylcholine were augmented and prolonged by 50-100%. Larger doses of methysergide (up to 200  $\mu$ g intra-arterially) caused a longer lasting block of 5-HT responses but still augmented and prolonged responses to acetylcholine. Methysergide was without effect by itself and did not change responses to stroking. The response of neural receptors in mouse duodenum to 5-HT differs from that of the rabbit saphenous nerve in that it is not blocked by methysergide (Drakontides & Gershon, 1968).

Although delay in the onset of responses to both histamine and 5-HT occurs in cats (Fjällbrant & Iggo, 1961; Keele, 1962), there was little delay in responses to either drug in the present experiments on rabbits.

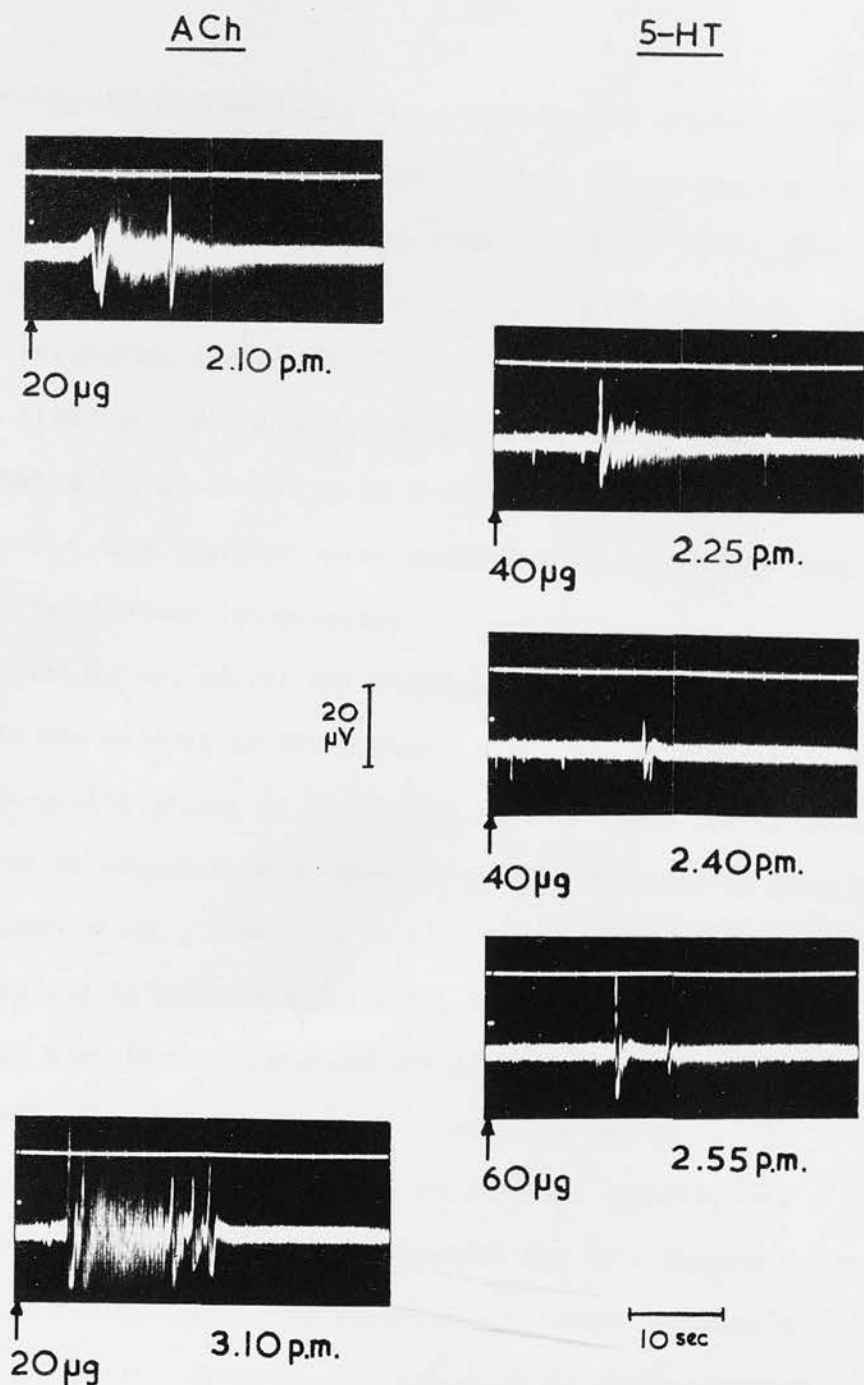


Fig. 11. Afferent discharge in the rabbit saphenous nerve evoked by acetylcholine and 5-HT. Note the tachyphylaxis to repeated injections of 5-HT, even when the dose was increased by 50%. No cross tachyphylaxis to acetylcholine was evident. In fact the response to acetylcholine was slightly augmented, but of similar duration, following 5-HT. (Large deflections are movement artifacts).

Bradykinin

On a weight basis bradykinin was a more potent stimulant than was acetylcholine (Fig. 12). In doses of 20  $\mu$ g bradykinin always produced a strong afferent discharge, even in animals which, for one reason or another, responded only poorly to acetylcholine. An approximate estimation indicated that bradykinin was at least twice as potent a stimulant as was acetylcholine on a weight basis. In one experiment doses up to 200  $\mu$ g of bradykinin were injected, and these produced a very vigorous nerve discharge accompanied by body movement and respiratory irregularity.

Bradykinin did not affect the response to stroking, and no tachyphylaxis was evident in its action.

The nociceptive action of bradykinin injected into the splenic artery of dogs is antagonised by acetylsalicylic acid and by phenylbutazone (Guzman et al., 1964; Lim et al., 1964), as is the bronchoconstrictor effect in guinea-pigs (Lewis, 1961). It was therefore of interest to test these substances for any antagonistic action against the effect of bradykinin on the saphenous nerve.

Solubilising agents are necessary to dissolve aspirin, and citrates of sodium or potassium were selected for this purpose since the alternative surface active agents were considered unsuitable for this type of study. However, 10% solutions of these citrates were rejected as they elicited a massive stimulant effect. Tests made with solutions of the citrates alone showed that a 2% potassium citrate solution was most suitable from the point of view of minimal stimulant effect coupled with solubilisation of an appropriate dose of aspirin. This strength of potassium citrate (2%) is approximately isotonic with blood and enabled a maximum amount of 10 mg/ml of





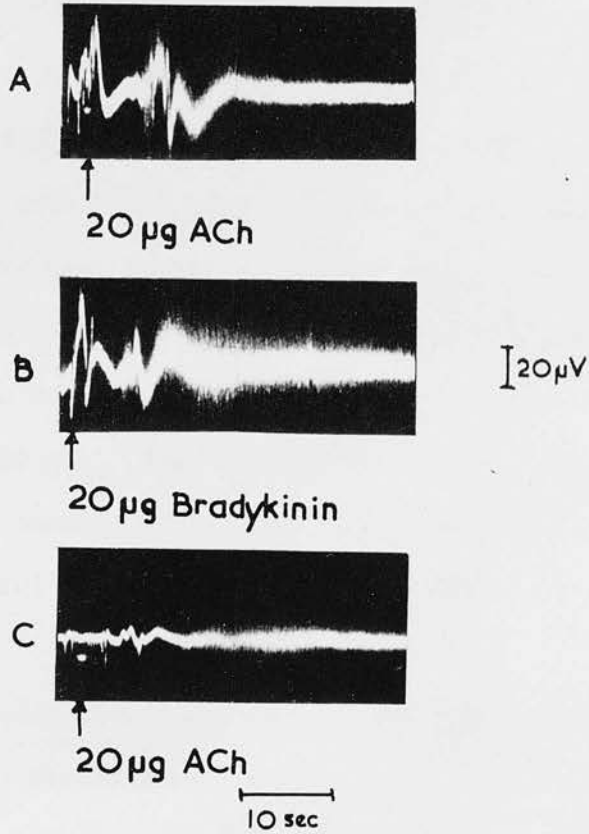


Fig. 12. Afferent discharges evoked in the rabbit saphenous nerve by intra-arterial injections of 20 µg acetylcholine and 20 µg bradykinin. 15 min elapsed between injections. Note the vigorous response to bradykinin despite the weak response to acetylcholine in this preparation. The poor response in C was due to the preparation and not drug interaction. (Large deflections are movement artifacts: such movement was very common following bradykinin injections).

aspirin to be dissolved. Since the injection volume was restricted to 0.3 ml, the maximum dose of aspirin that could be injected was 3 mg.

0.3 ml of 2% potassium citrate solution (without aspirin) produced a weak afferent discharge lasting about 10 sec and only slightly reduced or did not affect subsequent responses to bradykinin or to acetylcholine, even after three successive doses of the solubilising agent had been injected at 5 min intervals. When the same volume of potassium citrate solution contained 3 mg of aspirin, responses to bradykinin were markedly depressed but not abolished for up to 30 min. However, the same dose of aspirin completely blocked responses to acetylcholine for up to 45 min, and recovery to control levels then gradually occurred over the next 2 hr.

Neither the diluent alone, nor the diluent plus aspirin affected responses to stroking in any way.

Sodium salicylate in a dose of 5 mg given in isotonic solution (2.53%) did not alter responses to bradykinin, acetylcholine or stroking.

Phenylbutazone in doses up to 2 mg intra-arterially was without effect on responses to acetylcholine or stroking, but transiently depressed activity evoked by bradykinin.

### Angiotensin

In some preparations angiotensin in a dose of 20  $\mu$ g produced a weaker, but slightly more prolonged discharge than did an equal dose of acetylcholine. In other preparations angiotensin was without effect even in doses of 75  $\mu$ g. The effect of angiotensin was slower in onset than that of acetylcholine. When spontaneous nerve activity

TABLE 1

Sensitivity of the rabbit saphenous nerve endings to stimulant drugs

DRUG	Approximately equivalent effective doses $\mu\text{g}$	Approximately equivalent effective doses $\mu\text{M}$	Dose Ratio on molar basis Carbachol = 1	Antagonised by
Carbachol	30	0.1642	1	$\text{C}_6$ , tubocurarine & mecamlamine and local anaesthetics
Acetylcholine	20	0.1101	0.67	$\text{C}_6$ , tubocurarine & mecamlamine and local anaesthetics
Acetylthiocholine	>30	>0.1037	0.63	-
Methacholine	200	1.0220	6.22	Atropine & hyoscine
Pilocarpine	200	0.7372	4.49	Atropine & hyoscine
Nicotine	10	0.0201	0.12	$\text{C}_6$ & tubocurarine
TMA	200	1.8248	11.11	$\text{C}_6$ & tubocurarine
DMPP	100	0.3413	2.08	$\text{C}_6$ & tubocurarine
Histamine	20	0.0651	0.40	Mepyramine
5-HT	40	0.0987	0.60	Methysergide
Angiotensin	>20	>0.0194	0.12	-
Bradykinin	<10	<0.0094	0.06	Aspirin but not selectively

was strong, angiotensin markedly reduced it, whether or no it produced a stimulant effect itself (Fig. 13). Stroke reactions were not affected by angiotensin (Fig. 13), but an acetylcholine response evoked within 1 or 2 min of the angiotensin injection was decidedly curtailed, although it was fully restored 15 min later.

#### Potassium chloride

Potassium chloride was occasionally employed as a non-specific stimulant in the sense that its action does not involve specific drug receptors, but presumably depends simply on its ability to depolarize excitable membranes.

In intra-arterial doses of 2-5 mg and above, given in isotonic solution, KCl produced a brisk discharge. Tachyphylaxis developed when it was injected at intervals of ten minutes or less, but provided a sufficient time was allowed, constant responses were produced.

Doses of 10 mg or above had a pronounced depressant effect on the responses to stroking and to acetylcholine for up to 30 min or more after injection. This depressant effect was even more pronounced when KCl was injected in hypertonic solution.

#### Anticholinesterase drugs

Physostigmine (20-40  $\mu$ g), neostigmine (10-40  $\mu$ g) and ambenonium (5  $\mu$ g), injected intra-arterially, were without effect by themselves and did not affect the response to stroking. Nor did these drugs produce any change in the evoked compound action potential of the saphenous nerve recorded antidromically, and the possible significance of this result is referred to in the Discussion.

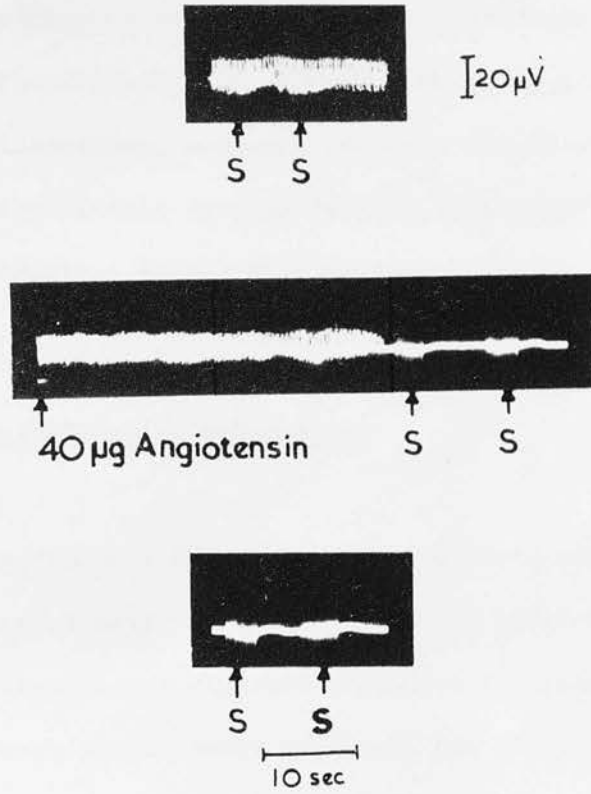


Fig. 13. Effect of angiotensin ( $40\ \mu g$ ) on spontaneous activity in the saphenous nerve. In this experiment angiotensin was without marked stimulant effect, but depressed spontaneous activity within 40 sec without depressing responses to stroking (S). The lowest trace was recorded 2 min after the injection of angiotensin (not shown). Slightly re-touched.

All three anticholinesterase drugs in the above doses potentiated acetylcholine. Augmented responses to acetylcholine were produced for about 30 min after neostigmine or ambenonium, and for a somewhat longer period (about 45 min) after physostigmine. Responses to nicotine, methacholine, histamine or bradykinin were not changed in the presence of the anticholinesterase drugs. The absence of effect on responses to methacholine, which is a substrate for acetylcholinesterase, suggests that the effect of an intra-arterial injection of this drug is largely terminated by diffusion and redistribution, rather than by hydrolysis by acetylcholinesterase.

#### Acetylcholine antagonists

##### Atropine and Hyoscine

In the first 8 experiments the rabbits were atropinised (atropine 2 mg/kg intravenously) at the start of the surgical procedure. In these experiments vigorous responses to acetylcholine or carbachol were subsequently produced, but there was no reaction to methacholine. In a subsequent experiment, control responses to acetylcholine (20  $\mu$ g) were obtained before giving atropine intravenously. Any depression of responses to acetylcholine after atropine was impossible to distinguish from the slight variation in control responses that occurred in any case.

When injected intra-arterially, atropine or hyoscine, in doses of 20  $\mu$ g and above, blocked the weak responses to methacholine (200  $\mu$ g) and to pilocarpine (200  $\mu$ g), but were without effect on responses to acetylcholine (20  $\mu$ g), to stroking or to histamine (20  $\mu$ g). However, responses to 200  $\mu$ g acetylcholine were slightly reduced by these doses of atropine or hyoscine. Even huge doses of

atropine or hyoscine (1-2 mg) failed to affect responses to stroking, suggesting that the doses used were well below the local anaesthetic level.

In 3 out of 5 experiments, with doses of 40  $\mu\text{g}$  of atropine intra-arterially, there was a brief depression, within 2 min, of responses to 20  $\mu\text{g}$  acetylcholine. With doses of 200  $\mu\text{g}$  of atropine intra-arterially, responses to acetylcholine (20  $\mu\text{g}$ ), carbachol (30  $\mu\text{g}$ ), DMPP (150  $\mu\text{g}$ ) and nicotine (25  $\mu\text{g}$ ) were completely blocked for 15 min and gradually returned to control levels during the next 15 min. It seemed likely that these large doses of atropine were producing a transient block of nicotinic receptors, because in the same experiments, responses to bradykinin (20  $\mu\text{g}$ ), or to KCl (10 mg) were unaltered while responses to nicotinic agents remained completely blocked.

In the remaining 2 experiments, doses of atropine even up to 1 and 2 mg intra-arterially, failed to block responses to acetylcholine (20  $\mu\text{g}$ ), although they were slightly reduced. In the same experiments, responses to histamine (20  $\mu\text{g}$ ) were also slightly reduced to a similar degree.

#### Hexamethonium

Hexamethonium in doses up to 10 mg intra-arterially was without effect by itself, and did not change the responses to stroking, to bradykinin (20  $\mu\text{g}$ ) or to KCl (10 mg). In doses of 1 or 2 mg intra-arterially or 3-5 mg/kg intravenously, hexamethonium abolished the responses to acetylcholine (20  $\mu\text{g}$ ), carbachol (30  $\mu\text{g}$ ), nicotine (20  $\mu\text{g}$ ) and DMPP (100  $\mu\text{g}$ ). Block of the responses to carbachol, nicotine and DMPP was relatively short-lasting, full recovery occurring within about 30 min. Block of the responses to acetyl-

choline was longer lasting, more than an hour elapsing before full recovery (Fig. 14). In this series of experiments it was observed that an injection of bradykinin hastened the return of the response to acetylcholine after blockade by hexamethonium. For example, in an experiment in which bradykinin was injected after hexamethonium, the response to acetylcholine returned to normal in less than 30 min. In the same experiment, hexamethonium blocked acetylcholine for over an hour when bradykinin was not injected.

Hexamethonium, in a dose of 2 mg intra-arterially, did not reduce the discharge produced by methacholine (200  $\mu$ g).

#### Mecamylamine

In a dose of 200  $\mu$ g injected intra-arterially, mecamylamine was without effect on responses to stroking, to KCl (10 mg) or to methacholine (200  $\mu$ g), but blocked the response to acetylcholine (20  $\mu$ g) for about 90 min.

#### Tubocurarine

A dose of 1 mg of tubocurarine intra-arterially was also without effect on responses to stroking and to KCl, but blocked responses to acetylcholine for about 60 min.

#### Tetraethylammonium (TEA)

In a dose of 5 mg injected intra-arterially, TEA blocked the response to acetylcholine (20  $\mu$ g) for about 20 min, but did not affect responses to stroking or to KCl (5 mg). In smaller doses (200-250  $\mu$ g), TEA itself produced a weak afferent discharge in the saphenous nerve. This effect, which was blocked by hexamethonium, was not produced by any dose of the other acetylcholine antagonists. Tetraethylammonium has been reported to produce a



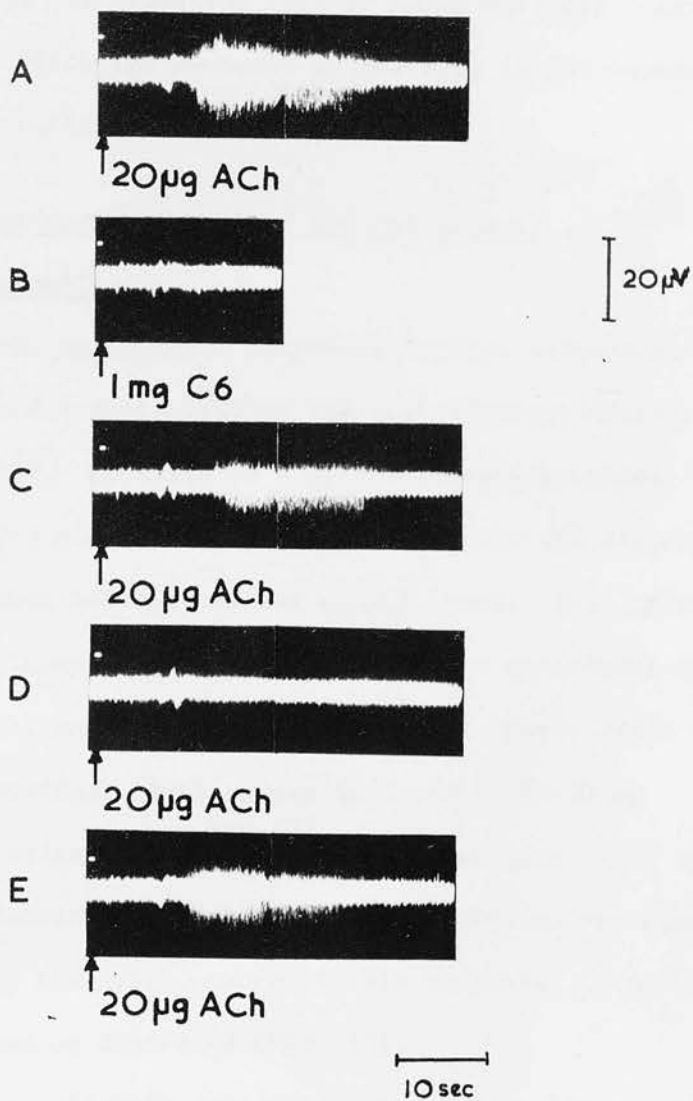


Fig. 14. Effect of hexamethonium on afferent discharges produced in the rabbit saphenous nerve by 20  $\mu$ g acetylcholine.

A Control response to acetylcholine.

B shows the lack of response to hexamethonium.

C shows the slight diminution in acetylcholine response 2 min after hexamethonium.

D shows complete block 15 min later.

E shows partial recovery 1 hr after the introduction of hexamethonium.

tingling sensation in the hands and feet of human subjects (Laurence, 1966), and the weak discharge produced by the drug in the saphenous nerve is probably a reflection of this effect.

### Sympathomimetic amines and antagonists

#### Adrenaline and noradrenaline

In doses of 10-20  $\mu\text{g}$  injected intra-arterially, adrenaline and noradrenaline produced a short-lasting and weak afferent discharge in the saphenous nerve. In doses of 2  $\mu\text{g}$  and above adrenaline transiently (up to 2-3 min after injection) depressed the response to stroking, which then returned to its normal level. This effect was not obvious with noradrenaline; in fact, in one experiment there was a transient slight enhancement of the stroke response after each injection of noradrenaline. Both amines in doses of 10-20  $\mu\text{g}$  produced a biphasic effect on responses to acetylcholine. For up to 2-3 min after injection the response to acetylcholine was clearly augmented, but during the subsequent 30-45 min responses to acetylcholine were abolished or depressed (Fig. 15).

Noradrenaline was slightly less active than adrenaline in all cases where their effects were qualitatively similar.

In doses of 0.5-1 mg phentolamine itself depressed responses to acetylcholine for up to 45 min. In doses of 250  $\mu\text{g}$ , this effect of phentolamine was weak, and this dose was used in subsequent experiments involving sympathomimetic amines. After phentolamine (250  $\mu\text{g}$ ), adrenaline or noradrenaline (20  $\mu\text{g}$ ) no longer produced an afferent discharge on injection, nor did they produce the biphasic change in acetylcholine responses described above.

Pronethalol in doses of 250  $\mu\text{g}$  did not alter any of the responses to adrenaline or noradrenaline. At this dose level,

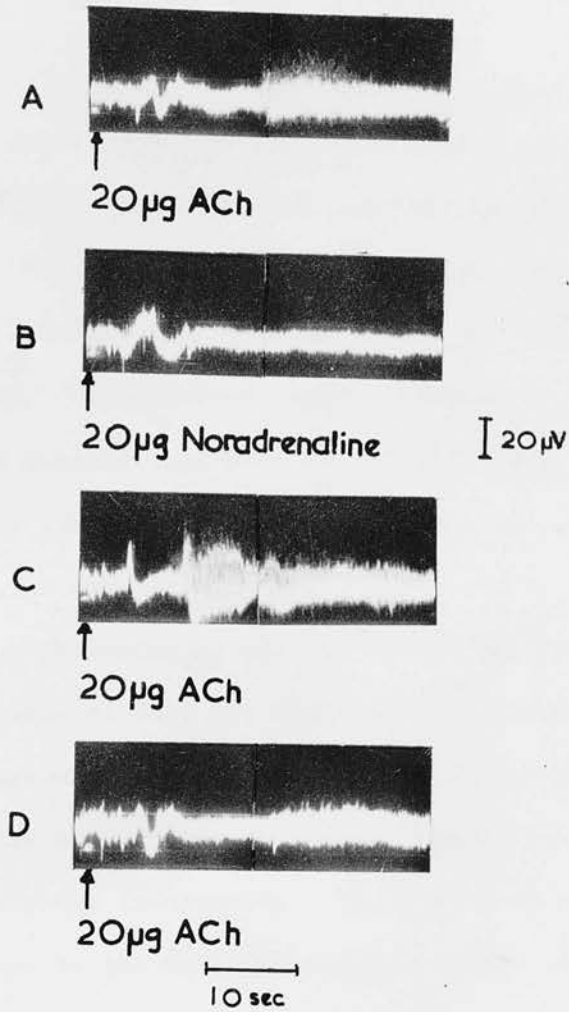


Fig. 15. Interaction between noradrenaline and acetylcholine on the rabbit saphenous nerve. 2 min after noradrenaline the response to acetylcholine (C) was quicker in onset, more vigorous and more prolonged. 30 min later (D) the response to acetylcholine was curtailed. Note the weak discharge induced by noradrenaline alone (B).

pronethalol itself did not affect responses to stroking or to acetylcholine.

### Isoprenaline

Isoprenaline, in doses of 20-200  $\mu\text{g}$ , occasionally produced a transient and weak afferent discharge, but was without effect on the response to stroking. Responses to acetylcholine were prolonged by about 40% when tested 2-15 min after isoprenaline, and returned to normal within 30-40 min. Isoprenaline, unlike adrenaline and noradrenaline, did not depress responses to acetylcholine, but it regularly produced some inhibition of any background spontaneous activity in the nerve.

In doses of 250  $\mu\text{g}$  phentolamine did not affect the responses to isoprenaline. Pronethalol (250  $\mu\text{g}$ ) abolished any responses to isoprenaline and prevented its interaction with acetylcholine.

Lignocaine was also tested three times for its influence on the adrenaline-acetylcholine interaction. The maximum dose used (350  $\mu\text{g}$ ) was more potent in its local anaesthetic action than the effective dose (250  $\mu\text{g}$ ) of pronethalol, as judged by the guinea pig wheal test. There was no change in the modified acetylcholine response following adrenaline, so it is clear that any effect of pronethalol is independent of its local anaesthetic activity.

### Local anaesthetic and adrenoreceptor blocking drugs

Because of current interest in the actions of  $\beta$ -adrenoreceptor blocking drugs, the opportunity was taken to test them for local anaesthetic activity on the saphenous nerve preparation. The effects of dichloroisoprenaline, pronethalol and propranolol were compared with those of the local anaesthetic drugs procaine,

lignocaine, and propanadid. Local anaesthetic activity has previously been reported with both pronethalol and with propranolol (Gill & Vaughan-Williams, 1964; Vaughan-Williams, 1966).

Procaine in doses below 2 mg, and lignocaine in doses below 1 mg were without effect on spontaneous firing, and on the responses to stroking, acetylcholine or KCl. Procaine in doses of 2-3 mg and lignocaine in doses of 1-1.5 mg blocked responses to acetylcholine, to KCl and to stroking for periods which varied in different experiments from 5 min to 30 min. This difference in potency between lignocaine and procaine accords well with published results summarised by Wiedling (1959) and with tests using motor nerve terminals (Usubiaga & Standaert, 1968).

With both local anaesthetic drugs, responses to acetylcholine or KCl were depressed by slightly smaller doses than those necessary to depress the response to stroking. Neither drug itself produced any reaction: nor did the drugs depress any spontaneous firing at these dose levels. Fig. 16 illustrates the effects of lignocaine on the response to stroking. Lignocaine was also tested by intravenous injection. In sub-lethal doses (up to 25 mg/kg given slowly), lignocaine was completely without effect on the responses of the saphenous nerve.

Propanadid is a general anaesthetic drug with local anaesthetic properties (Wirth & Hoffmeister, 1965). In doses of 4-5 mg, propanadid blocked the response to acetylcholine for about 15 min. A dose of 10 mg blocked responses both to stroking and to acetylcholine for 10-20 min and there was also a short-lasting (2-3 min) depression of spontaneous activity in the nerve.

