STUDIES ON SYNAPTIC TRANSMISSION IN SYMPATHETIC GANGLIA AND THE ADRENAL MEDULLA

by

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I declare that this thesis has been composed by myself, and is a record of work performed by myself. It has not been submitted previously for a higher degree.

This work was carried out within the Department of Pharmacology, University of Edinburgh, under the supervision of Dr. A. Ungar.

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C.G. Henderson

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LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abbreviations in text

A - Adrenaline
NA - Noradrenaline
CA - Catecholamines

SUMMARY

When one reviews the available literature it is apparent that many aspects of the pharmacology and physiology of the chromaffin cell and the postganglionic sympathetic neurone have not been fully investigated. The present project provides the first stage of a comparative study of sympathetic ganglionic transmission and adrenomedullary chromaffin cell function as well as the drawing together of biochemical, electrophysiological and pharmacological data from the previous literature.

Our study of sympathetic ganglia has combined a means of stimulating, in a physiological manner, different groups of sympathetic fibres, with a pharmacological identification of the cholinergic receptors, both nicotinic and muscarinic, involved in synaptic transmission under different experimental conditions. In order to build on previous work and to resolve a number of the uncertainties in the literature the study on adrenomedullary function has primarily involved an identification of the receptors present on the chromaffin cells of the cat and the dog. The results obtained, together with information taken from other peoples' work have allowed us to compare the synaptic transmission of vasomotor reflexes in the sympathetic ganglia and the adrenal medulla and its physiological relevance is discussed. Other observations have confirmed the marked species difference between chromaffin cell receptor populations between the dog and the cat as well as indicating a possible role for corticosteroids as direct-acting adrenomedullary secretagogues.

Particular attention has been paid to the study of muscarinic receptors in both tissues and we have postulated the existence of a second muscarinic receptor which apparently differs in structure from that present at postganglionic parasympathetic nerve endings. In addition a preliminary finding has been the hitherto unreported secondary vasodilatation of resistance vessels during vasomotor reflexes. We have studied this together with an investigation on the response of a capacitance vessel to vasomotor reflexes and several steps have been taken towards elucidating the mechanism underlying this vascular response.

INTRODUCTION

INTRODUCTION

A large volume of literature has been published concerning the morphology, biochemistry and physiology of both sympathetic ganglia and the adrenal medulla. Although much of this work has provided vital information about the mode of synaptic transmission in both these structures, little is known about the characteristics of transmission initiated by physiological rather than external stimuli. In the present study we have compared the pharmacology of the ganglionic transmission of different vasomotor reflexes within the lumbar sympathetic ganglia of the dog as a first step in the understanding of the pharmacology of the transmission pathways present within a ganglion. This has been extended to a preliminary study of the muscarinic receptors present both in the canine adrenal medulla and sympathetic ganglion, and with some realisation of their role in both these structures.

A feature of a number of previous publications has been the tacit assumption that observations on adrenomedullary function may be applicable to all species. It has become apparent that this concept is naive for both the adrenal medulla and the sympathetic ganglion, and with this in mind we have used a standardised catecholamine assay to compare dogs and cats in their basal catecholamine secretion, the role of corticosteroids in its basic control and the adrenomedullary response to some secretagogues.

The thesis commences with a review of literature which concentrates primarily on the pharmacology and physiology of the sympathetic ganglion and the adrenal medulla in an attempt to compensate for the lack of a review article in the last 5 - 10 years. Following this the general methods and the methods and results of each series of experiments is described. In the final section these results and their implications are discussed with an emphasis on the close comparison between adrenomedullary and sympathetic ganglionic function. During our studies on the ganglionic transmission of vasomotor reflexes an interesting vasodilator response was observed and further investigations of this phenomenon and of the response of capacitance vessels to vasomotor reflexes comprises Appendix 1.

LITERATURE REVIEW

SYMPATHETIC GANGLIA

Morphology

Sympathetic ganglia appear as fusiform lumps with clearly recognisable pre- and postganglionic rami. The neurones of the autonomic ganglia are readily affected by local or systemic drug administration as they are exposed to the common interstitial fluid through the gliocytes. Ganglion neurones are, in mammals, multipolar with several axons arising from the same cell soma. Three subdivisions of mammalian ganglion cells have been described; cells with diameter 33 - 35 μm, medium size cells, 25 - 32 μm diameter, and cells of diameter 15 - 22 μm (De Castro, 1932), of which 50 - 70% are medium sized. Their axons are generally unmyelinated and measure up to 1.5 μm, though in the cat Superior Cervical Ganglia, (S.C.G.) a population of myelinated postganglionic fibres has been observed.

The majority of preganglionic fibres are myelinated (2 - 3 μm diameter) and classified as B fibres with the rest non-myelinated C fibres although Bishop & Heinbecker, 1932; Eccles J.C., 1935a have subdivided B fibres into three subtypes. In most ganglia, the preganglionic fibres are outnumbered by the ganglion cells by varying amounts. When only myelinated fibres were considered, the ratio in the cat S.C.G. was 1:32 (Billengsly and Ranson, 1918) but later estimates which included unmyelinated fibres are between 1:11 and 1:17 (Wolf, 1941). In the cat S.C.G. most contacts are axodendritic associated with discernible synapses (Elfvin, 1963). In addition to the postganglionic fibres and neurones, small granule-containing cells are observed within some sympathetic ganglia (Matthews & Raisman, 1968: Jacobowitz, 1970) which seem to be the same as the small intensely fluorescent (SIF) cells previously observed (Norberg & Hamberger, 1964). These cells seem to show afferent and efferent synaptic contacts and for this reason, they may act as interneurones (Matthews & Raisman, 1969).

Identification of Synaptic Potentials

Eccles, J.C. 1935(a) observed slow potentials in ganglion cells from postganglionic recordings which seemed to consist of a late negativity and a late positivity. They differed in their latencies and duration and the threshold of preganglionic stimulation necessary to observe them.

The late positivity was much less sensitive to nicotine induced blockade and the two waves of potential were able to interact at the ganglion cell to produce facilitation or inhibition depending on the time course of adjacent preganglionic volleys. From this interaction at the level of the ganglion cell, they appeared likely candidates for the basis behind the central excitatory (c.e.s.) and inhibitory states (c.i.s.) (Eccles J.C., 1935(c)). The observed potentials showed the same characteristics and response to drugs as these central states which are reflections of the level of electrical excitability prevalent in ganglion cells at a specified time. Ganglion cell reactivity to a second stimulus seemed to depend on the resultant potential change produced by the independent c.e.s. and c.i.s. which may correlate with the observed late potentials. In the cat stellate ganglion, which differs from the S.C.G. in fibre population and action potential shape (Bronk, Tower, Solandt & Larrabee, 1938), potentials were observed, but at frequencies above 50 c/s where spike transmission has failed. This was also observed when action potentials were prevented by curare. Prolonged 'after-discharges' were noted after repetitive stimulation or 'tetanic stimulation' with prolongation of potentials by eserine or low frequency conditioning stimuli. This led J.C. Eccles to suggest that the after-discharges were the result of the prolonged action of the neurotransmitter whereas the initial depolarisation might be electrical coupling without transmitter action. In an attempt to identify the observed negative (N wave) and positive potentials (P wave) as composite synaptic potentials generated by the ganglion cells, without the large component as a result of after-discharge, Eccles, J.C. 1943 investigated the postganglionic potentials

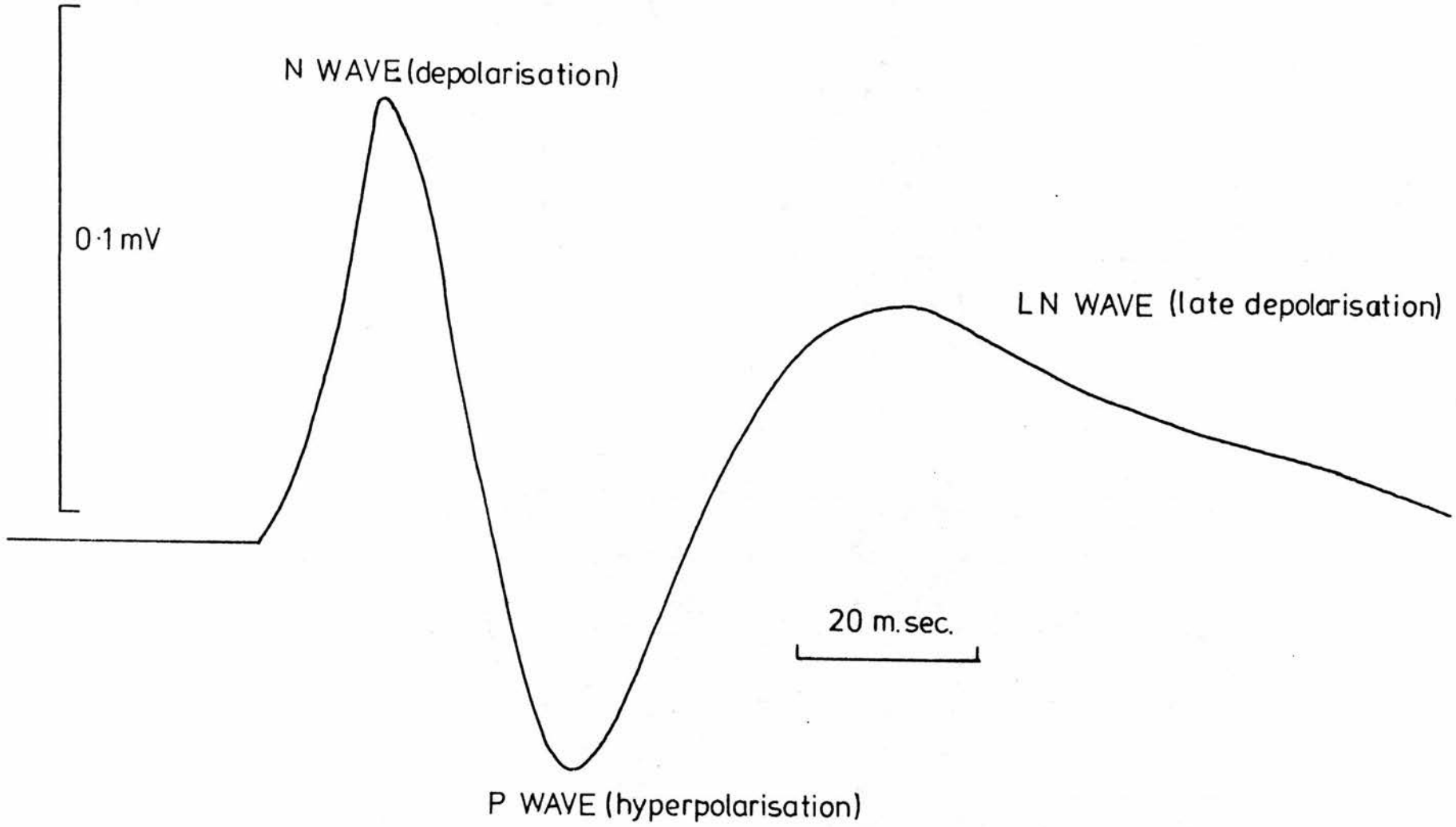


Fig. 1 A diagrammatic representation of externally recorded synaptic potentials from a rabbit S.C.G.

obtained following preganglionic test volleys to a cat stellate ganglion curarised to prevent action potential development. A summating synaptic potential was observed which was elongated in duration by eserine and it was concluded that the initiation of impulses in ganglion cells is due to a summation of these synaptic potentials. The N wave was confirmed as an external recording of summated synaptic potentials but the existence of a later positive potential (P wave) was attributed to a large after-discharge.

As a means of analysing ganglionic potentials more accurately, an isolated moist chamber technique was developed (Eccles, R.M., 1952(a)). This allowed different lead combinations to be used and a known concentration of drugs to be administered, once deterioration problems were overcome. In the first instance the rabbit S.C.G. was preferred because of the larger postganglionic trunk. Difference in the postganglionic action potential, from those in the cat suggest a different postganglionic fibre population, only non-medullated fibres being present in the rabbit. Using curare, transmission was blocked and a large synaptic potential recorded from a surface electrode. d-Tubocurarine (d-TC) blocked transmission but a synaptic potential (N wave) was observed but with a slower time course than in the blood bathed preparation (Eccles, J.C., 1943). After this a small positivity and a late negativity of approximately 0.2mV with several seconds duration was noted. (Fig 1, p 149) d-TC reduced the N wave but was without action on the late negativity (LN wave), which was especially obvious after 'tetanic stimulation'. The sensitivity of the P wave to d-TC suggested it had a large after-potential component. The LN wave was not observed in uncurarised ganglia where the P wave was prolonged. This investigation (Eccles, R.M., 1952(b)) provided the basis for many of the later studies on ganglionic cell synaptic potentials.

Fast Excitatory Postsynaptic Potential (Fast E.P.S.P./N wave)

In response to the acetylcholine liberated from the presynaptic nerve terminals an E.P.S.P. is generated in the ganglion cells

which can summate both temporally and spatially to produce spike discharge. The early negativity (N wave) observed with a surface electrode is the sum of the E.P.S.P.s generated in the individual ganglion cells. The intracellularly recorded E.P.S.P. (Eccles R.M., 1955) was seen to summate to form a spike potential. Stimulation frequencies greater than 35 c/s did not produce full size spike potentials but synaptic potentials were observed for each volley, suggesting an inhibitory influence on the cells. The synaptic delay of the fast E.P.S.P. seems to be 1.5 - 2m.sec. (Eccles, R.M., 1955; Christ & Nishi, 1971 (a)) whereas the externally recorded N wave in a curarised ganglion is slower in onset (10m.sec.) and decay (60m.sec.) but this may be accounted for by temporal dispersion. The amplitude of the fast E.P.S.P. increases with increasing membrane potential and declines as the membrane depolarises. It seems to be generated by an increase in Na^+ and K^+ conductance of the activated ganglion cell membrane which has been measured in rabbit S.C.G. (Woodward, Bianchi & Erulkar, 1969). The fast E.P.S.P. can be inhibited by nicotinic ganglionic antagonists and it is assumed to be produced as the result of acetylcholine's combination with a nicotinic receptor on the ganglion cell or dendrite (Nishi, 1970; Christ & Nishi, 1971 (b)).

Slow E.P.S.P. or LN wave

A late negativity (LN wave) observed by Eccles R.M., 1952 (b) was noted by Eccles R.M. & Libet, 1961 in a rabbit S.C.G. with a preganglionic stimulation frequency of 60 - 80 c/s following treatment with dihydro β erythroidine. It was removed by botulinus toxin and reduced by atropine and anticholinesterase but increased following 'tetanic stimulation'. As it was observed in the absence of a spike potential it was not an after-potential. Libet, 1964 confirmed that this maintained atropine sensitive LN wave occurred in normal ganglia after 'tetanic stimulation' and felt that it might account for much of the long lasting post-tetanic potentiation observed in ganglion cells. Evidence exists correlating the externally recorded LN

wave with an intracellularly recorded E.P.S.P. observed in rabbit S.C.G. cells (Libet & Tosaka, 1966). The slow E.P.S.P. measured in rabbit S.C.G. cells or cat coeliac ganglion cells has a latency of 200 - 300m.sec. and following preganglionic 'tetanic stimulation' at 50 c/s for 1 - 5 seconds, the slow potential may last as long as 20 seconds, which is similar to the prolonged negative surface potential recorded by Libet, 1964. This long delay was explained by Eccles and Libet, 1961 as diffusion of transmitter to a more peripheral site but this does not agree with the observed depressant action of anticholinesterases on the slow E.P.S.P. though a concomitant increase in an inhibitory potential might explain this.

It was felt that these synaptic delays might be represented by increased time for transmitter release, longer diffusion or a slow response of the postsynaptic membrane. The third possibility fits the observation that no decrease in membrane resistance occurs following the muscarinic action of acetylcholine on curarised rabbit S.C.G. cells (Libet and Kobayashi, 1969; Kobayashi and Libet, 1970). Other evidence for an electrogenic process for the generation of the slow E.P.S.P. comes from the depressant action of metabolic inhibitors and its depression by membrane hyperpolarisation which enhances the fast E.P.S.P. (Kobayashi and Libet, 1968). Recent evidence from intracellular recordings of membrane permeability in isolated rabbit S.C.G. cells supports strongly the concept of a generation of slow potentials in these cells without current flow across the membrane (Kobayashi, Hashiguchi and Ushiyama, 1978).

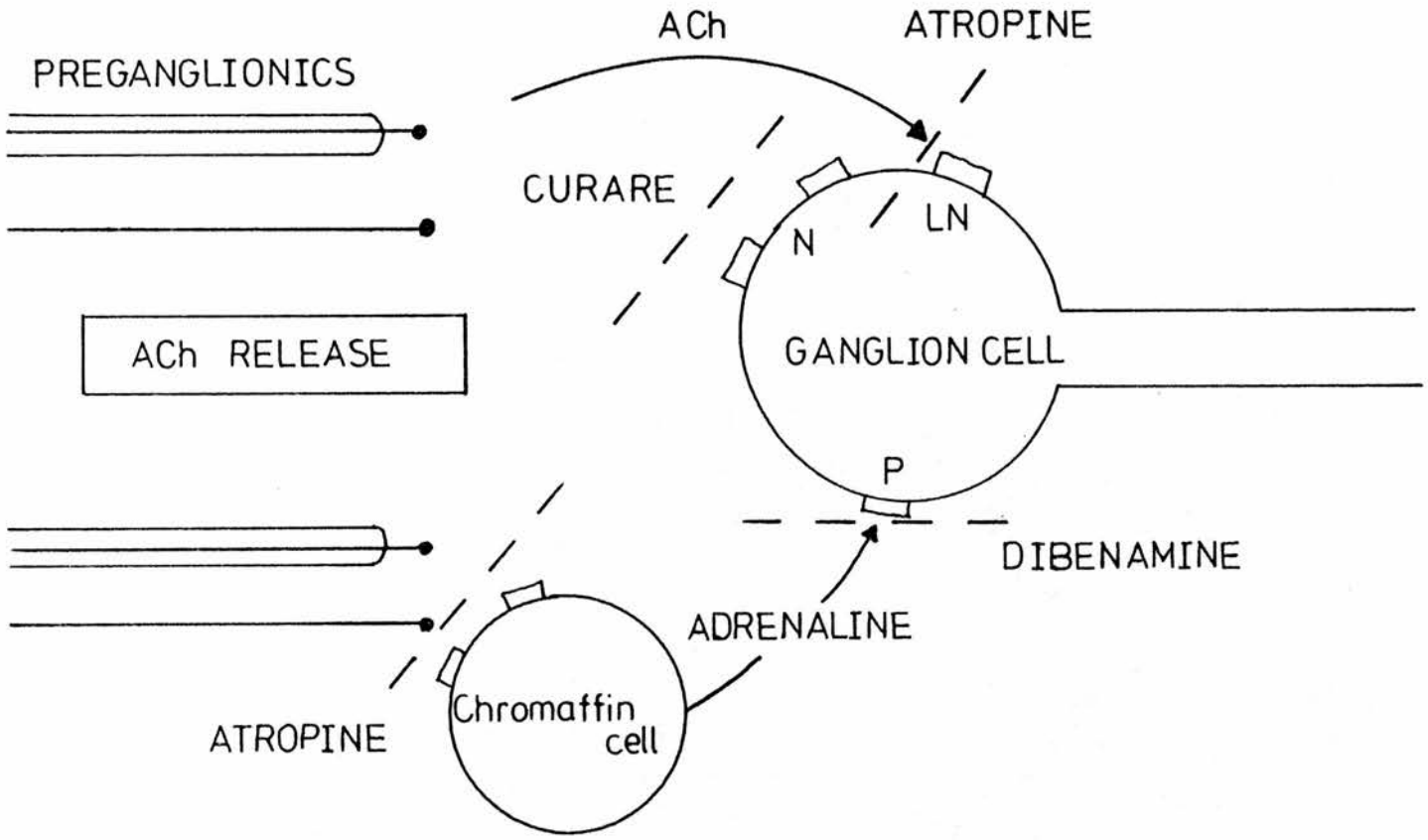
In summary, one is left with evidence for an atropine sensitive, muscarinic E.P.S.P. recorded from single ganglion cells after periods of high frequency preganglionic stimulation, the mechanism of which is, as yet, undefined.