Dichloroisoprenaline in doses of 500  $\mu$ g and above produced a

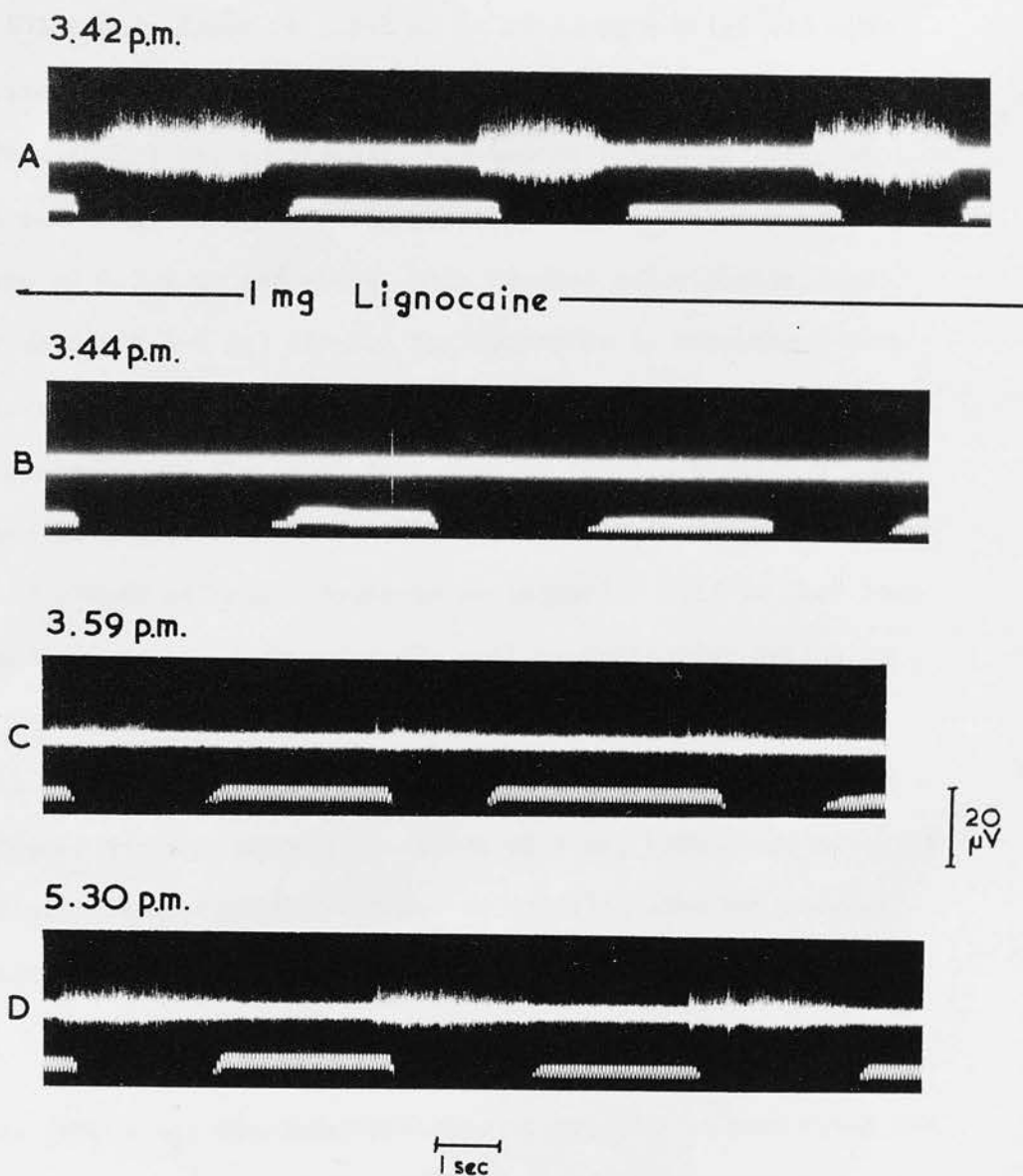


Fig. 16. Effect of lignocaine (1 mg intra-arterially) on the afferent discharges in the rabbit saphenous nerve produced by lightly stroking the skin (marked by the breaks in the time trace). Note that responses to stroking were abolished (B) within 2 min of giving lignocaine. 17 min later (C) stroke responses were just discernible but even  $1\frac{3}{4}$  hr later (D) had not fully returned to control levels.

weak response on injection similar to that produced by isoprenaline (page 63) and in doses of 1.5-2 mg it produced a brief (15 min) depression of the responses to acetylcholine and to stroking.

Pronethalol and propranolol blocked responses to acetylcholine and to stroking. The two drugs possessed about the same potency. In doses of 0.5-1 mg and above, both blocked acetylcholine and larger doses (1.5-2 mg) blocked the responses to stroking. They therefore resembled the local anaesthetics in their action and were comparable to the local anaesthetics in this respect. They differed from the local anaesthetics in that both drugs themselves produced a weak afferent discharge on injection, and in that they appeared to possess some selective anti-acetylcholine action in doses below the local anaesthetic level.

The  $\alpha$ -receptor blocking drugs phentolamine and dibenamine were tested for comparison. In doses of 2 mg, both drugs slightly and briefly depressed the response to acetylcholine but neither affected the response to stroking.

#### Guinea Pig Wheal Method

At this stage the local anaesthetic action of these drugs was tested, for comparison, using the guinea pig wheal method of Bülbring & Wajda (1945).

The potency ratios determined at the dose level needed to produce 50% local anaesthesia, and taking propranolol (the most potent drug) as unity were: pronethalol 1.4, dichloroisoprenaline 1.5, lignocaine 1.8. The graphs of Figs. 17 and 18 express the results for potency and duration of the effect of these four drugs. The detailed figures are given in the Appendix (Table B).

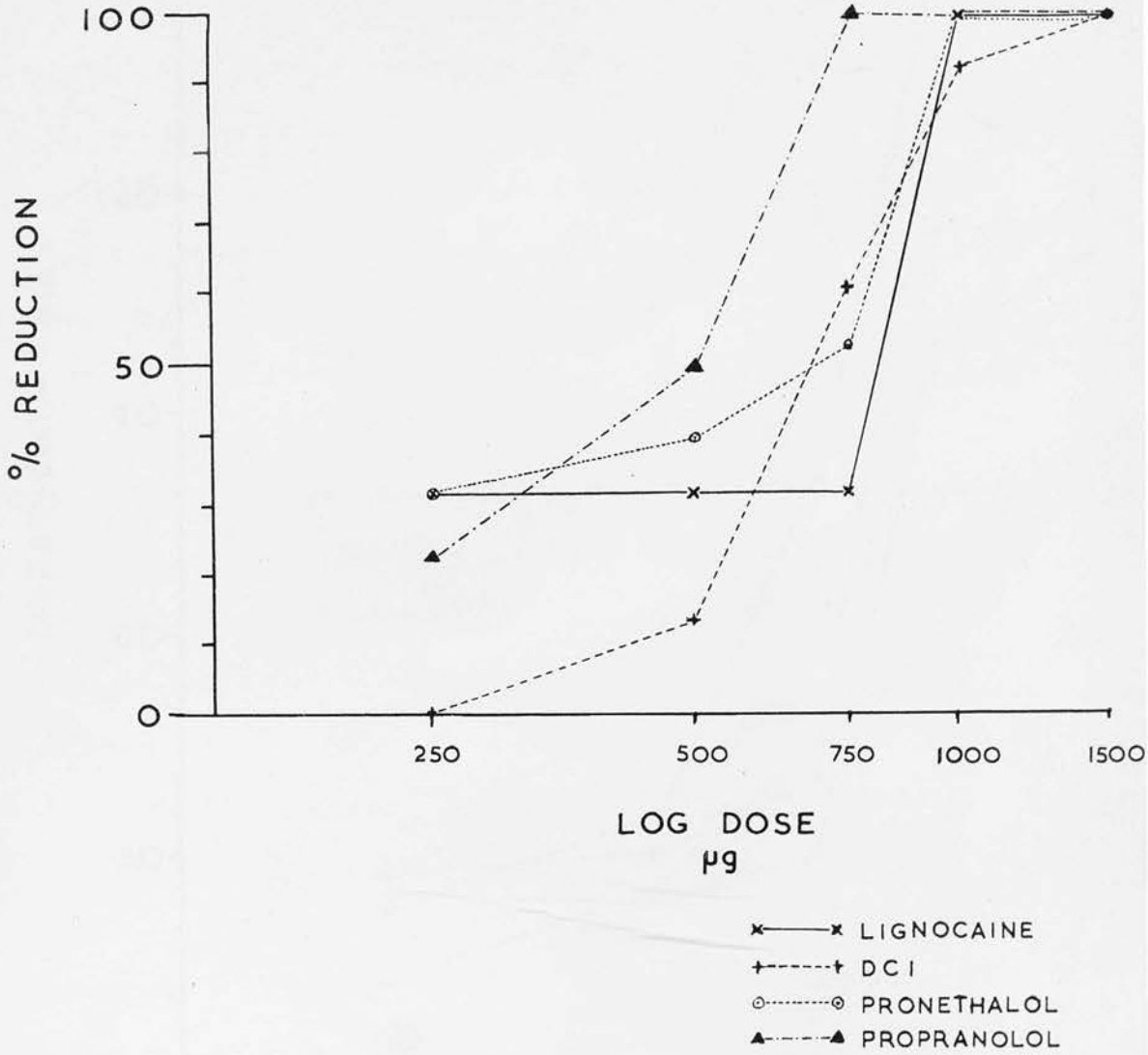


Fig. 17. Local anaesthetic potencies determined by the modified guinea pig wheal test (at 20 min - see p. 30)



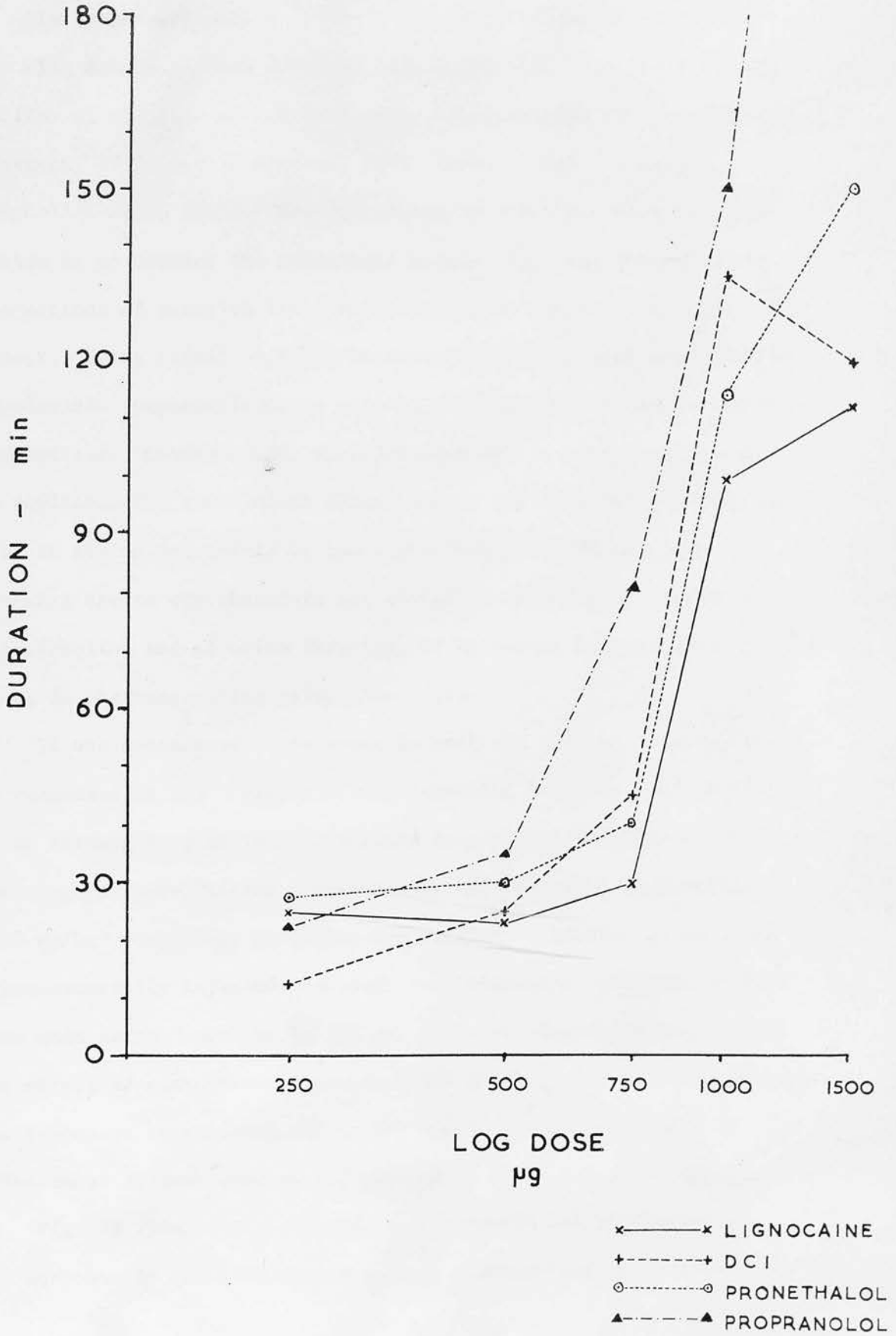


Fig. 18. Duration of local anaesthesia determined by the modified guinea pig wheal test.

Guanethidine

In sympathetically-innervated tissues, adrenergic neurone blocking agents abolish both the sympathomimetic effects of acetylcholine or nicotine and the responses to adrenergic nerve stimulation (Jarrett, 1962; Burn & Gibbons, 1964; Akubue, 1966; Burnstock, Campbell & Rand, 1966). However, these observations give no information as to whether the adrenergic neurone blocking drugs prevent the actions of acetylcholine or nicotine by a specific blocking effect. Ferry (1963) obtained evidence indicating that acetylcholine depolarizes sympathetic nerve endings causing them to release their transmitter. Consequently, acetylcholine may be considered merely to duplicate the stimulating electrodes in the sense that both give rise to action potentials in the nerve trunk. Adrenergic neurone blocking agents may therefore act either by blocking the action of acetylcholine and nicotine directly, or by acting at some later stage in the transmitter release mechanism.

It was considered of interest to test the effect of guanethidine on responses of the fibres of the saphenous nerve to acetylcholine in an attempt to gain further insight into the interaction of these two drugs on nerve tissue. Intravenous injections of guanethidine (5-8 mg/kg) completely abolished the afferent discharge produced by intra-arterially injected acetylcholine, carbachol, nicotine or DMPP, even when large doses (up to 200  $\mu$ g) of these stimulants were given; the effect of guanethidine persisted for the duration of the experiment. The responses to stroking and to KCl however, were unimpaired by intravenous guanethidine in all sub-lethal doses (up to 30 mg/kg).

Fig. 19 illustrates the effect of guanethidine in abolishing the response to acetylcholine but leaving unimpaired the response to

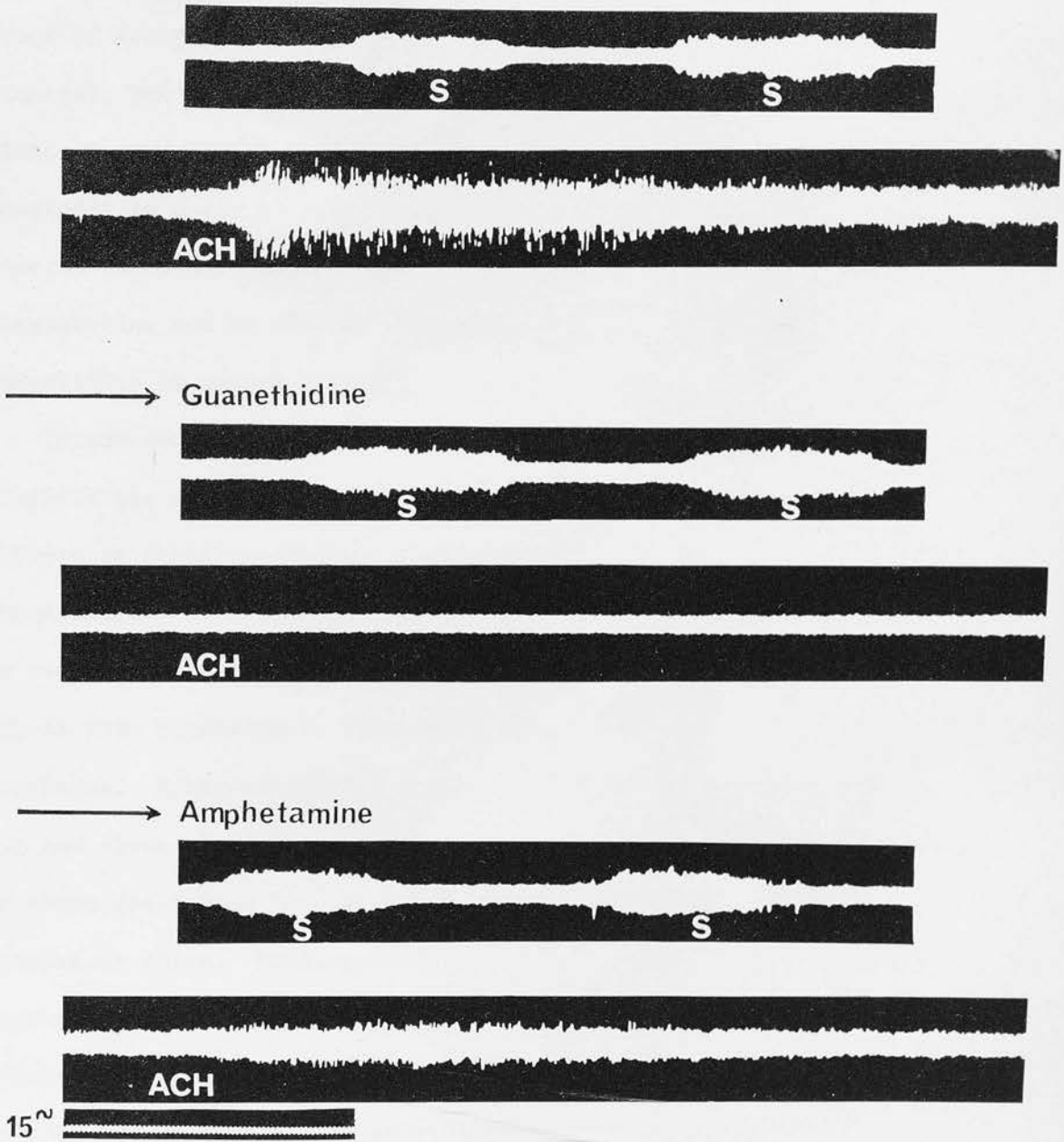


Fig. 19. Afferent discharges in the knee branch of the saphenous nerve evoked by lightly stroking the skin (S) and by intra-arterially injected acetylcholine (ACH, 20  $\mu$ g). The middle two records show the unimpaired stroke response and the absence of the acetylcholine response 10 min after the intravenous injection of guanethidine (6 mg/kg). The lower two records show the stroke response and the partially restored acetylcholine response 30 min after the intravenous injection of dexamphetamine (0.6 mg/kg).

stroking. As would be expected, guanethidine also abolished the effect of acetylcholine in depressing the stimulus-evoked C fibre potential, but did not change the effect of stroking. Similar effects were produced by guanethidine when it was injected intra-arterially in doses of 1 mg. Larger doses of guanethidine (5-10 mg) injected intra-arterially blocked the response to stroking, to acetylcholine and to KCl; at this dose level the effects of guanethidine therefore resembled those of the local anaesthetics.

Intravenously injected dexamphetamine in doses one-tenth to one-fifth the size of the guanethidine dose used, restored the response to acetylcholine to a large extent although it never returned fully to the control level (Fig. 19). The restoration of the response occurred gradually to reach its maximum in about 30 min; it then persisted at this level for the remainder of the experiment. Intra-arterially injected dexamphetamine in doses of 1 mg and above also produced some reversal of the guanethidine block but these doses were approaching in size those necessary by the intravenous route. Smaller doses of dexamphetamine injected intra-arterially were without effect, possibly because they were diluted to sub-effective concentrations in the general circulation before the slow restoration of the guanethidine-blocked response could occur.

In the same intravenous or intra-arterial doses, dexamphetamine also often produced some enhancement of responses to acetylcholine and KCl in the absence of guanethidine, and hastened recovery from the blocks produced by hexamethonium or lignocaine. However, these effects of dexamphetamine differed from those produced in the presence of guanethidine in that they reached their maximum within

1 to 2 min of injection and persisted throughout the effect of only one injection of acetylcholine or KCl.

The mean general arterial blood pressure ( $\pm$  the average deviation about the mean) of 5 rabbits under urethane anaesthesia was  $99.8 \pm 10.2$  mm Hg. Guanethidine, in doses of 6 mg/kg intravenously, produced a mean fall to  $64.4 \pm 14.5$  mm Hg. In experiments on 3 other rabbits, the mean blood pressure was  $91.6 \pm 12.2$  mm Hg, and hexamethonium in doses of 4 mg/kg intravenously produced a mean fall to  $40 \pm 6.6$  mm Hg. These doses of guanethidine and hexamethonium completely abolished responses to acetylcholine within 3 min. Isoprenaline in doses of 10-15  $\mu$ g/kg intravenously produced a fall in blood pressure about equal to that produced by hexamethonium and, although shorter in duration, it persisted beyond 3 min at which time there was no change in the response to acetylcholine.

DISCUSSION OF SECTION 1

Although the rabbit saphenous nerve, like that of the cat and other species (Heinbecker, O'Leary and Bishop, 1933; Gasser, 1950; Douglas & Ritchie, 1960), contains both A and C fibres, the branch innervating the skin over the knee, at least in the Californian Albino strain of rabbits used, was found electrophysiologically to consist almost exclusively of C fibres and this was the branch used for recording throughout the experiments. C fibres were therefore involved in all recorded drug responses, and this was confirmed in the case of acetylcholine by showing that the afferent discharge produced extinguished the evoked antidromic C fibre potential. The sensory terminals of these fibres were shown to be mechanoreceptors activated by movements of the hair. Touching the skin, after cohesion of the fur with liquid paraffin, failed to evoke an afferent discharge, as did pinching the skin, pulling the fur, or application of heat. The discharge produced by lightly stroking the fur, like that produced by acetylcholine, extinguished the evoked antidromic C fibre potential, and thus it seems probable that, under the conditions of the experiments, both stroking and drug responses involved the same fibres, and that stroking could therefore be used as a valid test for the functional integrity of the receptors and neurones involved.

In early experiments, a number of unsuccessful attempts were made to produce an apparatus for applying a constant and controlled mechanical stimulus. Jets of air from a respiration pump produced inadequate hair movement, and devices with electric motors interfered with the oscilloscope records. An apparatus involving a clockwork motor was effective in some experiments, but the sensitivity of

different preparations varied widely and it was not possible to alter the strength or the area of application of the stimulus when using this method. In the end, mechanical devices were abandoned in favour of stroking the fur by hand. With practice, the ability to reproduce sufficiently comparable afferent discharges was quickly acquired.

Conclusions concerning the relative effectiveness of drugs at receptor sites are complicated, in experiments involving intravascular injection, by lack of knowledge concerning diffusion barriers which may affect the penetration of some drugs to their site of action more than that of others. For this and other reasons, some drugs, although effective, may have a slower onset of action than others, and with intra-arterial injection they may pass into the venous return without having had adequate time to reach effective concentrations in the region of the receptor sites. Furthermore, some drugs may have additional effects on the blood flow to the skin which could increase or decrease their penetration to, and time of contact with, the receptor sites. Although gross movements of the animal, evoked reflexly through stimulation of peripheral receptors of the saphenous nerve, were prevented by sectioning the nerve in the thigh, some drugs (e.g. neostigmine) in large doses caused limb movements and muscle fasciculations which gave rise to mechanical artefacts in the electrical records. These were a nuisance but did not lead to errors in interpretation, since such artefacts were readily distinguished and were present only when the muscle movement was obvious to the eye. Records including movement artefacts caused by the act of injection, rather than by drug action, have been discarded. Attempts were

made to obviate some of these difficulties by killing the animal and perfusing the skin vessels at constant rate with an oxygenated physiological salt solution to which drugs could be added when necessary. However, in 6 experiments of this type it was found impossible to maintain a viable preparation for a sufficient length of time. The skin and surrounding tissues quickly became oedematous and responsiveness to both stroking and drugs rapidly diminished and disappeared within a variable time, which in all cases was too short to allow useful results to be obtained. Since the sensory receptors concerned, presumably the basket-like arrangements around hair follicles, were activated by stroking the fur, it is possible that drugs causing contraction of the smooth arrector pili muscles could cause an afferent discharge, not by acting directly on the sensory neurone, but as a secondary effect of hair movement. This possibility has been discussed by others (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas & Ritchie, 1959; Paintal, 1964). With some drugs, e.g. adrenaline, it is difficult to exclude this possibility entirely, although it appears unlikely. With the recording system used, it was necessary, when stroking, to produce a gross disturbance of the position of the hairs before an afferent discharge was evident, but close observation failed to detect hair movement after injection of any of the drugs used. Nevertheless, these limitations of the method used should be borne in mind when assessing the validity of the results obtained.

### Receptors

The results obtained with agonists and the appropriate specific antagonists demonstrated the presence of separate receptor sites sensitive to acetylcholine, histamine, 5-HT and bradykinin. The



cholinoceptive receptors, activated by relatively small doses of acetylcholine and carbachol, were mainly of the nicotinic type, since responses to these agonists were blocked by hexamethonium, mecamlamine, tetraethylammonium and tubocurarine, and unaffected by small intra-arterial doses of atropine or hyoscine, or even by large intravenous doses. Furthermore, nicotine, DMPP and TMA produced an afferent discharge resembling that produced by acetylcholine. Gray (1959) concluded that the cholinoceptive receptors in the cat saphenous nerve were also essentially nicotinic. Large doses of atropine injected intra-arterially did briefly block responses to these "nicotinic" agents. This block was specific in the sense that responses to KCl or bradykinin were not depressed. Large doses of atropine have been shown to block the actions of nicotinic drugs at other sites and to produce ganglion block (Feldberg & Vartiainen, 1934; Marrazzi, 1939; Abdon, 1940; Dutta, 1949; Konzett & Rothlin, 1949; Fink & Cervoni, 1953; Giotti, 1954; Bainbridge & Brown, 1960; Quilliam & Shand, 1964).

The results indicated that muscarinic cholinoceptive sites are also present in the rabbit saphenous nerve; large doses of methacholine and pilocarpine produced an afferent discharge which was unaltered by hexamethonium or mecamlamine but blocked by atropine or hyoscine in doses too small to abolish responses to nicotinic agents. The large doses of methacholine and pilocarpine required suggest that these muscarinic sites are few in number or relatively inaccessible. Brown & Gray (1948) failed to detect muscarinic sites in the cat saphenous nerve, possibly because the doses they used were below the threshold level, but Widdicombe (1954) found that large doses of pilocarpine elicited responses

from pulmonary stretch receptors. Presumably large doses of acetylcholine will excite both nicotinic and muscarinic receptors, but with this drug the more pronounced effects on nicotinic receptors largely overshadow any other effect. The effects of methacholine and pilocarpine were unlikely to be secondary to the contraction of the arrector pili muscles since there was no obvious hair movement, and these muscles are adrenergically innervated, there being no evidence that they will respond to muscarinic agents.

Responses to angiotensin were weak and it is impossible to exclude the possibility that its effects were secondary to some other action. In any case, the weakness and variability of the responses to angiotensin indicates that any receptor sites present on the nerve endings must be sparse and that they probably play little part in modulating sensory perception involving mechanoreceptors of the rabbit saphenous nerve.

Catecholamine effects mediated by both  $\alpha$  and  $\beta$  receptors were demonstrated. While it is difficult to exclude the possibility that these effects, particularly the initial stimulant effect produced by adrenaline and noradrenaline were secondary to actions on smooth muscles, such as the arrector pili muscles, it nevertheless remains possible to explain them in terms of their known actions on nerve. It seems unlikely that their interaction with acetylcholine could be related to vascular effects, since the joint effect of adrenaline and noradrenaline on skin vessels is vasoconstriction and this should hinder the access of acetylcholine to the sensory nerve endings, and therefore reduce the afferent discharge produced. In fact the opposite proved to be the case, the acetylcholine-

induced discharge being initially enhanced by the amines. Adrenaline and noradrenaline have been shown to lower the threshold of excitation of nerve (Bülbring & Whitteridge, 1941; Goffart & Holmes, 1962) and a similar effect in the saphenous nerve could account for the initial enhancement of the discharge produced by acetylcholine. Surprisingly, at the same time the membrane potential of nerve is raised (Goffart & Holmes, 1962), and if this hyperpolarization develops more gradually and outlasts the early threshold lowering effect, it could account for the secondary depression of the response to acetylcholine. The present experiments showed that these actions of adrenaline and noradrenaline were mediated by  $\alpha$ -receptors.

Isoprenaline enhanced the duration of the acetylcholine-induced discharge, and it is possible that this effect is secondary to its vasodilation action. However, the potentiation by isoprenaline was slow in onset (2 min) and lasted up to 30-40 min, and it is unlikely that its vasodilation action lasted as long. Isoprenaline has been shown to depolarize ganglion cells (De Groat & Volle, 1965; Volle, 1966b), and a similar depolarizing action on sensory nerve endings may explain its ability to augment the response to acetylcholine. This effect was shown to be mediated by  $\beta$ -receptors.