Late, Late Negative Wave (LLN Wave)

Recordings from deeply nicotinised or curarised ganglia have shown evidence of early and late discharges following supramaximal

Fig 2

A model for the structural arrangement of a sympathetic ganglion. (After Eccles and Libet, 1961).



ACh - acetylcholine

preganglionic 'tetanic stimulation'. The late discharge seemed to be insensitive to atropine and may represent the response to a non-cholinergic LLN potential. The existence of such a potential in mammalian ganglia is controversial and depends on one's interpretation of results. (Chen, 1971).

Slow Inhibitory Postsynaptic Potential (Slow I.P.S.P./P Wave)

The externally recorded LN wave is usually preceded by a P wave which may have been similar to that observed by Eccles, J.C., 1935 though one must allow for an after-potential component in his readings. In rabbit S.C.G. the P wave was abolished by dibenamine (Eccles, R.M. and Libet, 1961) and reserpine (Libet, 1962). They postulated that an adrenergic substance was released from an intraganglionic chromaffin cell and that this hyperpolarised the ganglion cell membrane. Intracellular recordings confirm the existence of an I.P.S.P. in ganglion cells where spiking has been inhibited by curarisation (Libet and Tosaka, 1966) and this may correlate with the externally recorded P wave.

The proposed model of an intraganglionic interneurone of Eccles, R.M. and Libet, 1961 (see Fig 2, p 7a) has received much support with much evidence produced in an attempt to correlate structural and biochemical as well as electrophysiological data with a model for transmission of this type. Since the biphasic response to muscarinic agonists (Takeshige and Volle, 1962) can be converted to only an enlarged late depolarisation by low Ca^{++}/high Mg^{++} solutions (Libet, 1970) and that the recorded P wave could be abolished by administration of dibenamine, reserpine and atropine, it was proposed that an interneurone must exist. It seemed that a possible candidate for this structure was the small intensely fluorescent (S.I.F.) cells observed in some mammalian ganglia by a number of morphologists (Eränkö and Eränkö, 1971).

S.I.F. cells were observed scattered throughout the rabbit S.C.G. and the fluorophore was identified as dopamine with no evidence

of dopamine B hydroxylase being present (Fuxe, Goldstein, Hokfelt, and Joh, 1971). The S.I.F. cells and their apparent terminations seem to surround all ganglion cells which would be necessary as Libet and Tosaka, 1969 observed a slow I.P.S.P. in all the cells they recorded from. The slow I.P.S.P. after 40 c/s supramaximal preganglionic stimulation increased then decreased after eserine, decreased after alpha-methyl-para-tyrosine, which inhibits dopamine synthesis, and decreased after a conditioning stimulus of 30 c/s supramaximally every 10 seconds for 30 minutes. Libet and Owman, 1974 noted that all these procedures depleted the S.I.F. cell dopamine content. Histochemical analysis following these procedures and the addition of bethanechol showed a loss of dopamine which seemed to correlate with a decrease in I.P.S.P. The addition of dopamine did increase the dopamine content of the tissue and may have increased the slow I.P.S.P. To summarise, a number of criteria are used as evidence for S.I.F. cells acting as ganglionic interneurones. These consist of non-cholinergic transmitter present in ganglia, catecholamines acting similarly to produce s-I.P.S.P. (Libet and Kobayashi, 1969) and the presence of catecholamines in the S.I.F. cell. Synapses have been observed between preganglionic fibres and S.I.F. cells (Matthews and Nash, 1970; Matthews, 1971; Libet and Owman, 1974) and there is a detectable release of dopamine from S.I.F. cells following preganglionic stimulation, and also changes in slow I.P.S.P. which correlate with changes in S.I.F. cell dopamine content after receptor blockade (Libet and Owman, 1974).

One must be aware that the authors have not established whether these effects may only occur in the rabbit superior cervical ganglion as the S.I.F. cells of other ganglia contain catecholamines other than dopamine (Elfvin, Hökfelt, Goldstein, 1975), with some ganglia e.g. cat inferior mesenteric or guinea-pig S.C.G. lacking discernible slow I.P.S.P. (Libet, 1970; Crowcroft and Szureweski, 1971), and absence of synaptic contacts with the S.I.F. cells (Ostberg, 1970; Elfvin, 1971). Difficulty in interpretation may also arise from the measurement of results against a steadily declining slow I.P.S.P. with reductions in the ganglion cells' response to drugs with time, thus

making direct comparisons of electrophysiological and biochemical data difficult. Thirdly, the physiological significance of these results can be questioned in view of the stimuli required to elicit these effects as supra-maximal stimulation at 40 c/s is well above the physiological range unless, of course, the number of stimuli is important, a point not investigated. This large stimulus may only produce potential changes which are measurable in the ganglion cells, whereas far smaller changes may be functionally significant.

Transmission of Impulses

The synaptic potentials recorded from ganglion cells may be involved in the generation of spike discharges from these cells, e.g. the fast E.P.S.P. summates to produce the initial spike, but the main role of the slow synaptic potentials may be facilitatory and inhibitory rather than the initiation of transmission. To understand further the physiology of the sympathetic ganglia, one must consider the transmission of pre-ganglionic impulses and its modification by drugs.

Initially, it was suggested that the response of a ganglion cell was a repetitive spiking for each preganglionic impulse impinging on it. This was contradicted by Bronk, 1939 and Sato and Schmidt, 1973 who, using single fibre analysis, confirmed that each preganglionic impulse was matched by a postganglionic spike up to a frequency of 20 c/s whereupon a more frequent and prolonged discharge is observed. Temporal and spatial summation can occur, but a maximal preganglionic synchronous stimulus is not greatly modified by the ganglion cell if single volleys are considered though facilitatory and inhibitory effects can occur. Two methods of investigating sympathetic transmission have been devised though these methods have both usually been applied to solely the S.C.G.. Kibjakow, 1933 (see Fig. 3, p10a) devised the commonly used, in situ, isolated S.C.G. perfusion method where vascular isolation of the S.C.G. must be complete so that an artificial circulation can be maintained. This can lead to a decrease in sensitivity to some drugs (Trendelenburg 1956(a); Hancock

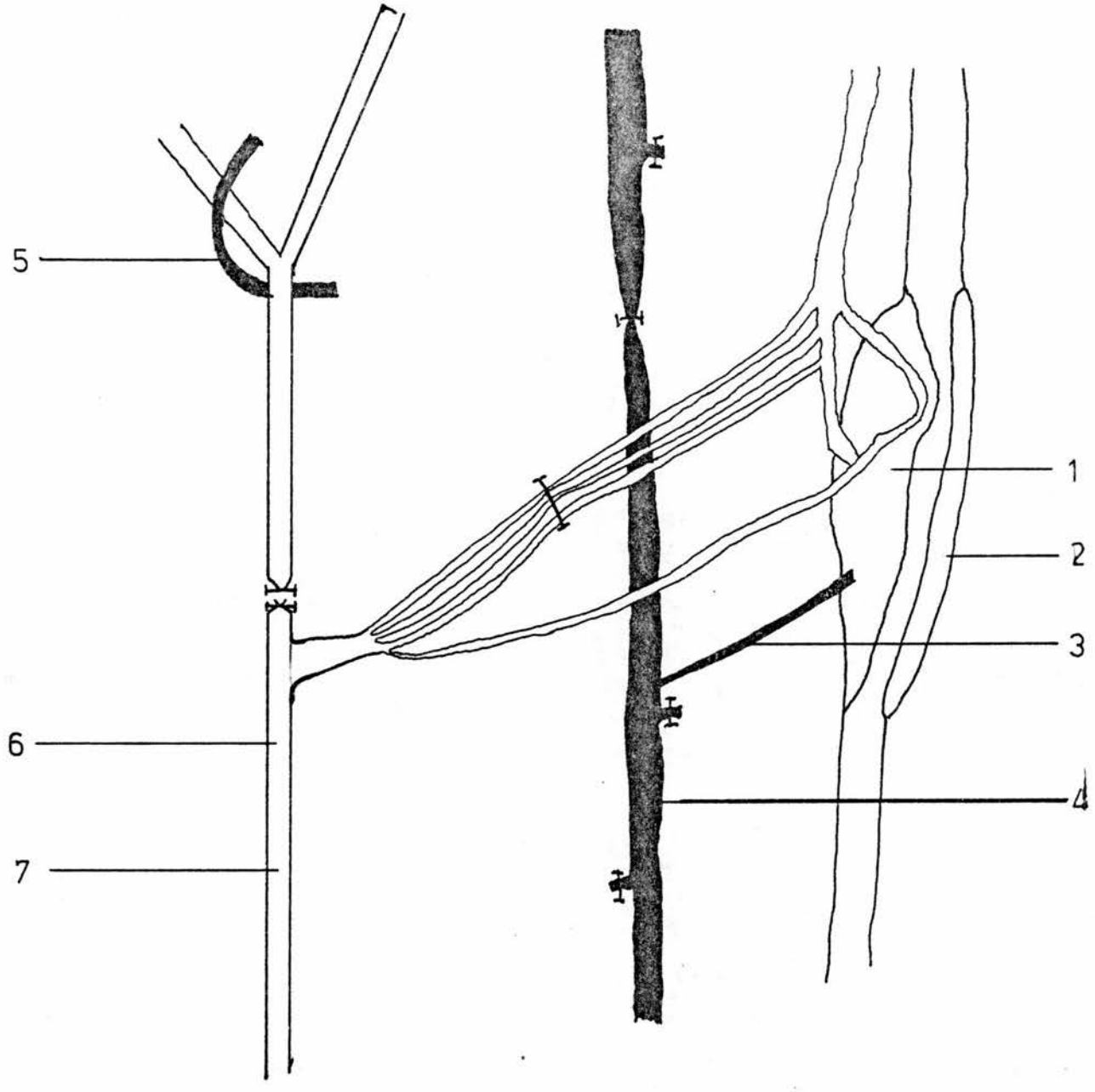


Fig 3

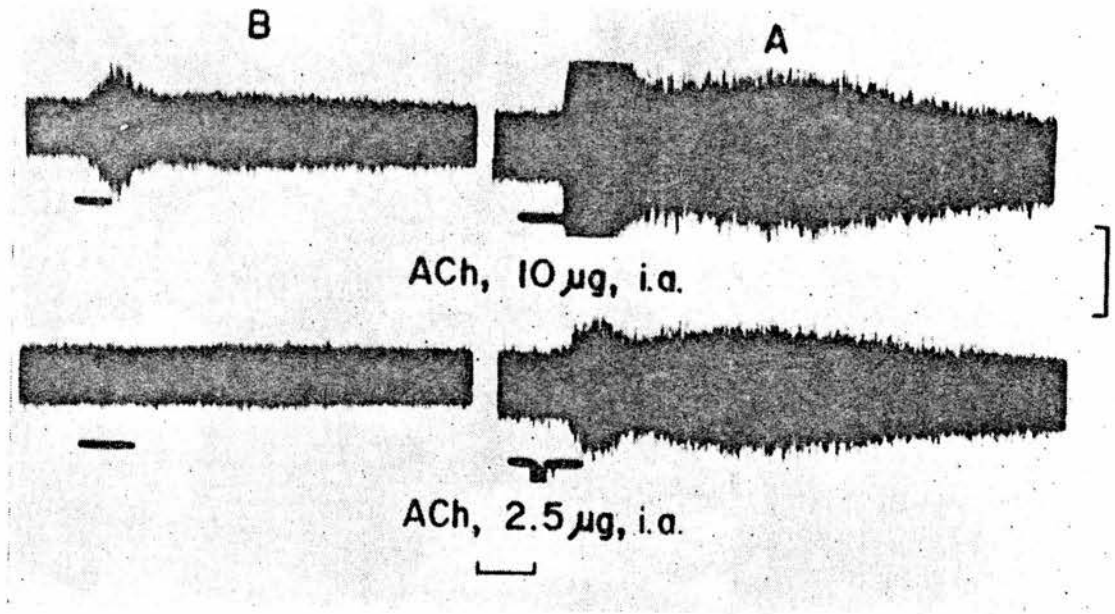
A method for the perfusion and administration of drugs to a Superior Cervical Ganglion (After Kibjakow, 1933). 1 - Superior Cervical Ganglion 2 Nodose ganglion 3 - Vein from SCG 4 - Jugular vein 5 - Glossopharyngeal N. 6 - Site of insertion of cannula 7 - Common Carotid artery.

and Volle, 1970). A much simpler method (Trendelenberg, 1959) involves close arterial injections to the S.C.G. with an intact circulation, whilst the external carotid artery is occluded, allowing the drug to reach the S.C.G. The response of the ganglion cells is assessed either by measurement of the effector organ response, nictitating membrane contractions, or by direct electrical recording from the postganglionic nerve trunk (Volle, 1962(a)). Both methods have disadvantages in that the quantitative assessment of nictitating membrane responses may be altered by drugs acting postganglionically to alter noradrenaline release,or on the sensitivity of the muscle to noradrenaline. The other difficult assumption is that the postganglionic fibres innervating the nictitating membrane are similar to other postganglionic fibres and the cells from which they arise are similar in their pharmacology to other ganglion cells which may be false (Koss and Rieger, 1976). The more direct measurement of postganglionic activity avoids these problems and has the advantage of allowing simultaneous surface recording of ganglion cell potentials but has the major disadvantage of excluding quantitative measurement. It is against this background of experimental limitations that the action of pharmacological agents on ganglionic transmission must be considered.

Acetylcholine

In 1914, Dale concluded that acetylcholine had a 'nicotinic' action in its ability to stimulate ganglia. It was confirmed as the ganglionic neurotransmitter by Feldberg and Gaddum, 1934 and in addition acetylcholine seemed able to lower the threshold of cells, or stimulate the cells of lower threshold, as it produced an asynchronous discharge from ganglion cells associated with an increase in the spike amplitude to low frequency preganglionic volleys (Bronk, 1939). Whilst there is good evidence for the effects of acetylcholine being predominantly nicotinic, some muscarinically mediated effects have been observed under certain experimental conditions. Volle and his co-workers and Trendelenburg and his co-workers have studied the action of acetylcholine on the superior cervical

Fig. 4 The "unmasking" of the muscarinic action of Acetylcholine as a ganglion stimulant. (From Takeshige and Volle, 1963 (a)).



Postganglionic responses to acetylcholine (ACh) of normal superior cervical ganglion of the cat before (B) and after (A) the administration of eserine (50 µg, i.a.).

Top row of records: postganglionic firing evoked by ACh (10 µg, i.a.) before (B) and after (A) eserine. Bottom row of records; postganglionic firing evoked by smaller dose of ACh (2.5 µg, i.a.). Vertical and horizontal calibrations are 10 µV and 2 seconds, respectively.

ganglion of the cat. Without any pretreatment, the close arterial injection of acetylcholine caused a short burst of postganglionic firing which was suppressed by hexamethonium and other nicotinic antagonists. This short and 'early' postganglionic discharge seems to represent a nicotinic action of acetylcholine on the ganglion cells. An additional late low amplitude discharge, 'late firing', was observed in response to acetylcholine injections under the following conditions:-

Preganglionic Stimulation (Takeshige and Volle, 1962; Volle, 1962(b).)

Repetitive stimulation of the preganglionic trunk at 30 c/s supramaximally for one minute produced a 'late firing' postganglionically which was unaltered by d-Tubocurarine (d-TC) but was abolished by atropine (0.5 - 1.0 µg intra-arterially). This antagonism by atropine could be overcome by preganglionic stimulation at 60 c/s for one minute,by eserine (100 µg i.a.), or by KCl i.a.. Antidromic stimulation did not enhance responses to acetylcholine but procaine reduced the potentiation.

Previous Administration of an Anticholinesterase (Takeshige and Volle, 1962; Takeshige and Volle, 1963(a)).

When eserine was present, the threshold for the 'late response' was far lower than for the 'early response' and the 'unmasked late response' could be antagonised by atropine (0.5 - 1.0 µg i.a.). Higher doses of eserine produced an asynchronous discharge and after neostigmine the response to acetylcholine was not blocked by d-TC in a dose inhibiting completely the response to preganglionic stimulation. This contrasted with the work of Mason, 1962 who observed blockade of neostigmine's action by d-TC but using the nictitating membrane to observe responses.

Denervation of Ganglion (Takeshige and Volle, 1963(b))

Following denervation of the S.C.G. the nerve terminals and cholinesterase within them has degenerated. When denervation is complete

acetylcholine produces a bimodal response with the early part blocked by hexamethonium or d-TC and the late response blocked by atropine. In animals, where regeneration is presumed to have occurred, only the early response was observed and it was sensitive to nicotinic antagonists. This suggested to the authors the development of more receptors of a muscarinic type peripheral to the sympathetic area or a change in the cell excitability produced by denervation. In a recent study (Burt, 1978) no evidence of an increased receptor population was obtained from analysis of antagonistic binding data.

Nicotine - Desensitization

During the initial phase of a depolarising block by nicotine, the ganglion cells fail to respond to any agonists. Responses to non-nicotinic agonists were restored and enhanced during the late non-depolarising phase of nicotine block (Trendelenburg, 1966(a)). The muscarinic nature of acetylcholine's action at this stage in the block by nicotine is clear from the long latency of the response which is characteristic of muscarinic agents. The flat dose response curve now present to acetylcholine being similar to that produced by muscarinic agonists whereas the previous acetylcholine dose response curve was steep. Also morphine is active in reducing the response to acetylcholine where it is inactive against nicotine. It was also observed that all these procedures which were shown to alter the ganglionic responses to the muscarinic agonist McNA343 (Fig 6, p16a) also affect acetylcholine similarly. (Trendelenburg and Jones, 1965).

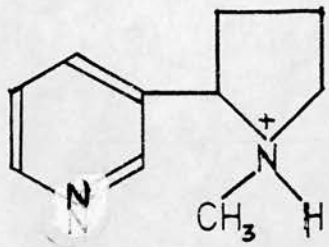
Nicotinic Drugs (Fig 5, p13a)

The oldest known ganglion stimulant is nicotine (Schmiedeberg, 1870; Langley and Dickinson, 1889). It was shown to depolarise ganglion cells in the cat S.C.G. (Paton and Perry, 1953) and the rabbit (Eccles, R.M., 1956; Pascoe, 1956). When 'end-organ' responses are considered a 'bell-shaped' dose response relationship is obtained, a phenomenon

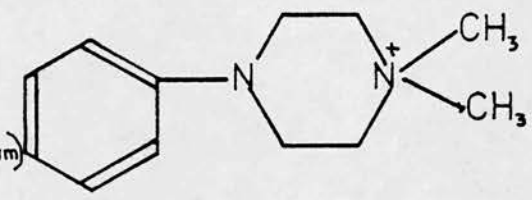
Fig 5

The structures of commonly used nicotinic agonists.

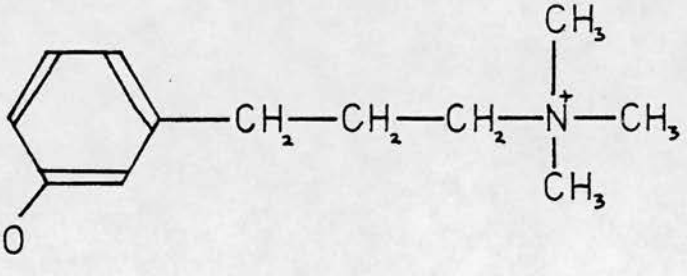
NICOTINE
(nicotinium ion)



D.M.P.P.
(dimethylphenylpiperazinium)



H.P.P.T.M.A.
(m-hydroxyphenylpropyl
tri methylammonium)



described by Van Rossum, 1962 as 'auto-inhibition'. Paton and Perry, 1953 concluded that nicotine prevented the action of trimethylammonium and acetylcholine on ganglion cells during both the period of time that the cells were depolarised and for a period after this. This 'late block' was deduced by Paton and Perry to be competitive. During the depolarising phase of nicotine block potassium chloride is inactive but during the late non-depolarising phase, the potassium chloride is again active. This late phase of nicotine block seems to be insurmountable and hence not competitive (Trendelenburg, 1966(a)) and seems to be of a desensitising type as described by Rang and Ritter, 1970. Further work on ganglion preparations has confirmed the action of a new muscarinic antagonist which appears to have a selective action on desensitised nicotine receptors, (Fisher, Weinstock, Eliash, Gitter and Cohen, 1977) and similarly some drugs seem to prevent desensitisation by stabilising the 'active' receptor conformation (Kirpekar & Prat, 1978).