#### Physiological significance

The results showed that although the sensory discharge produced by acetylcholine was augmented by prior treatment with anticholinesterase drugs and was abolished by hexamethonium, mecamylamine, tetraethylammonium, tubocurarine and guanethidine, none of these drugs (except huge "local anaesthetic" doses of the last-named) in any way modified the response to stroking the fur. These results therefore support the arguments of Harvey, Lilienthal & Talbot (1941),

Douglas (1954), Paintal (1956), Gray & Diamond (1957), Gray (1959), Douglas & Ritchie (1962) against the suggestions by Liljestr and (1954), Davis (1961), and Koelle (1962a, b) that there is necessarily a cholinergic link in the initiation of sensory impulses from the skin. A similar argument against an essential chemical link involving histamine, 5-HT or bradykinin may also be advanced, since the relevant antagonists abolished the effects of these substances without modifying the responses to stroking. Nevertheless, naturally occurring substances such as acetylcholine, histamine, 5-hydroxytryptamine, bradykinin or  $K^+$ , all of which have been shown capable of initiating or modifying sensory discharges, may well be concerned with the modulation of sensory perception in life, or even with the initiation of painful stimuli as a result of injury or disease (Rosenthal, 1964). Bradykinin, the most potent substance examined in the present tests, may be particularly important in this respect. The possible role of such substances in the mediation of painful stimuli has been fully discussed by Keele & Armstrong (1964). Although it was found possible to inhibit the action of bradykinin on the saphenous nerve by prior treatment with aspirin and related substances, this effect was found to be non-specific, since responses to acetylcholine were also inhibited and to a more pronounced degree. This finding raises the question as to whether the antagonistic action of aspirin on painful stimuli evoked from the spleen by bradykinin (Guzman et al., 1964; Lim et al., 1964) is as specific as has been thought to be the case. A similar doubt has also been expressed by Aarsen (1966) from his studies of bradykinin on guinea pig lungs.

#### Anticholinesterase drugs

In the somatic motor nerve system, neostigmine and other anti-

cholinesterase drugs have long been known to convert the reaction to a single nerve shock into a repetitive response, which is detectable orthodromically in the innervated muscle, and antidromically in the ventral root (Masland & Wigton, 1940; Feng & Li, 1941; Lloyd, 1942; Eccles et al., 1942; Riker et al., 1957; Riker et al., 1959a, b; Werner, 1960a, b; 1961; Blaber & Bowman, 1963; Randić & Straughan, 1964). Some authors, notably Riker and his co-workers (Riker & Wescoe, 1946, 1950; Riker, 1960; Riker et al., 1957; Riker et al., 1959a; Werner, 1960a, b, 1961), have deduced that this effect of these drugs is unrelated to their anticholinesterase action and is the result of a direct action on the non-myelinated motor nerve terminals, probably in the region of the first node of Ranvier. The rabbit saphenous nerve resembles a motor nerve to the extent that it contains myelinated fibres and receptor sites to acetylcholine near its terminals. However, it differs from a somatic motor nerve in that acetylcholine is neither released from it, nor apparently plays any physiological role in initiating its response. It was therefore of interest to examine the action of anticholinesterase drugs on the evoked compound action potential of the whole saphenous nerve. The anticholinesterase drugs were injected intra-arterially into the region of the nerve endings and found to be completely without effect on the configuration of the evoked compound action potential; there was no sign of repetitive firing in their presence. Thus, if an analogy is permissible, this result suggests that repetitive firing in the nerve is induced by anticholinesterase drugs only when acetylcholine is released in close proximity to the nerve endings as it is in the case of motoneurons. The results therefore add support to the idea

by some workers (Masland & Wigton, 1940; Eccles et al., 1942; Randić & Straughan, 1964; Bowman, Goldberg & Raper, 1969) that the action of anticholinesterase drugs in inducing antidromic repetitive firing in motor nerve is due to the preserved transmitter stimulating the nerve endings, and is not the result of a direct action of the anticholinesterase drug.

#### Local anaesthetic drugs

The local anaesthetic drugs (lignocaine, procaine and propanadid) and drugs with local anaesthetic activity (guanethidine, dichloro-isoprenaline, pronethalol and propranolol) were the only drugs that non-selectively blocked the responses to stroking, as well as to chemical agents, including KCl. There was some evidence that responses to chemical agents were blocked slightly sooner or by slightly smaller doses of the local anaesthetics, than were responses to stroking. This can possibly be explained on the grounds that the site of action of injected stimulant drugs is more accessible to injected local anaesthetic drugs than is the sensory receptor itself, and that activity evoked from the receptor may bypass or "jump" the blocked region in the initial stages, until the number of afferent fibres blocked or the areas of their membranes affected are sufficient to abolish all responsiveness. However, the difference was in fact marginal and tests of this type enabled a local anaesthetic effect to be distinguished from the more specific type of drug receptor antagonism.

This test for local anaesthetic action may be considered more specific than others such as the guinea pig wheal test or the frog plexus (Burn, 1952) methods in which it is possible that actions other than local anaesthesia may prevent the observed response.

It also has the advantages over the frog plexus and rabbit cornea (Koppanyi & Karczmar, 1958) methods in that it is possible to measure duration of effect and to administer more than one drug to the same animal. Withrington & Zaimis (1961) used a similar test in cats and showed that it can be designed to give reasonably accurate quantitative data. In the present experiments only rough quantitative data were obtained. The rank order of potency of the compounds studied was similar to that obtained in the guinea pig wheel test and was as follows: propranolol = pronethalol > lignocaine = dichloroisoprenaline > procaine > propanadid. It was of interest that dichloroisoprenaline, pronethalol and propranolol each produced a weak afferent discharge before blocking on intra-arterial injection. It is not known whether any reflection of this sensory stimulation has been experienced in clinical practice with propranolol.

#### Guanethidine

The selective depressant effect of intravenous guanethidine on responses to acetylcholine and related drugs was unlikely to have been the result of its hypotensive action for several reasons. (1) Doses of isoprenaline, which produced a fall in blood pressure equal to or greater than that produced by guanethidine, did not prevent the responses to acetylcholine. (2) Intra-arterially injected guanethidine also blocked responses to acetylcholine, yet by this route of injection little change in the general arterial blood pressure was produced. (3) In one experiment, the animal died before any antagonistic drugs had been administered. In this experiment, an afferent discharge in response to stroking and to intra-arterially injected acetylcholine continued to be produced

for over 60 min after death.

The results emphasise the difference between the action of guanethidine and that of local anaesthetic drugs, which always blocked responses to stroking. They therefore support the view of Rand & Wilson (1967) that the known local anaesthetic action of guanethidine (Bein, 1960; Green, 1960) is unlikely to contribute to its adrenergic neurone blocking action. However, large doses of guanethidine injected intra-arterially did resemble lignocaine in that they blocked the response to KCl and touch as well as that to acetylcholine, and it therefore remains possible that a selective sequestration of guanethidine by adrenergic nerve terminals (Bisson & Muscholl, 1962; Boura & Green, 1965; Chang, Costa & Brodie, 1965) may cause it to reach a concentration sufficient to act as a local anaesthetic at this site, while remaining below local anaesthetic concentrations elsewhere.

Guanethidine specifically blocked the action of acetylcholine-like drugs on fibres of the saphenous nerve in doses which did not impair the nerve's function in other respects. This result adds to the accumulating evidence (Bein, 1960; Maxwell et al., 1960; Gertner & Romano, 1961; Dixit, Gulati & Gokhale, 1961; Burn & Seltzer, 1965) that an anti-acetylcholine action of guanethidine may be an important aspect of its effects, and indicates that the action of guanethidine in abolishing the effect of acetylcholine or nicotine on adrenergic fibres is likely to be the result of a direct antagonistic action. The fact that guanethidine did not affect the normal function of the fibres in the saphenous nerve where acetylcholine is believed not to play a physiological role might, by inference, be interpreted to mean, in support of Burn &



Rand (1959), that acetylcholine is involved at sites where guanethidine does impair C fibre function, that is, at adrenergic nerve endings.

Like the adrenergic neurone blocking action of guanethidine (Day, 1962; Day & Rand, 1962), the anti-acetylcholine action of the drug on the saphenous nerve was slowly but permanently reversed by dexamphetamine. Dexamphetamine also produced some enhancement of responses to KCl and acetylcholine in the absence of guanethidine, and hastened recovery of acetylcholine responses after blockade by hexamethonium. However, these latter effects were abrupt and short-lasting in comparison with the reversal of the guanethidine effect and were probably a consequence of an improvement in peripheral blood supply resulting from the pressor effect of dexamphetamine.

It is difficult entirely to exclude an improved blood supply as a factor in the reversal of the guanethidine effect, but the slow onset and long duration of the antagonism make it unlikely that this was an important contribution. Reversal by dexamphetamine of adrenergic neurone blockade produced by guanethidine is not related to circulatory changes, since it also occurs in isolated preparations (Day, 1962; Day & Rand, 1962).

Guanethidine has been shown to possess ganglion blocking activity (Bein, 1960; Maxwell et al., 1960; Gertner & Romano, 1961; and the next section of this thesis) and the similar results obtained with guanethidine and hexamethonium in the present experiments suggest that the ability of guanethidine to block the action of acetylcholine on sensory fibres may have been a reflection of its ability to block nicotinic receptors. However, the ganglion blocking action of guanethidine differs from its action on sensory fibres and |

from its adrenergic neurone blocking action in that, though readily demonstrable in isolated tissues it is difficult to demonstrate in vivo, requires large doses to produce it and is relatively short-lasting (Boura & Green, 1965). Furthermore, as shown in the next section of this thesis, the ganglion blocking action is not reversible by dexamphetamine. For these reasons it is suggested that the anti-acetylcholine action of guanethidine on fibres in the saphenous nerve is not related to its ganglion blocking action, but may be a reflection of its adrenergic neurone blocking activity.

SECTION 2

Isolated superior cervical ganglion of the rat

RESULTS

When the meniscus was swept over the ganglion in the absence of drugs, a small potential difference was present in most preparations between the body of the ganglion and the stump of the post-ganglionic trunk. At the start of an experiment the surface of the body of the ganglion was found to be up to 3 mV positive with respect to the post-ganglionic trunk. Fig. 20 illustrates an example of the potential difference recorded from a resting ganglion. The potential was presumably a demarcation potential due to injury of the post-ganglionic trunk at the point of severance (Pascoe, 1956). This potential diminished with time (Fig. 20).

Depolarizing drugs produced a deflection of the trace in the direction opposite to that occurring in the absence of drugs. The effect of the depolarizing drug was recorded as the difference (in mV) between the deflections in the presence and in the absence of the drug, and for this measurement a point on the trace (which represents a particular point on the surface of the ganglion) was chosen and adhered to throughout any one experiment. The point chosen was one that was easily recognized, e.g. point 'X' in Fig. 22, and not necessarily the point of maximal deflection in the control sweep. Control sweeps were made before the addition of each drug. During the course of an experiment lasting 8 hours or so, the extent of the deflections recorded when sweeping the ganglion in the absence of drugs diminished slowly (Fig. 20). However, the difference between the deflections produced by the same dose of a depolarizing drug and the control deflection recorded before each addition of the drug remained remarkably constant throughout an experiment.

The shape of the deflection produced by a depolarizing drug, as the meniscus was swept over the ganglion, varied from experiment

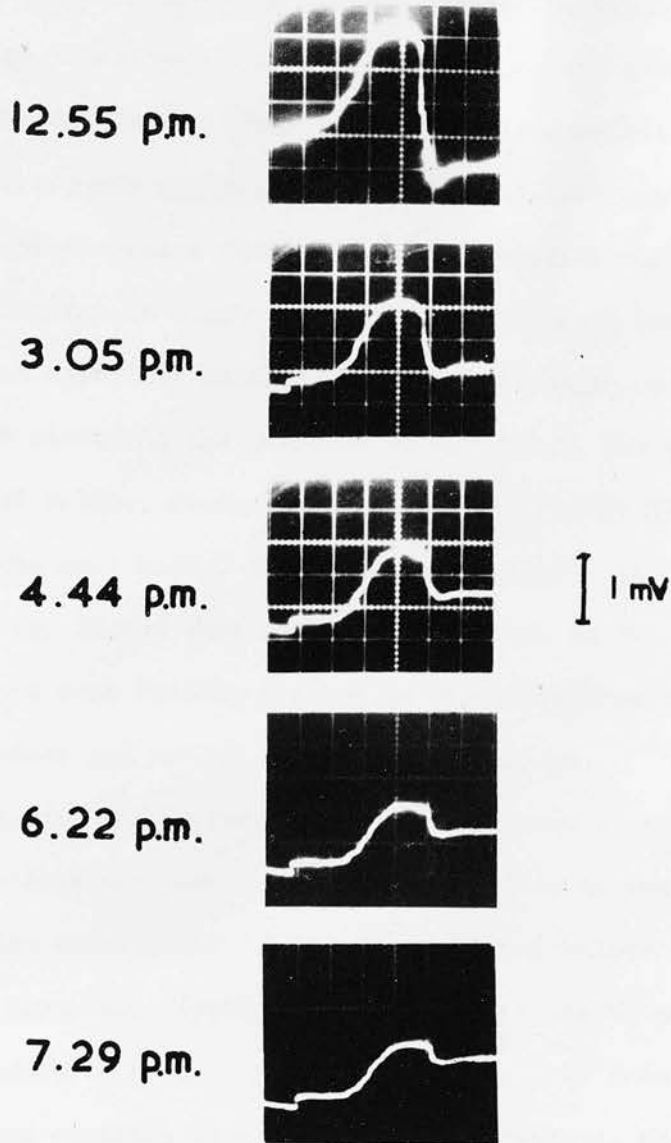


Fig. 20. Rat superior cervical ganglion. Trace of the resting positive potential from the surface of the ganglion. The trace was swept from left (post-ganglionic) to right (pre-ganglionic) trunk. The gradual decline in the size of the resting potential over a period of  $6\frac{1}{2}$  hr is clearly seen.

to experiment. Rough sketches made of over 50 ganglia suggested that there was some correlation between the shape of the ganglion and the form of the depolarization trace. Thus the greatest depolarization corresponds approximately to the thickest part of the ganglion and this suggests that the maximal depolarization corresponds to the point at which the density of the cell bodies is greatest. This was tested by histological examination in one experiment. After recording the response to carbachol, the ganglion was fixed in formol saline, stained and cut at 5  $\mu$ . It yielded 71 intact sections, the cell bodies in which were counted. The results are presented in Fig. 21 and show that the variations in the depolarization trace were broadly related to the variations in the density of cell bodies and to the shape of the ganglion.

Another check made was to record control responses to carbachol in several preparations and then to crush the ganglion at one point, by means of mosquito forceps shielded with polythene, before recording another carbachol response. (Great care had to be taken to avoid tearing the delicate pre- or post-ganglionic trunks away from their ligatures during the crushing operation). After crushing, the depolarization produced by carbachol was absent or much reduced in the area corresponding to the damaged neurones. Damage to the ganglion also cancelled out the demarcation potential recorded from the point of crushing. This indicates that the drug response is dependent on the integrity of the neurones.

### Carbachol

Carbachol was chosen as the reference drug in preference to acetylcholine because of its stability to cholinesterase. In the presence of carbachol, the depolarization of the ganglion was almost

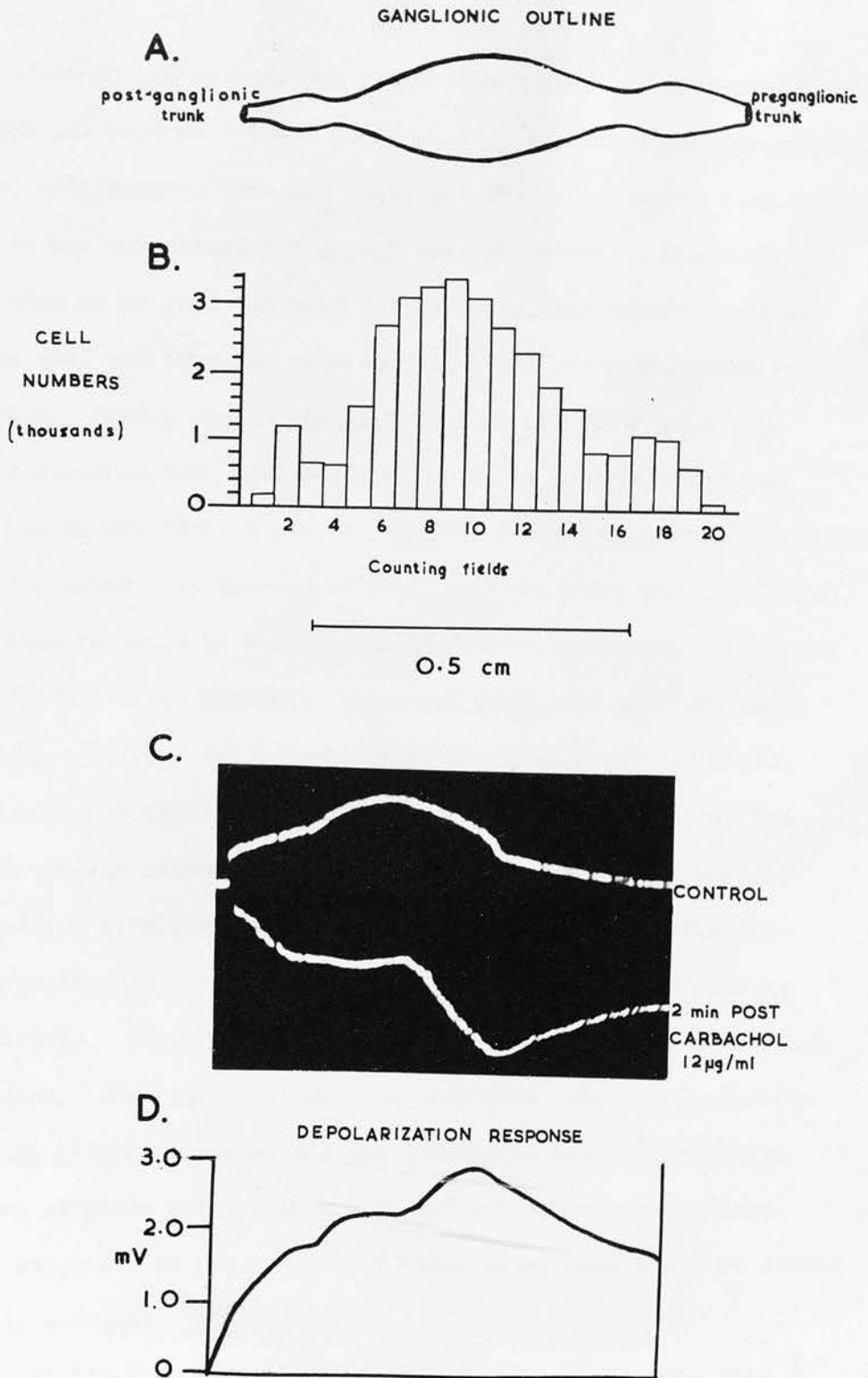


Fig. 21. Rat superior cervical ganglion. Composite diagram showing shape of the ganglion (A), histogram of cell numbers estimated by histological reconstruction of the ganglion (B), the ganglionic potential before (upper trace) and after carbachol ( $12 \mu\text{g/ml}$ ) (C), and the actual potential obtained by measuring the distance between the two traces of depolarization (D). The maximal degree of depolarization broadly corresponds to the region of greatest cell density, which in turn is related to the widest part of the ganglion.

maximal after 30 sec and maximal after 1 to 2 min. In most experiments the effect was read at 2 min. Occasionally, if the effect was maximal at 1 min, this contact time was used, but whatever contact time was chosen, it was maintained throughout that experiment. Once the contact time to be used had been decided upon, the depolarization value was read off from the selected point and the preparation was then washed. During the 15 min period after the first wash, the potential recorded from the same point usually swung through two phases. During the first 2 min, a diminished and waning depolarization was still present. By the end of the third min after washing it had usually been replaced by some degree of hyperpolarization, which was maximal 3-5 min after washing. This then gradually subsided to be followed by a further small depolarization which persisted until the next wash, 15 min after the first. After the second wash the potential usually returned to the original control level, but occasionally a slight degree of depolarization persisted despite repeated washing and this became the new base-line for subsequent drug additions. Fig. 22 illustrates a complete record of a response to carbachol. With the first dose of carbachol, the depolarization was usually slightly greater and the subsequent hyperpolarization on washing slightly smaller than with all subsequent equal doses. However, responses to the second and later doses were found to remain remarkably constant.

Fig. 23 illustrates a log dose/depolarization response line to doses of carbachol ranging from 0.04 to 40  $\mu\text{g/ml}$ . The results were subjected to a multiple regression programme test for linearity using an Elliott L.S.17 computer. This showed that 94.23% of the variation was accounted for by a linear relationship. A concentration



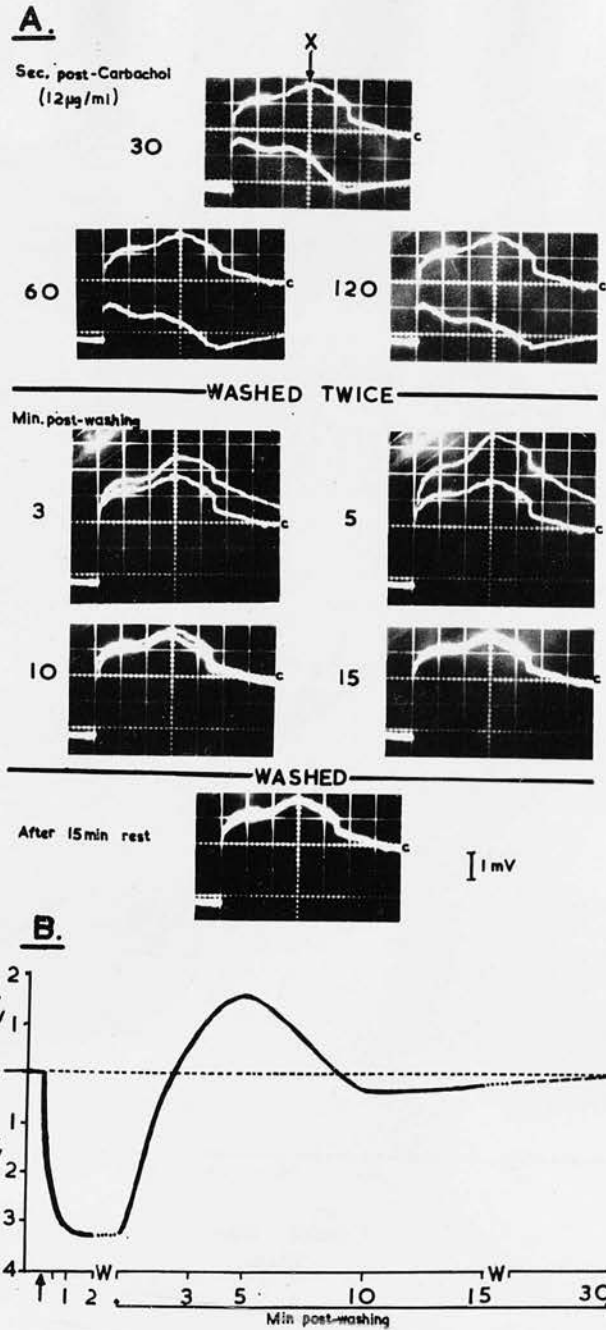


Fig. 22. Rat superior cervical ganglion. Complete cycle of responses to a single dose of carbachol (12  $\mu\text{g}/\text{ml}$ ).

A. The resting (control) trace (c) is superimposed on each succeeding record. The identifiable peak 'X', corresponding to a neuronally dense region of the ganglion was the point selected for readings to be made throughout.

B. The changes in potential measured at 'X' expressed in graphic form. After 2 min (W) the preparation was washed twice, and it was washed once more 15 min later.

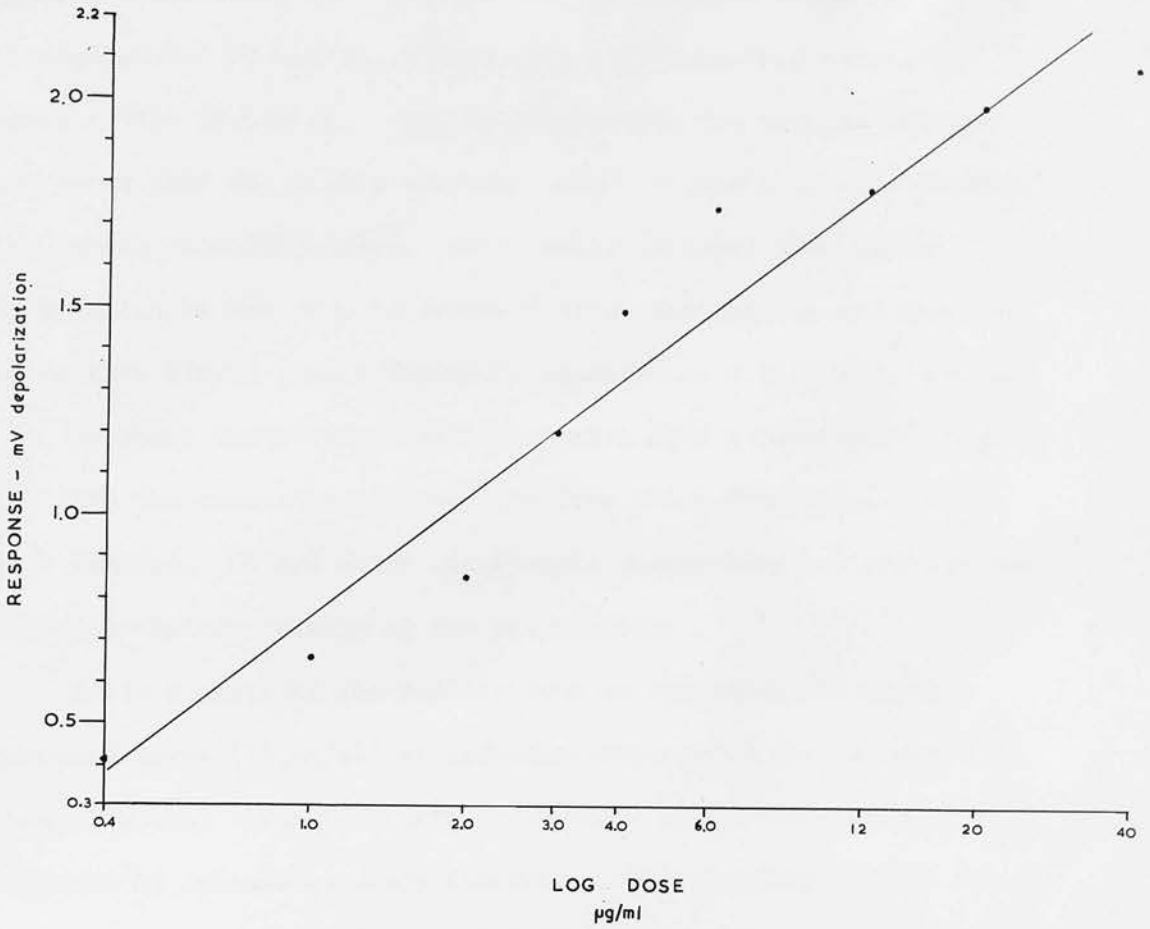


Fig. 23. Rat superior cervical ganglion. Log dose/depolarization response line showing responses to carbachol from 0.04 to 40  $\mu\text{g/ml}$ . Elliott LS 17 computer analysis confirmed the straight line tendency.

of 12  $\mu\text{g/ml}$  was chosen as a suitable standard dose to give a sub-maximal response for all subsequent experiments.

At this stage an experiment was carried out to test the necessity for desheathing the ganglion. A preparation was set up without desheathing and subjected to two standard doses (12  $\mu\text{g/ml}$ ) of carbachol. It was then taken down and desheathed before re-testing with carbachol. Fig. 24 illustrates the results obtained and shows that the values recorded after desheathing were greater than those obtained before. This result suggests that better penetration of the drug is achieved after desheathing and that the connective tissue sheath therefore constitutes a diffusion barrier. Fig. 25 shows histological sections taken from a desheathed ganglion and from the contralateral ganglion from which the sheath had not been removed. In all other experiments desheathing was carried out routinely before setting up the preparation.