This stimulation followed by blockade appears to be an action unique to nicotine but somewhat similar effects, with perhaps a different underlying mechanism, have been seen with other nicotinic agonists. Gebber and Volle, 1966 found that tetramethylammonium (T.M.A.) produced a triphasic effect with a blockade of transmission corresponding to a hyperpolarisation. This block could be prevented by any agent which increased the sensitivity to acetylcholine, or by acetylcholine itself, and this was reversed by atropine. The hyperpolarisation was reduced by ouabain which did not alter the action of methacholine or adrenaline. Thiocholine type drugs seem to resemble tetramethylammonium in this action which is not attributable to a muscarinic action being very different from the action of methacholine. Dimethylphenylpiperazinium (DMPP), since its first described action on ganglion cells (Chen, Portman and Wickel, 1951), has been used as a typical nicotinic agonist. It does not produce a late non-depolarising phase of ganglion block and has far less muscarinic action that T.M.A. (Trendelenburg, 1961(a)).

Carbachol was postulated as acting indirectly by releasing acetylcholine from preganglionic nerve terminals (Volle and Koelle, 1961). McKinstry and Koelle, 1967 confirmed this since the action on denervated compared to innervated preparations was less pronounced and the release of acetylcholine seemed to occur in Ca free solutions and was abolished by hexamethonium. Carbachol may have an indirect component in its action but it is difficult to provide conclusive evidence.

It appears that nicotinic agonists all act by a common mechanism to produce synaptic potentials within a ganglion cell which summate to produce discharge (Eccles, R.M., 1956). This action would seem to be maintained for 5 - 12 minutes and this may result from an increase in cell excitability, in addition to cell discharge. Nicotine can depress the externally recorded N wave without depressing transmission suggesting that cells are depolarised and cannot be further excited. As the nicotine concentration increases, the N wave falls and the spike is reduced as a depolarising block occurs. Associated with this is a large increase in the P wave which probably reflects an after-hyperpolarisation as a result of the depolarised membrane potential. Although nicotinic agonists act directly on sympathetic ganglion cells, they may also release noradrenaline from peripheral sympathetic nerve endings as may a number of other agonists (Lindmar, Löffelholtz and Muscholl, 1968).

Most commonly used nicotinic antagonists, d-tubocurarine, hexamethonium, mecamylamine and chlorisondamine seem to have no action on the resting ganglion cell membrane potential or on the threshold for generation of a potential following direct stimulation. They seem to act as non-depolarising blockers by a competitive antagonism at nicotinic receptors on the ganglion cells (Lees and Nishi, 1972). Hexamethonium and d-tubocurarine seemed devoid of presynaptic actions but mecamylamine decreases transmitter release by a presynaptic action making its use as a nicotinic ganglion blocker more controversial.

Muscarinic Drugs

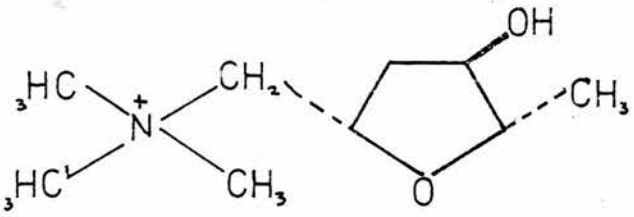
Pilocarpine is known to enhance transmission in the S.C.G. by an action which was found to be atropine sensitive, (Dale and Laidlaw, 1912; Koppanyi, 1932; Marazzi, 1939), but there is evidence from much later work for a specific muscarinic action involving a distinct population of muscarinic receptors on the ganglion cells. The response of some ganglion preparations was variable and using effector organ responses, an extremely flat dose response curve was obtained making potency determinations difficult. As well as actions on the cat S.C.G., some muscarinic agonists have been shown to stimulate the cat and dog stellate ganglia to increase myocardial rate and force (Flacke and Gillis, 1968; Aiken and Reit, 1969; Hilton, 1978). Direct recording from the postganglionic nerve trunk has shown increased activity after methacholine (Takeshige and Volle, 1963 (a)) and after McNA343 (Fig 6) (Jaramillo and Volle, 1967 (a)).

McNA343, muscarine, oxotremorine and AHR602 (Fig 6) (Franko, Ward, Alphin, 1963) all gave a low amplitude asynchronous firing which was sensitive to atropine. This firing measured postganglionically may correlate with changes in surface potential observed with these agonists. McNA343 and AHR602 and pilocarpine (Jaramillo and Volle, 1967(a), (b)) produced a maintained depolarisation of the ganglion cells which was slow in onset, (2 - 4 sec.), and lasted 60 - 90 seconds. Unlike the depolarisation produced by the other two agonists, the pilocarpine response was sensitive to atropine. Methacholine, muscarine and oxotremorine all gave biphasic ganglion potential changes of a type similar to those attributed to the muscarinic action of acetylcholine. The biphasic ganglion potentials following these drugs are like the biphasic, postganglionic firing also observed to be atropine sensitive, whereas the maintained depolarisation produced by McNA343 and AHR 602 is not atropine sensitive and only the asynchronous firing produced by the former drug is altered by atropine. Bethanechol is said to be only able to hyperpolarise ganglion cells (Libet, 1970) but this seems only a

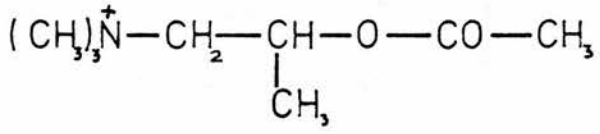
Fig 6

The structure of the commonly used muscarinic agonists. This includes the two "selective" muscarinic ganglion stimulants McNA343 and AHR 602.

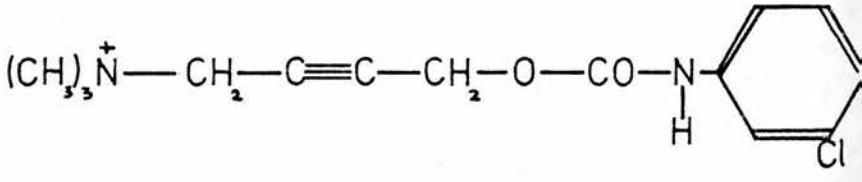
(+)-MUSCARINE



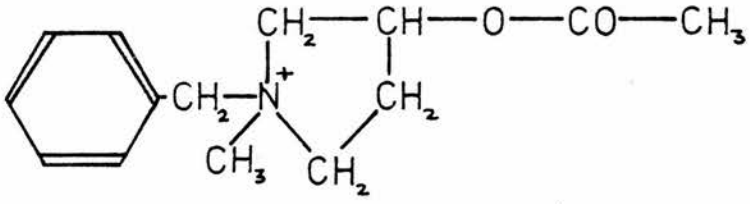
ACETYL-β-METHYLCHOLINE



McN A 343



AHR 602



characteristic of the first dose administered to any preparation, the effect not being reproducible. The actions of muscarinic agonists can be enhanced by a number of procedures which also 'unmask' the muscarinic action of acetylcholine. Previous preganglionic stimulation supramaximally at 25 c/s for 5 seconds, produces a longlasting potentiation of the responses to McNA343, pilocarpine and AHR602 (Jones, 1963; Trendelenburg and Jones, 1965; Smith, 1966) as does denervation of the ganglia (Ambache, Perry and Robertson, 1956; Jones, 1963; Green, 1965). When one produces a non-depolarising 'late phase' block by nicotine, muscarinic agonists show a potentiated response (Trendelenburg, 1957; 1966(a), (b); Jones, 1963) with the potentiation being prevented by hexamethonium. This implies that this facilitation was the result of nicotine's action on the ganglion cells.

Though subthreshold doses of muscarinic agonists facilitated transmission of submaximal preganglionic impulses through the S.C.G. of the cat (Jones, 1963; Murayama and Unna, 1963; Smith, 1966) higher doses can produce inhibition of transmission. Takeshige, Papanno, De Groat, Volle, 1963 observed an atropine sensitive depression of transmission following exposure of cat S.C.G. to acetylcholine or methacholine. The reduction in postganglionic firing was accompanied by a hyperpolarisation measured from a surface electrode. In the case of methacholine this coincided in time course, with the reduction of firing. Reserpine did not reduce this depression suggesting an absence of catecholamine release from an interneurone, though the reserpine concentration may have been insufficient. McNA343 and AHR602 also depressed transmission (Jaramillo and Volle, 1967(a), (b)) but no hyperpolarisation was observed and this depression of transmission was not atropine sensitive. Furmethides seemed to have similar actions.

The evidence of an atropine sensitive, muscarinic stimulation of the sympathetic ganglion cells seems conclusive though one is left in some doubt as to whether the mode of action of McNA343, AHR602 and furmethides may involve a mechanism other than, or in addition to,

muscarinic receptor activation. The potency of these agonists as ganglion stimulants in comparison to their actions on tissues, containing parasympathetic, postganglionic muscarinic receptors has been used as evidence of a selective action on muscarinic receptors in the ganglia differing from those traditionally observed. The non-specific stimulant action of these 'selective muscarinic ganglion stimulants' may make interpretations of this kind suspect without evidence from antagonist studies.

To summarise, one can distinguish a muscarinic action of a cholinergic agonist from a nicotinic action by its selective sensitivity to antagonists, by the flat dose response curve obtained from experiments where end organ response is measured (Trendelenburg, 1966(b); Flacke and Gillis, 1968; Hilton, 1977), by the inability to produce tachyphylaxis with maintained administration unlike nicotinic agents, by their ability to stimulate ganglion cells during the late phase of nicotinic blockade and by their susceptibility to blockade by cocaine, morphine, methadone and $CaCl_2$. In the literature the existence of nicotinic and muscarinic receptors in the sympathetic ganglia chosen for study has been confirmed, but there would appear to be in addition to these receptors, a population of non-cholinergic receptors as other agonists can act on ganglion cells.

Non-Cholinergic Drugs

Drugs Increasing Ganglionic Activity

Various non-cholinergic agonists have been found to stimulate sympathetic ganglion cells with characteristics very similar to the actions of muscarinic agonists. 5-hydroxytryptamine (5-HT) (Trendelenberg, 1956) histamine (Trendelenburg, 1954), angiotensin (Lewis and Reit, 1965) and bradykinin (Lewis and Reit, 1965) all produce increases in ganglionic cell activity which are not antagonised by cholinergic antagonists but do show receptor specificity as witnessed by the action of selective antagonists. There is some evidence that they may share a common mechanism of action at some stage in the generation of ganglion cell action potentials as all non-nicotinic agonists are antagonised by morphine, cocaine,

Fig. 7

SCHEMATIC REPRESENTATION OF EFFECTS OF VARIOUS DRUGS ON THE STIMULATION OF CAT S.C.G. BY GANGLION STIMULATING AGENTS

	T.M.A. Nicot. D.M.P.P.	Musc.	Hist.	5HT	Angiot.	Bradykinin	KCL
Non depol. gang. block	IN	0	0	0	0	0	0
Nicotine							
depol. phase	IN	IN	IN	IN	IN	IN	IN
non. depol. phase	IN	POT	POT	POT	POT	IN	0
followed by C_6	IN		R E T U R N T O N O R M A L				0
Cocaine	0	IN	IN	IN	IN	IN	0
Morphine/Methadone	0	IN	IN	IN	IN	IN	0
Atropine	0	IN	0	0	0	0	0
Antihistamine	0	0	IN	0	IN ?	0	0
Methysergide	0			IN			
Facilitation by preganglionic stim.	0	POT	POT	POT	POT	POT	0

IN - Reduction of end-organ response to this agonist.

POT. - Potentiation of response to this agonist.

O - no alteration of response to this agonist.

T.M.A. - tetramethylammonium

D.M.P.P. - dimethylphenylpiperazinium

Nicot. - nicotine

Musc. - Muscarinic agonists.

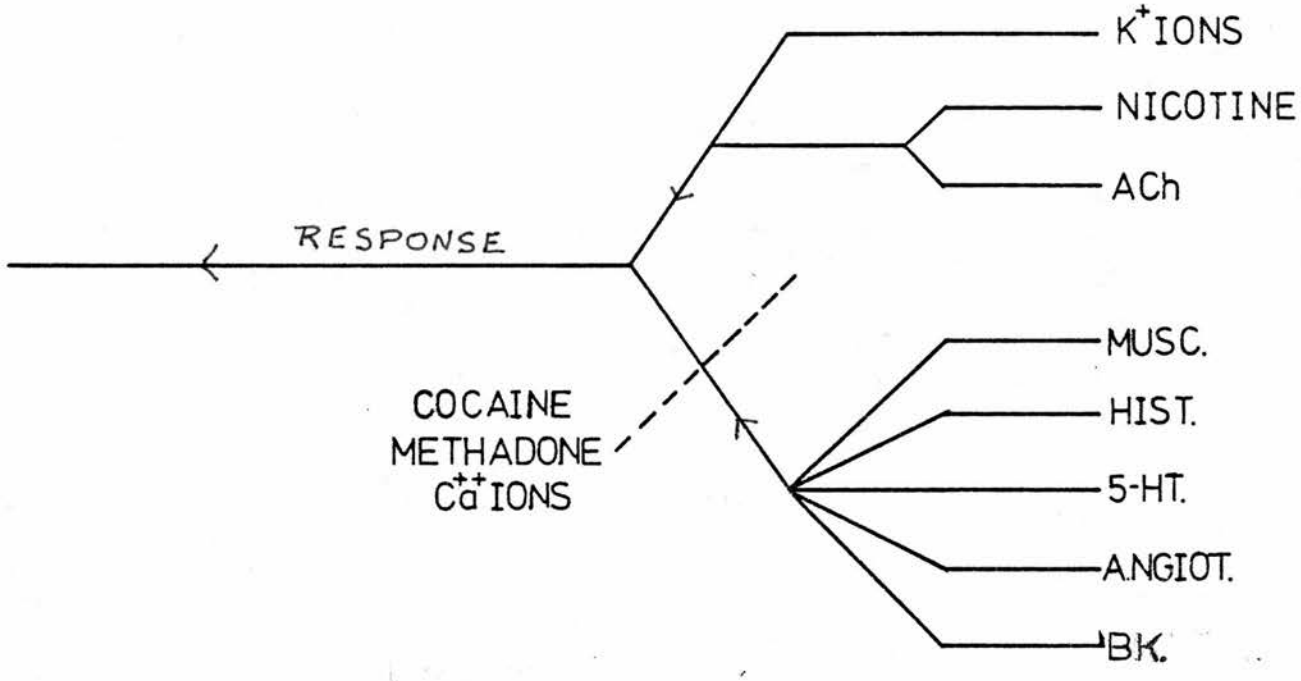
5-HT. - 5-hydroxytryptamine

Angiot. - angiotensin II

C_6 - hexamethonium

Fig 8

A diagramatic representation of the possible common mechanism of action on all non-nicotinic agonists on ganglion cells.



ACh:ACETYLCHOLINE MUSC.-MUSCARINE HIST.-HISTAMINE
BK.-BRADYKININ 5-HT.-5-HYDROXYTRYPTAMINE ANGIOT.-ANGIOTENSIN

methadone and $CaCl_2$ (Smith, 1966(b)) and they also exhibit cross-tachyphylaxis (Trendelenberg, 1954; 1956; Lewis and Reit, 1965; Smith, 1966 (b)). (Fig 7, Fig 8, p 18a, 18b.) Whenever cross-tachyphylaxis occurs to one of these non-nicotinic agents, these drugs not only fail to produce spike discharge, they also fail to facilitate transmission (Iorio and McIsaac, 1966). These non-cholinergic agonists also appear to share with muscarinic agonists the hexamethonium-sensitive enhancement of their responses during late phase block by nicotine.

The Action of Metal Ions and Selective Actions of Other Drugs

Pappano and Volle, 1962 noted that Ca^{++} ions were able to produce a depolarisation of a cat S.C.G. perfused with Locke's solution. This surface recorded depolarisation was hexamethonium sensitive but was not accompanied by any postganglionic firing unlike the usual response to nicotinic agonists. Ba^{++} mimicked this action of calcium ions and also appeared to 'unmask' the late firing after acetylcholine ascribed to a muscarinic action of the drug (Takeshige and Volle, 1964(a)). This depolarisation seemed also to be associated with a depression of the normal depolarisation produced by potassium chloride. With these effects in mind, a comparison of the action of Ca^{++} ions with that of atropine was attempted (Takeshige and Volle, 1964(b)). In all cases 4.5 mM $CaCl_2$ inhibited, in a similar way to atropine, the muscarinic actions of acetylcholine ('unmasked' by prior treatment) or methacholine. $CaCl_2$ was also able, like atropine, to reverse the blockade of transmission produced by acetylcholine, methacholine or tetramethylammonium. It has also been observed that all the actions of all tested non-nicotinic agents on the S.C.G. of the cat can be inhibited by $CaCl_2$, methadone, morphine or cocaine (Trendelenberg, 1967). The selectivity of these agents and the observations of Pappano and Volle, 1966, that the depolarisation produced by acetylcholine applied exogenously to a perfused S.C.G. is mediated by Ca^{++} ions in the absence of sodium ions, at which time ganglionic transmission is abolished, suggests that the mechanism of synaptic potential generation following the administration of acetyl-

choline and possibly other non-cholinergic agonists involves a mechanism different from the generation of an action potential during normal 'nicotinic' transmission. This would support the hypothesis of an electrogenic mechanism connected to muscarinic receptor occupation unlike the conductance changes arising from nicotinic receptor occupation. Pappano and Volle, 1966 noted that the acetylcholine depolarisation was sensitive to d-TC suggesting to the authors a nicotinic action but later work has shown (Hancock and Volle, 1970) that in a salt-solution-perfused S.C.G. the selectivity of nicotinic and muscarinic antagonists is lost by an as yet undiscovered mechanism.

The molecular action of divalent cations on ganglion cells is not well documented but the depolarisation observed appears to involve presynaptic transmitter release, (McLachlan, 1977) but the 'membrane stabilising' acetylcholine antagonist action of these ions is by an action, one presumes, on the generation of ganglionic potentials which may have a mechanism common to all non-nicotinic agonists but markedly different from that to nicotinic agents.

The Modulating Action of Catecholamines

A dopaminergic or adrenergic interneurone is postulated as producing an inhibitory influence on ganglion cell firing, but exogenous catecholamines show other actions which may be of equal or greater physiological significance.

Adrenaline when applied exogenously was observed to block transmission through the S.C.G. (Marazzi, 1939(b); Eccles and Libet, 1961; Pardo, Cato, Gijow, and Alonso de Florida, 1963). Paton and Thompson, 1953 proposed that adrenaline reduced acetylcholine output as well as depressing postganglionic sensitivity. This depressant action of adrenaline appeared to contrast with the enhancement of transmission and acetylcholine's action observed after lower catecholamine doses (Kewitz and Reiwert, 1952; Malmejac, 1955). De Groat and Volle, 1966 resolved this by obtaining evidence of two distinct sites of action

of catecholamines on the cat S.C.G. cells. The inhibition of transmission was antagonised by α blockers and the enhancement of transmission by β blockers. This has recently been confirmed (Brown and Caufield, 1979; Brown, Caulfield and Kirby, 1979).

The inhibitory action of catecholamines may not necessarily involve the hyperpolarisation observed by several workers as low concentrations of noradrenaline and adrenaline decreased the release of acetylcholine from preganglionic nerve endings and were without an action on the postsynaptic sensitivity to acetylcholine (Christ and Nishi, 1967, 1971(b)). This presynaptic action of catecholamines seems to be involving dopamine receptors. The facilitation observed by De Groat and Volle, 1966 was in the presence of an α blocker and may be due to an adrenaline mediated depolarisation in the absence of hyperpolarisation or by a presynaptic action.

Dopamine has two actions on the sympathetic ganglion cells. It has been observed to depress ganglionic transmission (Weir and McLennan, 1963) and to decrease acetylcholine liberation (Dun and Nishi, 1974) but it also can produce a maintained enhancement of the muscarinic generation of the slow E.P.S.P. This enhancement was most pronounced in the rabbit S.C.G. and seemed to involve no change in postsynaptic potential but might reflect a release of dopamine from a second type of S.I.F. cell, namely that lacking efferent synapses. (Libet and Tosaka, 1970; Libet, 1976). It is conceivable that noradrenaline or adrenaline might have similar actions in other ganglia.

Cyclic Nucleotides

Associated with the actions of catecholamines and muscarinic agonists,cyclic nucleotides have been postulated as mediating these effects at a cellular level.

cAMP

Preganglionic stimulation, 10 c/s supramaximally, and dopamine when administered to isolated rabbit S.C.G. increase the cAMP concentration up to five-fold. (McAfee, Schorderet and Greengard, 1971; Kalix, McAfee, Schorderet and Greengard, 1964). The former increase can be prevented by atropine (2 x 10^{-6} M) and the latter by α blockers but not by hexamethonium (5 x 10^{-4} M) or β blockers. The dopamine appears to increase adenyl cyclase activity in the postganglionic neurones which are the site of dopamine mediated hyperpolarisation. Monobutyryl cAMP seemed to cause hyperpolarisations in postganglionic neurones when measured by sucrose gap techniques (Kosterlitz, Lees and Wallis, 1968; McAfee and Greengard, 1972). Based on this evidence and indirect evidence that acetylcholine may act through an interneurone, the action being antagonised by α blockers, tetracaine and low Ca^{++} medium, all of which have been shown to alter postsynaptic responses to acetylcholine alone, it was proposed that release of dopamine from the S.I.F. cell interneurone produces a hyperpolarisation of the postganglionic cell mediated by cAMP. From later evidence this theory becomes less feasible as muscarinic agonists are more potent increasers of adenyl cyclase activity than dopamine, it seems β receptor stimulation increases cAMP concentrations not α receptor actions (Brown and Caulfield, 1979) and in contrast to earlier results, no hyperpolarisation of intracellularly recorded potentials from single rabbit S.C.G. cells have been found in presence of dibutyryl cAMP (Kobayashi, Hashiguchi and Ushiyama, 1978). It does appear that biochemically observed cAMP increases following dopamine or preganglionic stimulation may reflect a physiological event as the maintained enhancement of the slow E.P.S.P. by dopamine is mimicked by adenine cyclic nucleotides (Kobayashi et al, 1978).

From this evidence the role of the S.I.F. cell as an interneurone may still be considered controversial and the histochemically observed S.I.F. cells may have a modulating role without being actually

associated with preganglionic synapses and the hyperpolarisation observed may be purely muscarinically mediated or may not exist physiologically being replaced by an after-hyperpolarisation resultant from spike discharge.

cGMP

It seems cGMP may mediate muscarinic actions in several tissues (Greengard, 1976). Intracellular recording from single rabbit S.C.G. cells has shown that the depolarising action of cGMP mimics that of muscarinic agents producing the slow E.P.S.P. in that no change in membrane conductance occurs. This evidence, together with results showing increases in postganglionic cGMP after acetylcholine action may provide some initial evidence for a role for cGMP in the cellular production of the slow E.P.S.P. but at this stage, this view is speculative.

Posttetanic Potentiation

Recording from single postganglionic fibres arising from cells in the stellate ganglion of the cat, Larrabee and Bronk, 1947 observed increases in spike height of a single transmitted volley after a repeated conditioning stimulus. This facilitation was increased as the number of conditioning stimuli was increased and was maximal after 30 c/s supramaximal stimulation for 5 - 10 seconds. As antidromic stimuli were ineffective and no facilitation of the response to acetylcholine was observed, they assumed that 'posttetanic potentiation' arose as a result of a change in properties of the presynaptic endings. Libet, 1964 observed two components of posttetanic potentiation, one of which was atropine sensitive and would appear to generate spike discharge in recruited cells associated with an increased LN wave. As has been reported by a number of workers, a previous period of preganglionic nerve stimulation does 'unmask' the muscarinic action of acetylcholine. Brimble and Wallis, 1974 have expanded this study of facilitation in the rabbit S.C.G. and have noted 'early' and 'late

facilitation' depending on the stimuli used. Some of these facilitations especially after trains of stimuli of varying thresholds at 30 c/s have been shown to be atropine sensitive. It seems that, following trains of preganglionic stimuli classed as 'tetanic' by authors, a prolonged facilitation of ganglion spike potentials occurs presumably representing recruitment of subliminal cells. The mechanism of this recruitment of cells remains to be fully elucidated but may involve a change in presynaptic sensitivity causing an increased release of transmitter per stimulus as well as changes and enhancement of transmitter action postsynaptically.

After-Hyperpolarisation

When a S.C.G. from rabbit, rat or kitten has been depolarised by preganglionic stimulation or exogenous cholinergic agonists, a large after-hyperpolarisation is observed (Kosterlitz, Lees and Wallis, 1968; Brown, Brownstein and Scholfield, 1969; Lees and Wallis, 1974). This seems to be a property of the ganglion cells and the amplitude depends more on the duration than the degree of depolarisation. This after-hyperpolarisation does not seem to depend on increases in K^+ permeability which may account for the P wave (Kosterlitz et al, 1968), or chloride ions (Cl^-) but seems associated with the level of sodium influx during the previous depolarisation. The after-hyperpolarisation is sensitive to ouabain, glucose-free media and K^+ concentrations and may be explained by an electrogenic sodium pump which produces a coupling ratio $Na^+ - K^+$ which is not unity and depends on K^+ for its action. A pump mechanism of this type (Rang and Ritchie, 1968) has been observed in the rat S.C.G. (Brown, Brownstein and Scholfield, 1972). The large increase in this after-hyperpolarisation after preganglionic 'tetanic' stimulation has led to the suggestion inferred from experiments on amphibian ganglia that presynaptic receptors may modify the transmitter outflow to overcome this and allow normal transmission. In this context a role for nicotinic receptors might be postulated in mammalian ganglia similar to that documented in

amphibians (Nishi, 1970), though this type of receptor might mediate the postulated indirect release of acetylcholine produced by some cholinergic agonists. (Koelle, 1962).

Summary

From the experimental evidence reviewed, it is clear that the predominant transmission of impulses through the sympathetic ganglia investigated involves the release of acetylcholine from preganglionic nerve terminals closely associated with nicotinic receptors. These may be present on the postsynaptic cell soma or dendrites with most mammalian ganglia showing predominantly axodendritic connections. The structural morphology of most sympathetic ganglia with different preganglionic fibres, associated with different cell groups, showing very varying conduction velocities and thresholds leads to the possibility of a sympathetic ganglion acting as an integrating as well as a transmission centre. The large divergence of preganglionic fibres on to the postsynaptic cells is associated with the large subliminal fringe observed in the ganglion studied, where a number of stimuli must interact to produce a large enough summated synaptic potential to initiate spike discharge from the cell. This accounts for the facilitatory and inhibitory effects observed.

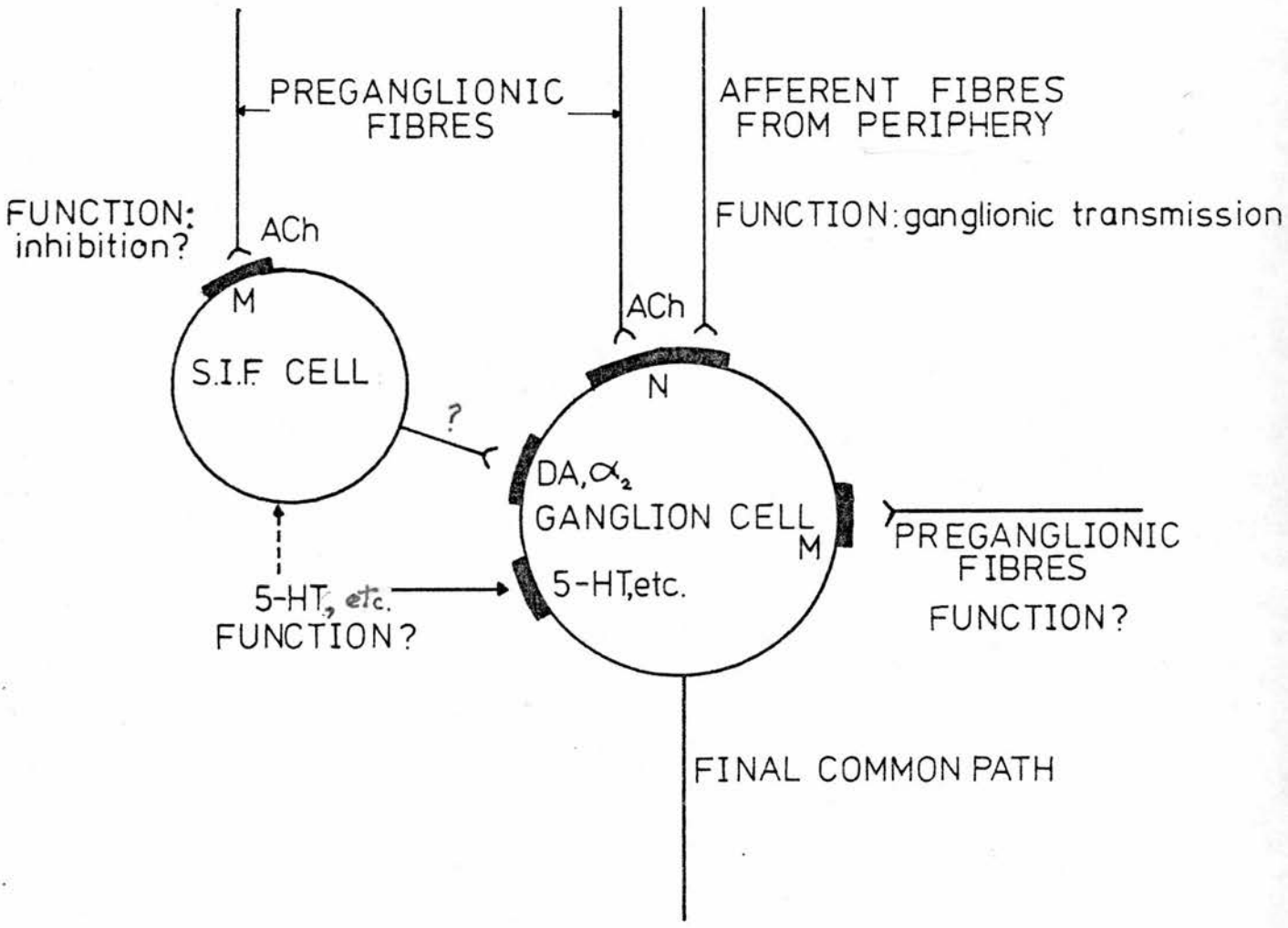
In addition to this nicotinic receptor mediated transmission, muscarinic effects have been observed. These manifest themselves as a slow I.P.S.P. which may be easily submerged by the after-hyperpolarisation observed after spike generation, and a slow E.P.S.P. The former has been ascribed to the involvement of a ganglionic interneurone, either adrenergic or dopaminergic, the evidence of which has been reviewed. The slow E.P.S.P. can also be observed under conditions of normal or blocked transmission and is similarly atropine sensitive and resistant to nicotinic blockade. The evidence has been accumulated to form a more advanced hypothetical model of ganglion cell transmission based on that of

Eccles and Libet, 1961. (Fig 9, p 26a)

Various points make the appropriateness of this model to sympathetic ganglionic transmission, as a whole, rather questionable. Many of these effects are extrapolated from one ganglion to another where the structure and physiological function of sympathetic ganglia is very variable within the same species and between species. As an example, the evidence for a functional ganglionic interneurone is derived from studies on the rabbit S.C.G., and a number of other ganglia when investigated do not show inhibitory potentials and do not contain discernible S.I.F. cells. The conditions under which these slow potentials, notably the slow I.P.S.P., are observed, are very unphysiological involving high frequency supramaximal synchronous preganglionic stimulation. One does not know whether these potentials can be observed even from surface recordings in situations of true physiological sympathetic transmission. Thirdly, the model suggests that the muscarinic receptors, on which exogenous drugs or endogenous acetylcholine acts, are some distance from the subsynaptic area and that diffusion phenomena account for the latency of the observed potentials. Recent evidence implies that the generation of these potentials may involve an electrogenic process which may alone account for the delay.

The role of these potentials and the many unusual phenomena observed, e.g. the enhancement of non-nicotinic effects by nicotinic desensitisation and the presynaptic potentiation effects, remain unexplained. Although a muscarinic transmission of supramaximal preganglionic stimuli, which is frequency dependent, can be observed following nicotinic blockade, it seems that during normal transmission, the I.P.S.P. and slow E.P.S.P. may not summate to produce an action potential within a ganglion cell. These potentials may have solely evolved to act as a way of controlling cell excitability to underly the facilitatory or inhibitory effects conditioned by subliminal cell recruitment. Beyond a cellular level, one does not know the molecular mechanism behind slow synaptic potential generation and the

Fig. 9 The proposed structure of a sympathetic ganglion.



DA=dopamine receptor, 5-HT=5-hydroxytryptamine receptor, α_2 = α_2 receptor, N=nicotinic receptor, M=muscarinic receptor, ACh=acetylcholine, S.I.F. = small intensely fluorescent, etc. = otherpossible agonists. p18.

mechanism induced by different agonists following receptor occupation. The mechanism of action of non-cholinergic drugs remains largely uninvestigated as does their possible physiological importance, even though receptors mediating their action are present on the ganglion cells.

Against this background of morphological, electrophysiological and biochemical studies a much neglected field of study has been the characterisation of sympathetic ganglion transmission when preganglionic firing rates are increased by physiological stimuli. In a small number of studies, the increase in sympathetic firing generated has been associated with a residual non-nicotinic atropine sensitive transmission observed after nicotinic blockade. Muscarinic antagonists have proved inactive when administered alone in reducing transmission of physiologically or electrically stimulated increases in preganglionic spiking. A study of selective physiological stimulation which might lead to differences in the sympathetic fibre population activated had not been attempted. Even though one must be aware of the dangers of extrapolation to other ganglia and other species, it was felt the knowledge of the pharmacology of sympathetic ganglionic synaptic transmission and its role in the control of different regions of the circulation might be advanced by a study of this type.

THE ADRENAL MEDULLA

Morphology

Cells within the mammalian adrenal medulla are arranged in small groups, but the location of either noradrenaline (NA) or adrenaline (A) cells varies greatly among the species. Each cell appears to be associated with a capillary and under the electron microscope, numerous storage vesicles are observed adjacent to the free surface of the cell. The organelles of chromaffin cells and respective capillary and nerve endings are so distributed that the cell is polarised. (Fig. 10) Only the basement membrane separates the large extracellular spaces from the intracellular cleft and large molecules are free to circulate around the cell (Holtzman and Dominitz, 1968).

Adrenaline cells under osmium staining after glutaraldehyde fixation, contain a homogenous granule population only separated into two groups by electron density (D'Anzi, 1969). Noradrenaline cell granules are far more heterogeneous in size and are more electron dense than those predominating in A cells. Small granules are also observed which are less electron dense and may be dopamine granules (Lishajko, 1968) or may be granules at some stage in development (Coupland, 1965).

The interest in electron microscope studies arises from assumptions based on earlier fluorescence microscopy which allowed one purely to distinguish cell types with a predominance of either amine and this was used as evidence for there being two different cell types, morphologically similar, but containing different catecholamines. It has not been conclusively excluded from electron microscope studies that the above evidence may indicate chromaffin cells containing both A and NA granules but then one would have to postulate a selective system of release at a granular, not cellular level and a selective uptake of adrenaline into adrenaline granules, as the methylating enzyme is cytoplasmic, for which there is at present no evidence.

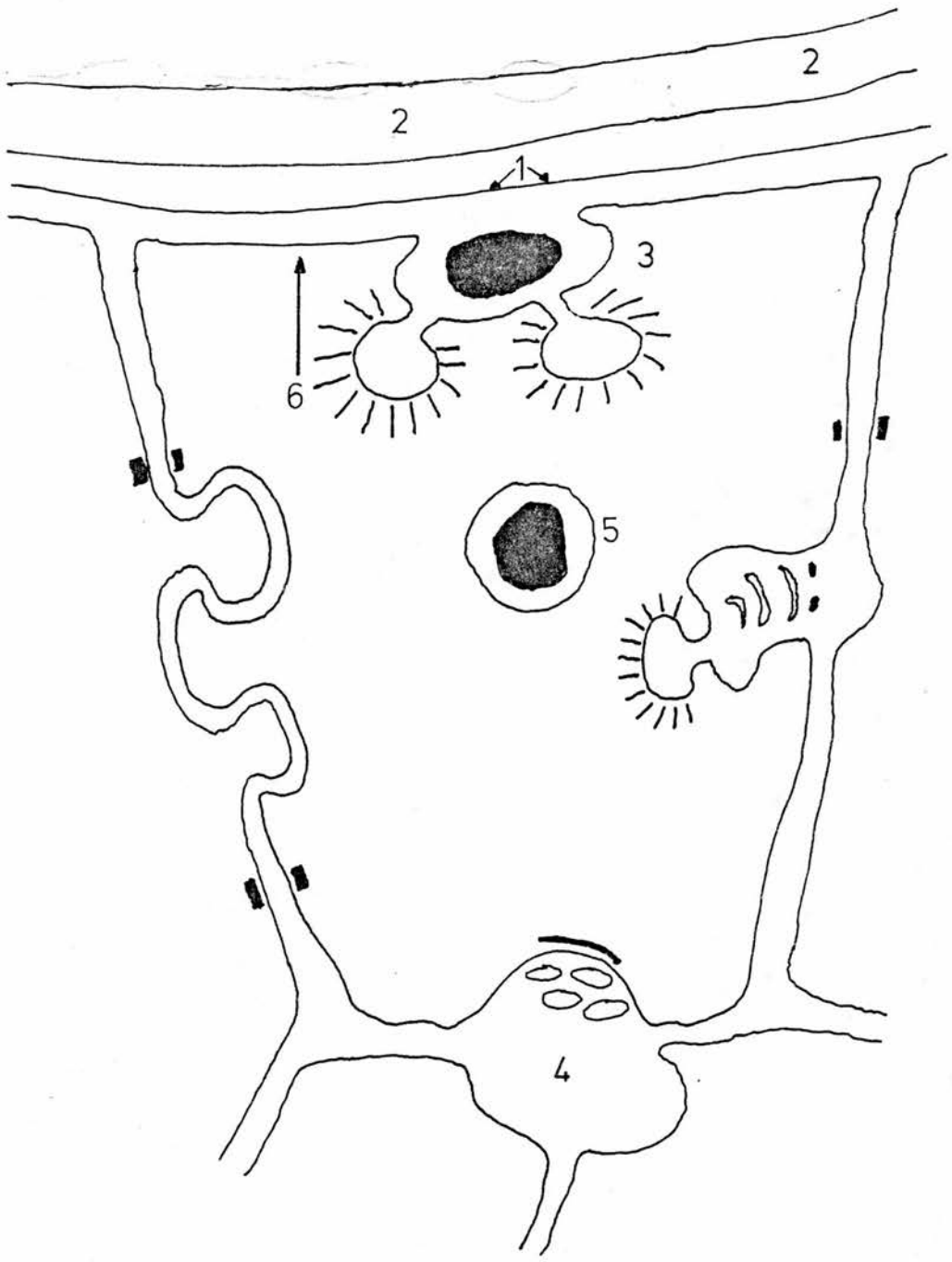


Fig. 10

An illustration of the structure of an adrenal chromaffin cell viewed under the Electron Microscope. 1 - Basement lamina 2 - Capillary 3 - Coated pits (fusion of vesicle with cell membrane) 4 - Nerve ending. 5- CA storage vesicle 6- cell membrane. (After Grynszpan-Winograd, 1975)