Table 2 presents the results from an experiment in which 8 standard doses (12  $\mu\text{g/ml}$ ) of carbachol were added over a period of about 5 hours. Over this period the value of the depolarization produced by successive doses fluctuated only slightly, and in the last test gave the same value of 3 mV at 2 min after addition, as it did in the first. The mean value  $\pm$  the standard deviation was  $2.89 \pm 0.23$  mV. The hyperpolarization values recorded 3 min after washing also remained fairly constant, although the variation was greater than that seen with the depolarization values. The mean hyperpolarization value  $\pm$  the standard deviation at 3 min after washing was  $0.84 \pm 0.22$  mV. In this experiment the level of hyperpolarization after the last dose was slightly higher than after the second dose of the series. In other experiments, the second dose

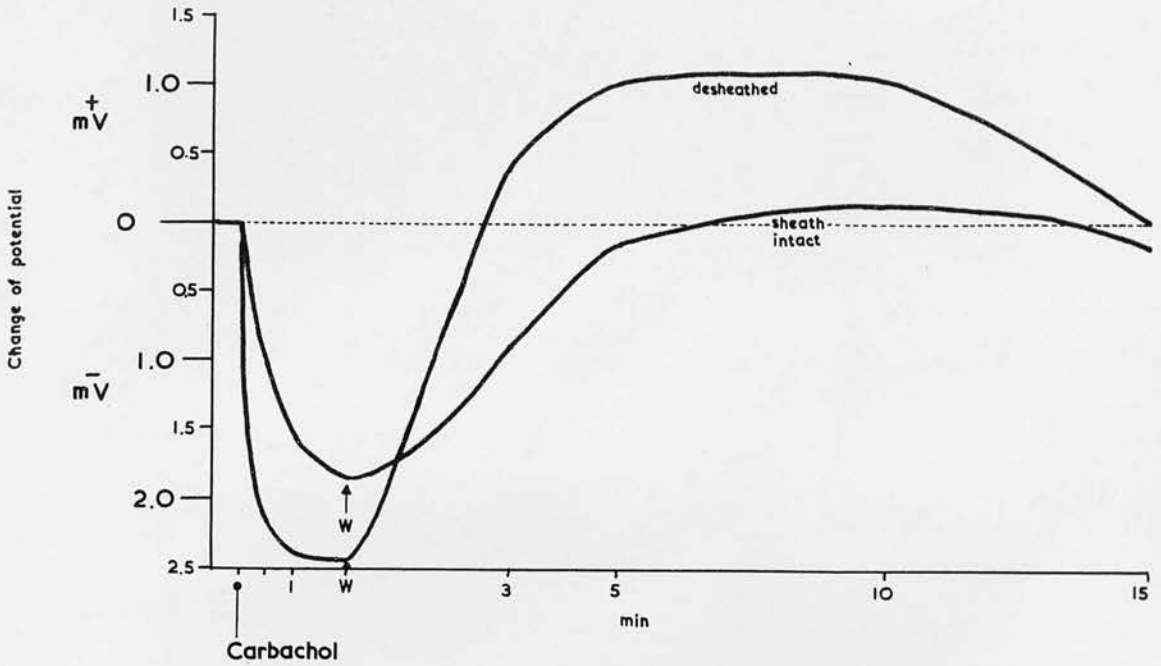
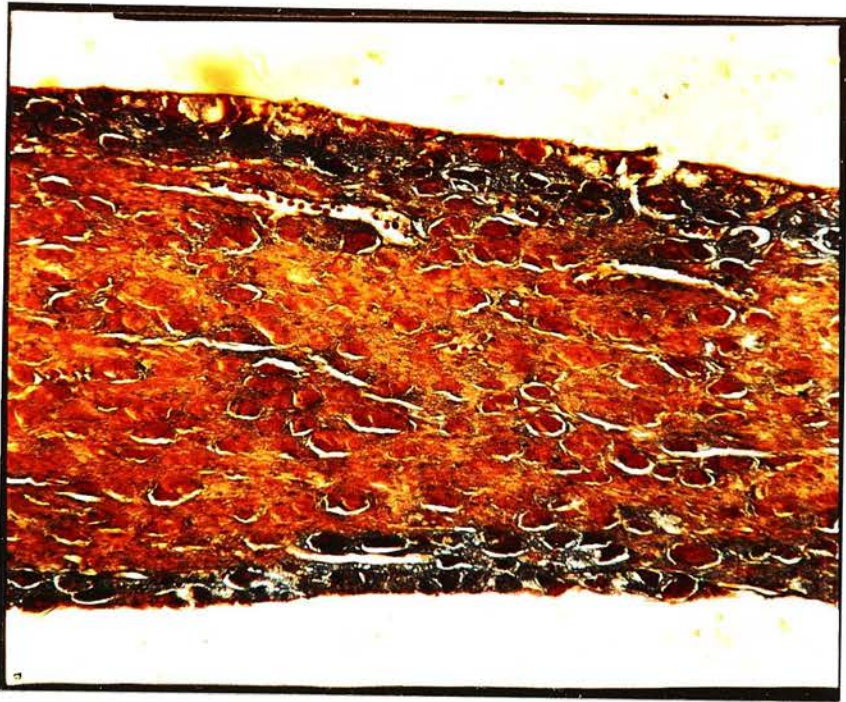


Fig. 24. Rat superior cervical ganglion. Responses to carbachol ( $12 \mu\text{g}/\text{ml}$ ) in a ganglion before and after desheathing. The onset and extent of depolarization and hyperpolarization phases were clearly enhanced after desheathing, and this applied particularly to the (secondary) positive phase. W indicates double washing of the preparation.



Left ganglion

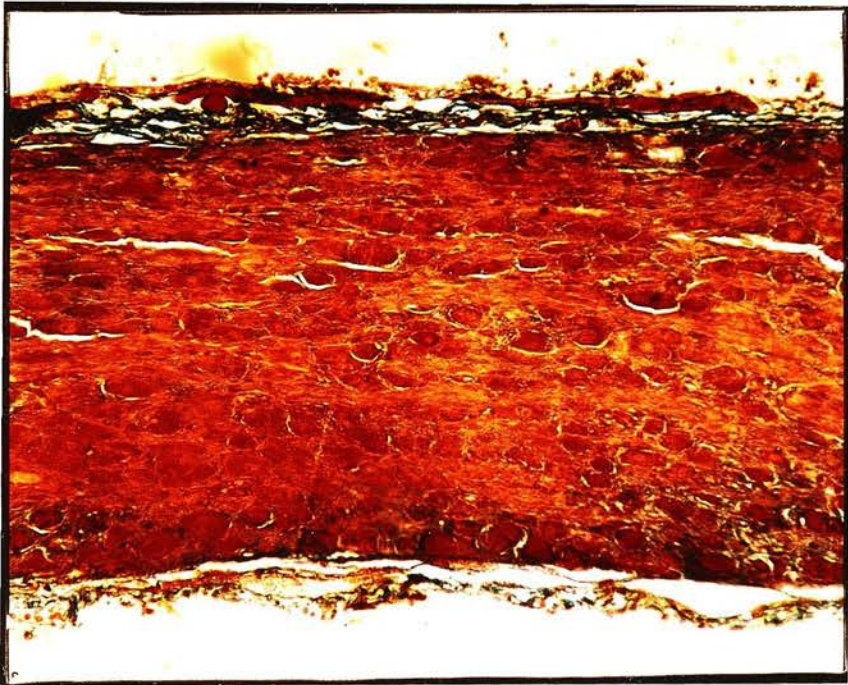


Fig. 25. Rat superior cervical ganglion. Section of left and right ganglia from the same rat stained with acid fuchsin. The left ganglion was stripped of its connective tissue sheath prior to fixation, and the close proximity of cell bodies (stained red) to the surface is clearly seen. The cell bodies should not be confused with the vascular tissue present in the sheath of the right superior cervical ganglion which have stained similarly.

TABLE 2

Carbachol

CARBACHOL APPLICATION	1	2	3	4	5	6	7	8
30 sec post carbachol	- 1.70	- 1.65	- 2.00	- 1.85	- 2.00	- 1.70	- 2.30	- 2.05
60 sec post carbachol	- 2.30	- 2.30	- 2.55	- 2.10	- 2.70	- 2.55	- 2.90	- 2.70
120 sec post carbachol	- 3.00	- 2.80	- 2.90	- 2.40	- 3.05	- 2.85	- 3.15	- 3.00
W A S H								
3 min post washing	+ 0.60	+ 0.80	+ 1.00	+ 0.85	+ 0.75	+ 0.80	+ 0.90	+ 1.05
5 min post washing	+ 0.20	+ 0.35	+ 0.50	+ 0.20	+ 0.15	Normal	+ 0.10	+ 0.20
10 min post washing	- 0.50	- 0.35	- 0.20	- 0.30	- 0.30	- 0.30	- 0.30	- 0.20
15 min post washing	- 0.70	- 0.35	- 0.20	- 0.30	- 0.40	- 0.30	- 0.35	- 0.20
W A S H								
REST PERIOD (mins)	30	30	30	30	15	15	15	-

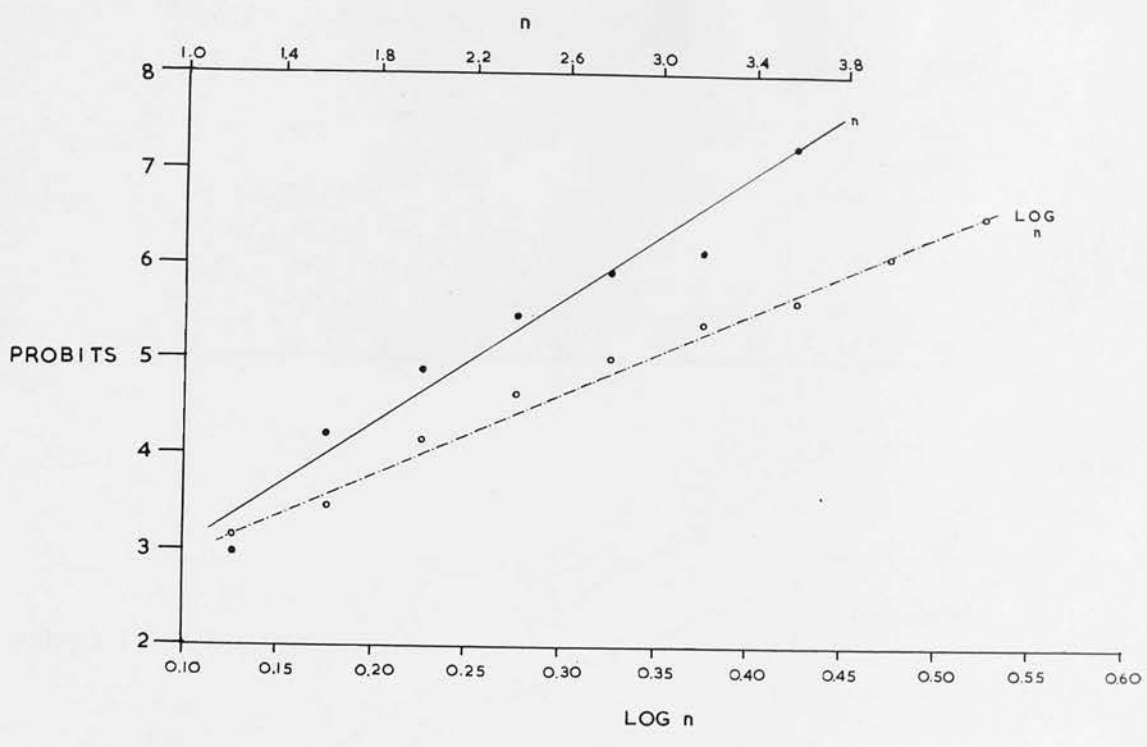
Dose of carbachol = 12  $\mu$ g/ml.

Potential changes (in mV) produced by a succession of standard doses of carbachol.

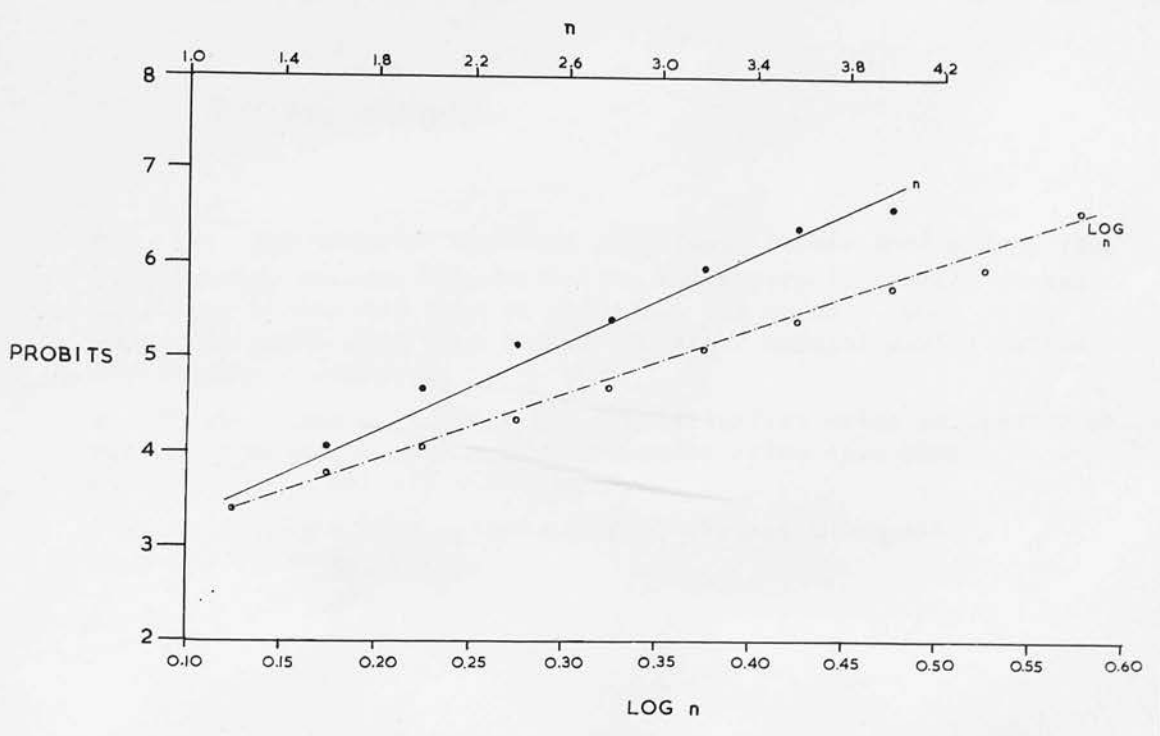
of the series gave the greatest hyperpolarization value, and the value then often declined slightly with successive doses during the course of an experiment. However, all tests showed that the values for successive control doses were sufficiently constant to permit conclusions regarding interactions with other drugs.

An attempt was made to assess whether the post-washing hyperpolarization in different experiments was constantly related to the extent of the depolarization produced by carbachol. A sample of 128 additions of carbachol (12  $\mu\text{g/ml}$ ) to ganglia which had settled down (i.e. in which the post-washing hyperpolarization had been activated by successive doses) was taken and defined on the basis of the ratio of depolarization: hyperpolarization, taking the latter as unity.

The sample was made up of ninety three results in which the depolarization exceeded 2 mV (the significant level arbitrarily selected by Brown, 1966a) and thirty five with results below 2 mV. These two groups were analysed both separately and together. In the larger sample the mean value of depolarization/hyperpolarization was 2.34 : 1 (S.D. = 0.64) and in the smaller one 2.52 : 1 (S.D. = 0.95). Combining the results gave a mean of 2.39 : 1 (S.D. = 0.73). All three sets of observations were statistically satisfactory in that more than 95% of the results lay within the range of the mean  $\pm 2 \times \text{S.D.}$  They were also subjected to probit analysis and plotted as probit against number of observations (n) in ratio graphs ranging from 1.0 to 4.6. The results are shown in Fig. 26a, b and c, in which  $\log n$  is also recorded: the points lie very close to a straight line, suggesting that for carbachol, there is a relationship between the extent of the depolarization



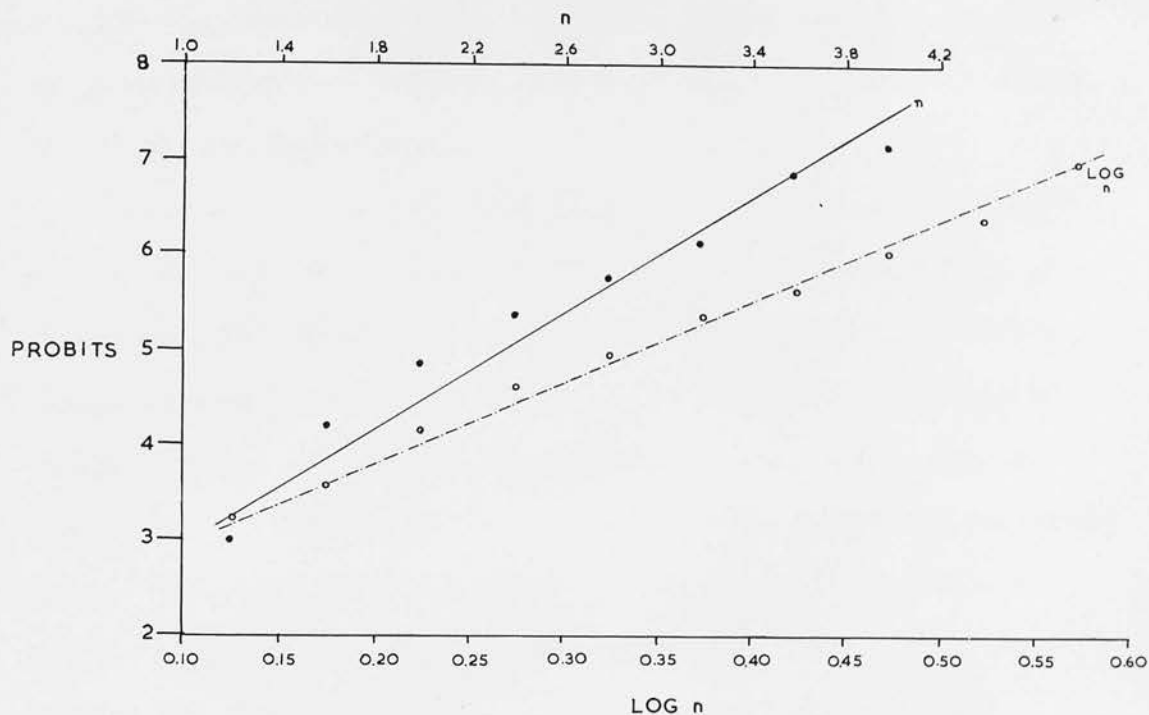
a. 93 observations



b. 35 observations

Fig. 26. For legend see p.100.





**C. 128 observations**

Fig. 26. Rat superior cervical ganglion. Probit analysis of the relationship between depolarization and hyperpolarization phases following a standard dose of carbachol ( $12 \mu\text{g/ml}$ ). Only one value was taken from each experiment after initial equilibration had obviously occurred.

- a. 93 experiments in which the depolarization value exceeded 2 mV.
- b. 35 experiments with a depolarization value less than 2 mV.
- c. The sum of all 128 experiments.

A clear straight line relationship is obvious throughout.  
(n = no. of experiments).

and that of the hyperpolarization. It is also clear that ganglia giving a depolarization value less than 2 mV are acceptable, and need not be discarded. In later experiments this ratio was used as a quick check that the ganglion under test was apparently normal. It is noteworthy that, without exception, the ratio fell well within the limits set by the analysis.

A further use for the consistency of this ratio was suggested by the fact that if either of its components (hyperpolarization or depolarization) varied, due to drug intervention, the ratio should change in a marked fashion. It was fully appreciated that such a change in ratio did not show which component was variant, but it means that, provided the initial carbachol ratio lay within the normal range, any marked change would have a significance. If this was capable of repetition then the intervention of the drug could be implicated. This approach obviates unnecessary repetition of observations in order to satisfy statistical requirements.

#### Acetylcholine

Acetylcholine was tested in 9 experiments. In the absence of anticholinesterase drugs a concentration of 12  $\mu\text{g/ml}$  of acetylcholine produced only about 10% of the depolarization in response to the same dose of carbachol. Even in concentrations of 40  $\mu\text{g/ml}$ , acetylcholine produced only about 40% of the depolarization produced by carbachol (12  $\mu\text{g/ml}$ ). There was never any evidence of a post-washing hyperpolarization produced by acetylcholine in the absence of anticholinesterase drugs even when the extent of the depolarization produced equalled that produced by a concentration of carbachol which did give rise to a post-wash hyperpolarization (Fig. 27). In place of the post-wash hyperpolarization there was a secondary phase of

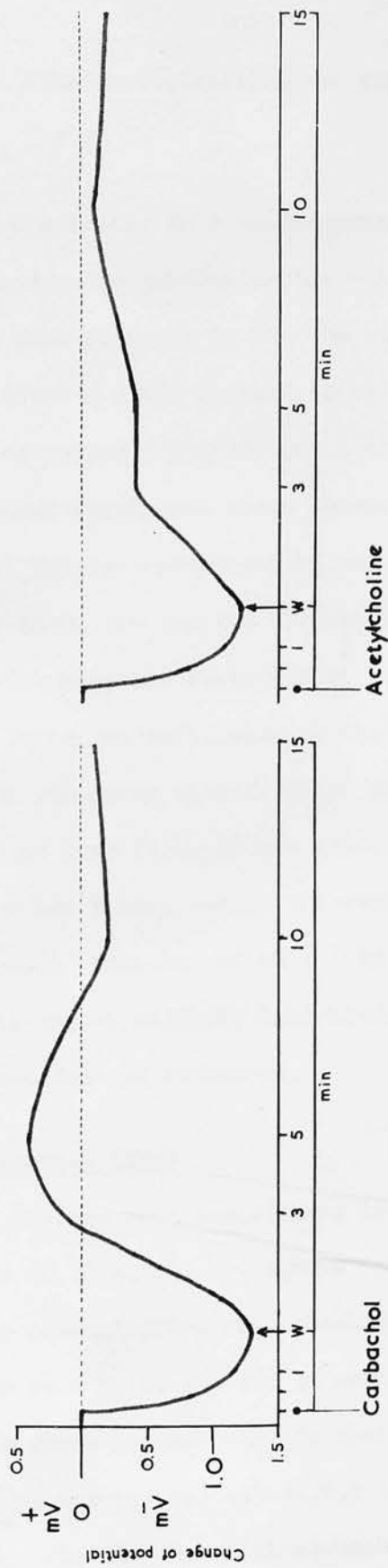


Fig. 27. Rat superior cervical ganglion. Approximately equivalent depolarization evoked by doses of carbachol (12  $\mu\text{g}/\text{ml}$ ) and acetylcholine (35  $\mu\text{g}/\text{ml}$ ). The complete lack of a positive potential following acetylcholine is well illustrated. W = wash.

depolarization after acetylcholine was removed from the bath.

### Nicotine

Nicotine was tested in 3 experiments. In a concentration of 12  $\mu\text{g/ml}$  it produced a depolarization which was consistently between 80 and 90% of that produced by the same dose of carbachol in the same experiment. After a 2 min contact time, the preparation was washed and the depolarization gradually waned to be replaced by a slowly developing hyperpolarization which sometimes exceeded that produced by carbachol. The hyperpolarization was evident within 15 min and persisted for 60-90 min despite further washing at 15 min intervals (Fig. 28). With nicotine there was no late depolarization phase. At this dose level, no tachyphylaxis was evident in the nicotine depolarization responses whether fresh additions were made immediately after the second wash (i.e. 17 min after the previous addition of nicotine) or after a long enough interval for full recovery (90 min) had been allowed. Nicotine at this dose level (12  $\mu\text{g/ml}$ ) was either without effect on, or slightly augmented, the depolarization produced by a subsequent dose of carbachol.

### Tetramethylammonium (TMA)

TMA was studied in 7 experiments in a range of doses. In a concentration of 12  $\mu\text{g/ml}$ , TMA always produced a greater depolarization than the same concentration of carbachol. Roughly equivalent doses were shown to be 8  $\mu\text{g/ml}$  TMA and 12  $\mu\text{g/ml}$  carbachol. Responses to TMA differed from those to carbachol in that the maximal depolarization produced by TMA was present within 0.5 to 1 min and was beginning to wane at 2 min, the time at which carbachol depolarization reached its maximum. This difference may reflect a more rapid penetration of

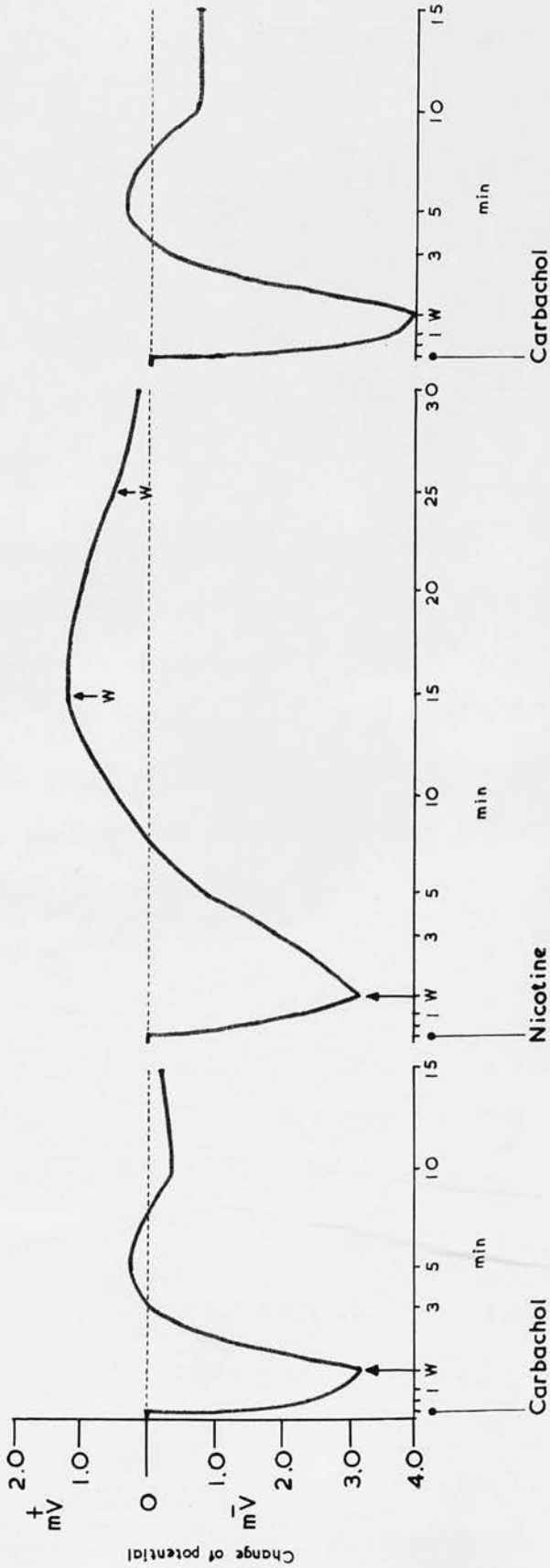


Fig. 28. Rat superior cervical ganglion. Effect of nicotine (12  $\mu\text{g}/\text{ml}$ ). The control dose of carbachol elicited a negative potential of 3.8 mV, which was followed by an unusually small positive potential. The depolarization due to nicotine was 3.2 mV, but its onset was slower than that of carbachol. The positive potential developed slowly, and eventually reached a maximal value which was 37.5% of the depolarization one (c.f. 7% in the case of carbachol). The third trace shows the effect of carbachol following nicotine. Both negative and positive potentials were slightly enhanced, but the value of the latter was only 7.5% of the initial depolarization. W = wash.

the tissue by the smaller TMA molecule and a more transient drug receptor complex.

Another difference noted with TMA was that the post-wash hyperpolarization value associated with a given level of depolarization was always greater by 10-20% than that produced by carbachol, and, as with nicotine, there was no late depolarization phase. Fig. 29 compares responses to TMA and to carbachol in a typical experiment.

#### 1,1-Dimethyl-4-phenylpiperazinium (DMPP)

DMPP in a concentration of 10  $\mu\text{g/ml}$  produced a depolarization about equal to that produced by the standard concentration of carbachol (12  $\mu\text{g/ml}$ ), but the post-wash hyperpolarization produced by DMPP, although slower in developing, was considerably larger and longer lasting than that produced by carbachol (Fig. 30). In a concentration of 40  $\mu\text{g/ml}$ , DMPP produced a pronounced depolarization which waned during the application of the drug. With this concentration of DMPP, the post-wash hyperpolarization was slower in reaching its maximum and was no greater in extent than that produced by the smaller dose. As with nicotine and TMA, the late depolarization phase was absent.

#### Acetylthiocholine

Acetylthiocholine was tested in 3 experiments in doses of 8-12  $\mu\text{g/ml}$ . The responses produced resembled those produced by carbachol both in depolarization and post-wash hyperpolarization phases. Acetylthiocholine was somewhat less potent than carbachol and at the standard dose level of 12  $\mu\text{g/ml}$  gave responses equivalent to 70-85% those of carbachol. Acetylthiocholine was thus considerably

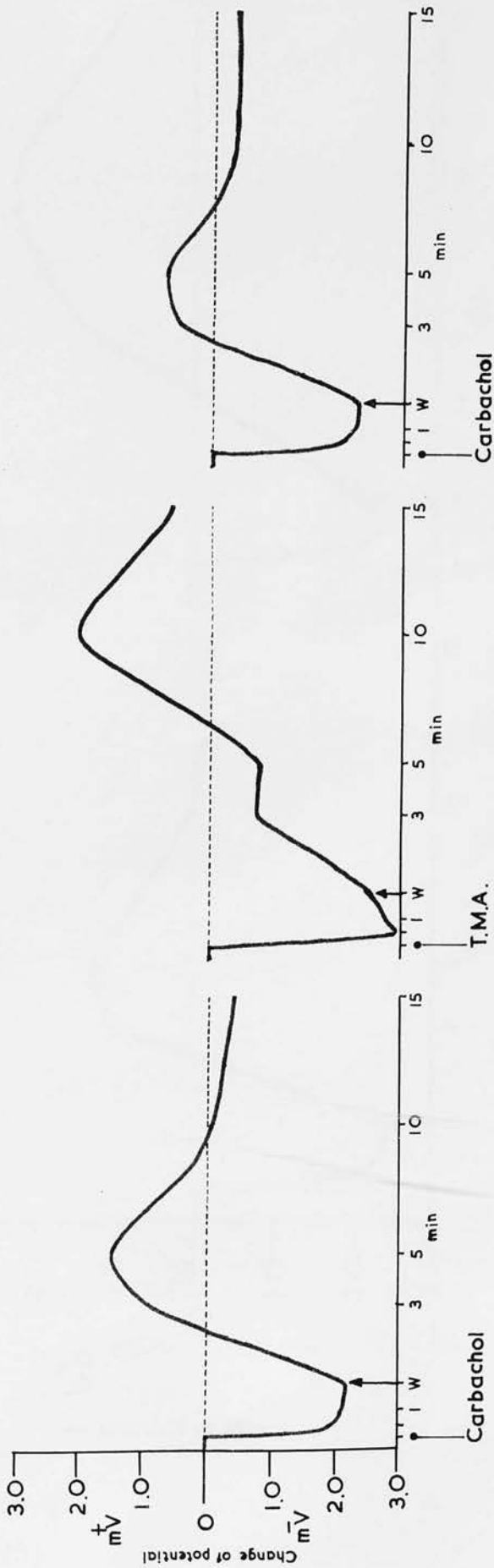


Fig. 29. Rat superior cervical ganglion. Tetramethylammonium (TMA). The effect of equal doses ( $12 \mu\text{g/ml}$ ) of carbachol and TMA. TMA produced a more rapid and vigorous response, although it was not sustained for the full 2 min exposure time. After washing (W) the TMA depolarization declined slowly, but the subsequent hyperpolarization was more vigorous (71% of the depolarization value) than the carbachol one (52% of the depolarization value). The following dose of carbachol showed a near normal depolarization value, but the positive potential was relatively curtailed, its size being only 31% of the depolarization one.