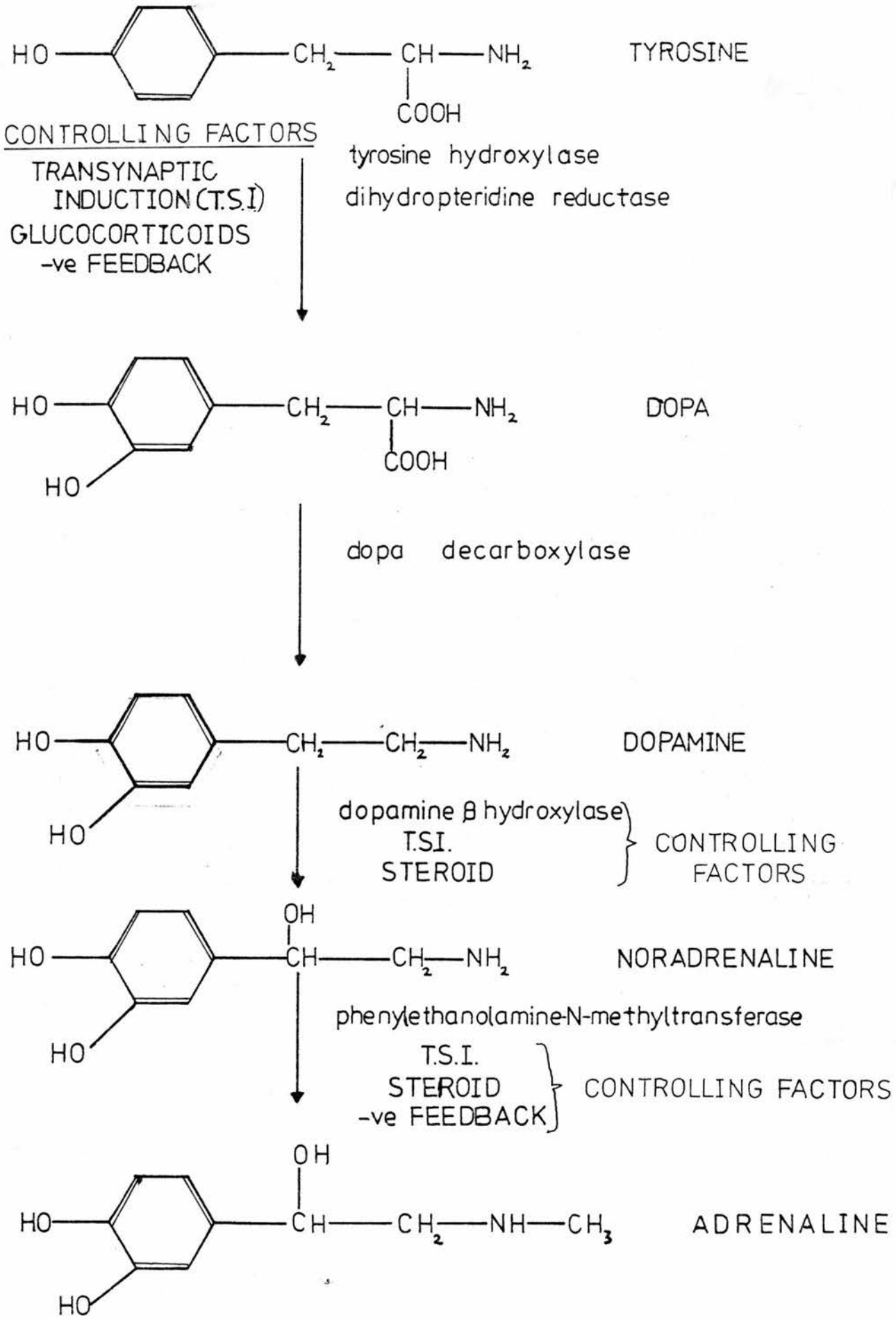
By sucrose gradient centrifugation, the chromaffin granules can be isolated and biochemical investigation of the purified granules has been rigorous. The biochemical constituents are as illustrated (Fig 14, p47a) and one should note the high percentage of water soluble substances which must be prevented from diffusing across the chromaffin membrane by some mechanism (Winkler, 1976). Once again in support of the morphological evidence, chromaffin granules in homogenates of the adrenal medulla do not form a single population and different NA and A storage granules can be isolated, though whether they come from different cells is not known. Two different granule types store A and different ratios of adenine nucleotides and soluble dopamine B hydroxylase, (DBH to CA), are observed. It would appear that though a more readily interpretable basis for secretory mechanisms is one where the level of control is cellular the actual morphology and biochemistry of the granules may suggest a more complex differentiation within the chromaffin cells with implications of stimulus secretion coupling being initiated at a more molecular level. Present techniques do not allow one to distinguish and interpretation becomes more complex beyond a cellular separation of adrenaline and noradrenaline.

Synthesis - Controlling Influences

Within the adrenal chromaffin cells, catecholamine synthesis has been characterised by numerous authors (Fig 11) (for review, Kirshner, 1975). Three levels of control seem to exist, of which end product inhibition would seem to control acute changes in synthesis, whereas the other two controlling mechanisms transynaptic induction and humoral influences mediate more long-term changes. The rate limiting enzyme in the synthesis of noradrenaline has been found to be tyrosine hydroxylase which has been shown to be greatly inhibited by high doses of noradrenaline suggesting a concept of end product inhibition (Weiner, 1970) and in a similar manner, changes in phenylethanolamine-N-methyl transferase, (P:N:M.T.) activity have been observed following the addition of noradrenaline, just in

Fig 11

The biosynthetic pathway for the production of noradrenaline and adrenaline.



excess of that necessary for maximum enzyme activity (Fuller and Hunt, 1967).

These acute changes in enzyme activity are almost certainly subordinate to the long-term control of adrenomedullary catecholamine biosynthesis which is mediated by both neural and humoral mechanisms.

Transynaptic Induction

A variety of pharmacological and physiological stimuli which increase splanchnic nerve activity will cause a delayed rise in tyrosine hydroxylase activity after approximately 24 hours. This has been confirmed to be a nicotinically mediated effect prevented by denervation and blocked by nicotinic antagonists (Patrick and Kirshner, 1971). Slotkin and his colleagues have pioneered work in this field and have shown that transynaptic induction of both tyrosine hydroxylase and, to a lesser extent, dopamine B hydroxylase occurs in rats following the administration of any drug which increases splanchnic nerve activity. Similar experiments in neo-natal animals have confirmed enzyme induction in parallel with increases in glandular catecholamine content mediated through nicotinic receptors present before functional splanchnic innervation has developed (Rosenthal and Slotkin, 1977).

The relationship between increases in tyrosine hydroxylase activity and dopamine B hydroxylase activity is not well established. The lack of correlation between tyrosine hydroxylase activity and depletion of catecholamine seems to exclude any explanation of the above observations as removal of end product inhibition. It seems that the transynaptic induction of tyrosine hydroxylase and other catecholamine synthesis enzymes provides a mechanism by which the adrenomedullary catecholamine synthesis adapts to a prolonged stimulation of the secretory mechanism.

Humoral Control

In most species the chromaffin cells are in close contact with blood containing a high level of corticosteroid as a result of the adrenomedullary vasculature. (Fig 12) The blood supply shows two distinct vascular networks. The 'arteriae medullae' penetrate the cortex before dividing into the capillary plexus within the medulla whereas the venous drainage from the cortex is in parallel with this to form a portal system containing steroid-rich blood. A large volume of literature based on studies in hypophysectomised animals and administration of steroids to extra-chromaffin tissue has confirmed a role for glucocorticoids in the synthesis of P.N.M.T., the final methylating enzyme (see Review, Wurtman, 1972). These humoral influences on P.N.M.T. seem to parallel neural influences (Ciaranello, Dornbusch and Barchas, 1972). Cortical influences on the rate limiting enzyme tyrosine hydroxylase have also been reported (Kvetnansky, Gewirtz, Weise and Kopin (1970) and again this would appear to complement the reported transynaptic induction. In the adrenal, tyrosine hydroxylase induction following dibutyryl cyclic AMP has been reported (Gewirtz, Kvetnansky, Weise and Kopin, 1971). Guidotti and Costa, 1973 have suggested that cAMP from the cortex produced as a result of the action of ACTH may activate tyrosine hydroxylase induction. In later work, they were able to show the phosphorylation of a protein kinase by cAMP from the adrenal cortex. (Guidotti and Costa, 1976).

When one considers these results, the secretory action of corticosteroids observed in the present study within the canine adrenal medulla together with the previous evidence of secretory stimulation by corticosteroids and ACTH in the rat (Roffi, Jost and Redon, 1966) may shed a different light on one's interpretation of the results. As P.N.M.T. is very sensitive to end product inhibition, as is tyrosine hydroxylase and the species investigated have in almost all cases been the rat and the dog, both of which show a catecholamine secretory response to corticosteroids, it is possible that the induction of P.N.M.T. and tyrosine

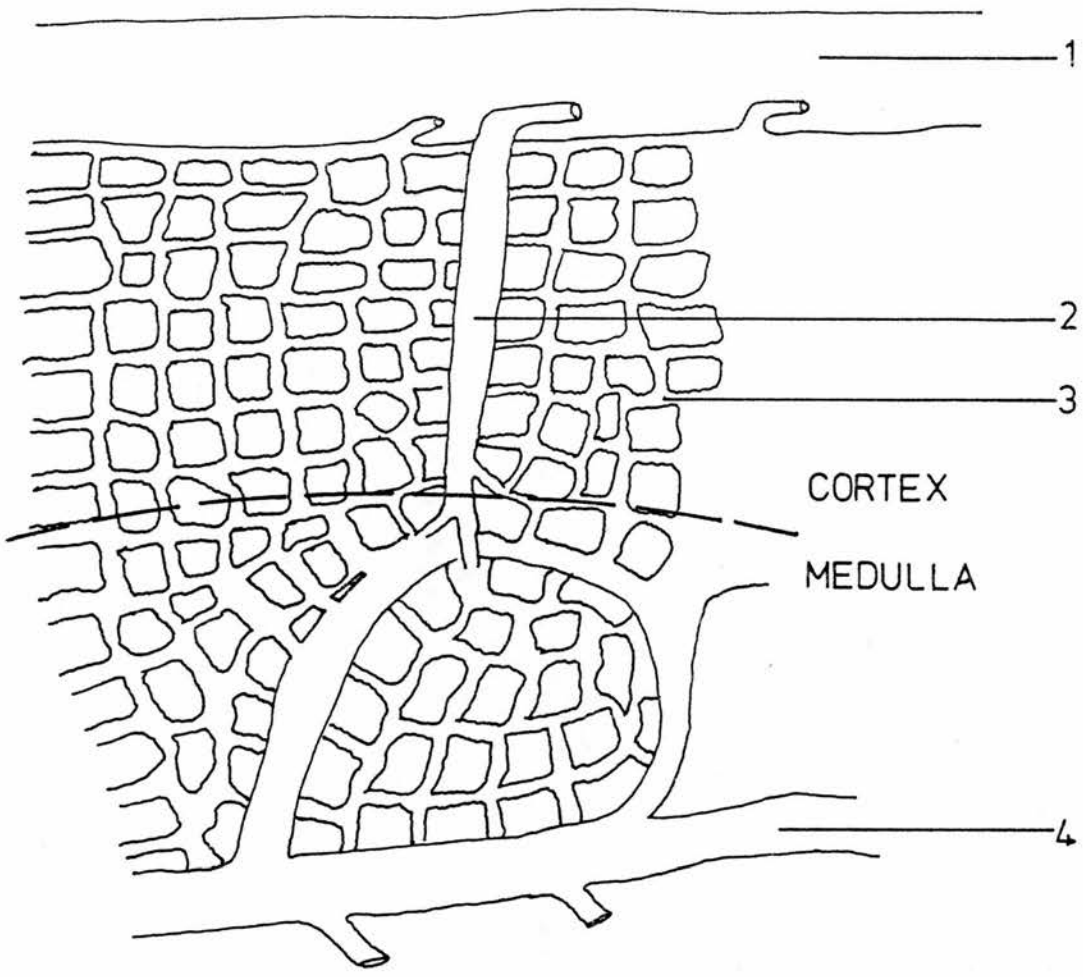


Fig. 12

The blood supply of the adrenal cortex and medulla in the dog.

1 - Capsule 2 - Medullary capillaries 3 - Cortical capillaries

4 - Central collecting V.

hydroxylase by glucocorticoids might be explained by removal of end product inhibition. No comprehensive study of enzyme activities following chronic administration of other secretagogues has been performed and they might also show enzyme induction.

Secretion from the Chromaffin Cells

A large variety of physiological and pharmacological stimuli can promote secretion of catecholamines from the adrenal medulla but much is still not known about the interaction of stimuli in a physiological situation and much still awaits discovery regarding the mechanism of secretion at a cellular level, which will be of great consequence in the understanding of other secretory cells.

Splanchnic Nerve Stimulation

The anatomic discreteness of adrenal glands allowed their recognition by the Ancients but not until the pioneering work of Oliver and Schafer, 1895; Dreyer, 1898; Tscheboksaroff, 1910) was their secretory role observed. In 1913, Elliot confirmed that adrenal chromaffin cells develop from the same lineage as sympathetic ganglion cells and are innervated with 'preganglionic' sympathetic fibres. These fibres run mainly in the ipsilateral splanchnic nerve:- greater, lesser and least and a complex study of adrenal innervation was completed by Young, 1939. Greater splanchnic nerve stimulation produces twice the catecholamine release of that from the other nerves in both the cat (Marley and Prout, 1965) and the dog (Malmejec, 1964). Maturation of the splanchnic-adrenal junction occurs at different times in species being functional before birth in the sheep (Comline, Silver and Silver, 1965) but not in the calf (Silver, 1960) or the rat (Rosenthal and Slotkin, 1977).

The electophysiological properties of synaptic transmission at the splanchnic-adrenal junction have only been sparsely investigated. Marley and Prout, 1965 in anaesthetised cats with a bioassay for catecholamine output in adrenal-lumbar venous blood observed both spatial and temporal recruitment of splanchnic nerve stimulation. It seems

a large subliminal fringe is present in the adrenal medulla and overlap of innervation appears to occur which was observed as increased sensitivity after partial denervation (Simeone, 1938). Histological studies confirm that a single splanchnic neurone innervates clusters of chromaffin cells and that individual cells may receive innervation from several neurones. It was found that hexamethonium raised the threshold for release by 2 - 3 fold but temporal and spatial summation still occurred, but the data presented is limited. Eserine lowered the stimulation threshold and increased temporal and spatial recruitment. The present experimental techniques do not allow one to distinguish between a presynaptic or postsynaptic mechanism underlying recruitment. One can conceive the former involving more quanta of acetylcholine per impulse or the latter, perhaps an accumulation of intracellular free Ca^{++} as influx outpaces inactivation. In the rat, similar observations are reported by Yoshizaki, 1973, but in the majority of other studies, a supramaximal stimulation of the splanchnic nerves has been employed.

Where an assay for catecholamine which is differential has been used, two other controversial points are raised. Holland and Schümann, 1956 noticed no depletion of the catecholamine contents of cat adrenal glands following maintained splanchnic nerve stimulation. They felt that this reflected a rapid resynthesis but this has been contradicted by Butterworth and Mann, 1957 and Eade and Wood, 1958 who could not confirm the earlier results. It seems the difference in the experimental methods may account for this as Eade and Wood used a selective fluorimetric assay, whereas Holland and Schümann compared the pressor response of a solution of 50% A/50% NA with that obtained after gland stimulation and secondly they correlated content with that of the right unstimulated gland but at the end not the beginning of the experiment where loss from their 'control' gland might influence their results. It seems in the light of more recent biochemical evidence that biosynthesis takes several days (Viveros, Arqueros, Connet, Kirshner, 1969), and it is most unlikely that rapid resynthesis can occur.

The second point of interest is whether selective secretion of catecholamines can occur following splanchnic nerve stimulation. It has become clear that this is a species dependent phenomenon but authors did and do persist in relating results obtained, following splanchnic nerve stimulation, cholinergic and other drugs to those from other workers without regard to the species used.

Outschoorn, 1952; Von Euler and Folkow, 1953 using cat glands, in situ, with a differential bioassay detection found that electrical stimulation of the splanchnic nerves did not alter the percentage of adrenaline in adrenal venous blood. Similar results were obtained in the dog by Hermann, 1951; Houssay and Rapela, 1953; Malmejac, Neverre and Gross, 1953. These studies were performed at a single stimulation frequency and more intensive studies have been performed in cats (Klevins and Gebber, 1970) and dogs, (Mirkin, 1961). In both cases some measure of selective release was observed. The former workers observed an increase in adrenal venous adrenaline percentage from 30% at 1 c/s to 60% at 10 c/s where the adrenaline percentage in the glandular catecholamine content was 70% making adrenaline mobilisation feasible. Mirkin, 1961 obtained similar results in dogs, where again the adrenaline percentage increased with increasing frequency of nerve stimulation. One finds that these results do correlate with earlier studies where medullary A % fell after prolonged splanchnic nerve stimulation (Bülbring and Burn, 1949) but one must consider the correlation between gland content and selective release a false one and a comparative study of adrenal gland content and catecholamine secretion requires investigation as earlier assay methods were often not sensitive enough to allow conclusions to be made, as the measured selective catecholamine output was extremely variable.

Central Stimuli

A number of central stimuli have been employed to evoke catecholamine secretion from the adrenal medulla as a result of increases

in splanchnic nerve activity. The stimuli used have often been attempts to mimic the stimuli physiologically important in controlling the adrenal medulla in its role in homeostasis.

Cannon and De La Paz, 1911 showed that secretion from the adrenal medulla contributed to signs of emotional excitement. Following the work of Houssay and Molinelli, 1924, and Tournade and Chabrol, 1925 with cross-perfusion techniques for the measurement of released catecholamines following bulbar stimulation, Brücke, Kaindl and Mayer, 1951; Redgate and Gellhorn, 1953 and Folkow and Von Euler, 1954 confirmed that hypothalamic stimulation can cause a marked increase in adrenomedullary catecholamine secretion in cats and dogs. In addition, the latter two groups of workers confirmed that depending on electrode position and stimulus strength, selective release of NA or A could occur. The non-selective release obtained following splanchnic nerve stimulation, might be due to stimulation of all fibres, whereas a central representation of the secretory chromaffin cells may occur, which is differentiated. Von Euler and Folkow, 1958 confirmed that cortical influences can exert a higher inhibitory level of control on the hypothalamus. Central influences seem very important in the regulation of adrenal catecholamine output as the highest circulating plasma catecholamine levels are observed during mental activity or emotional stress (O'Hanlon, 1965). Anxiety situations increase A plasma levels preferentially but increased mental activity may increase NA levels, though one does not know the importance of overflow from sympathetic nerve endings.

Various changes in the animal's environment can also cause increased adrenal secretion. Cold and heat stress both cause marked secretions of catecholamines. Klepping, Tanche and Cier, 1957 observed an increased catecholamine output in dogs when subjected to cold stress but in view of our present findings, a component of this was probably mediated by increased adrenocortical secretion. Metabolic acidosis may also cause increased secretion but a component

of this may be a direct action on the chromaffin cells in addition to the centrally induced increase in splanchnic tone. (Higashi, 1966). In the dog, it is clear that hypercapnic acidosis can directly stimulate the adrenal medulla, (Nahas, Mather, Wargo, Adams, 1954) but a large central component is involved. (Morris and Millar, 1962).

Haemorrhage and Hypotension

Both cause a marked increase in adrenal medullary secretion which can usually be correlated with the drop in blood volume (Satake, 1931). In the cat, Feuerstein and Gutman, 1971; Feuerstein, Boonyaviroj, Gutman, Khosla, and Bumpus, 1977 have observed an increased adrenal venous catecholamine secretion in haemorrhage associated with a fall in A% from 70% to 30%. The increase in secretion could be antagonised by an angiotensin antagonist or splanchnic denervation leading the authors to postulate that, in the cat, the increase in circulating angiotensin levels observed during hypotension cause an increased splanchnic nerve firing via a central action. Associated with this, one would expect the primary response to hypotension to be a decrease in baroreceptor and an increase in chemoreceptor activity which, according to the results of Critchley, Ungar and Welburn, 1973 should increase adrenal secretion. With the marked decrease in carotid sinus pressure, one would expect an increased NA output in the cat as a result of the baroreceptor reflex and this may explain the decreased A percentage observed by Feuerstein et al, 1977 in adrenal venous blood, though the authors are at variance with regard to their resting adrenal venous adrenaline percentage.

Hypoglycaemia

Insulin depletes adrenomedullary hormones for 8 hours following injection (Burn, Hutcheon and Parker, 1950; Slotkin and Kirshner, 1971) with the depletion depending on increased splanchnic nerve activity (Duner, 1953). A component of the response to hypo-

glycaemia may be attributed to the secretagogue action of glucagon (Scian, Westermann, Verdesca, Hilton, 1960). It would appear that hypoglycaemia favours adrenaline release and few peripheral venous studies have been sufficiently accurate to allow one to distinguish between a selective release of adrenaline or an increased adrenomedullary secretion in an animal secreting a high percentage of adrenaline thus altering the peripheral venous adrenaline percentage (Vendsalu, 1960; Haggendal, 1963; Christenson, 1975). In the cat where adrenal venous levels have been measured by bioassay Dunèr, 1953 and Feuerstein and Gutman, 1971, have observed increases in the adrenaline output preferentially but with widely differing resting adrenaline percentages. Similar results are obtained in the sheep and it appears a selective stimulation of the adrenaline-containing cells occurs.