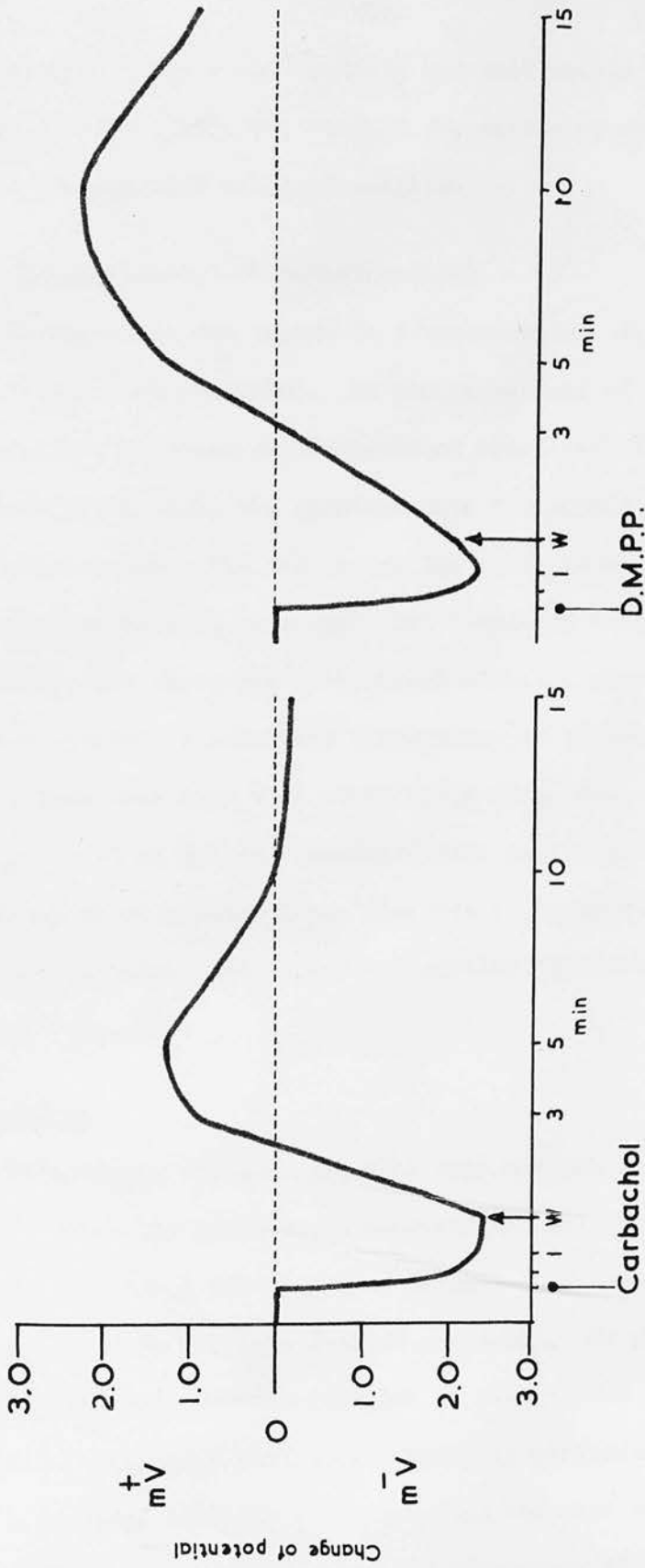


Fig. 30. Rat superior cervical ganglion. 1,1-dimethyl-4-phenylpiperazinium (DMPP). Comparison of the responses to carbachol (12  $\mu\text{g}/\text{ml}$ ) and DMPP (10  $\mu\text{g}/\text{ml}$ ). The depolarization induced by DMPP was nearly as large as that following carbachol, but it was not sustained for the full 2 min. As with TMA the negative potential was slow to disappear but was followed by a more pronounced hyperpolarization than that produced by carbachol. In this case its value equalled the preceding hyperpolarization, and was more than 1 mV larger than the carbachol-induced positive potential. A subsequent dose of carbachol was normal in all respects. W = wash.



more powerful than acetylcholine, and this result supports that of Gebber & Volle (1965) who studied the action of acetylthiocholine on the cat superior cervical ganglion.

#### Methacholine (Acetyl- $\beta$ -methylcholine)

Methacholine was tested in 6 experiments. It was considerably less potent than carbachol. In concentrations of 40, 80 and 120  $\mu\text{g/ml}$ , methacholine produced depolarizations which were 17%, 19-21% and 35-44% of those produced by the standard dose (12  $\mu\text{g/ml}$ ) of carbachol in the same preparation. The depolarization produced by methacholine was succeeded on washing by a rapid but temporary return towards normality, but there was no hyperpolarization phase. Then, without further washing, a secondary depolarization occurred which was more marked than that seen with other drugs (Fig. 31). A dose of carbachol that produced an initial depolarization equivalent to that produced by 120  $\mu\text{g/ml}$  of methacholine, also failed to give rise to a post-wash hyperpolarization, and a late but smaller depolarization phase was present (Fig. 31).

#### Pilocarpine

Pilocarpine was more powerful than methacholine in its ability to depolarize the ganglion, a measurable result being produced by 12  $\mu\text{g/ml}$ , although the response, at this dose level, was only 20-30% that produced by the same dose of carbachol. No post-wash hyperpolarization followed the addition of pilocarpine in doses of 12 or 24  $\mu\text{g/ml}$ . Instead a prolonged negativity persisted after washing.

A previous addition of pilocarpine affected subsequent responses to carbachol. When pilocarpine had been added 32 min before carbachol and had been washed out 30 min previously, the depolarization produced

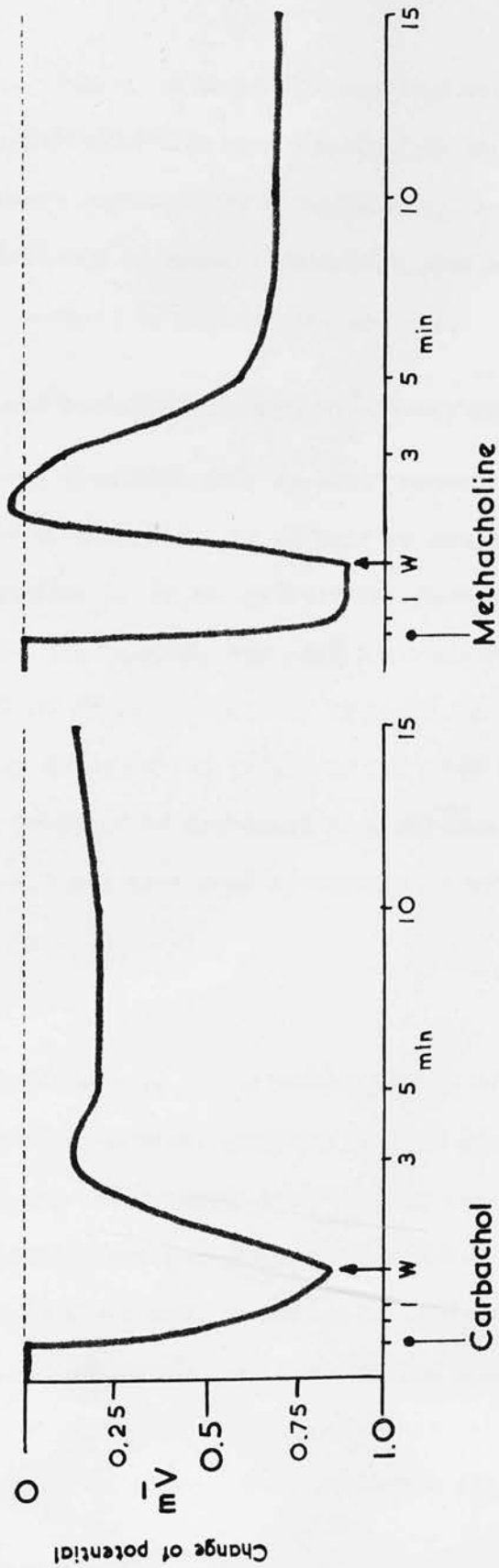


Fig. 31. Rat superior cervical ganglion. Methacholine. Comparison of the effects of doses of carbachol ( $2 \mu\text{g}/\text{ml}$ ) and methacholine ( $120 \mu\text{g}/\text{ml}$ ) needed to evoke a comparable depolarization effect. Following methacholine the recovery to normal after washing was transient and succeeded by a strong secondary negative potential.

by carbachol was reduced by about 20% compared with the control, whereas the hyperpolarization was augmented by about 30% (Fig. 32). In some experiments depolarization produced by small doses of carbachol was followed by hyperpolarization, but equivalent depolarization produced by pilocarpine was not.

McN A343 (4- $\overline{m}$ -chlorophenylcarbamoyloxy $\overline{r}$ -2-butylnyltrimethylammonium chloride)

This drug was available only in small amounts and could be tested only in 2 experiments. It is said to stimulate muscarinic receptors on ganglion cells selectively (Roszkowski, 1961). In concentrations of 12-24  $\mu\text{g/ml}$ , McN A343 gave depolarization values of 40.5% and 60% of those produced by 12  $\mu\text{g/ml}$  of carbachol. No hyperpolarization followed the effect of McN A343 although equal depolarizations produced by carbachol were followed by hyperpolarization. McN A343 did not give rise to changes in subsequent responses to carbachol.

Histamine

Histamine was added in concentrations up to 48  $\mu\text{g/ml}$ . Unlike those of the superior cervical ganglion in cats (Lewis & Reit, 1966) the responses of the rat ganglion were weak or non-existent and when a small depolarization was produced, it diminished with repeated additions. Histamine was without effect on responses to carbachol. The weak responses to histamine were unaffected by concentrations of mepyramine up to 40  $\mu\text{g/ml}$ , even though these concentrations produced a more than 75% block of responses to carbachol (12  $\mu\text{g/ml}$ ).

5-Hydroxytryptamine (5-HT)

In the present experiments 5-HT was more powerful than histamine and in a concentration of 36  $\mu\text{g/ml}$  produced a depolarization value of

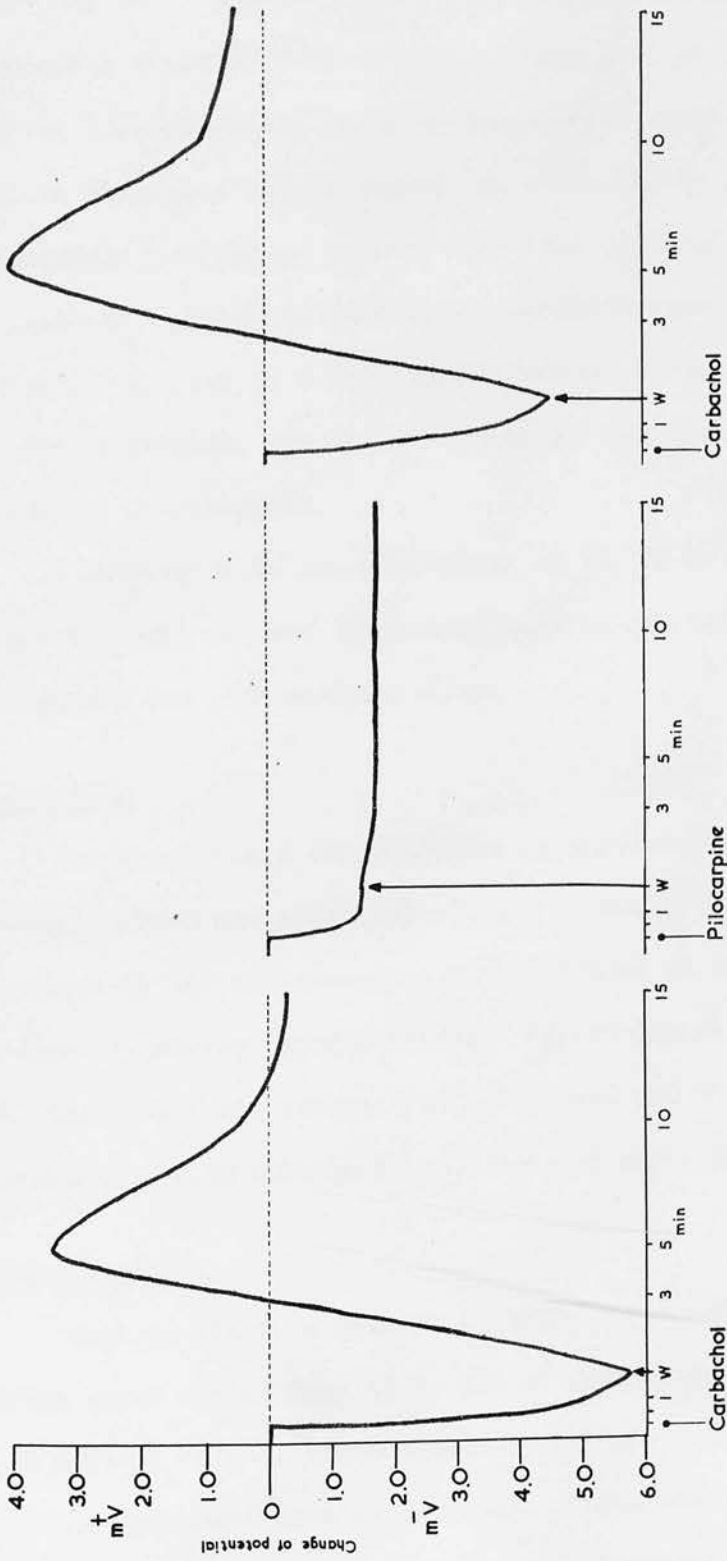


Fig. 32. Rat superior cervical ganglion. Effect of pilocarpine. Responses to equal doses ( $12 \mu\text{g}/\text{ml}$ ) of carbachol and pilocarpine. The depolarization produced by pilocarpine was approximately 25% that of carbachol and was slow to recover. The third trace shows the effect of carbachol after the normal 15 min recovery interval. The negative potential was 78% of the pre-pilocarpine control, but the positive potential exceeded the control one. (The hyperpolarization induced was nearly 90% of the size of the carbachol control depolarization; the comparable figure for the control hyperpolarization was 59%). The values were nearly normal in the succeeding cycle (not shown). W = washing.

20-25% of that produced by the standard dose of carbachol (Fig. 33a). Increasing doses of 5-HT did not produce greater depolarizations. Smaller concentrations produced even weaker depolarizations but despite this they were followed, on wash-out, by a detectable hyperpolarization which was greater than that produced by a concentration of carbachol giving an equivalent depolarization (Fig. 33b). 5-HT did not give rise to a late depolarization phase after the post-wash hyperpolarization, nor did it change the responses to subsequent additions of carbachol.

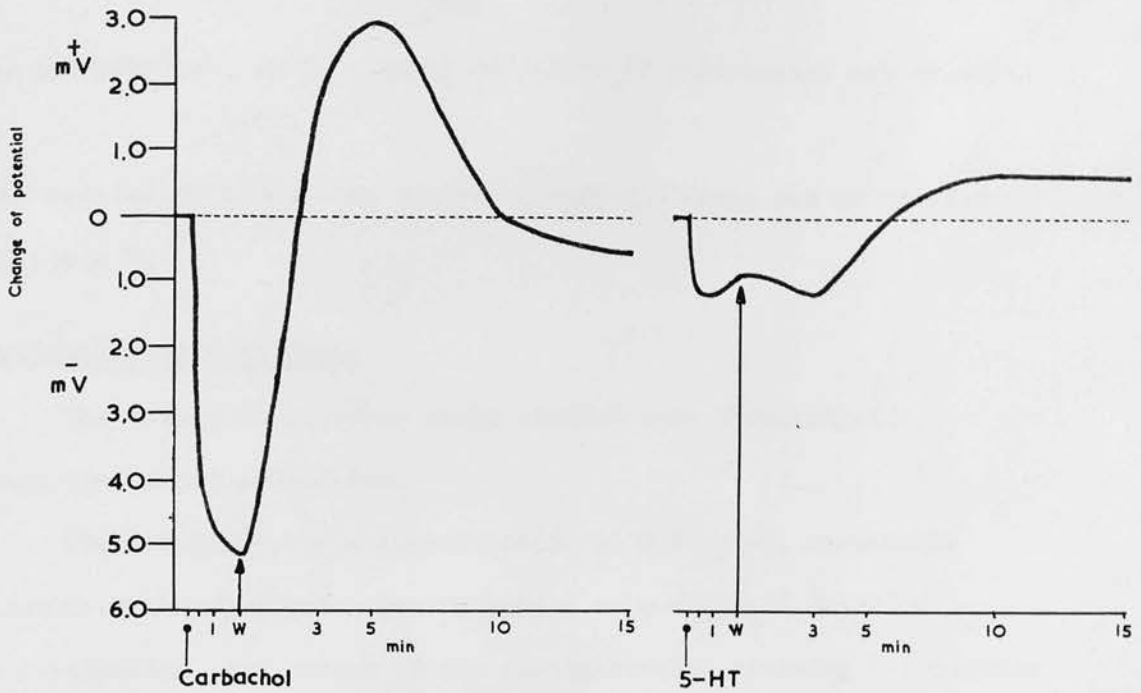
Methysergide in concentrations of 10, 20 or 40  $\mu\text{g/ml}$  given 10 min prior to 5-HT, did not alter responses to the latter drug. Mepyramine (40  $\mu\text{g/ml}$ ) was also without effect.

#### Bradykinin

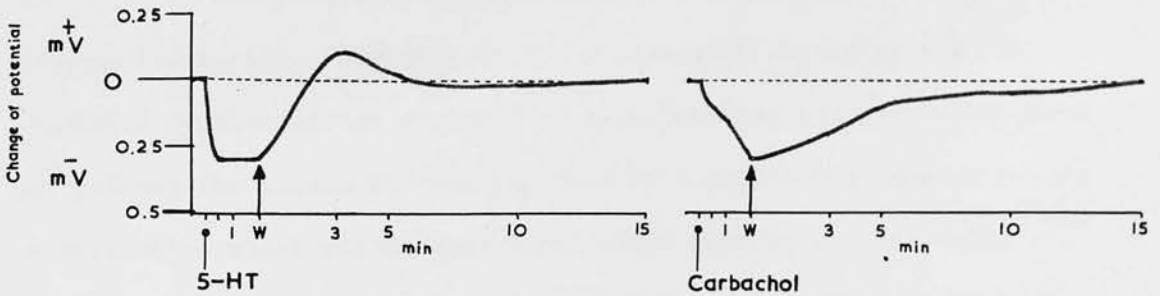
Bradykinin in a concentration of 4  $\mu\text{g/ml}$  produced a depolarization response which was about 10% of that produced by carbachol (12  $\mu\text{g/ml}$ ). Increase in the concentration of bradykinin up to 20  $\mu\text{g/ml}$  did not produce a greater depolarization. Hyperpolarization after wash-out did not follow the response to bradykinin and there was no sign of interaction with subsequent additions of carbachol.

#### Angiotensin

Angiotensin in a dose of 1.2  $\mu\text{g/ml}$  produced a depolarization value which ranged from 17 to 32% of that produced by carbachol (12  $\mu\text{g/ml}$ ). Higher concentrations of angiotensin, up to 12  $\mu\text{g/ml}$ , did not produce a greater response. There was no post-wash hyperpolarization and no interaction with subsequent additions of carbachol. Maximal depolarization was achieved after 1 min contact of angiotensin with the ganglion and thereafter there was a slight waning of response.



a.



b.

Fig. 33. Rat superior cervical ganglion. 5-hydroxytryptamine (5-HT).

a. Effect of doses of carbachol ( $12 \mu\text{g/ml}$ ) and 5-HT ( $36 \mu\text{g/ml}$ ). The negative potential following 5-HT was 23% that of carbachol, and showed signs of failing by 2 min. Following washing (W), despite the relatively small depolarization, there was a definite positive potential, the value of which reached 59% that of the depolarization.

b. Responses to equivalent depolarizing doses of carbachol ( $40 \text{ ng/ml}$ ) and 5-HT ( $12 \mu\text{g/ml}$ ). With this small dose of carbachol the development of the negative potential was slow, and there was no positive potential after washing (W). 5-HT, however, produced a more stable depolarization, followed by a small hyperpolarization, the value of which was about 30% of the size of the negative potential.

No tachyphylaxis to subsequent additions of angiotensin was evident.

The results obtained with the aforementioned drugs are summarized in Table 3.

#### Anticholinesterase drugs

The anticholinesterase drugs studied were physostigmine, neostigmine and ambenonium.

Physostigmine, in a concentration of 0.5  $\mu\text{g/ml}$ , strikingly augmented the depolarization responses to a range of doses of acetylcholine, the extent of the depolarization reaching its maximum within 30 sec of adding the acetylcholine. The graph of Fig. 34 illustrates potentiation of acetylcholine by physostigmine.

In the absence of anticholinesterase drugs, the depolarization produced by acetylcholine was not followed, on washing out, by a hyperpolarization. However, in the presence of physostigmine, the enhanced depolarization produced by acetylcholine rapidly disappeared on washing the tissue and was replaced by a relatively massive hyperpolarization which was maximal 1 min after washing and was still present, although somewhat reduced, at 3 and 5 min after washing. The hyperpolarization produced by acetylcholine under these conditions greatly exceeded that produced by carbachol, even when the amount of depolarization produced by carbachol was the greater. Fig. 35 illustrates responses to acetylcholine in the absence and in the presence of physostigmine, and compares them with a response to carbachol. Table A (Appendix) gives the potential measurements from another experiment. On removing the physostigmine from the bath, enhanced depolarization produced by acetylcholine, although reduced, remained evident for about a further 2 hours, but the hyperpolariza-

TABLE 3

Sensitivity of the isolated superior cervical ganglion of rat to stimulant drugs

DRUG	Approximately equivalent effective concentrations $\mu\text{g/ml}$	Approximately equivalent effective concentrations $\mu\text{M/ml}$	Concentration ratio on molar basis Carbachol = 1	Antagonised by
Carbachol	6	0.0328	1	Largely by hexamethonium, tubocurarine & mecamlamine, partially by atropine & hyoscine
Acetylcholine	24	0.1320	4.02	"
Acetylthiocholine	10	0.0346	1.05	"
Methacholine	80	0.4088	12.46	-
Pilocarpine	14	0.0516	1.57	Atropine & hyoscine
McN A343	12	0.0377	1.15	Atropine & hyoscine
Nicotine	6	0.0120	0.37	C6, tubocurarine & mecamlamine
TMA	5	0.0456	1.39	Mecamlamine
DMPP	5	0.0157	0.48	"
Histamine	48	0.1563	4.77*	Not antagonised by mepyramine
5-HT	36	0.0888	2.71	Not antagonised by methysergide
Angiotensin	12	0.0116	0.35*	-
Bradykinin	20	0.0189	0.58*	-

\* These drugs elicited very small responses, which were apparently not dose-related.



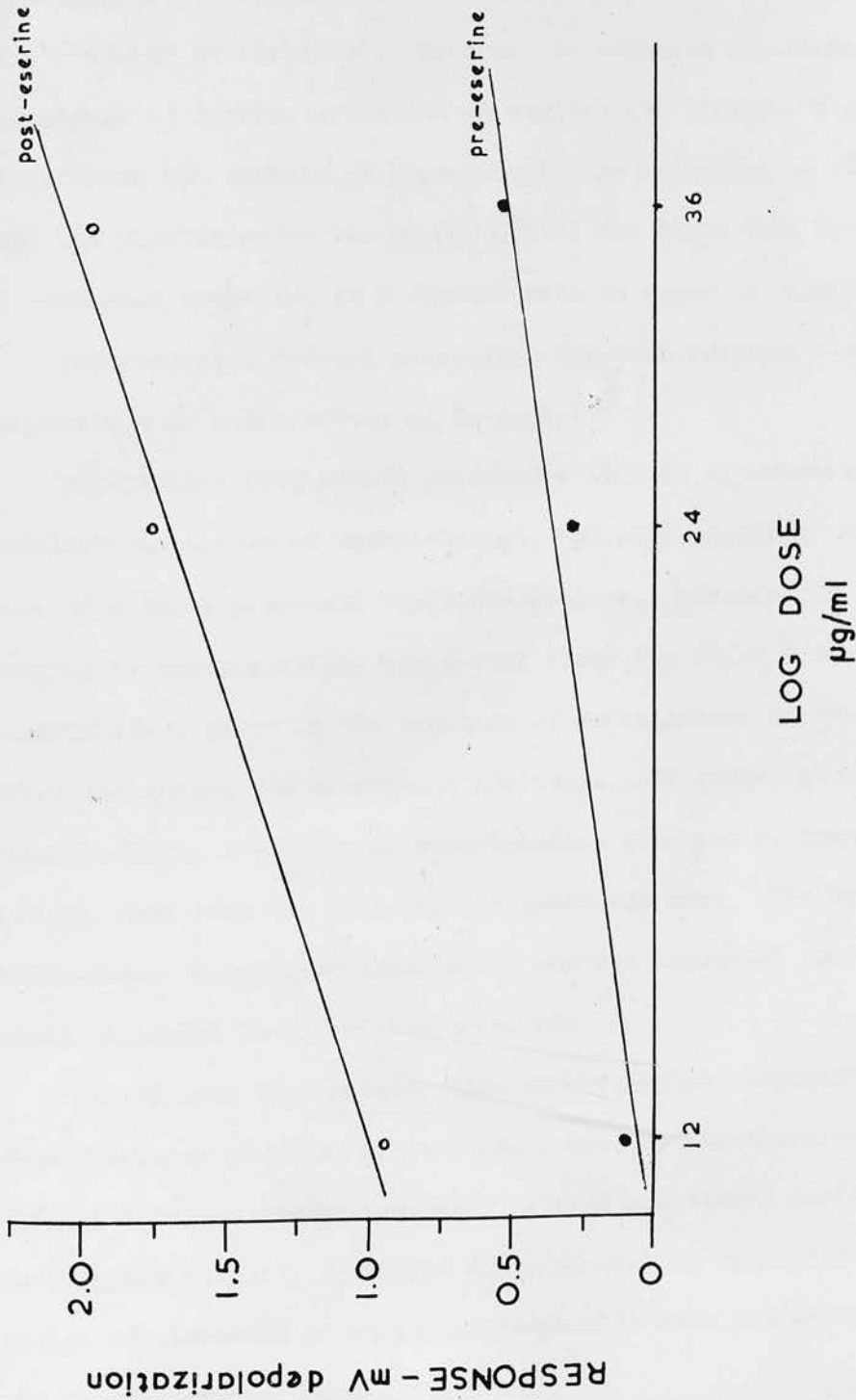


Fig. 34. Rat superior cervical ganglion. Log dose/response lines for a ganglion treated with varying doses of acetylcholine (12, 24, 36  $\mu\text{g/ml}$ ) alone and in the presence of physostigmine (eserine - 0.5  $\mu\text{g/ml}$ ). The slightly greater increase in potency and consequent divergence of the lines with the higher doses after eserine may reflect the total time of exposure to the anticholinesterase, since the lower doses were always tested first.

tion on washing was absent (Fig. 35).

In contrast to its effect on acetylcholine responses, physostigmine (0.5  $\mu\text{g/ml}$ ) depressed (by about 30%) the depolarization produced by carbachol. However, it enhanced (by about 35%) the degree of hyperpolarization on washing the tissue. Fig. 36 illustrates the effects of physostigmine on responses to carbachol. When the physostigmine was washed out of the organ bath the responses to carbachol recovered at a similar rate to those to acetylcholine.

Physostigmine did not potentiate the weak responses of the preparation to methacholine or to angiotensin.

Neostigmine (0.5  $\mu\text{g/ml}$ ) produced a similar augmentation of the depolarizing action of acetylcholine, and also caused it to give rise to a large post-wash hyperpolarization. However, the post-washing hyperpolarization was absent after the first dose of acetylcholine given in the presence of neostigmine; it appeared only after the second and subsequent additions. On removing neostigmine from the bath, responses to acetylcholine returned to normal more quickly than they did when physostigmine was used. The hyperpolarization phase disappeared immediately and the augmented depolarization waned to normal levels within 30-40 min.

Neostigmine (0.5  $\mu\text{g/ml}$ ), like physostigmine, depressed the depolarization produced by carbachol, but, unlike physostigmine, it did not increase the post-washing hyperpolarization; instead neostigmine slightly depressed the post-washing hyperpolarizing action of carbachol to an extent comparable with the depression of the depolarization phase.

Neostigmine did not affect responses to methacholine or pilocarpine, and slightly depressed those to acetylthiocholine.

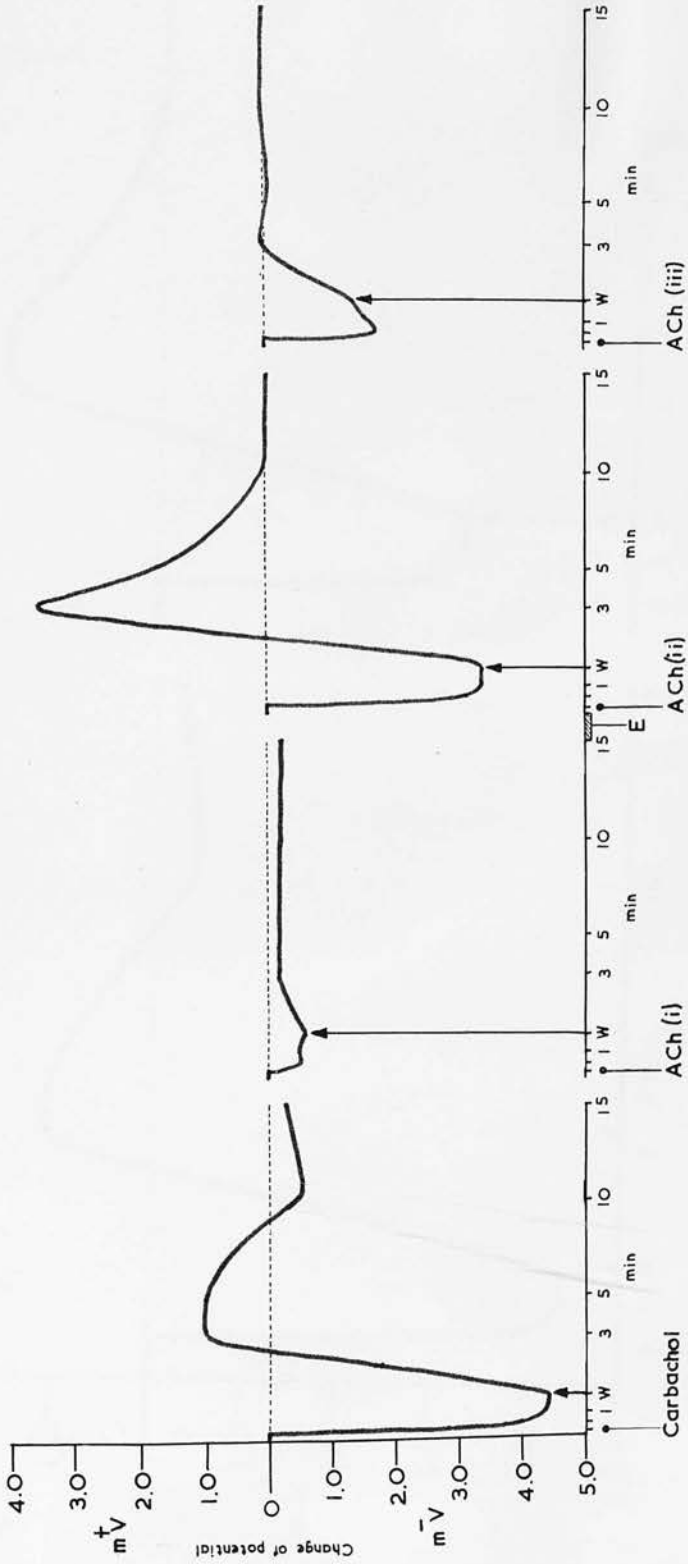


Fig. 35. Rat superior cervical ganglion. Influence of physostigmine (eserine) upon responses to acetylcholine. Acetylcholine ( $12 \mu\text{g/ml}$ ) alone produced a small depolarization which was approximately 14% of the size of that produced by the same dose of carbachol; there was no positive potential after washing (W). Following exposure to eserine ( $0.5 \mu\text{g/ml}$ ) acetylcholine produced a depolarization which was 77% of the carbachol control; the value of the positive potential exceeded the negative one. ACh (iii) was the response to  $12 \mu\text{g/ml}$  acetylcholine 47 min after ACh (ii) and after washing out the eserine; the positive potential was virtually extinguished, though the depolarization potential was still about 41% that of the carbachol one.

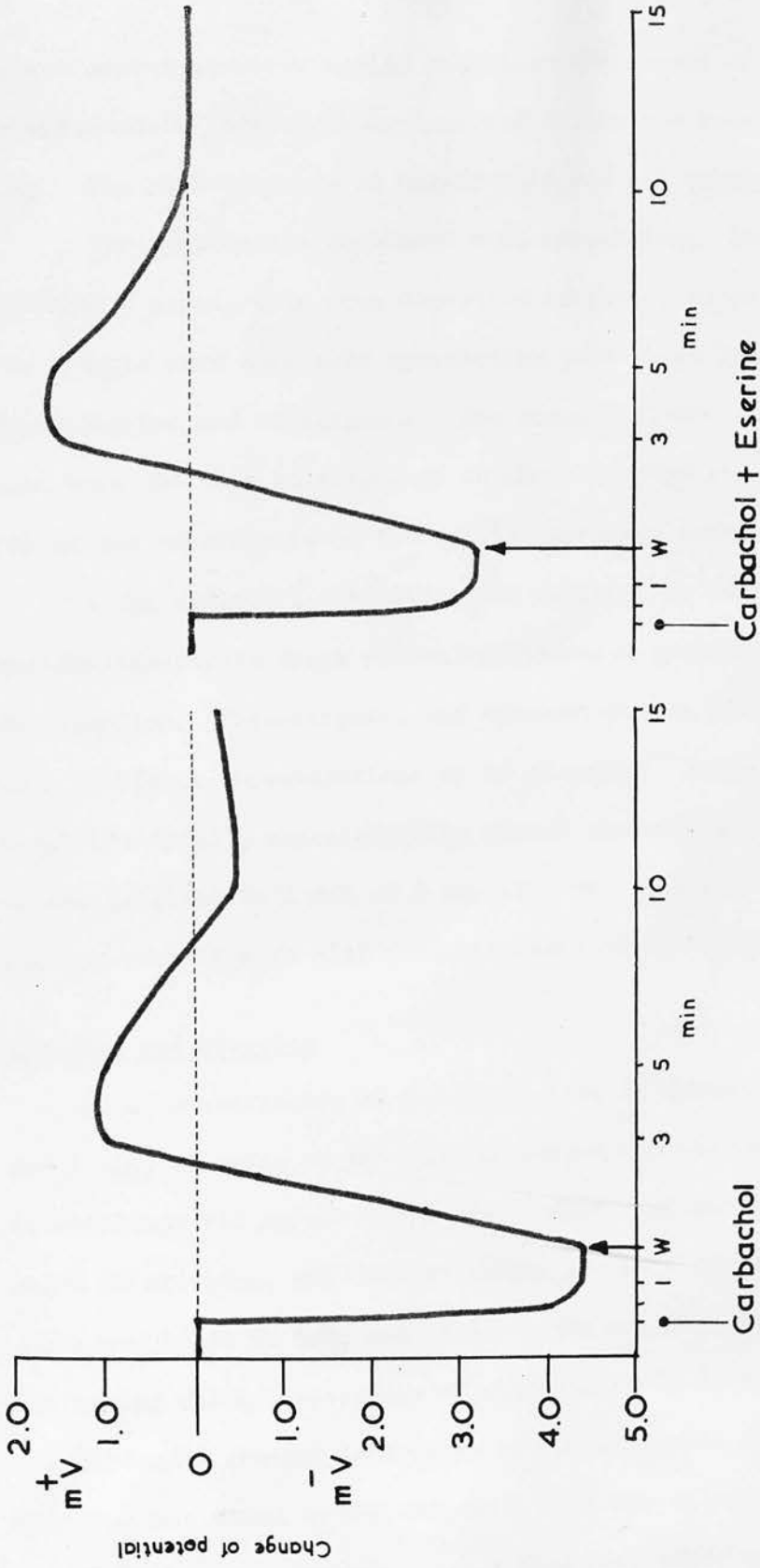


Fig. 35. Rat superior cervical ganglion. Responses to carbachol ( $12 \mu\text{g/ml}$ ) alone, and in the presence of eserine ( $0.5 \mu\text{g/ml}$ ). In the presence of the anticholinesterase the depolarization response was slightly reduced, but the post wash (W) hyperpolarization was enhanced, and the second negative potential was absent.

In one experiment neostigmine prevented the waning of the response to angiotensin that normally occurred during the presence of the drug. The peak response to angiotensin was not changed by neostigmine.

A few experiments were made with ambenonium. In concentrations of 0.2-0.4  $\mu\text{g/ml}$ , this drug augmented responses to acetylcholine but the changes were much less spectacular than those produced by physostigmine and neostigmine. The concentrations of ambenonium used bore the same relationship to those of physostigmine as they did in the experiments on the rabbit saphenous nerve.

In the effective concentrations referred to above, none of the anticholinesterase drugs themselves produced potential changes in the ganglion. Physostigmine and ambenonium were also without effect even in higher concentrations up to 10  $\mu\text{g/ml}$ . However, at this level (10  $\mu\text{g/ml}$ ), neostigmine by itself produced some depolarization of the ganglion in 2 out of 5 experiments. Mason (1962) recorded similar results with high concentrations of neostigmine.

#### Atropine and Hyoscine

In a concentration of 0.1  $\mu\text{g/ml}$  left in contact with the tissue for 5 min, atropine or hyoscine depressed the depolarization produced by carbachol (12  $\mu\text{g/ml}$ ) by 25-30%. After a 10 min exposure to 0.1  $\mu\text{g/ml}$  of atropine, the depolarization produced by carbachol was depressed by 30 to 50%, and this was the maximal effect obtainable; increasing the concentration of atropine to 1, 2, 6 or 12  $\mu\text{g/ml}$  did not produce a greater inhibition of the carbachol response. When atropine was added to the reservoir of Krebs solution so that it was replaced when the carbachol was washed out, there was a slight increase in the extent of the post-washing hyperpolarization and the secondary depolarization was absent (Fig. 37). When the

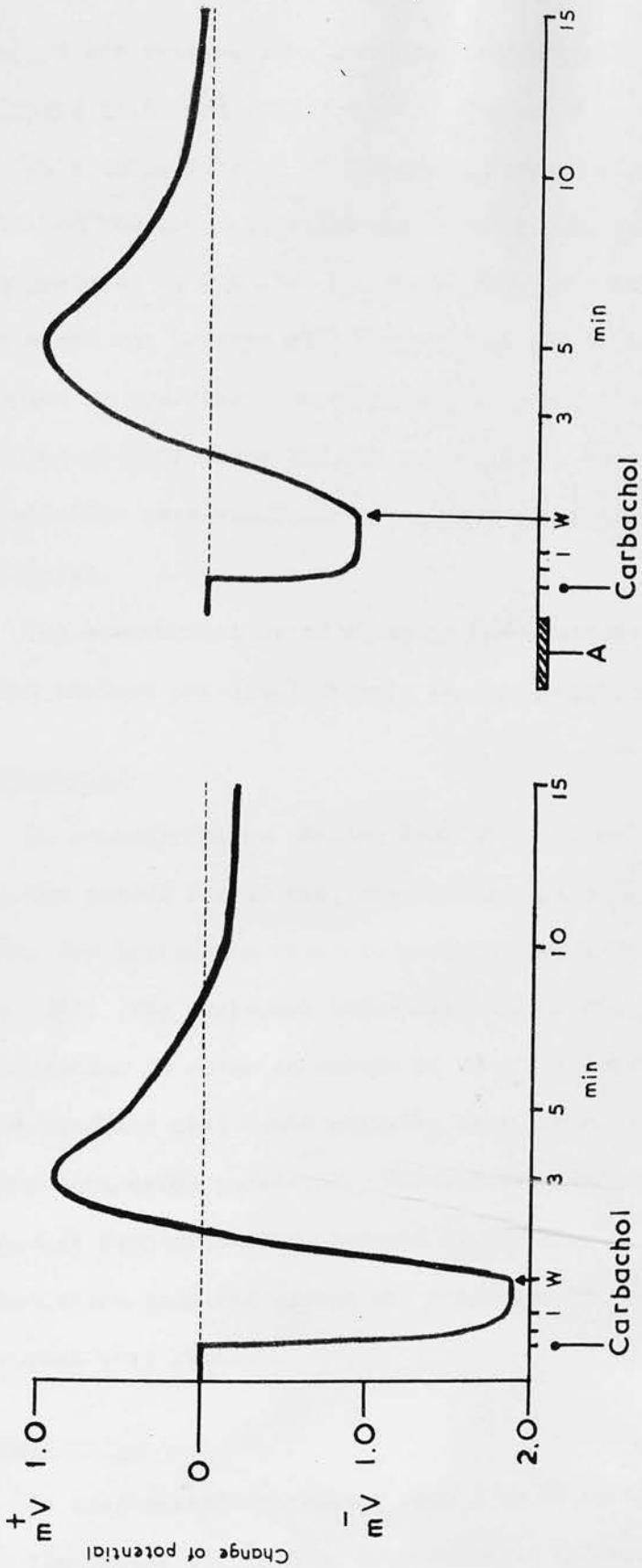


Fig. 37. Rat superior cervical ganglion. Influence of atropine ( $1 \mu\text{g/ml}$ ) upon the reaction to carbachol ( $12 \mu\text{g/ml}$ ). The atropine (A) was allowed to act for 10 min prior to the addition of carbachol, but was present throughout the rest of the experiment as it was added to the reservoir of Krebs solution. The negative potential was decreased by about 50% but the hyperpolarization was potentiated. Prior to atropine the positive potential value was 42% of the negative one, but after atropine the value was 111%. W = washing.

atropine was removed from the bath, responses to carbachol slowly recovered to normal over a period of about 2 hr.

At a concentration of 0.1  $\mu\text{g/ml}$ , atropine or hyoscine almost abolished the depolarization produced by pilocarpine and depressed that produced by McN A343 by about 50%. No post-wash hyperpolarization phase was evident with these drugs either before or after atropine or hyoscine. Depolarization produced by acetylthiocholine was depressed by about 20% but surprisingly the weak responses to methacholine were unaffected even with concentrations of atropine up to 1  $\mu\text{g/ml}$ .

The concentrations of atropine and hyoscine used did not themselves produce potential changes in the ganglion.

#### Tubocurarine

In concentrations ranging from 20 to 80  $\mu\text{g/ml}$  left in contact with the tissue for 10 min, tubocurarine produced a 45 to 75% depression of the depolarization response produced by 12  $\mu\text{g/ml}$  of carbachol (Fig. 38). The post-wash hyperpolarization was completely blocked by tubocurarine in doses in excess of 20  $\mu\text{g/ml}$ , even when a degree of depolarization that would normally have given rise to a post-wash hyperpolarization persisted. Tubocurarine did not block the late secondary depolarization produced by carbachol. On washing out the tubocurarine from the tissue the responses to carbachol recovered to normal very rapidly.

#### Hexamethonium

In concentrations ranging from 5 to 50  $\mu\text{g/ml}$  left in contact with the tissue for 10 min, hexamethonium depressed the depolarization produced by 12  $\mu\text{g/ml}$  of carbachol by 80% (Fig. 39). This

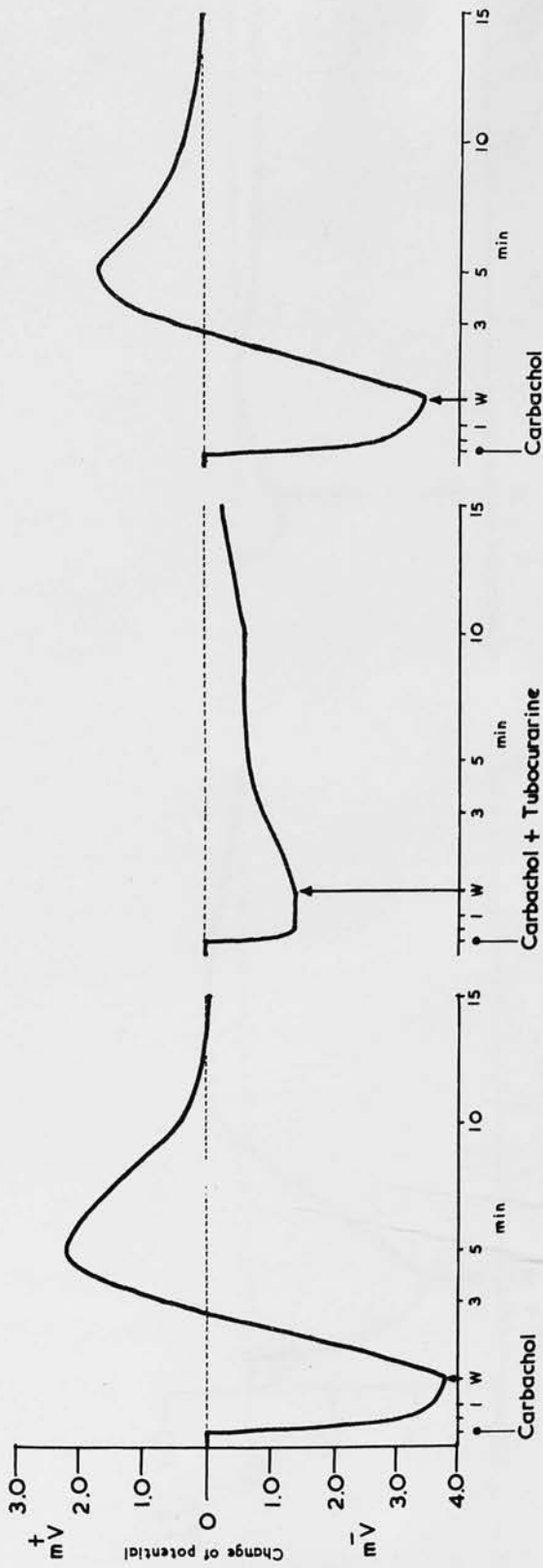


Fig. 38. Rat superior cervical ganglion. The effect of tubocurarine ( $40 \mu\text{g}/\text{ml}$ ) introduced 10 min prior to carbachol ( $12 \mu\text{g}/\text{ml}$ ). After tubocurarine the negative potential produced by carbachol was reduced and the positive one was completely absent. After washing (W) and 15 min rest the response to carbachol alone was more than 90% of the pre-tubocurarine control, and both phases displayed normal proportionality.



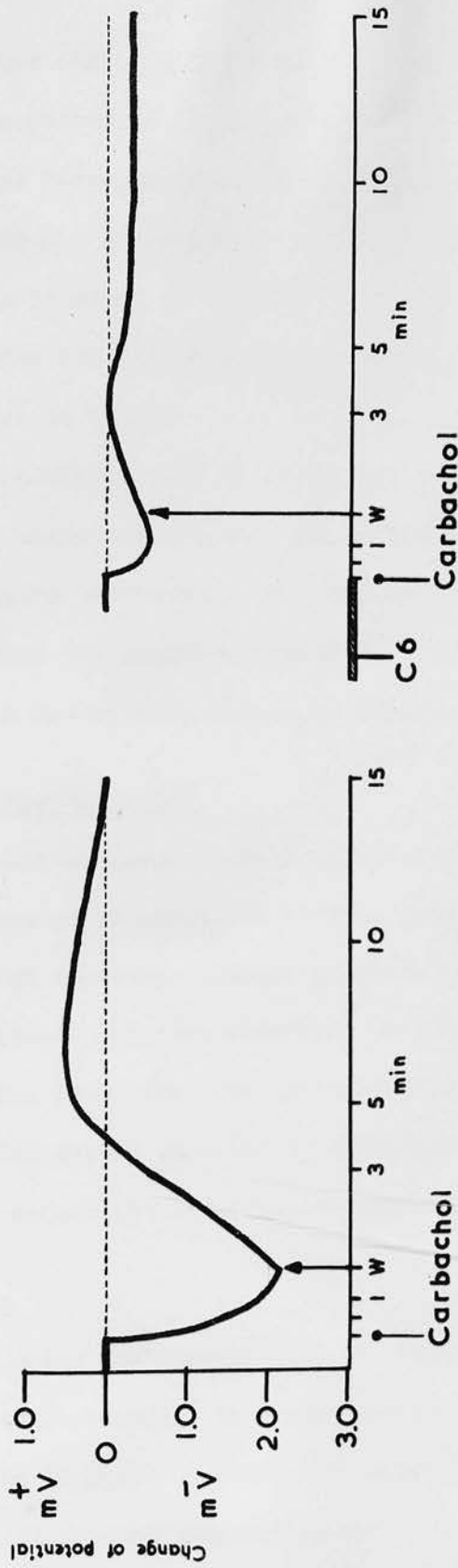


Fig. 39. Rat superior cervical ganglion. The effect of standard doses of carbachol ( $12 \mu\text{g}/\text{ml}$ ) before and after a 10 min exposure to hexamethonium ( $40 \mu\text{g}/\text{ml}$ ). W = washing.

result confirms those of Brown (1966b) who used both kitten and rat ganglia. The post-wash hyperpolarization was variably affected by lower doses of hexamethonium but was regularly abolished by concentrations of 40  $\mu\text{g/ml}$  and above. The late depolarization was more marked in the presence of hexamethonium. An increase in the period of contact with hexamethonium beyond 10 min resulted in a slightly greater degree of block.

In a concentration of 40  $\mu\text{g/ml}$  hexamethonium depressed the depolarizing action of nicotine (12  $\mu\text{g/ml}$ ) by 60% and abolished the post-wash hyperpolarization. The same concentration of hexamethonium failed to alter the ganglion response to tetramethylammonium. Hexamethonium was without effect on responses of the ganglion to 5-HT.

#### Tetraethylammonium (TEA)

TEA itself produced a small depolarization of the ganglion in concentrations of 12  $\mu\text{g/ml}$ . A similar slight stimulant effect of TEA on the cat superior cervical ganglion was described by Acheson & Pereira (1946). In concentrations of 12-24  $\mu\text{g/ml}$  in contact with the tissue for 10-15 min, TEA depressed the initial depolarization and hyperpolarization produced by carbachol (12  $\mu\text{g/ml}$ ) by 10-20%, but did not reduce the late depolarization.

#### Mecamylamine

Mecamylamine was consistently the most effective antagonist of the action of carbachol. In a concentration of 12  $\mu\text{g/ml}$  added 2 min previously to 12  $\mu\text{g/ml}$  of carbachol it produced a 79 to 85% depression of the initial depolarization response and abolished the post-wash hyperpolarization without reducing the late depolarization (Fig. 40). Its effect was persistent, full recovery of the initial depolarization

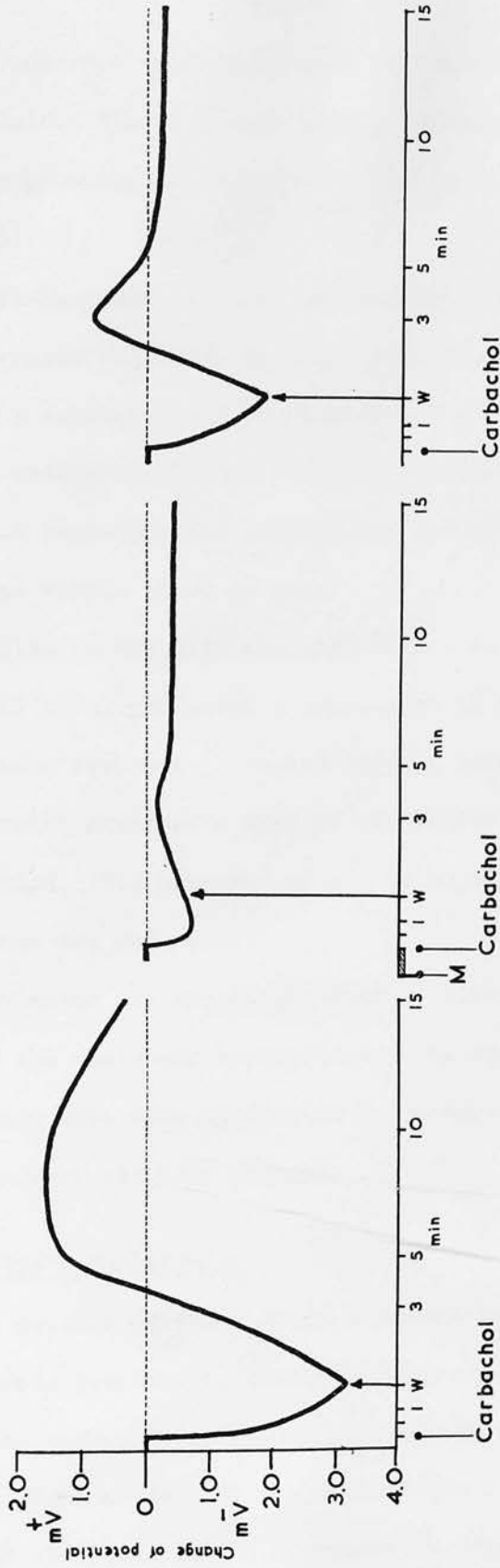


Fig. 40. Rat superior cervical ganglion. Reaction to carbachol ( $12 \mu\text{g/ml}$ ) alone and in the presence of mecamlamine ( $12 \mu\text{g/ml}$ ). The slow recovery is illustrated by the third trace which was induced by carbachol ( $12 \mu\text{g/ml}$ ) tested 47 min after the removal of mecamlamine from the bath. W = wash.

phase not occurring until about 2-3 hr after first removing it from the bath fluid. The post-wash hyperpolarization returned more quickly, reappearing in response to the second or third addition of carbachol.

Mecamylamine was the only ganglion blocking drug that consistently depressed responses to TMA. A concentration of 12  $\mu\text{g/ml}$  of mecamylamine depressed the depolarization phase produced by TMA by 62-72% and abolished the post-wash hyperpolarization. Responses to TMA returned rapidly after washing out the mecamylamine and were fully normal within about 30 min.

Mecamylamine was also the most effective antagonist of DMPP, a dose of 12  $\mu\text{g/ml}$  producing a depression of over 90% in the DMPP depolarization response. Despite this, a definite hyperpolarization persisted which attained a size of two thirds of the depressed depolarization. The presence of any hyperpolarization under such circumstances was unique.

Mecamylamine was completely without effect on both the depolarization and the post-wash hyperpolarization response to 5-HT. Responses to bradykinin were also unaffected by mecamylamine, but those of angiotensin were slightly reduced.

#### Atropine plus hexamethonium

Since neither of these drugs alone produced complete block of the ganglionic response to carbachol, two experiments were performed in which the antagonistic action of mixtures was tested.

Hexamethonium alone in a concentration of 20  $\mu\text{g/ml}$  produced a 19% and 25% reduction in the responses to carbachol (12  $\mu\text{g/ml}$ ) in the two experiments. In the same two experiments, atropine (2  $\mu\text{g/ml}$ ) alone produced 37% and 14% reductions.

Mixtures of the two antagonists in the above concentrations produced, on both occasions, a reduction in the depolarization produced by carbachol in excess of 60%. It was interesting to note that on washing out the mixture, complete recovery of the carbachol response occurred immediately. This was surprising in view of the fact that recovery after atropine alone occurred gradually over about 2 hours following washing of the tissue.

In three additional experiments the same effects were produced by low doses (0.1  $\mu\text{g/ml}$ ) of atropine in conjunction with hexamethonium, and the rapid recovery on removal of the antagonist drugs was again noted.

The combination of hexamethonium (40  $\mu\text{g/ml}$ ) with atropine in both low (0.1  $\mu\text{g/ml}$ ) and high (2  $\mu\text{g/ml}$ ) doses was tested on the same ganglion in one experiment, and a block of about 65% was recorded in each case.

#### Sympathomimetic amines

Adrenaline in concentrations of 12-36  $\mu\text{g/ml}$  and above regularly produced a small (about 0.1 mV) hyperpolarization of the ganglion which declined gradually over a period of 2 min without washing the tissue. Carbachol (12  $\mu\text{g/ml}$ ) added 2 min after adrenaline without washing the tissue, but at a time when the adrenaline-evoked hyperpolarization had disappeared, produced a slightly greater depolarization than when added alone. Noradrenaline produced effects similar to those of adrenaline, but was slightly less potent.