Hypoxia and Asphyxia

The most investigated physiological stimuli causing an increased adrenomedullary secretion are hypoxia and asphyxia. One must distinguish between whole body hypoxia usually employed by most authors, and a local selective chemoreceptor stimulation. Since Cannon and Hoskins, 1911, acute whole body hypoxia induced by N_2 breathing or tracheal clamping has been observed to increase adrenomedullary catecholamine secretion. In the cat, a preferential secretion of adrenaline was said to occur (Redgate and Gellhorn, 1953) but no preferential release in the dog (Houssay and Rapela, 1953). Though most of the response will be due to a centrally induced increase in splanchnic nerve activity, a component of direct stimulation of the chromaffin cells may occur (Bulbring, Burn and D'Elio, 1948). As a large increase in secretion is only observed after prolonged asphyxia (Von Euler and Folkow, 1953; Celander, 1954; Baugh, Cornett and Hatcher, 1959) a component of the maintained release in dogs would appear to be mediated by maintained increases in adrenocortical steroid production in a similar manner to the prolonged response to

maintained chemoreceptor stimulation (Critchley and Ungar, 1975). This mechanism in the light of our present work may only account for the response in the dog and certainly does not seem to be significant in the hypoxic calf, (Bloom, Edwards and Hardy, 1977) where large increases in adrenal venous corticosteroid levels are not accompanied by changes in catecholamine output. With techniques employing whole body hypoxia, one is unsure of the site of action, with brain stem, peripheral chemoreceptor and direct stimulation of adrenomedullary cells, all interacting to produce increased secretion. With this in mind, experiments have been performed where the stimulus is localised to the peripheral chemoreceptors in the region of the carotid body (Heymanns and Neil, 1958; Anichkov and Belinki, 1963; Critchley, Ungar and Welburn, 1973).

The latter workers found the release of catecholamine into adrenal venous blood to be non-selective in the dog, but preferential release of adrenaline was observed in the cat contrasting with the preferential release of noradrenaline observed during the carotid baroreceptor reflex. It seemed that carotid chemoreceptor stimulation can produce a reflex rise in catecholamine secretion as a result of increased splanchnic nerve activity with a reinforcement mechanism involving increased corticosteroid production, though the existence of this mechanism in species other than the dog is unconfirmed.

Adrenomedullary Secretagogues

Acetylcholine

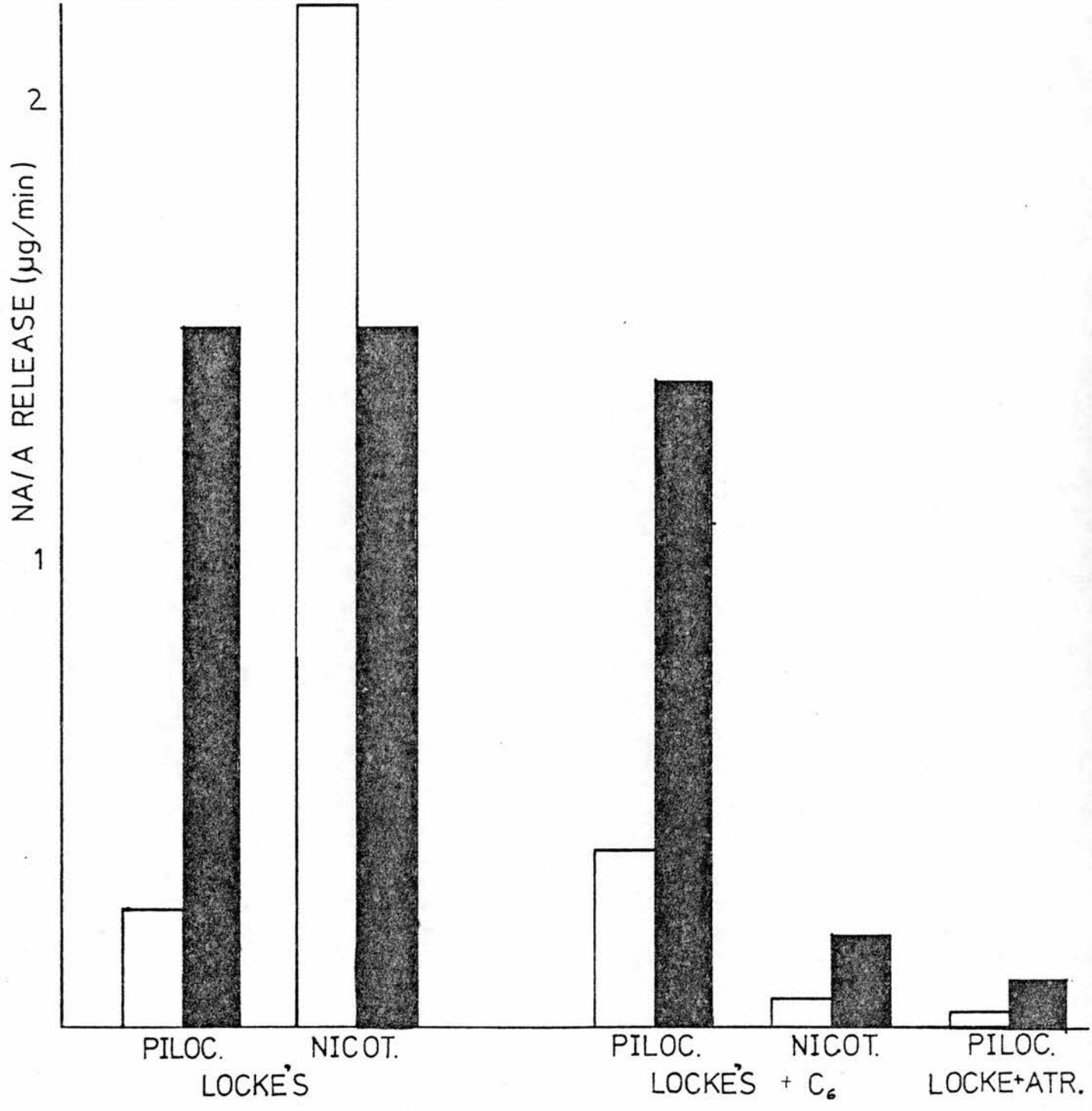
Since Feldberg, Minz and Tzudzimura, 1934 confirmed that acetylcholine was the transmitter at the splanchnic-adrenal junction, much work has focused on acetylcholine as an adrenomedullary secretagogue.

In the cat, Douglas and Poisner, 1965; Rubin and Miele, 1968 noted that acetylcholine produces a secretion of both amines consistent with an action on both chromaffin cell types. Their results are also consistent with the predominant action of acetylcholine at this dose (10^{-5} g/ml) being nicotinic. Miele, 1969 found that acetylcholine (6×10^{-6} M) was active in causing adrenomedullary secretion even after nicotinic blockade with a rise in the adrenaline percentage in this residual release. This implied that the non-nicotinic component of acetylcholine's action was a muscarinic activation of the chromaffin cells containing adrenaline. In a study correlating the catecholamine secretion produced by equiactive doses of nicotinic agonists, acetylcholine or splanchnic nerve stimulation, increasing frequency of stimulation gave releases with a higher adrenaline percentage than those with lower frequency and that the former were similar to the adrenaline percentage observed after exogenous actylcholine. These differences between endogenous and exogenous acetylcholine might be explained by the noradrenaline cells having a lower threshold for stimulation, or the muscarinic component increasing as the frequency increases but this was not confirmed as no antagonists were used.

In the dog, a similar situation occurs, exogenous acetylcholine being inhibited by hexamethonium or atropine alone or a combination of both drugs, dependent on dose (Kayaalp and Türker, 1969). As abundant cholinesterase is present in splanchnic terminals, chromaffin cells and interstitial cells (Palkama, 1967; Lewis and Shute, 1969) this may greatly alter the response to acetylcholine. In two studies (Trendelenburg, 1967; Miele and Rubin, 1968) the predominant action of acetylcholine in the presence of an anticholinesterase was muscarinic as it was antagonised by atropine but not by hexamethonium and the acetylcholine induced response was potentiated during the nicotine desensitisation. The selective action of acetylcholine may well be a function of access to the receptors or arise from the fact that low doses of acetylcholine produce muscarinic rather than nicotinic actions on tissues.

Fig 13

The action of cholinergic agonists as adrenomedullary secretagogues in the cat. (After Douglas & Poisner, 1965) Open bars represent secreted noradrenaline and the closed bars adrenaline.



Cat adrenal gland perfused in situ with Locke solution containing pilocarpine (piloc.) or nicotine (nicot.) in the presence or absence of hexamethonium (C_6) or atropine (atr.).

Nicotinic Drugs

Pharmacological studies, in vivo, and in vitro, have classified the cholinergic receptors present in the adrenal medulla. The receptor population is species dependent and this has had an important bearing on the interpretation of results obtained following physiological stimuli where hitherto differences had proved difficult to reconcile.

In the cat, the nicotinic receptors present in the adrenal medulla (Dale, 1953) are of a type similar to those present in sympathetic ganglia as witnessed by pharmacological studies. In 1961, Douglas and Rubin,1961 (b),using cat glands vascularly isolated, and perfused, in situ, with Locke's solution, confirmed the catecholamine releasing action of nicotine, (T.M.A. = tetramethylammonium, Fig 5, p13a), lobeline and D.M.P.P. This has been confirmed by a number of workers. When a differential assay of catecholamines has been employed, nicotine (2×10^{-5} g ml^{-1}) has been said to produce a secretion similar to that at rest in percentages of NA and A (Douglas and Poisner, 1965 (Fig 13, p39a);Rubin and Miele, 1968). Rubin and Miele, 1968 did observe that low doses of nicotine released predominantly NA and they suggested that higher doses of nicotine may be non-selective and act on other receptors on the adrenaline containing cells but this hypothesis is at odds with Douglas and Poisner's observation that hexamethonium 10^{-4} g/ml fully abolished the response to nicotine. In blood-perfused experiments, close arterial injections to adrenal glands, in situ, produced an elevated adrenal venous catecholamine concentration with approximately 50% NA. The two studies, Klevans and Gebber, 1970 and Feuerstein and Gutman, 1971 obtained completely differing resting secretions with 70% adrenaline in the former study and 40% adrenaline in the latter. It seems from the results discussed that nicotine in the cat may act on either solely the noradrenaline cells or both cell types and the interpretation of results leading to this conclusion depends on the widely differing resting secretions observed in blood-perfused and in Locke's solution perfused, in situ, experiments.

In the dog, the situation is equally confused, with Vogt, 1965

and Kovacic and Robinson, 1967,both obtaining selective release of noradrenaline following nicotine or D.M.P.P. but Kayaalp and McIsaac, 1968,and Critchley, Tibenham, Ungar, Waite and West, 1975 obtaining similar resting catecholamine percentages but no selective release in response to nicotinic agonists m-hydroxyphenylpropyl T.M.A. (H.P.P. T.M.A.)(Fig 5, p13a) or D.M.P.P. although similar incremental catecholamine release was observed.

The action of nicotine has been investigated in a number of other species. In the rat, Yoshizaki, 1973 obtained increases in catecholamine induced by nicotine and unaltered by splanchnicotomy though a component of nicotine's action may involve increases in splanchnic activity (Patrick and Kirshner, 1971). In bovine glands, nicotine and carbachol also cause secretion with no change in the percentage of the two amines (Schneider, 1969). Although non-nicotinic agents can cause catecholamine secretion, it seems that the sole action of acetylcholine is nicotinic at the bovine adrenal medulla and that muscarinic drugs are inactive. This has been confirmed by histochemical, binding and pharmacological release studies (Wilson & Kirshner, 1977). Similarly, in some avian species, notably the chicken (Ledbetter and Kirshner, 1975) only nicotinic receptors are present on the chromaffin cells of the adrenal medulla whereas all other species investigated are responsive to muscarinic agonists.

Common to its action on sympathetic ganglion cells, nicotine is also able to produce a 'dual block' when acting on chromaffin cells. During the early phase of block, a depolarisation is produced which prevents the action of any agonists though one does not know whether drugs not requiring depolarisation as an initial stage in stimulus secretion coupling are active during this period. After the response to non-nicotinic agonists has returned the chromaffin cells are still insensitive to nicotinic agonists and this 'late phase' of block is assumed to represent desensitisation of the receptors (Rang and Ritter, 1970).

Muscarinic Agonists

Dale's classification of the adrenomedullary receptors as nicotinic delayed awareness of the existence of muscarinic receptors on the chromaffin cells. Nicotinic antagonists appeared completely to abolish adrenomedullary responses to nerve stimulation but experiments with muscarinic agonists were able to confirm atropine-sensitive increases in catecholamine secretion (Dale and Laidlaw, 1912; Feldberg, Minz and Tsudimura, 1934). Confirmation came from Trendelenburg, 1954 and the intensive study of Douglas and Poisner, 1965. Using a differential assay to analyse the secretion from cat adrenal glands, perfused in situ, they found with doses of agonists producing similar secretion rates that pilocarpine gave 96% adrenaline and muscarine 84% but nicotine and acetylcholine only 45%. This implied that pilocarpine acted solely on the adrenaline-containing cells without concomitant release of noradrenaline. Later work by Rubin and Miele, 1968 confirmed this observation. Realising that one source of error in the earlier work was the absence of data presented by the authors on resting catecholamine output and the normal percentage of adrenaline, we have repeated the experiments with cat adrenal glands perfused in vitro and observed in the incremental release a secretion containing predominantly adrenaline in response to the muscarinic agonist acetyl B. methylcholine. The results are far less marked than those seen by earlier workers suggesting a selective action may not be as complete as was previously suggested. The selective muscarinic ganglion stimulants, McNA343 and AHR602 (see Fig. 6, p 16a) seem to differ with respect to an adrenomedullary stimulant action, the former being active in the cat, but the latter inactive even at a high dose. This might imply a difference between the muscarinic receptor type present in the adrenal medulla and superior cervical ganglion of the cat.

In the dog, muscarinic agonists are also active (Kovacic and Robinson, 1967; Kayaalp and McIsaac, 1968; Tsujimoto and Nishikawa, 1975) but no preferential secretion is observed which is in line with the response to physiological stimuli though suggestions of selective release after nicotinic agonists have been made. In the rat, muscarinic agonists

are also active but in the in situ studies of Yoshizaki, 1975, the secretion in response to pilocarpine was greatly diminished by adrenal denervation. This seems to reflect a common failing of in situ studies where, if one does not denervate, a large component of the action of muscarinic agonists is due to the baroreceptor reflex induced increase in splanchnic activity produced by the fall in systemic blood pressure seen after administration of muscarinic drugs.

The observation that muscarinic agonists can stimulate catecholamine release in a number of species raised questions whether muscarinic receptors may play a role in synaptic transmission in the adrenal medulla. In the cat, Lee and Trendelenburg, 1967 observed increased adrenomedullary secretion following splanchnic nerve stimulation at 30 c/s supramaximally during the 'late phase' of nicotinic blockade and this response was atropine sensitive. They were unable to show either a residual response to splanchnic nerve stimulation after hexamethonium or any action of atropine when administered alone. This implied that atropine-sensitive (muscarinic) transmission of splanchnic nerve impulses in the cat adrenal medulla could only be observed under the special conditions of a 'desensitising block' by nicotine, a procedure previously discovered to increase the sensitivity of the adrenal medulla to non-nicotinic agents. Klevans and Gebber, 1970 in anaesthetised cats have observed incomplete blockade of adrenal medullary secretion following splanchnic nerve stimulation with hexamethonium bromide 2 - 4 mg/kg I.V. but atropine alone was inactive. Their differential assay suggested that higher frequency stimulation increased the percentage of adrenaline released but this increase was not altered by hexamethonium which markedly inhibited the overall response. It seems the adrenaline-containing cells were less sensitive to splanchnic nerve stimulation despite the major synaptic transmission being nicotinic.

In the dog, non-nicotinic components of the response to splanchnic nerve stimulation have been observed, this response being reduced by atropine (Kayaalp and McIsaac, 1969). In this study, atropine alone 0.5 mg/Kg did reduce the response to 10 c/s stimulation of the splanchnic nerve but only this frequency (10c/s) showed a significant alteration.

This action was not observed by earlier workers (Feldberg, Minz, Tsudimura, 1934). Klepping, 1956 and Mirkin, 1961 have observed a secretion of catecholamines from the dog adrenal medulla in response to splanchnic nerve stimulation which is frequency dependent with no alterations in the adrenaline percentage from the resting secretion (67% in both studies). Mirkin at 20 c/s observed a sudden increase in adrenaline secretion though the overall catecholamine output was lower than at 10 c/s but interpretation of this result, together with reductions observed in adrenaline release by Rapela, 1956 at 40 c/s and a more marked reduction in adrenaline rather than noradrenaline after cold exposure or nerve secretion, (Mirkin, 1961,) is difficult.

No reference is made by either author to vascular changes within the gland following splanchnic nerve stimulation and although changes in ratio of adrenaline to noradrenaline after cold exposure or nerve section may reflect removal of fibres specifically associated with adrenaline cells in a manner similar to that found in the cat, it is doubtful whether useful information would be gained from supramaximal stimulation of this type, without a more careful analysis of spatial and temporal summation or the use of antagonist drugs to investigate changes in receptor activation at higher frequencies.

Histamine

As with other non-cholinergic secretagogues, it is doubtful whether histamine in physiological or pathophysiological concentrations reaches stimulant levels.

Szczygielski, 1932, Staszewska-Barczak and Vane, 1965 found histamine to produce adrenomedullary secretion in cats and dogs. In the cat, the response was not altered by splanchnicotomy and was predominantly adrenaline, whereas in the dog, a large component of the action appeared to be secondary to the peripheral vasodilatation produced by histamine which presumably initiated a baroreceptor reflex reinforcing any direct adrenomedullary stimulant action. The latter study involved

a superfusion bioassay with carotid blood from which one would expect most of the noradrenaline to have been removed due to passage through the lungs (Vane, 1969), and was as such, unsuitable for differential analysis. All other workers using cat glands, in vitro (Smith, 1970), or, in situ, (Rubin and Miele, 1968), have observed large increases in the adrenaline percentage in the secretion following drug dosage which is consistent with the view that only the adrenaline-containing cells possess histamine receptors. In the dog, De Schaepdryver, 1959 and Staszewska-Barczak and Vane, 1965 report increases in adrenaline outflow after histamine but Robinson and Jochim, 1960 reported increases in the noradrenaline percentage. The histamine receptor involved would appear to be of the H_1 type as conventional antihistamines antagonise the secretory action. In the rat, histamine is a secretagogue but a differential assay has not been performed. (Yoshizaki, 1975).

5-Hydroxytryptamine, Peptides (Bradykinin, Angiotensin)

5-hydroxytryptamine (5-HT) and peptides are also potent secretagogues. Angiotensin and Bradykinin are more potent than 5-HT but all three drugs produced marked secretion from the cat adrenal medulla following close arterial injections (Reid, 1952; Lecomte, 1953; Lewis and Reit, 1966). In cats, a selective release of adrenaline appears to occur in response to angiotensin or bradykinin (Comline, Silver and Sinclair, 1968) and the same has been observed in dogs, though the change in adrenaline percentage is small but significant (Robinson, 1967). α,ω-bis(dimethylammonium-acetaldehyde diethylacetal) diacetyl biphenyl dibromide (D.M.A.E.) pp′ seems able to potentiate the action of angiotensin on chromaffin cells in a similar manner to its potentiation of angiotensin's action on vascular smooth muscle and that angiotensin I, II and III may interact with a common adrenomedullary receptor seems likely (Aackerly, Sarstedt and Peach, 1977).