Isoprenaline, in concentrations of 12-24  $\mu\text{g/ml}$ , produced a slight depolarization of the ganglion whereas a larger concentration (40  $\mu\text{g/ml}$ ) produced a small (0.2 mV) hyperpolarization. In the

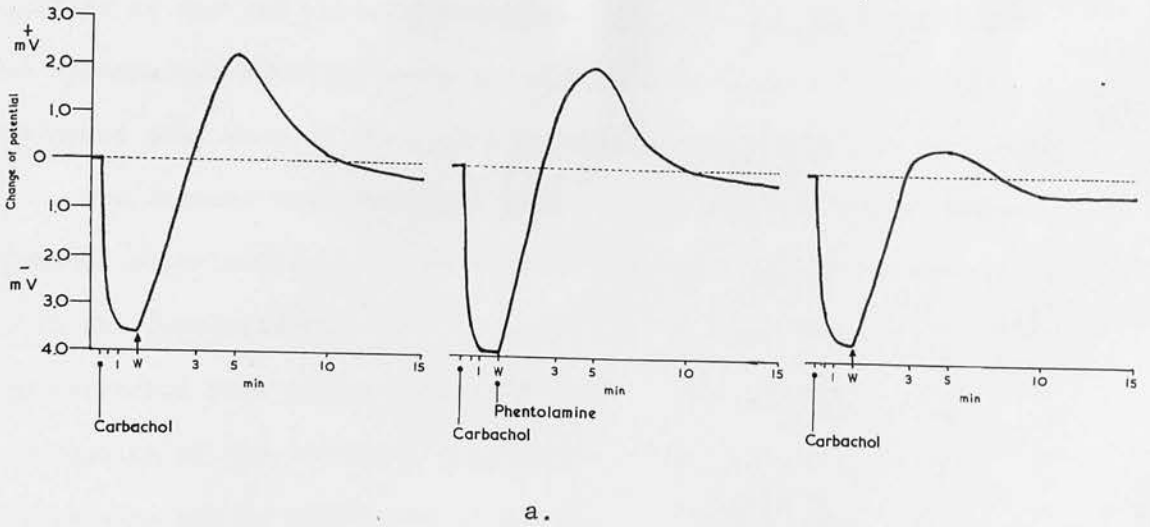
presence of a hyperpolarization produced by isoprenaline (40  $\mu\text{g/ml}$ ), the depolarization produced by carbachol was slightly reduced by an amount equivalent to the increase in potential produced by isoprenaline.

#### $\alpha$ and $\beta$ -Adrenoreceptor blocking drugs

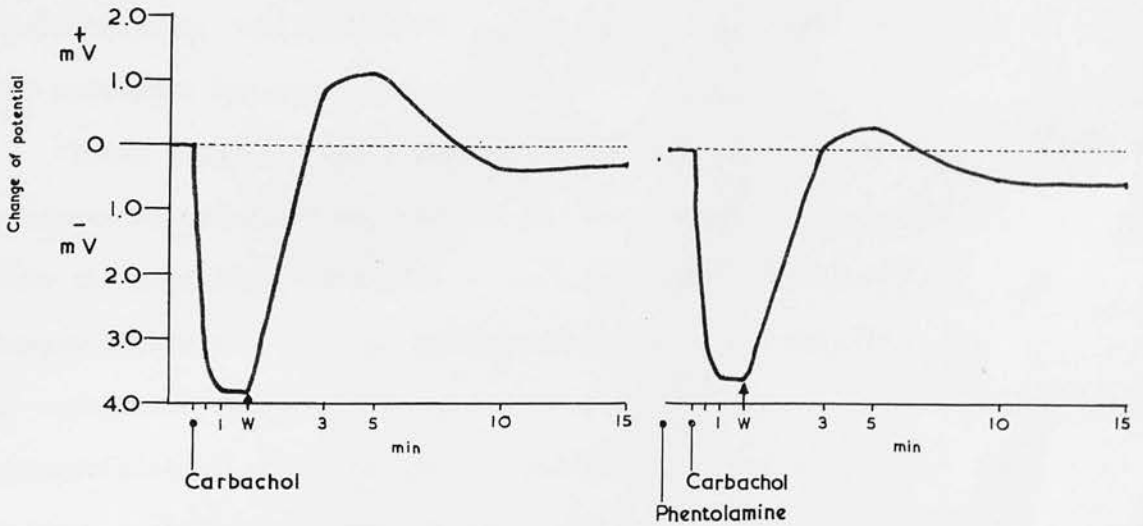
Phentolamine in concentrations of 10-20  $\mu\text{g/ml}$  was without effect by itself and did not alter the depolarization produced by carbachol (12  $\mu\text{g/ml}$ ). However, when added immediately after carbachol was washed out, phentolamine produced a 40-45% depression of the post-wash hyperpolarization produced by the subsequent addition of carbachol (Fig. 41.i).

When added 2 min before carbachol, phentolamine depressed the hyperpolarization produced after the carbachol was washed out (Fig. 41.ii) but did not alter the depolarization response to carbachol.

Dibenamine produced effects essentially similar to those elicited by phentolamine. When dibenamine (20  $\mu\text{g/ml}$ ) was added to the bath immediately after washing out the carbachol it at once prevented the appearance of the post-wash hyperpolarization. The depolarization produced by the carbachol simply waned gradually to the control level over the course of 15 min. When the same concentration of dibenamine (20  $\mu\text{g/ml}$ ) was added to the tissue first, the depolarization produced by the subsequent addition of carbachol (12  $\mu\text{g/ml}$ ) two minutes later was depressed by about 50% as also was the post-wash hyperpolarization. With this regime of dosage, washing out the carbachol would also wash out most of the dibenamine, so that the depressed post-wash hyperpolarization occurred in the absence of most of the dibenamine. Combining the



a.



b.

Fig. 41. Rat superior cervical ganglion. Influence of phentolamine.

a. The effect of phentolamine ( $20 \mu\text{g/ml}$ ) introduced into the bath at the end of a 2 min exposure to carbachol ( $12 \mu\text{g/ml}$ ). There was a slight curtailment of the positive potential, but after 15 min contact with phentolamine this tendency was amplified in the succeeding carbachol cycle, despite the absence of the blocking agent at this time. Note that the depolarization value of the last cycle was quite normal. W = wash.

b. Phentolamine ( $20 \mu\text{g/ml}$ ) introduced 2 min before carbachol ( $12 \mu\text{g/ml}$ ) severely restricted the hyperpolarization phase. The depolarization value was normal.

results of the two types of experiment showed that, in the presence of dibenamine, depolarization produced by carbachol was depressed by about 50%, whereas the post-wash hyperpolarization was abolished.

The diluent used for the dibenamine was 20% ascorbic acid. Control experiments showed that in the presence of the diluent alone both the depolarization and the post-wash hyperpolarization produced by carbachol were depressed by about 50%. Thus the whole of the depression of the carbachol depolarization in the presence of dibenamine can be attributed to the diluent rather than to the drug. However, only part of the depression of the post-wash hyperpolarization can be attributed to the diluent and the results therefore indicate that, like phentolamine, dibenamine selectively depresses the post-wash hyperpolarization.

These results suggest that the post-wash hyperpolarization produced by carbachol may involve the liberation of catecholamine from the ganglion. Adrenaline itself was shown to produce some hyperpolarization, and the ability of phentolamine and dibenamine to antagonise the effect of adrenaline was therefore tested. The hyperpolarizing effect of added adrenaline was much weaker than that following the wash-out of carbachol, but this could possibly be due to poor penetration of the tissue by exogenous adrenaline.

When tested against the effects of the sympathomimetic amines no clear result emerged due to the small effects attributable to the amines. 1  $\mu\text{g}/\text{ml}$  of adrenaline produced a slight hyperpolarization on addition to the preparation, and enhanced the carbachol depolarization as described previously: the impression gained was that both of these features were missing after a 2 min exposure to 2  $\mu\text{g}/\text{ml}$  of phentolamine.



The  $\beta$ -receptor blocking drugs, pronethalol, propranolol and dichloroisoprenaline were also studied. All three produced essentially similar effects on the responses to carbachol. In concentrations of 40  $\mu\text{g/ml}$  added 1 or 2 min before carbachol, all three blocking drugs produced a 30-40% depression of both the depolarization and the post-wash hyperpolarization induced by carbachol. The effects of propranolol and DCI were slightly greater and more difficult to reverse by washing than were those of pronethalol. Since DCI is a weaker local anaesthetic than pronethalol, the effect on carbachol responses is unlikely to be entirely due to a local anaesthetic action but may be partly the result of the same effect which produced the selective reduction in the afferent discharge evoked by acetylcholine in the rabbit saphenous nerve.

Carbachol responses in the presence of 1  $\mu\text{g/ml}$  of adrenaline were slightly reduced by 4  $\mu\text{g/ml}$  of pronethalol, but the effect did not differ from the reduction of carbachol responses produced in the absence of adrenaline. It therefore seems unlikely that effects mediated through  $\beta$ -adrenoreceptors are implicated in the post-wash hyperpolarization produced by carbachol.

#### Local anaesthetic drugs

In concentrations of 40  $\mu\text{g/ml}$ , cocaine, lignocaine and procaine depressed both the depolarization and post-wash hyperpolarization produced by carbachol (12  $\mu\text{g/ml}$ ). Cocaine and lignocaine each produced a 25-30% depression of the depolarization response after 1 min contact with the tissue. Increasing the contact time to 2 and 5 min gave rise to 30-40% and 45-50% depressions of the carbachol depolarization, but longer contact times up to 20 min did not result in depressions greater than 50%. The post-wash hyperpolarization

produced by carbachol was completely abolished after previous contact of the ganglion with either of these local anaesthetics for 2 min or more. The longer the contact time, the slower the recovery to control values with successive additions of carbachol.

Procaine produced qualitatively similar results to those produced by cocaine and lignocaine, but it was less active at the same concentration (40  $\mu\text{g/ml}$ ) as illustrated in Table 4.

TABLE 4

Comparison of percentage block of depolarization response to carbachol after exposure for varying times to 1 mg of local anaesthetics

Contact Time (mins)	Procaine	Lignocaine	Cocaine
1	16	28	27
2	35	32	38
5	37	48	47
10	44	45	50
20	35	-	50

With procaine, a 10 min exposure time was necessary to eliminate the post-wash hyperpolarization induced by carbachol. The maximum depression of carbachol depolarization was produced after a 10 min contact with the ganglion; after 20 min the depression was somewhat less than at 10 min possibly due to hydrolysis of the procaine.

If this was so, it was probably not catalysed by pseudocholinesterases present in the ganglion, since neostigmine pretreatment neither

enhanced the response to procaine, nor prevented the fall-off with prolonged contact times. In fact neostigmine slightly antagonised the depressant effect of procaine. The fall-off in the effect of procaine is therefore probably simply due to aqueous hydrolysis of the drug in the bath fluid.

Propanadid in a concentration of 80  $\mu\text{g/ml}$ , added 2 min before carbachol, produced about a 50% depression of the depolarization and abolished the hyperpolarization. When the propanadid was washed out of the bath immediately before adding the carbachol, the depolarization response to carbachol reached the normal control value, but the post-wash hyperpolarization remained absent, to reappear 17 min later, after the next addition of carbachol.

#### Guanethidine

In a concentration of 40  $\mu\text{g/ml}$ , guanethidine produced a  $74.3 \pm 2.5\%$  depression of the depolarization produced by carbachol in 9 experiments. The post-washing hyperpolarization was abolished and failed to reappear with the next dose of carbachol even though the depolarization produced by the carbachol had returned to about 95% of the control value (Fig. 42). Thus guanethidine seemed to possess a more persistent antagonistic effect to the post-wash hyperpolarization than to the depolarization effect. However, with smaller doses of guanethidine (4-10  $\mu\text{g/ml}$ ), depolarization and post-wash hyperpolarization were about equally depressed and recovered equally quickly.

Dexamphetamine in concentrations of 4-16  $\mu\text{g/ml}$  was without effect by itself and did not alter responses to carbachol. Nor did it modify the effects of guanethidine when added from 2 to 10 min before, together with, or 2 min after the guanethidine.

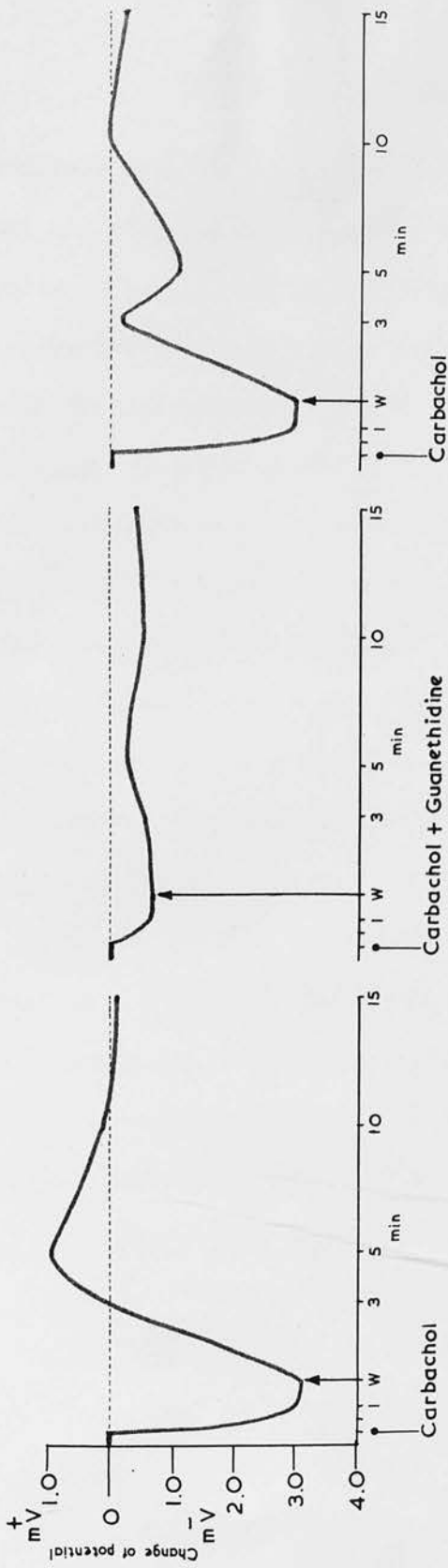


Fig. 42. Rat superior cervical ganglion. The effect of guanethidine (40  $\mu\text{g}/\text{ml}$ ) introduced 2 min prior to carbachol (12  $\mu\text{g}/\text{ml}$ ). The depolarization phase was reduced by 79% and the positive potential was completely lacking. In the subsequent carbachol cycle also shown the positive potential was still absent, despite the nearly complete recovery of the depolarization value. W = wash.

DISCUSSION OF SECTION 2

The findings that the extent of the depolarization produced by carbachol was greatest in that part of the ganglion shown histologically to contain the greatest density of cell bodies, and that depolarization was dependent on integrity of the neurones, indicate that the recorded response is analogous to the in vivo ganglionic action of the drug rather than being an artefact due to some type of surface activity. Nevertheless, the preparation has serious limitations consequent upon the lack of blood supply and the fact that penetration of the drugs to their sites of action is dependent on their ability to cross diffusion barriers.

Desheathing the ganglion was shown to remove one diffusion barrier, but results obtained with the various drugs indicated that the external sheath is probably not the only diffusion barrier involved. Thus, for example, tetramethylammonium, possibly because it is a small ion, produced a relatively more rapid and powerful depolarization than did other drugs that are normally considered more effective when given in vivo. That tetramethylammonium reached sites in the ganglion that were not accessible to other drugs was suggested by the observation that its action resisted blockade by strongly ionised quaternary drugs such as hexamethonium and tubocurarine, despite the fact that the concentrations of these drugs used readily antagonised equivalent responses produced by other depolarizing drugs. However, the secondary amine, mecamlamine, did block responses to tetramethylammonium suggesting that mecamlamine was capable of blocking sites inaccessible to hexamethonium and tubocurarine. It thus appears that the ability of the drugs to

penetrate into the isolated ganglion is dependent on at least two properties - molecular size and lipid solubility. Small molecules such as tetramethylammonium, or lipid soluble molecules such as mecamylamine penetrate more readily than do larger, highly ionised molecules such as carbachol, hexamethonium or tubocurarine. Similar criteria do of course hold in vivo, but the situation is exaggerated in the absence of a blood supply, and the wide variation in these properties of different drugs must be borne in mind, and may account for part of the differences in rate of onset and effect of different drugs, and for some of the discrepancies observed. For these reasons this preparation is considered unsuitable for quantitative measurements concerned with drug receptor kinetics, although useful qualitative information as to the presence or absence of different types of drug receptor may be derived from it.

### Receptors

As expected, and known since the work of Langley & Dickinson (1889), nicotinic receptors were found to be the most prevalent as shown by the depolarizations produced by acetylcholine, carbachol, tetramethylammonium, dimethylphenylpiperazinium and nicotine itself, and by the specific antagonism of these responses by mecamylamine, hexamethonium and tubocurarine.

The ganglion is clearly rich in cholinesterase as evidenced by the strong potentiation of acetylcholine produced by anticholinesterase drugs. Ambenonium, a specific inhibitor of acetylcholinesterase (Koelle, 1957), was effective in potentiating acetylcholine, indicating that acetylcholinesterase is present. Although a more potent inhibitor of acetylcholinesterase than are physostigmine and neostigmine, the degree of potentiation produced by ambenonium was

less than that produced by the other anticholinesterases. This result could indicate that much of the enzyme present is pseudo-cholinesterase. However, it could also be a reflection of the relative inability of the bisquaternary ambenonium to penetrate the ganglion compared with the tertiary amine physostigmine or the monoquaternary neostigmine.

According to Volle & Koelle (1961), McKinstry, Koenig, Koelle & Koelle (1963), and McKinstry & Koelle (1967a, b) much of the ganglion depolarizing activity of carbachol is the result of its stimulating pre-synaptic terminals and causing the release of acetylcholine. If this were so in the isolated rat ganglion, carbachol should be potentiated by anticholinesterase drugs. The observation that it was not indicates that carbachol acts directly on post-synaptic receptors in this preparation.

That muscarinic receptors are probably also present in the ganglion, as they are in sympathetic ganglia of other species (see Introduction), was indicated by the depolarization responses to acetyl- $\beta$ -methylcholine, pilocarpine and McN A343, and by the fact that responses to the last two drugs named were antagonised by low concentrations of atropine or hyoscine. The finding that responses to acetyl- $\beta$ -methylcholine were not antagonised by atropine or hyoscine is a discrepancy for which no obvious explanation is apparent. Acetylcholine and carbachol probably stimulated both types of receptor since their effects were partially blocked either by hexamethonium or tubocurarine, or by low concentrations of atropine or hyoscine. Furthermore, mixtures of atropine with hexamethonium or tubocurarine were more effective in antagonising responses to acetylcholine or carbachol than was any concentration

of any one of the antagonists. The observation that mecamlamine, despite being devoid of atropine-like action, produced a more complete block of responses to carbachol than did the quaternary ganglion blocking drugs, may not be entirely due to its greater ability to penetrate diffusion barriers. This drug possesses ganglion blocking activity (Stone, Torchiana, Navarro & Beyer, 1956) but also possesses additional actions (Bennett, Tyler & Zaimis, 1957) including a local anaesthetic action. These additional activities may contribute to its ganglionic effect, so that responses evoked via both nicotinic and muscarinic receptors may be depressed.

The late depolarization that followed the post-wash hyperpolarization produced by carbachol appeared to be a continuation of the muscarinic component of the initial depolarization phase, and may correspond to the LN (late negative) potential of Eccles & Libet (1961). Thus, it was blocked by atropine but not by tubocurarine, hexamethonium or mecamlamine; it was not produced by drugs possessing only a nicotinic action (tetramethylammonium, nicotine, dimethylphenylpiperazinium), but was very pronounced with drugs possessing only a muscarinic action (acetyl- $\beta$ -methylcholine, pilocarpine).

As in ganglia from other species (see Introduction), evidence was obtained for at least a sparse population of receptors sensitive to histamine, 5-hydroxytryptamine, bradykinin and angiotensin. The depolarizations produced by these drugs were less pronounced than those evoked by carbachol, but whether this was the result of the presence of only a few receptors, of a low efficacy of the drug-receptor combination or of inability of the drug to penetrate



the tissue, is not known. However, if poor ability to penetrate were the explanation it might be expected that after a very prolonged contact time a greater response would be produced. This was not found to be the case and it is therefore more likely that a small receptor population or a low efficacy is the explanation. Species difference is probably a factor in responsiveness to these agents, since the cat superior cervical ganglion responds much more powerfully (Robertson, 1954; Trendelenburg, 1954; Lewis & Reit, 1965, 1966). In the cat superior cervical ganglion Lewis & Reit (1965, 1966) found angiotensin, on a molar basis, to be the most potent stimulant known. In the rat ganglion it was more potent than bradykinin, 5-hydroxytryptamine or histamine, but was considerably less potent than the nicotinic agents.

It was of interest that the response to angiotensin was partially blocked by atropine or mecamylamine, and, in the one experiment in which it was studied, the waning of the response to angiotensin, which normally occurred in the contact period, was prevented by neostigmine. These results suggest that, in this preparation, angiotensin may act partly indirectly by releasing acetylcholine from presynaptic nerve endings, although further experiments are required to test this possibility.

#### Post-wash hyperpolarization

The most obvious explanation of the hyperpolarization that followed depolarization produced by carbachol when the ganglion was washed, is that it is related to and dependent upon the depolarization, and represents a rebound overcompensation by the cell membranes once they are released from the depolarizing drug

action. Other workers (Pascoe, 1956; Mason, 1962; Brown, 1966a) have recorded the same effect in the isolated ganglion, and a similar biphasic change in membrane potential occurs in the cat superior cervical ganglion in vivo (Maefely, Hürliman & Thoenen, 1967). Bennett (1966) described a similar rebound hyperpolarization in guinea pig intestinal muscle after removal of depolarizing drugs. An alternative possibility, but one still relating to the primary depolarization, is that the hyperpolarization is analogous to the positive after-potential that follows the spike potential, particularly in sympathetic C fibres (Douglas & Ritchie, 1962). However, if this is so it is surprisingly large, since the change constituting the positive after-potential following an axon spike is normally only a minute fraction of the potential change constituting the spike itself.

On the other hand, it is possible that the hyperpolarization is an independent response arising through a mechanism unrelated to the depolarization. It may be that the occurrence of both types of action overlap, the one opposing the other, until the more powerful but transient depolarizing action is terminated by washing the preparation. The more tenacious hyperpolarizing action would then be unmasked. Although inconclusive, there was some evidence in favour of this possibility. If the hyperpolarization arose from a rebound recovery process then, for a given degree of depolarization, it might be expected to reach the same extent in the same preparation, no matter which drug produced the initial depolarization. However, this was not found to be so. Thus: (1) In the absence of anticholinesterase drugs, acetylcholine did not give rise to hyperpolarization, although hyperpolarization followed a

matching depolarization produced by carbachol. After anticholinesterase treatment, acetylcholine was considerably more powerful than carbachol in producing hyperpolarization in relation to the initial depolarization. (2) Tetramethylammonium, and especially dimethylphenylpiperazinium, produced a more powerful hyperpolarization than did carbachol in relation to a given initial level of depolarization. (3) In contrast, pilocarpine and McN A343 did not give rise to hyperpolarization, although hyperpolarization followed an equivalent degree of depolarization produced by carbachol. (4) Tubocurarine, hexamethonium, and mecamlamine blocked the hyperpolarization more readily than the depolarization produced by carbachol. Hyperpolarization was abolished by these antagonists at a stage when a degree of depolarization, normally big enough to be followed by hyperpolarization, still persisted. (5) Atropine slightly depressed the depolarization produced by carbachol, but at the same time enhanced the post-wash hyperpolarization. (6) Phentolamine and dibenamine depressed the post-wash hyperpolarization produced by carbachol without affecting the depolarization. (7) Guanethidine abolished both the depolarization and the hyperpolarization produced by carbachol, but with a suitable concentration of guanethidine, the antagonism of the hyperpolarization was the more persistent. (8) 5-Hydroxytryptamine produced a greater hyperpolarization than that produced by a concentration of carbachol giving an equivalent depolarization.

The results obtained with guanethidine and the  $\alpha$ -adreno-receptor blocking drugs, phentolamine and dibenamine, suggest that the post-wash hyperpolarization produced by carbachol may have been

due to the action of catecholamine released by carbachol. Catecholamines are known to be present in sympathetic ganglia (Reinert, 1963; Eränkö & Härkönen, 1964; Clementi et al., 1966), and adrenaline and noradrenaline have been shown to hyperpolarize ganglion cells by acting on  $\alpha$ -adrenoreceptors (De Groat & Volle, 1965, 1966, a, b; Volle, 1966b). In the present experiments, adrenaline and noradrenaline were only very weakly active in hyperpolarizing the rat ganglia but this may have been due to diffusion barriers restricting the access of the exogenously administered catecholamines to the site of the  $\alpha$ -receptors. The weak hyperpolarization that was produced was abolished by  $\alpha$ -receptor blocking drugs.

The appearance of the post-wash hyperpolarization produced by carbachol and related drugs appeared to be associated with nicotinic rather than with muscarinic receptors. Thus, the effect was produced by nicotine, tetramethylammonium or dimethylphenylpiperazinium but not by methacholine, pilocarpine or McN A343; it was blocked by tubocurarine, hexamethonium and mecamylamine but not by atropine or hyoscine. This is compatible with a mechanism involving release of catecholamine, since in other tissues from which amines may be released (heart, skin, gut, blood vessels, nictitating membrane, adrenal medulla) similar nicotinic receptors appear to be involved (Burn & Rand, 1958, 1959). The post-wash hyperpolarization recorded in the rat ganglion thus differs from the ganglionic P (positive) potential recorded by Eccles & Libet (1961) and by Brown (1966c) in the cat in vivo, since the P potential in the cat preparation was blocked by atropine, but not by tubocurarine. However, Jaramillo & Volle

have recently (1968) shown that hyperpolarization may be induced by some nicotinic drugs in the cat superior cervical ganglion in vivo.

The post-wash hyperpolarization produced by 5-hydroxytryptamine may have been the result of a direct action of this drug; it was probably unrelated to that produced by nicotinic agents or by catecholamines.

GENERAL DISCUSSION

Despite the species difference, the results demonstrate a considerable degree of qualitative pharmacological similarity between the sensory nerve endings of the rabbit saphenous nerve and the cells of the rat superior cervical ganglion. A comparison of Tables 1 and 3 illustrates the extent of the similarity. Both were affected by catecholamines, histamine, 5-HT, bradykinin, angiotensin and acetylcholine, and the results indicated that in both tissues, responses to acetylcholine were mediated by both nicotinic and muscarinic receptors, the former predominating. Quantitative differences in responsiveness were evident and the rank orders of potency of the compounds differed in the two tissues. Apart from the fact that different tissues were used, variations in potency might be expected as a result of species difference, since the responsiveness of the same tissue to a particular agent may vary widely in different species. For example, according to Lewis & Reit (1965) angiotensin and bradykinin are powerful stimulants of the cat superior cervical ganglion, angiotensin (on a molar basis) being the most powerful of all stimulants studied. However, though effective on the rat ganglion, neither of these agents was as powerful as were the nicotinic agents. In the rabbit saphenous, however, bradykinin was the most powerful drug studied.

Other peripheral nervous structures in which responsiveness to acetylcholine and related drugs has been demonstrated include nerve endings of cutaneous, slowly adapting pressure receptors (Fjällbrant & Iggo, 1961), non-myelinated cutaneous receptors in

general (see Paintal 1964 for review), mammalian thermal receptors of the tongue (Dodt, Skouby, Zotterman, 1953), carotid chemoreceptors and baroreceptors (Landgren, Liljestrang & Zotterman, 1952; Diamond, 1955; Gray & Diamond, 1957; Landgren, Skouby & Zotterman, 1953; Witzleb, 1959), somatic motor nerve endings (Hubbard, Schmidt & Yokota, 1965; Riker, 1966; Standaert & Adams, 1965), sympathetic nerve endings in the spleen (Ferry, 1963) and the axons of lobster nerves (Dettbarn & Davis, 1963).

Acetylcholine and related drugs induce a discharge from muscle spindle endings (Hunt, 1952; Granit, Skoglund & Thesleff, 1953), but so far it has not been possible to decide whether this effect is entirely secondary to contraction of intrafusal fibres or partly due to a direct action on the nerve endings (Paintal, 1964). In the spinal cord, acetylcholine and related drugs have been shown to stimulate the Renshaw cell (Curtis & Eccles, 1958; Curtis, Phillis & Watkins, 1961; Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956), and numerous neurones in various parts of the brain have been shown to be responsive, although others are apparently resistant (see for example, Eccles, 1964b; Crossland, 1967).