Local Anaesthetics

In a similar manner to Mg^{++} ions, tetracaine (3×10^{-6} M - 3

x 10^{-4} M) greatly inhibited the release of catecholamines in response to Ca^{++} reintroduction or 56 mM KCl. These inhibitory effects could be reversed by increasing the extra cellular Ca^{++} and similar depressions of acetylcholine induced secretion were observed (Rubin, Feinstein, Jaanus and Paimre, 1967). Jaanus, Miele and Rubin, 1967 extended this observation and concluded that local anaesthetics can block calcium movement in the adrenal medulla in a manner which parallels their inhibition of monovalent cations in electrically excitable tissue. A block of the acetylcholine induced secretion was also observed but a poor correlation between the ability of a local anaesthetic to inhibit calcium movement and its anticholinergic action suggests a possible extra action of local anaesthetics at the cholinergic receptors.

Miele and Rubin, 1968 in a further study noted that tetracaine, phenacaine and benzocaine are less able to decrease the secretion elicited by acetylcholine than that induced by KCl and it seems that in cat glands, this may be associated with the nicotinic and muscarinic stimulant action of acetylcholine. In summary, three actions of local anaesthetics on the chromaffin cell are observed. These are prevention of secretion by inhibition of calcium influx (Miele and Rubin, 1968; Rahwan, Borowitz and Miya, 1973), interaction with the cholinergic nicotinic receptor if the local anaesthetic contains a free tertiary amino group, and a selectivity of blockade of some secretagogues without full inhibition of a cholinergic response. This last effect may result in a preferential blockade of the nicotinic actions of acetylcholine with markedly less action on muscarinic mediated secretion, as witnessed by the resistance of pilocarpine to tetracaine administration and the change in adrenaline percentage when acetylcholine was administered to tetracaine treated cat adrenal glands (Rubin and Miele, 1968).

The Mechanism of Catecholamine Secretion from the Chromaffin Cell. Alternative Hypotheses

Cellular investigation techniques, notably electron microscopy and cell biochemistry, have enabled hypotheses to be tested and the possible

mechanism of stimulus-secretion coupling at a cellular level to be ascertained.

In 1964, Franzen postulated secretion of catecholamines by a holocrine mechanism but this and a variety of mechanisms involving secretion from the cytoplasm have been proved inaccurate in the light of biochemical evidence that cytoplasmic enzymes are not released and that only soluble vesicle contents are extruded into the extracellular space. Based on biochemical evidence, Blaschko and Welch, 1953; Carlsson, Hillarp, Hokfelt, 1957 proposed that the chromaffin granules released catecholamines into the cytoplasm whereupon they diffused out across the cell membrane.

Some morphological evidence, (Smitten, 1965) favours this view but the present biochemical evidence is against this hypothesis as is the morphological evidence of exocytosis profiles during secretion.

Several authors have confirmed the findings of De Robertis and Vaz Ferreira, 1957 that indentations in the chromaffin cell membrane are observed often with the electron dense core of the granule clearly visible (Coupland, 1965; Graf, 1966; Diner, 1967). Based on morphological studies alone, one might postulate expulsion of the intact granule from the chromaffin cell but biochemical data refute this and form much of the evidence for the concept of exocytosis. If the storage vesicle was to secrete its contents into the extracellular space, all soluble components should appear in the venous effluent but one would not expect any vesicle membrane components to be present in the extracellular space with retention of cytoplasmic components and other cell organelles. An extensive knowledge of the chemistry of the different cell compartments was required to establish what substances might act as endogenous tracers.

It has since been confirmed that the soluble contents of the chromaffin granules:- catecholamine, adenosine triphosphate (ATP) (Hillarp and Thieme, 1959; Douglas, Poisner and Rubin, 1965; Banks, 1966), Chromogranin A (Banks and Helle, 1965; Kirshner, N. and Kirshner, A.G., 1971) are released but without the concomitant release

Fig. 14 The soluble contents of adrenal chromaffin cells and the biochemical markers used to characterise subcellular components.

Fig. 14A

COMPOSITION OF BOVINE ADRENAL GRANULE

CONSTITUENT	% TOTAL DRY WEIGHT
Soluble content	
CA	20.5
Adenine nucleotide	15
Protein	27
Calcium	0.1
Magnesium	0.02
MEMBRANE	
Phospholipid	17
Cholesterol	5
Protein	8
Calcium	0.06
Magnesium	0.02

From Borowitz 1970, Bioch. Ph. 19, 2475 and Hillarp (1959), Acta Phys. Scand, 271, 279.

SPECIFIC CONSTITUENTS

Component	Constituent
Soluble content	Dopamine β Hydroxylase (DBH)
	Chromogranin A (CHROMG. A)
Membrane	Dopamine β Hydroxylase
	(Chromomembrin A)
	Chromomembrin B
	Cytochrome b 559
	Mg^{++}-dependent ATPase
	Flavoprotein, lysolecithin

Fig. 14B

ENDOGENOUS TRACERS OF SUBCELLULAR COMPARTMENTS IN ADRENOMEDULLARY CELL

Plasmalemma	Adenyl Cyclase
Cytoplasm	LDH
	TOH
	PNMT
Chromaffin Vesicle Soluble	CA
	DBH
	CHROMOGRANIN A
	ATP
Particulate	DBH
	Phospholipid
	Lysolecithin
Mitochondria	MAO
	SUCC DH_2 ASE
Lysosome	Acid Phosphatase
	Phospholipase A
	Acid Ribonuclease
Microsome	Glucose 6 Phosphatase
	RNA

LDH - lactate dehydrogenase, TOH - tyrosine hydroxylase,
PNMT - phenylethanolamine-N-methyltransferase,
ATP - adenosine triphosphate, MAO - monoamine oxidase,
Succ DH_2 ASE - succinate de hydrogenase, DBH - dopamine β hydroxylase.

of the cytoplasmic markers Lactate dehydrogenase (L.D.H.) and phenyl ethanolamine-N-methyl transferase (P.N.M.T.). Following the gel electrophoreisis work of Schneider, 1969 where increased bands of protein were observed in the effluent from stimulated bovine glands, Viveros and his co-workers, 1968, 1969 confirmed that the soluble dopamine B hydroxylase was released on reflex stimulation of the adrenal medulla of an unanaesthetised rabbit, but that the membrane bound dopamine B hydroxylase (DBH) was retained. He confirmed that an 'all or none' release appeared to occur as the ratio of DBH to amines did not increase as secretion occurred. Partial secretion would be expected to increase the enzyme to catecholamine ratio in some vesicles but only two populations of vesicles were observed: filled and normal, and enzyme alone in granules with lower buoyant density, i.e. empty granules. Morphological evidence of coated pits observed with exocytosis profiles (Malamed, Poisner, Trifaro, Douglas, 1968) suggested retention of the vesicle membranes but there is debate whether the vesicle membrane is used whole, or partially digested, as only microvesicles are reformed prior to filling and resynthesis of their contents. It seems secretion may occur for several hours in the absence of catecholamine or protein precursors thus suggesting secretion from a readily available pool of stored amine (Patrick and Kirshner, 1971). Some reports of rapid resynthesis after stimulation have not been confirmed by more direct measurement but levels of the synthesis enzymes may be subject to a number of levels of control which may be altered by secretion.

The Mechanism of Stimulus/Secretion Coupling

Before much of the biochemical and morphological evidence for the probable secretory mechanism had been established, Douglas and his co-workers (Douglas, 1968) had postulated a mechanism for the release of catecholamines from the adrenal medulla analogous to that present in skeletal muscle as excitation-contraction coupling. Ever since Houssay and Molinelli, 1928 showed that calcium was important for the release of catecholamines from the adrenal medulla, it had become increasingly obvious that calcium in some form was the key to the control of adreno-

medullary secretion and within the framework of stimulus-secretion coupling, calcium seems to play the vital linking role in the molecular events involved, regardless of which hypothesis one favours.

Ionic Mechanisms Involved in Stimulation-Secretion Coupling

Originally Douglas and Rubin, 1961, 1963 observed release of catecholamines from the adrenal medulla of cat glands perfused, in situ, with salt solutions in response to acetylcholine in conditions where sodium ions, Na^{+} were removed and where only calcium ions, Ca^{++} and sulphate ions, $SO_4^{=}$ were present in the medium. The catecholamine secretion evoked by acetylcholine increased with increasing external calcium ion concentration, $[Ca^{++}]_e$ and other secretagogues were shown to depend on Ca^{++} e.g. nicotinic agonists, muscarinic agonists (Poisner and Douglas, 1966), carbachol (Banks, 1965), histamine, 5-HT, peptides (Poisner and Douglas, 1966). Far more rigorous later work ensuring removal of extracellular Ca^{++} and prevention of Ca^{++} flux across the chromaffin membrane has been confirmatory (Pinto & Trifaro, 1976). Potassium ions, when the external concentration was increased, $[K^{+}]_e$ from 5.6 mM to 56 mM, also caused a calcium dependent increase in catecholamine secretion (Vogt, 1952; Douglas and Rubin, 1961).

It seems that rubidium and caesium can mimic potassium (Sorimachi, 1968). It was postulated that acetylcholine and K^{+} increased the influx of Ca^{++} across the chromaffin cell membrane. Increasing $[Ca^{++}]_e$ alone was without secretory action but following a period of perfusion with Ca^{++} free Locke's solution reintroduction of 2 mM $[Ca^{++}]_e$ evoked a large catecholamine secretion. Strontium ions act similarly to Ca^{++} and can mimic Ca^{++} ions in every way. Barium ions in addition to sharing the actions of strontium will induce secretion even when added to a normal 2 mM Ca^{++} solution. Magnesium ions Mg^{++} compete with any of calcium's actions, and may be overcome by increasing $[Ca^{++}]_e$. It has from this been postulated that these ions act on an internal calcium receptor (Douglas and Poisner, 1964), or by facilitating or competing with calcium entry by an action at the chromaffin cell membrane.

Interaction of Ions Regulating $[Ca^{++}]_i$

Short term sodium depletion enhances the secretion of catecholamines and the adrenomedullary response to some secretagogues (Douglas and Rubin, 1963) but maintained low $[Na^+]_e$ solutions inhibit the response of bovine adrenal glands (Banks, Biggins, Bishop, Christian and Currie, 1969). Low $[K^+]_e$ also enhances the response to acetylcholine. It has been postulated that the energy for Na^+/Ca^{++} exchange across the chromaffin cell membrane depends on the sodium gradient and any action to abolish this gradient such as lowering $[Na^+]_e$ or removal of K^+ to decrease sodium pump activity will decrease Ca^{++} extrusion thus increasing secretion as one proposes that intracellular Ca^{++} controls secretory activity. Ouabain may also act in a similar manner (Banks, 1966). As maintained decreases in $[Na^+]_e$ actually progressively inhibit secretion, it has been proposed that intracellular Na^+ may regulate calcium influx but secretion is still observed if $[Ca^{++}]_e$ is increased suggesting a facilitatory rather than regulatory role (Banks, et al, 1969). The role of monovalent and divalent cations in stimulus-secretion coupling is complex and they may act to determine the intracellular free calcium ion concentration, though the site of action of intracellular calcium may also be another possible regulatory site of their action.

Stimulus-Secretion Coupling

Depolarisation - The Initial Stimulus?

The difficulty of microelectrode intracellular recording, in situ, (Matthews, 1967; Fawcett, 1969) led to the development of chromaffin cell tissue culture where, in gerbil chromaffin cells, electrode implantation proved possible under visual control into positively identified cells (Douglas, Kanno and Sampson, 1967; Biales, Dichter and Tischler, 1976). The low resting membrane potential, 25 - 30 mV seems to be explained by the high permeability to Na^+ and depolarisation by acetylcholine varied with $[Na^+]_e$ and $[Ca^{++}]_e$ over a wide range indicating an inward current of both cations with Na^+ quantitatively more important. It is assumed

this is associated with changes in membrane permeability but the absence of data on conductance changes does not exclude an electrogenic process. Chromaffin cells may be electrically inexcitable to depolarising pulses (Cannon and Rosenblueth, 1937; Kanno and Douglas, 1967) but a recent study (Biales, Dichter and Tischler, 1976) has shown chromaffin action potentials. Depolarisation is insufficient in itself to evoke secretion as witnessed by (1) depolarisation to acetylcholine or K^+ but no secretion in absence of Ca^{++} (2) chromaffin cells in absence of Ca^{++} depolarise but secretion falls, (3) excess Mg^{++} prevents secretion but not depolarisation (4) secretion is not observed after tetrocaine or amethocaine but depolarisation occurs. It seems from this and other evidence, such as calcium flux studies (Pinto and Trifaro, 1976) that the key stage in the initial stimulus to the chromaffin cell is the influx of Ca^{++}. This can occur without depolarisation in conditions such as transient $\left[Na^+\right]_e$ increase or cell depolarisation by K^+ (Douglas and Rubin, 1963). In addition, one might explain the facilitation and summation seen at the splanchnic-adrenal junction (Marley and Prout, 1965) as a summation Ca^{++} influx effects rather than a summation of synaptic potentials as observation of action potentials is controversial. One could obtain much more evidence from intracellular potential recording than catecholamine secretion to resolve these effects. The increase in membrane permeability to Ca^{++} may not be essential in the action of some secretagogues as a number are still active in Ca^{++} free solutions but confirmatory evidence from Ca^{++} flux studies has not been forthcoming.

Supporting evidence for Calcium as a Coupling Agent

The role of calcium as a coupling agent between stimulation via receptor sites on the chromaffin cell membrane and the secretory mechanism which-ever form it takes is still to be elucidated. Apart from ion flux measurements, much evidence supports the role of calcium and indicates some of the processes controlling the vital intracellular Ca^{++} concentration. It seems that ^{45}Ca retention and accumulation occurs within the chromaffin cells (Borowitz, 1969; Rubin, 1970) when they are

stimulated with acetylcholine and that this binding may depend on intracellular sodium levels (Banks, 1965; Goz, 1969). One must be wary of indirect measurements of Ca flux as it appears that though calcium influx may be of key importance in the action of most secretagogues, the actual levels of intracellular free ionised calcium may be very low compared to those stored and one awaits more direct measurements which may be obtained following the application of Ca microprobe analysis.

Exocytosis as a Secretory Mechanism - Alternative Hypotheses

The cellular mechanism of the adrenomedullary secretory process seems from the morphological and biochemical evidence to be by exocytosis, leaving one with necessity for fusion or association of the vesicle and cell membranes to allow expulsion of the chromaffin vesicular contents. The way in which this might occur and the role of calcium in initiating this process has been the subject of a number of hypotheses in the literature.

If secretory function depends on exocytosis, membrane fusion would seem a prerequisite unless a mechanism of membrane opening is postulated. It was proposed that this fusion might be by simple ionic bridging as the $\left[Ca^{++}\right]$ increased (Poisner and Trifaro, 1967). Ca^{++} ions do seem necessary to maintain adhesion of neighbouring cells but fusion does not occur and ionic bridging would not account for the inability of Mg^{++} to promote secretion. Matthews, 1970 proposed that granules exhibit Brownian motion and that increasing $\left[Ca^{++}\right]$ increases the probability of fusion with the chromaffin cell membrane due to the abolition of electrostatic repulsion. This view somewhat similar to that of Douglas, 1968 would not account for the action of microtubular disrupting agents unless they change membrane properties, or for the fusion of empty vesicles with the cell membranes suggested by the experiments of Dixon, Garcia and Kirkepar, 1975. Viveros, 1975 (Fig. 15) has modified this hypothesis to suggest that a secretagogue depolarises the membrane with an influx of Na^{+} and Ca^{++} leading to solvation of the cytosol and Brownian motion. Ca^{++} may increase the probability of fusion by charge changes or by changes in the plasma-

PROPOSED EXCITATION-SECRETION PATHWAY (VIVEROS 1975)

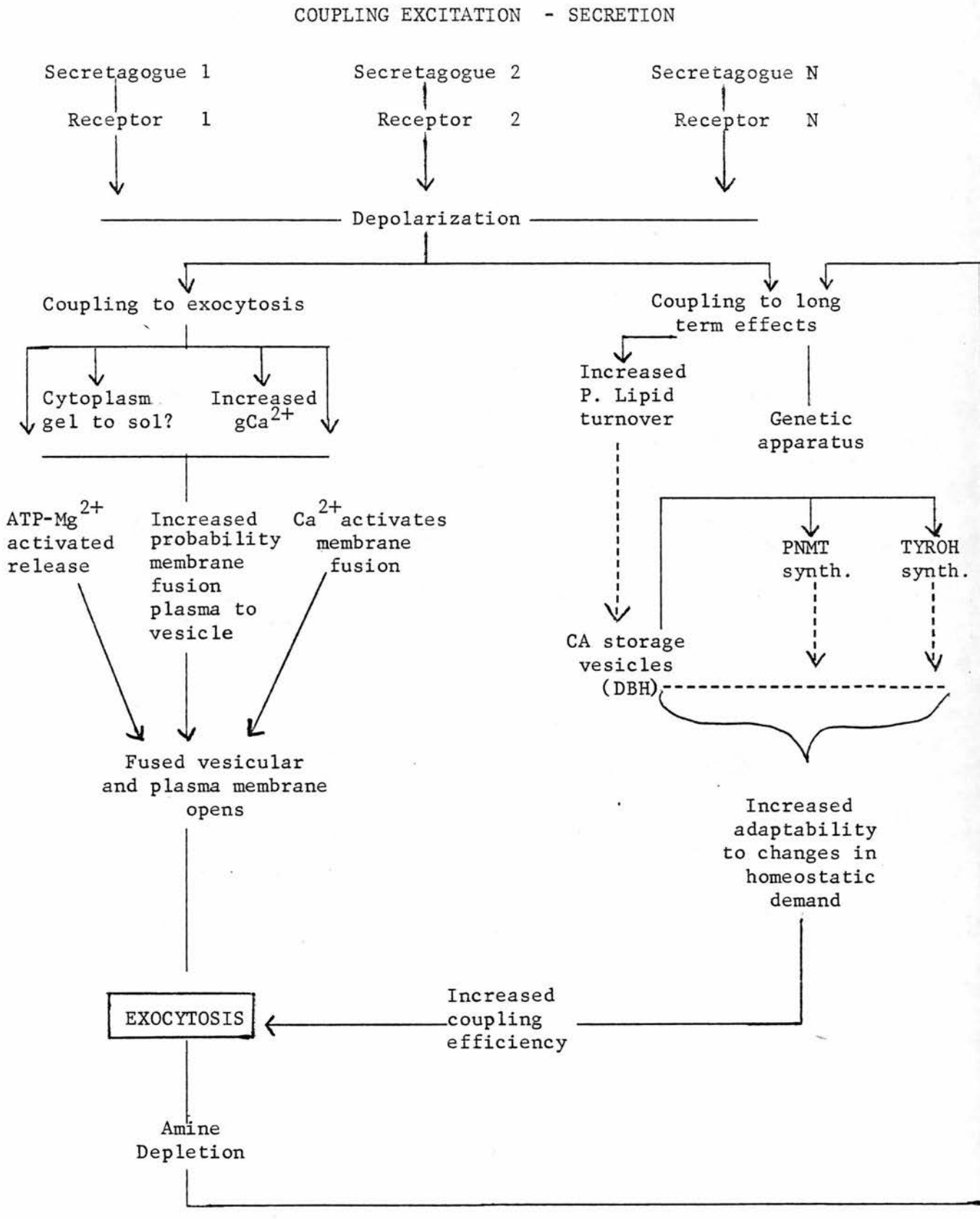


Fig 15

An hypothesis for stimulation-secretion coupling in the adrenal medulla (After Viveros, 1975). DBH- dopamine-β-hydroxylase. P.N.M.T.- phenylethanolamine-N-methyl transferase. TYROH - tyrosine hydroxylase

lemmal conformation at a specific site. This action might be mimicked by Ba^{++} or Sr^{++} but not Mg^{++} which compete. Breakdown of the complex within the vesicles may occur with the increase in osmotic pressure leading to expulsion of the vesicular contents. An inactivation system for Ca^{++} might exist which may be modified for example by $\left[Na^{+}\right]_i$. This hypothesis seems to fit most of the data obtained and provides steps at which drugs inhibiting secretion might act, which would adequately account for their action. It does not provide a role for suggestions of a contractile process or the release mechanism prevailing when 'empty' vesicles release DBH before resynthesis of the catecholamine /A.T.P./Mg complex (Dixon et al, 1975). As an alternative mechanism for Ca action in promoting membrane fusion, Simpson, 1968 adapting the results of Quarles and Folch-Pi, 1965 proposed that chromaffin granules might partition between phases, the cytosol or the lipid cell membrane, depending on the $\left[Ca^{++}\right]$. This hypothesis depends on data obtained in vitro, where the key Ca concentration for changes in partition was 5 mM and this may be so much higher than $\left[Ca^{++}\right]_i$ that physiological Ca may not reach levels sufficient to produce a fusion process of this type.