In all cases neuronal responses to acetylcholine-like drugs have been shown to be mediated by nicotinic receptors, but evidence for the presence of additional muscarinic receptors has been detected in some tissues, including thermal receptors of the tongue (Dodt, Skouby & Zotterman, 1953), sympathetic ganglion cells of the cat (Roszkowski, 1961; Pappano & Volle, 1963; Volle, 1967; Gebber & Snyder, 1968), dog (Flacke & Gillis, 1968), and of the rat (present experiments) and on the Renshaw cells (Curtis &

Eccles, 1958; Curtis & Ryall, 1966a, b). Recently it has been found that in the dog, muscarinic receptors, as well as nicotinic receptors, are present in adrenal medullary cells, which are embryologically analogous to post-ganglionic adrenergic fibres (Kayaalp & Türker, 1969). On the other hand, muscarinic receptors appear to be absent from muscle spindle endings, judging from the lack of responsiveness to muscarine (Verhey & Voorhoeve, 1963). However, it may be that larger doses than those used are required to excite them and it seems possible that muscarinic receptors may always co-exist with nicotinic receptors to some extent.

Catecholamines have also been found to affect a variety of neuronal membranes to a greater or lesser extent, and both  $\alpha$ -receptors, usually giving rise to hyperpolarization, and  $\beta$ -receptors, usually giving rise to depolarization, are involved. Apart from their effects in autonomic ganglia, sensory nerve endings and isolated C fibres, which have already been discussed, catecholamines have been shown to affect motor nerve endings in skeletal muscles. Indirect evidence indicates that the terminal membranes are hyperpolarized by adrenaline, and this effect results in facilitation of neuromuscular transmission (for reviews see Bowman & Raper, 1966; Bowman & Nott, 1969).

Angiotensin, bradykinin, histamine and 5-HT have been found to stimulate ganglion cells and a variety of sensory nerve endings (see Introduction), but the possibility of actions on somatic motor nerve endings and on the terminals of autonomic adrenergic and cholinergic neurones does not appear to have been investigated.

From the results obtained in the present experiments, together with published results by other workers, it seems



possible that all non-myelinated neuronal membranes may react in a qualitatively similar way to all the substances studied, provided the dosage is adequate, and this possibility should be borne in mind during pharmacological studies with these agents.



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APPENDIX A

ACh dose	12 $\mu\text{g/ml}$		24 $\mu\text{g/ml}$		36 $\mu\text{g/ml}$	
	Control	Post- eserine	Control	Post- eserine	Control	Post- eserine
30 sec post ACh	- 0.15	- 0.90	- 0.20	- 1.60	- 0.35	- 1.90
60 sec post ACh	- 0.20	- 0.75	- 0.30	- 1.60	- 0.50	- 1.75
120 sec post ACh	- 0.20	- 0.75	- 0.30	- 1.75	- 0.55	- 1.65
W A S H						
3 min post washing	- 0.10	+ 0.40	- 0.05	+ 1.10	0	+ 1.40
5 min post washing	- 0.10	+ 0.10	- 0.05	+ 0.80	0	+ 1.10
10 min post washing	- 0.05	- 0.15	0	Tr -	Tr -	Tr +
15 min post washing	0	Tr -	0	Tr -	Tr -	Tr -
Max dep <sup>n</sup> . (% of carbachol control)	9	38	13	75	23	81

Changes (in mV) in the rat superior cervical ganglionic potential induced by varying doses of acetylcholine in the absence, and presence, of eserine (0.5  $\mu\text{g ml}$ ).

APPENDIX B.

GUINEA PIG WHEELS

(i) Percentage block after 20 min.

Dose ( $\mu\text{g}$ )	250	500	750	1000	1500
DCI.	0	16.7	61.3	94.3	100
Lignocaine	33.0	33.3	33.3	100	100
Pronethalol	33.5	39.0	55.6	94.3	100
Propranolol	25.0	49.7	100	100	100

(ii) Recovery Times (min.)

Dose ( $\mu\text{g}$ )	250	500	750	1000	1500
DCI	10) 12.5 15)	25) 25 30) 25 20)	45) 43.7 60) 30) 40)	165) 135 120) 120)	120) 120 120)
Lignocaine	25) 25 25)	20) 23.3 25) 25)	15) 26.25 20) 40) 30)	75) 100 135) 90)	45) 112.5 180)
Pronethalol	25) 27.5 30)	35) 30 30) 25)	45) 42.5 45) 35) 45)	90) >105 >135) 90)	135) 150 165)
Propranolol	20) 22.5 25)	40) 35 35) 30)	90) 83.75 80) 80) 85)	105) >130 >135) >150)	>180) >255 330)

## APPENDIX C.

### Electrodes for rat superior cervical ganglion preparation

Silver/silver chloride/agar saline electrodes were prepared from 0.020" silver wire, two at a time. Seven inch lengths of wire were cleaned thoroughly with Duroglit, and then polished with clean cotton wool before being wound around a previously cleaned glass rod of  $\frac{1}{8}$ " diameter. The coil was then pulled out so that it fitted loosely into the glass electrode tube. During this process the wire was handled as little as possible.

It was next cleaned by dipping into absolute alcohol and then into ether. Approximately half an inch was cut off one end, and the other (upper) end was held in a spring-loaded slot in the electro-plating box (containing 0.9% NaCl and a silver wire acting as the cathode). A current of 0.1 mA per wire was passed for 24 hr, and the wires were then allowed to stand in the box for a further 48 hr.

Next, a solution of 3% agar in 0.9% saline was prepared, being heated gently until the agar dissolved and the liquid was still quite thick. Balsa wood plugs, approx.  $\frac{1}{2}$ " long, were shaped to fit snugly into the selected glass tubes, and agar saline was drawn up into the tubes, taking care to avoid the inclusion of any air bubbles. As soon as a tube was filled its wooden plug was fitted firmly into place, and the tube was secured in a beaker of saline at room temperature. Before the agar had time to set a wire was removed from the electroplating box, and lowered to the bottom of the electrode tube. It was essential that the wire should not be knocked during the transference, in order to safeguard the silver chloride film.

## Electrodes - 2.

After the agar had solidified the electrodes were connected together in pairs, and left for another 24 hr. At the end of this time the open end of the tube was sealed with Chatterton's compound in order to provide mechanical support for the wire. The electrodes were now ready for use.

Between experiments pairs of electrodes should be connected together and to earth. The system of having at least 5 electrodes constantly available, and selecting a suitable pair for each experiment proved to be more successful, as individual potential difference fluctuations do occur. With this method it was possible to use electrodes which had been prepared over 1 year previously, and yet whose potential difference could be as little as 50  $\mu$ V.

## INTERACTION BETWEEN ACETYLCHOLINE AND GUANETHIDINE ON SENSORY C FIBRES

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Intravenously or intra-arterially injected guanethidine and hexamethonium blocked the afferent discharge produced by acetylcholine in C fibres of the rabbit saphenous nerve, without impairing the response to touch or to KCl. In contrast, the local anaesthetic lignocaine was effective only by intra-arterial injection and all effective doses abolished responses to acetylcholine, KCl and touch. The results are discussed in relation to the adrenergic neurone blocking action of guanethidine.

### *Saphenous nerve*

compound action potential  
response to acetylcholine  
response to carbachol  
response to dexamphetamine  
response to dimethylphenylpiperazinium  
response to guanethidine

### *Saphenous nerve*

response to hexamethonium  
response to KCl  
response to lignocaine  
response to methacholine  
response to nicotine  
response to stroking

## 1. INTRODUCTION

In sympathetically-innervated tissues, adrenergic neurone blocking agents abolish both the sympathomimetic effects of acetylcholine or nicotine and the responses to adrenergic nerve stimulation (Jarrett, 1962; Burn and Gibbons, 1964; Akubue, 1966; Burnstock, Campbell and Rand, 1966). However, these observations give no information as to whether the adrenergic neurone blocking drugs prevent the actions of acetylcholine or nicotine by a specific blocking effect. Ferry (1963) obtained evidence indicating that acetylcholine depolarizes sympathetic nerve endings causing them to release their transmitter. Consequently, acetylcholine may be considered merely to duplicate the stimulating electrodes in the sense that both give rise to action potentials in the nerve trunk. Adrenergic neurone blocking agents may therefore act either by blocking the action of acetylcholine and nicotine directly, or by acting at some later stage in the transmitter release mechanism.

In an attempt to gain further insight into the interaction between guanethidine and acetylcholine-like drugs on nerve tissue, their effects on

sensory C fibres of the saphenous nerve have been studied.

## 2. METHODS

The experiments were performed on Californian albino rabbits anaesthetised with intravenous urethane (6 ml/kg of a 25% solution). The method of stimulating and recording from the saphenous nerve, and for injecting drugs intra-arterially was similar to the method used by Douglas and Ritchie (1960) in the cat. The saphenous nerve was exposed and crushed high in the thigh. Bipolar platinum recording electrodes were placed usually on the branch innervating the skin over the knee, and when necessary stimulating electrodes were placed in contact with the whole nerve trunk high in the thigh. The nerve was earthed between the stimulating and recording electrodes and was immersed in a pool of warm mineral oil (Heavy Liquid Paraffin, B. P.) held in place by raising the skin flaps. Intra-arterial injections were made retrogradely through a hypodermic needle cannula tied into the cut central end of the saphenous artery, pe-

ripherally to the branches supplying the skin over the knee. At the moment of injection the main femoral artery was temporarily occluded by pulling on a previously positioned loose ligature so that the injected fluid was forced into the branches supplying the skin over the knee. Intravenous injections were made through a cannula in a jugular vein. In some experiments, blood pressure was recorded from a common carotid artery. The nerve was stimulated when required by rectangular pulses of varying strength and duration. After differential amplification by a Tektronix (Type 122) pre-amplifier, nerve action potentials were displayed on a Tektronix (Type 502) oscilloscope and photographed on 35 mm film. The drugs used were acetylcholine chloride (Roche), guanethidine sulphate (Ciba), dexamphetamine sulphate (British Drug Houses), lignocaine hydrochloride (Duncan Flockhart), hexamethonium bromide (May and Baker), atropine sulphate (British Drug Houses), nicotine hydrogen tartrate (British Drug Houses), dimethylphenylpiperazinium (DMPP, Light and Co.), methacholine chloride (Light and Co.), carbachol chloride (British Drug Houses) and isoprenaline sulphate (Burroughs Wellcome). The drugs were dissolved in 0.9% wt/vol NaCl solution and the doses quoted refer to the base or to the cation. When these drugs were injected intra-arterially the volume of solution did not exceed 0.3 ml.

### 3. RESULTS

#### 3.1. *The compound action potential*

Fig. 1 illustrates the compound action potential recorded antidromically from the whole of

the saphenous nerve trunk. Weak stimulation selectively evoked a group of potentials from two groups of A fibres conducting at about 44 and 8 m/sec (fig. 1a, b). Increase in the strength of the stimulus evoked a large C fibre component conducting at about 0.9 m/sec (fig. 1c). Both A and C fibre components were also visible when the recording electrodes were placed on the saphenous nerve below the knee, but when placed on the nerve branch innervating the skin over the knee the A fibre component was very small, the main deflection being due to C fibres (fig. 2). In all experiments in which the effects of acetylcholine-like drugs, potassium chloride or stroking were studied, the recording electrodes were placed on the nerve branch to the skin over the knee so that the recorded responses were almost entirely those of C fibres.

#### 3.2. *Stroking and stimulating substances*

Lightly stroking the skin over the knee, or intra-arterial injection of acetylcholine in doses of 5-10  $\mu$ g and above produced a discharge of impulses in the knee branch of the saphenous nerve (fig. 3). Similar effects have been recorded in the cat saphenous nerve (Douglas and Ritchie, 1960). Stroking was effective in all experiments, but in about 20% of the experiments acetylcholine was without effect in doses up to 200  $\mu$ g. Throughout the total of 43 experiments in which the effects of both acetylcholine and stroking were studied, there appeared to be an inverse relationship between the two. When stroking produced a very vigorous discharge, the effects of acetylcholine were weak or apparently non-existent. Conversely, preparations which responded powerfully to small amounts of acetylcholine, responded only weakly to stroking. The

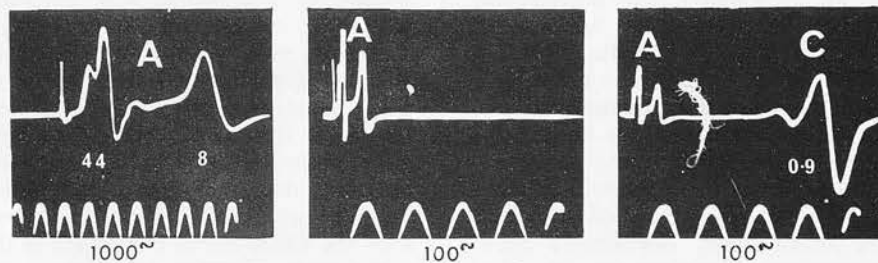


Fig. 1. Compound action potential; rabbit saphenous nerve. The nerve was stimulated with rectangular shocks and the compound action potential was recorded antidromically at a distance of 4 cm from the stimulating electrodes. *a* and *b* show the A fibre potentials evoked by a rectangular pulse of 100  $\mu$ sec duration. *b* is the same as *a* but with a slower time base to show the absence of a C fibre component with these stimulus parameters. For *c* the strength of the stimulus was doubled and the duration of the pulse was increased to 0.5 msec. With these stimulus parameters a large C fibre component was excited. The gain for *c* was half that for *a* and *b*. The numbers below the potentials denote the approximate conduction velocities in m/sec.



most suitable preparations were those which gave an intermediate response to both stroking and acetylcholine; this was so in 23 rabbits. The effects of acetylcholine were unaltered by atropine, and in most experiments in which acetylcholine was used the rabbits were previously injected with 1 mg/kg atropine intravenously. Methacholine did not induce an afferent discharge either before or after atropine in intra-arterial doses up to 200  $\mu$ g. The larger doses of methacholine were often lethal in unatropinised rabbits. Carbachol (5-10  $\mu$ g), nicotine (5-10  $\mu$ g) and DMPP (5-10  $\mu$ g) produced a brisk afferent discharge resembling that produced by acetylcholine. With all of these effective drugs, the first response was the greatest; the second and subsequent responses were smaller than the first but remained constant when doses were injected at intervals not exceeding 15 to 20 min.

Potassium chloride, in intra-arterial doses of 2.5 mg and above given in isotonic solution, produced a brisk discharge of impulses in all experiments in which it was studied. Tachyphylaxis to KCl injections developed when they were administered at intervals of 10 min or less, so that the third or fourth injection became ineffective.

Heparin solution (100 units/ml) was used occasionally in all experiments to wash out the cannula. In a few preparations, heparin evoked a weak afferent discharge. Saline solution (0.9% wt/vol NaCl), the diluent used for all drugs, was without effect in all experiments when injected intra-arterially in volumes up to 2 ml.

The collision technique of Douglas and Ritchie (1960) was used to check that the recorded discharge was travelling in C fibres. Antidromic C fibre potentials were evoked every 2 sec by stimulating the central end of the whole saphenous nerve trunk and were recorded with the recording electrodes in the usual position on the knee branch of the nerve. The discharge evoked by intra-arterial injection of 20  $\mu$ g acetylcholine rendered the C fibres refractory and so partially extinguished the stimulus-evoked potential (fig. 2). In these experiments the gain of the recording was too low to observe the acetylcholine-induced discharge clearly. A similar extinction of the C fibre potential was produced during lightly stroking the skin over the knee.

### 3.3. Guanethidine and hexamethonium

Intravenous injections of guanethidine (5-8 mg/kg) completely abolished the afferent discharge produced by intra-arterially injected acetylcholine, carbachol, nicotine or DMPP,

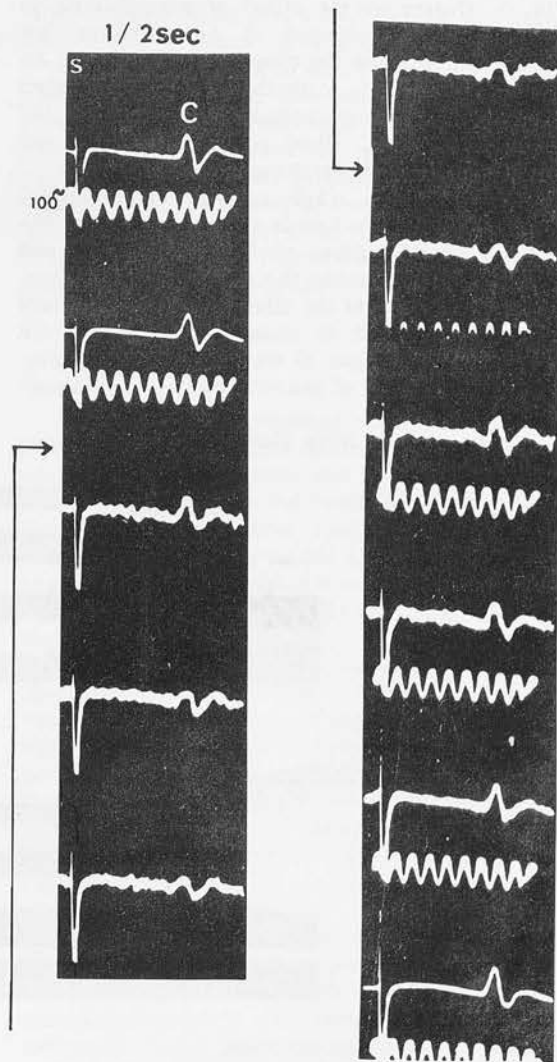


Fig. 2. Rabbit saphenous nerve stimulated once every 2 sec with rectangular pulses of 0.5 msec duration and of a strength greater than that necessary to evoke a maximal C fibre potential. The action potential was recorded antidromically from the knee branch of the saphenous nerve. This branch contains mainly C fibres. Acetylcholine (20  $\mu$ g) was injected intra-arterially for the period marked by the absence of the time trace and indicated by the arrows. Note that the C fibre potential was partially extinguished by the acetylcholine injection. S is the stimulus artifact.

even when large doses (up to 200  $\mu$ g) were given; the effect of guanethidine persisted for the duration of the experiment. The response to stroking, however, was unimpaired by intravenous guanethidine in all sub-lethal doses (up to 30 mg/kg).

Fig. 3 illustrates the effect of guanethidine in abolishing the response to acetylcholine but leaving unimpaired the response to stroking. As would be expected, guanethidine also abolished the effect of acetylcholine in depressing the stimulus-evoked C fibre potential, but did not change the effect of stroking.

Hexamethonium (3-5 mg/kg intravenously) resembled guanethidine in that it blocked the effects of acetylcholine, carbachol, nicotine and DMPP without changing the response to stroking. The time course of the effect of hexamethonium differed from that of guanethidine in that its blocking effect began to wear off in 20-30 min, whereas the effect of guanethidine was irreversible.

Similar effects were also produced by guane-

thidine and hexamethonium when they were injected intra-arterially in doses of 1 mg. Large doses of guanethidine (5-10 mg) injected intra-arterially blocked the response to stroking as well as that to acetylcholine, but hexamethonium had little effect on the response to stroking even in doses of this magnitude.

Neither guanethidine nor hexamethonium injected intravenously abolished the discharge produced by intra-arterially injected KCl. Large doses of guanethidine (5-10 mg) injected intra-arterially did abolish the response to KCl, but hexamethonium remained ineffective.

#### 3.4. Lignocaine

Intravenous injection of lignocaine in all sublethal doses (up to 25 mg/kg in divided doses)

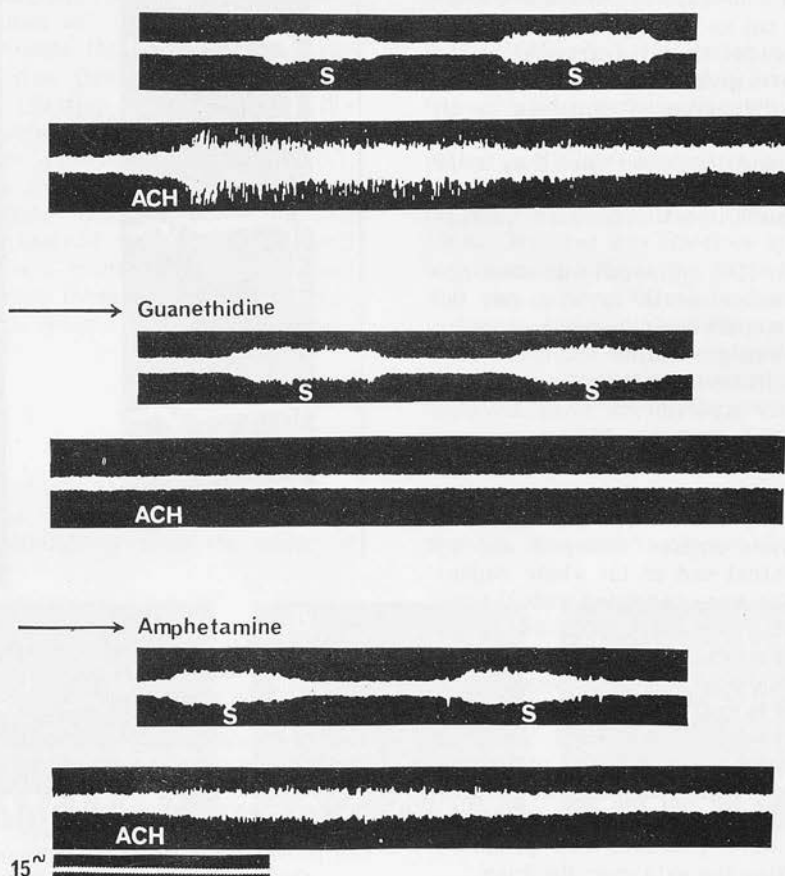


Fig. 3. Afferent discharges in the knee branch of the saphenous nerve evoked by lightly stroking the skin(s) and by intra-arterially injected acetylcholine (ACH, 20  $\mu$ g). The middle two records show the unimpaired stroke response and the absence of the acetylcholine response 10 min after the intravenous injection of guanethidine (6 mg/kg). The lower two records show the stroking response and the partially restored acetylcholine response 30 min after the intravenous injection of dexamphetamine (0.6 mg/kg).

was without effect both on responses to stroking and to intra-arterially injected acetylcholine, and therefore differed from guanethidine and hexamethonium. When injected intra-arterially, lignocaine again differed from guanethidine and hexamethonium in that all effective doses (1-4 mg and above) depressed responses to acetylcholine, to KCl and to stroking. There was some suggestion that with the smaller doses of lignocaine responses to the chemical agents were more affected than that to stroking, possibly because the site of initiation of the stroke response is less accessible to drugs carried by the circulation, but there was no resemblance to the wide differentiation seen with guanethidine and hexamethonium.

### 3.5. *Dexamphetamine*

Intravenously injected dexamphetamine in doses one-tenth to one-fifth the size of the guanethidine dose used, restored the response to acetylcholine to a large extent although it never returned fully to the control level (fig. 3). The restoration of the response occurred gradually to reach its maximum in about 30 min; it then persisted at this level for the remainder of the experiment. Intra-arterially injected dexamphetamine in doses of 1 mg and above also produced some reversal of the guanethidine block but these doses were approaching in size those necessary by the intravenous route. Smaller doses of dexamphetamine injected intra-arterially were without effect, possibly because they were diluted to sub-effective concentrations in the general circulation before the slow restoration of the guanethidine-blocked response could occur.

In the same intravenous or intra-arterial doses, dexamphetamine also often produced some enhancement of responses to acetylcholine and KCl in the absence of guanethidine, and hastened recovery from the blocks produced by hexamethonium or lignocaine. However, these effects of dexamphetamine differed from those produced in the presence of guanethidine in that they reached their maximum within 1 to 2 min of injection and persisted throughout the effect of only one injection of acetylcholine or KCl.

### 3.6. *Cardiovascular effects*

The mean general arterial blood pressure ( $\pm$  the average deviation about the mean) of 5 rabbits under urethane anaesthesia was  $99.8 \pm 10.2$  mm Hg. Guanethidine in doses of 6 mg/kg intravenously produced a mean fall to  $64.4 \pm 14.5$  mm Hg. In experiments on 3 other rabbits, the mean

blood pressure was  $91.6 \pm 12.2$  mm Hg and hexamethonium in doses of 4 mg/kg intravenously produced a mean fall to  $40 \pm 6.6$  mm Hg. These doses of guanethidine and hexamethonium completely abolished responses to acetylcholine within 3 min. Isoprenaline in doses of 10-15  $\mu$ g/kg produced a fall in blood pressure about equal to that produced by hexamethonium and, although shorter in duration, it persisted beyond 3 min at which time there was no change in the response to acetylcholine.

## 4. DISCUSSION

The selective depressant effects of intravenous guanethidine and hexamethonium on responses to acetylcholine and related drugs are unlikely to have been the result of their hypotensive action for several reasons. (1) Doses of isoprenaline which produced a fall in blood pressure equal to, or greater than, that produced by hexamethonium or guanethidine, did not prevent the response to acetylcholine. (2) Intra-arterially injected guanethidine or hexamethonium also blocked responses to acetylcholine, yet by this route of injection little change in general arterial blood pressure was produced. (3) In one experiment, the animal died before any antagonistic drugs had been administered. In this experiment, an afferent discharge in response to stroking and to intra-arterially injected acetylcholine continued to be produced for over 60 min after death.

The results confirm the view of Douglas and Ritchie (1960, 1962) that acetylcholine is not involved in the initiation of afferent impulses from mechano-receptors innervated by C fibres in the saphenous nerve, since the response to touch remained unimpaired when the response to acetylcholine had been blocked by guanethidine or hexamethonium. The effect of acetylcholine and related 'nicotine-like' drugs on C fibres of the rabbit saphenous nerve therefore probably represents a general pharmacological effect of these substances on parts of nerve membranes which are not protected by myelin; actions at all of these sites do not necessarily reflect a physiological function. Acetylcholine has long been known to depolarize autonomic ganglion cells, and a similar effect has been recorded from, or deduced from its effects on, various types of sensory receptors (Gray and Diamond, 1957; Gray, 1959; Douglas and Ritchie, 1960; Paintal, 1964), isolated vagal and sympathetic C fibres (Armstrong and Ritchie, 1960, 1961), sympathetic

nerve endings (Ferry, 1963), lobster nerve fibres (Dettbarn and Davis, 1963) and somatic motor nerve endings (Hubbard, Schmidt and Yokota, 1965).

The results also emphasise the difference between the action of guanethidine and that of a local anaesthetic drug, and support the view of Rand and Wilson (1967) that the known local anaesthetic action of guanethidine (Bein, 1960; Green, 1960) is unlikely to contribute to its adrenergic neurone blocking action. However, large doses of guanethidine injected intra-arterially did resemble lignocaine in that they blocked the response to KCl and touch as well as that to acetylcholine, and it therefore remains possible that a selective sequestration of guanethidine by adrenergic nerve terminals (Bisson and Muscholl, 1962; Boura and Green, 1965; Costa and Brodie, 1965) may cause it to reach a concentration sufficient to act as a local anaesthetic at this site, while remaining below local anaesthetic concentrations elsewhere.

Guanethidine specifically blocked the action of acetylcholine-like drugs on C fibres of the saphenous nerve in doses which did not impair the nerve's function in other respects. This result adds to the accumulating evidence (Bein, 1960; Maxwell, Plummer, Schneider, Povalski and Daniel, 1960; Gertner and Romano, 1961; Dixit, Gulati and Gokhale, 1961; Burn and Seltzer, 1965) that an anti-acetylcholine action of guanethidine may be an important aspect of its effects, and indicates that the action of guanethidine in abolishing the effect of acetylcholine or nicotine on adrenergic C fibres is likely to be the result of a direct antagonistic action. The fact that guanethidine did not affect the normal function of the C fibres in the saphenous nerve where acetylcholine is believed not to play a physiological role (Douglas and Ritchie, 1960) might, by inference, be interpreted to mean, in support of Burn and Rand (1959), that acetylcholine is involved at sites where guanethidine does impair C fibre function, that is, at adrenergic nerve endings.

Like the adrenergic neurone blocking action of guanethidine (Day, 1962; Day and Rand, 1962), the anti-acetylcholine action of the drug on the saphenous nerve was slowly but permanently reversed by dexamphetamine. Dexamphetamine also produced some enhancement of responses to KCl and acetylcholine in the absence of guanethidine, and hastened recovery of acetylcholine responses after blockade by hexamethonium. However these latter effects were abrupt and short-lasting in comparison with the reversal of

the guanethidine effect and were probably a consequence of an improvement in peripheral blood supply resulting from the pressor effect of dexamphetamine. It is difficult entirely to exclude an improved blood supply as a factor in the reversal of the guanethidine effect, but the slow onset and long duration of the antagonism make it unlikely that this was an important contribution. Reversal by dexamphetamine of adrenergic neurone blockade produced by guanethidine is not related to circulatory changes, since it also occurs in isolated preparations (Day, 1962; Day and Rand, 1962).

Guanethidine has been shown to possess ganglion blocking activity (Bein, 1960; Maxwell et al, 1960; Gertner and Romano, 1961), and the similar results obtained with guanethidine and hexamethonium in the present experiments suggest that the effect of guanethidine on sensory C fibres may have been a reflection of its ganglion blocking action. The ganglion blocking action of guanethidine differs from its action on sensory C fibres and from its adrenergic neurone blocking action in that, though readily demonstrable in isolated tissues, it is difficult to demonstrate *in vivo*, requires large doses and is relatively short-lasting (Boura and Green, 1965). However, the more potent and persistent effect on the saphenous nerve may be related to the fact that, in comparison with adrenergic neurones, the target tissue is composed of C fibres.

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