The concept of Ca^{++} enhancing the probability of fusion may seem sufficient but a number of groups have proposed the existence of a specific mechanism for the transport of vesicles to the vicinity of the chromaffin cell membrane and their subsequent release. Microtubules had been observed under the electron microscope associated with the chromaffin cell membrane (Plattner, 1969; Plattner, Hortnägl, Pfäller, Winkler, 1969) but they could not confirm the earlier observations of cytoplasmic microtubular systems (Coupland, 1965; Whittaker, 1968). Biochemical evidence supports the involvement of a microtubule system in secretion as disrupting agents such as colchicine prevented nicotine induced secretion (Poisner and Bernstein, 1971) but they are inactive against K^{+} evoked secretion (Douglas and Sorimachi, 1972; Trifaro, Collier, Lastowecka and Stern, 1972) though this does not exclude differences in the secretory mechanism between different secretagogues. Recently, actomyosin-like contractile protein has been characterised within the adrenal medulla (Trifaro and Ulpian, 1976; Johnson, McCubbin and Kay,

1977). It has been proposed that this may form the basis of a contractile mechanism with a role in the transfer of vesicles to the chromaffin cell membrane and it might additionally be involved in the opening of the vesicles and the cell membranes. (Whittaker, 1967; Berl, Puszkin and Nicklas, 1973). Rahwan and Borowitz, 1973 suggested that the catecholamines may be present as intratubular droplets and that Ca influx would expel the catecholamine by a contractile process. On the basis of existing morphlogical and biochemical evidence the functional role of a contractile mechanism seems feasible but microtubular or an actomyosin like contractile mechanism may indeed by the same thing, in view of the similarities between tubulin and actin (Schmitt, 1969).

The existence of actomyosin or tubulin within adrenal chromaffin cells, seems indisputable but its role in the secretory mechanism is hypothetical and one cannot readily confirm or disprove the alternatives. Recently, using the more specific marker for myosin, K^{+}-E.D.T.A.-A.T. Pase, Hesketh, Aunis, Pescheloche and Mandel, 1978 have been unable to confirm the association of myosin with the chromaffin granules and this may not support the hypothesis that neurosecretion involves vesicular myosin (Berl, Puskin and Nicklas, 1973).

Secretion Coupling at a Granular Level?

Isolated chromaffin granules seem to show an increased rate of amine release with exposure to acetylcholine (Blaschko, Hagen, Welch, 1955; Greenberg and Kopen, 1966) as well as increasing $\left[Ca^{++}\right]_e$ with a release of soluble vesicle contents in the same ratio as they are stored. (Oka, Ohuchi, Yoshida, Imaizumi, 1967). Berneis, Da Prada and Pletscher, 1969, 1970, observed that the catecholamine storage complex became unstable when $\left[Ca\right]_e$ was altered. Oka et al, 1965, 1967 noted that the release of catecholamines from isolated granules was doubled by A.T.P. and Mg^{++} addition. This may be some action of a granular membrane A.T. Pase which Ferris, Viveros, Kirshner, 1970 found could be blocked by drugs preventing acetylcholine-evoked release. This may provide the basis for yet another hypothesis for the action of calcium

ions but implying that the adrenomedullary response to stimuli is at a granular rather than a cellular level. Indeed the experiments of Oka et al, 1966 indicate the possible existence of separate noradrenaline and adrenaline containing granules which seemed to have distinct differences in their sensitivity to different secretagogues though naturally one does not know what effect biochemical separation techniques have on stimulus secretion coupling and whether the release resembles that occurring physiologically.

Secretagogues - Different modes of Action?

Three categories of secretagogues would appear to exist based on their action on stimulus secretion coupling and the different ways in which they may initiate the secretory mechanism.

Lysing agents seem to release cytoplasmic marker enzymes, as well as the soluble vesicle components. Acetaldehyde, which was found by Schneider, 1971, to release catecholamines in a calcium free solution, released catecholamines and adenine nucleotides without chromogranins or L.D.H. An increase in the catecholamine to A.T.P. ratio suggested an action of the vesicular membrane or storage system and is inconsistent with exocytosis.

Adrenomedullary secretagogues producing secretion by exocytosis are well documented and would appear to promote Ca^{++} influx followed by a secretory mechanism where the biochemical evidence supports exocytosis in the species studied.

Apart from these agonists, a number of drugs will produce catecholamine secretion from the adrenal medulla in calcium-free solutions which the authors assume involves an exocytotic mechanism. The action of indirectly acting sympathomimetic agents, for example, is controversial. Some authors observe secretion in Ca^{++} free solution (Phillipü and Schümann, 1966; Rahwan and Borowitz, 1972; Rahwan, Borowitz, Miya, 1973) whereas other workers claim the secretion is Ca^{++} dependent and can be

inhibited by Mg^{++} (Rubin and Jaanus, 1966, 1967). Rahwan, Borowitz and Miya, 1973 have proposed that under Ca^{++}-free conditions chlorpromazine mobilises mitochondrial calcium and d-amphetamine endoplasmic reticular Ca but the authors do not present biochemical evidence that the secretion observed is exocytosis. A secretion may have occurred not dependent on extracellular calcium but showing subcellular rearrangements of intracellular calcium and one cannot confirm the initiation of exocytosis where displacement of catecholamines from the storage granules into the cytoplasm may be possible, similar to the accepted action of these drugs in sympathetic neurones.

Role of Cyclic Nucleotides?

Phosphodiesterase inhibitors and dibutyryl 3'5' cyclic adenine monophosphate (dbc. AMP) can elicit catecholamine secretion from the adrenal medulla. (Poisner, 1970; Helle and Serck-Hannsen, 1975). Release of catecholamine does not seem to be associated with changes in adenyl cyclase activity when acetylcholine is the stimulant (Jaanus and Rubin, 1974). Stimulation of adrenal glands with a muscarinic agent results in an increase in phosphoinositol turnover (Trifaro, 1969) with a concomitant rise in cyclic guanosine monophosphate (c G.M.P.) which may have a role in stimulus-secretion coupling. Adenyl cyclase activity may have no action in nicotine induced secretion but enhancement of acetylcholine-evoked adrenomedullary secretion by adrenergic agonists (Malmejac, 1955) may be linked to B receptor-mediated rises in adenyl cyclase activity (Helle and Serck-Hannsen, 1975). Higher levels of exogenous catecholamines would seem to reduce catecholamine secretion possibly via an α receptor on the chromaffin cells (Boonyaviroj and Gurtman, 1977). The modulation of adrenal medullary secretion in this manner, may occur from endogenously released catecholamine, circulating catecholamines, or by the adrenergic neurones identifiable within the adrenal medulla (Prentice and Wood, 1974; Manueledis and Manueledis, 1975). The 'intracellular mechanism' of this facilitatory or inhibitory modulation awaits characterisation but it has been suggested that cAMP acts to mobilise intracellular stored calcium

though no evidence was presented that cAMP induced secretion (Peach, 1972) was by an exocytotic mechanism.

Termination of Secretory Response

Secretory cells appear to have developed mechanisms to maintain free ionised Ca at a low level and an active binding and uptake of Ca is seen into chromaffin cell microsomes (Borowitz, 1967; Poisner and Hava, 1970) and mitochondria (Poisner and Hava, 1970). The chromaffin granules themselves may play a role in calcium sequestration as uptake of Ca into the granules, and hence the catecholamine complex, has been observed (Borowitz, 1970). The uptake of Ca into the chromaffin granules may serve the dual purpose of terminating the secretory response and of participating in the synthesis and storage of new catecholamines. A component of the removal of calcium seems to involve the pumping of calcium out of the cell against a concentration gradient by way of Na/Ca pump which has been postulated by Banks, 1967.

Drugs or other factors which release intracellular Ca^{++} or block their sequestration would be expected to initiate catecholamine secretion, or potentiate, or prolong a secretory response. Indeed, caffeine, barium, thiocyanate, sulphydryl agents and ouabain (Poisner, 1970; Poisner and Hava, 1970; Rahwan, Borowitz and Miya, 1973) all of which are said to increase intracellular calcium by influencing intracellular calcium movements, evoke adrenomedullary secretion. One cannot confirm that this involves the normal exocytotic process but prevention of sequestration of calcium in this manner, may well lead to the initiation of true stimulus-secretion coupling.

Fatigue and Cross-Tachyphylaxis

Two phenomena have been observed following the exposure of adrenomedullary chromaffin cells to drugs. A decrease in secretion, fatigue, is observed in response to continued stimulation of the glands.

This decline in the secretory response has been observed during splanchnic nerve stimulation, acetylcholine infusion and after prolonged exposure to high $\left[K^{+}\right]_e$ and other drugs. Smith and Robinson, 1970 believed that depletion may occur as the result of a loss of the releasable pool of catecholamine but the observation that responses to acetylcholine or K^{+} were unaltered whilst the other response had declined due to continuous infusion of either drug makes depletion unlikely (Douglas and Rubin, 1963). It seems that 'desensitisation' may occur at a receptor level in the case of some drugs and antagonists of the nicotinic desensitisation can be obtained which do not alter the K^{+} declining response (Kirpekar and Prat, 1978). Baker and Rink, 1975 fully investigated the decline in response to acetylcholine and K^{+} at a cellular level and obtained results indicating an inactivation of Ca influx which only allowed a transient influx of Ca^{++}. This appeared to be due to the inactivation of a potential-sensitive Ca^{++} permeable channel which was time, and possibly potential, dependent. Cross-tachyphylaxis is also observed in the adrenal medulla between tyramine, phenylethanolamine, d-amphetamine and acetylcholine in the cat adrenal gland (Rubin and Jaanus, 1967(b)) but one does not know whether this is at a receptor or stimulus-secretion coupling level. Increasing the $\left[Ca^{++}\right]_e$ seems to overcome this effect and may reflect an inactivation at a cellular level preventing activation of the coupling mechanism by the other drugs, but why can K^{+} or Ba^{++} act when the response to acetylcholine has waned? There is a possibility of a different release mechanism or a difference in the stimulus produced by the drugs. Tyramine and other indirectly-acting sympathomimetics are claimed by other workers not to involve Ca^{++} influx. Baker and Rink, 1975 can explain the action of Ca reintroduction in being secretory by assuming a prior change in the chromaffin cell membrane properties during Ca free perfusion and the action of Ba^{++} ions, by substituting for Ca^{++} in Ca - Ca exchange, but they do not seem able to explain the persistence of an acetylcholine-evoked secretion in conditions of K^{+} depolarisation and fatigue, as by their hypothesis the Ca^{++} channel is inactivated. This again questions whether acetylcholine may act by another on more than one secretory mechanism.

Comparison Between the Adrenal Medulla and the Sympathetic Ganglia

In addition to the similarities in the anatomy and embryological development of the adrenal medulla and the sympathetic ganglion a number of functional similarities have been observed. The innervation of both structures is sympathetic preganglionic with fibres which innervate one or more ganglionic or chromaffin cells. In addition to this innervation, small intensely fluorescent cells, (S.I.F.) cells, have been observed by fluorescence and electron microscopy in many ganglia and the adrenal medulla of several species. Although these cells are described as having a functional role as interneurones within the superior cervical ganglion no investigation of a similar adrenal role is forthcoming. One cannot of course, exclude a modulatory role for these cells in both the ganglia and the adrenal medulla which has been suggested by a number of authors instead of, or in addition to, the interneurone hypothesis.

The predominant transmission of a supramaximal synchronous 'preganglionic' stimulus is nicotinic in both situations with the neurotransmitter confirmed as acetylcholine. Only a small residual non-nicotinic response is observed following dosage with nicotinic antagonists. When one considers the comparative electrophysiology of the ganglion and chromaffin cells similar facilitations and inhibitions are observed which seem to reflect similar spatial and temporal recruitments of the postsynaptic cells a large number of which are subliminal.

The residual non-nicotinic transmission and the pharmacological action of non-nicotinic drugs is similar in the ganglia and the adrenal medulla. The enhancement of non-nicotinic responses following nicotinic receptor desensitisation is identical in both situations as are the stimulant actions of the same agonists.

In general, stimuli producing increases in sympathetic vasoconstrictor tone e.g. a fall in carotid sinus pressure or a primary chemoreceptor reflex also increase adrenomedullary catecholamine secretion though differences in the strength of stimuli necessary to

elicit specific reflex responses may be observed. It has still not been confirmed whether the receptor differences between transmission of these different reflexes through the sympathetic ganglia, observed in the present study, are mimicked in the adrenal medulla.

At a receptor level various similarities may exist as witnessed by the apparent similarities of the nicotinic receptors on the ganglion cells and the chromaffin cells as opposed to those at the neuromuscular junction. The muscarinic agonists in general, are equiactive as ganglionic and adrenomedullary stimulants, within the same species, but some of the 'selective' ganglionic muscarinic agonists show less adrenomedullary stimulant action though this may be further evidence for a non-cholinergic component of the action of these drugs on ganglion cells. The response to muscarinic agonists is observed to be sensitive to the same antagonists in comparable doses but in our present study the first attempt has been made to determine quantitatively the affinity of antagonists for canine adrenomedullary muscarinic receptors. Whether these receptors are similar to those present on ganglion cells of the same species, or ganglion and chromaffin cells of other species remains undiscovered. Where the antagonism of responses to other non-nicotinic agents has been investigated no differences in the pharmacology of the sympathetic ganglia studied and the adrenal medulla have been observed.

In summary both the sympathetic ganglion and the adrenal medulla show a similar morphological structure with preganglionic fibres multiply innervating one or more postsynaptic cells. This structural similarity may explain the facilitatory responses observed as both the sympathetic ganglion and the adrenal medulla show a large subliminal fringe. The receptor populations on the cells seem to be identical but the selectivity of receptor populations on the noradrenaline and adrenaline-containing chromaffin cells noted in the cat has not been extended to the ganglia of the same species. Although the pharmacology is similar the mechanism underlying spike generation within postganglionic cells and catecholamine secretion from the chromaffin cell may differ greatly, one might propose that the facilitation observed may merely reflect changes

in the excitability of the cells, mediated in the ganglion cells by the level of depolarisation, and in the chromaffin cell possibly by the level of free intracellular Ca^{++} ions available to the coupling mechanism.

GENERAL METHODS

GENERAL METHODS

Whole Animal Experiments

The procedures used in the whole animal experiments were similar with regards to choice of anaesthesia, surgery and carotid perfusion method, though obviously some details vary according to the experiment performed. In this section, the general methods employed are illustrated and any detailed changes are referred to later in connection with particular experiments.

Choice of Anaesthesia

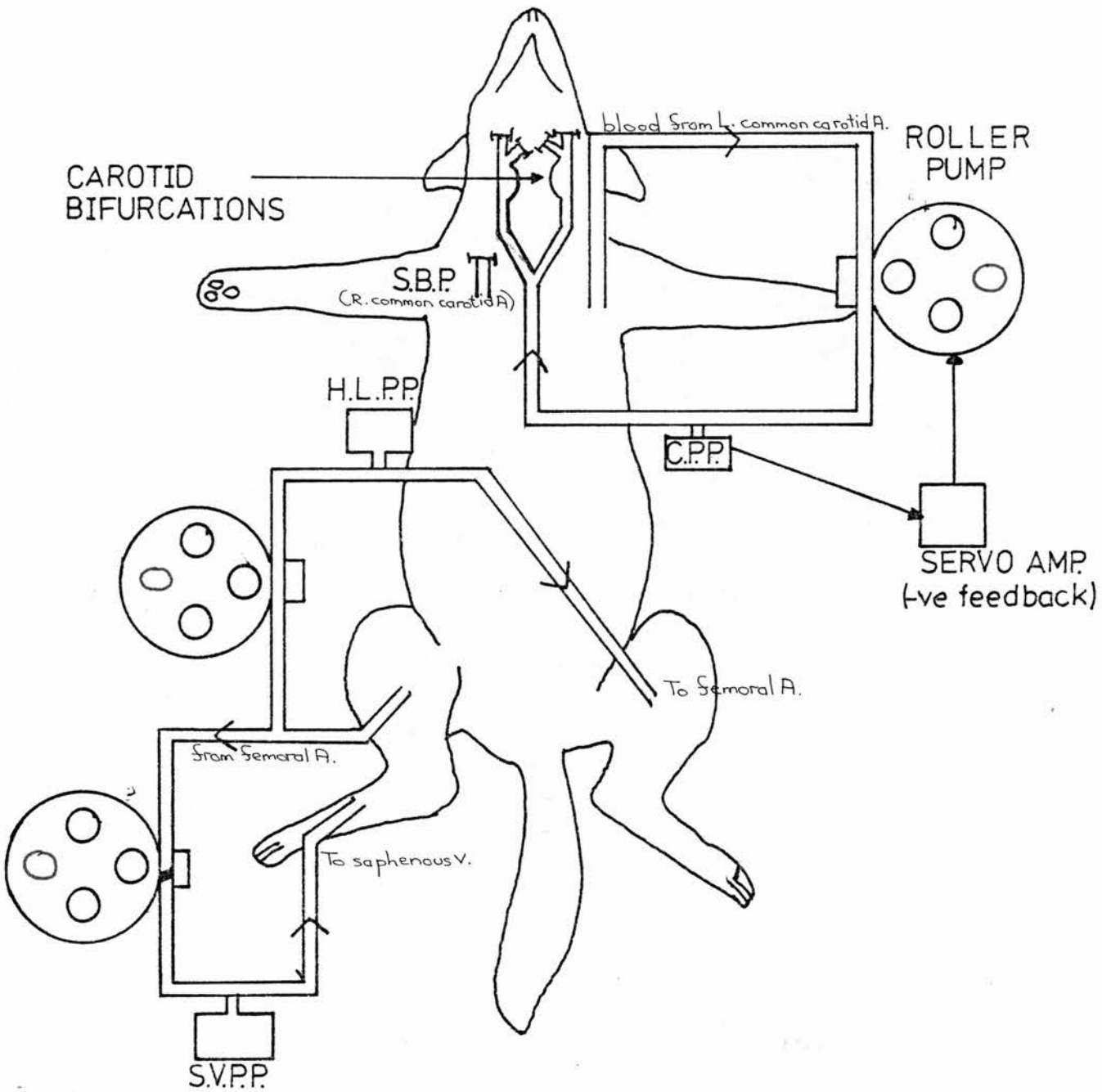
Sodium pentobarbitone (30 mg/Kg I.V.) was used to induce anaesthesia. Some barbiturates are known to depress ganglionic transmission but pentobarbitone is said to have little action at anaesthetic doses (Exley, 1954). We can confirm this finding as no change in the reflex response was seen to comparable baroreceptor or chemoreceptor tests immediately after maintenance doses of anaesthetic. The choice of an anaesthetic for adrenal collection experiments was more awkward as pentobarbitone is known to suppress secretion whereas chloralose-urethane greatly enhances secretion (Satake, 1931). Following previous work within our laboratory, it was felt that the depressant action of pentobarbitone was relatively minor at anaesthetic doses.

Carotid Surgery and Localisation of Stimulation

The carotid bifurcations were vascularly isolated by ligating the superior thyroid, internal carotid, occipital, external carotid and ascending pharyngeal arteries. Any other branches between the point of cannulation and the origins of the lingual arteries were ligated with only the lingual arteries remaining open to ensure an adequate flow to the bifurcations. The common carotid arteries were cannulated both ways in the neck, blood from one being perfused into both, towards the neck, using a Watson

Fig 16

The experimental set-up for the investigation of vasomotor reflexes.
S.B.P. - systemic blood pressure H.L.P.P. - hind-limb perfusion pressure
C.P.P. - carotid perfusion pressure, S.V.P.P. - saphenous vein perfusion pressure



Marlow MHRE Flow Inducer. Using the carotid perfusion circuit, one is able to maintain a constant pressure by taking a signal from a pressure transducer connected to a side arm of the perfusion circuit and passing the signal through a servo-amplifier to the perfusion pump, so that perfusion pressure can be set at will and held constant. (see Figure 16).

Removal of Secondary Influences

Both vagosympathetic trunks were cut in the neck and the animal artificially ventilated to remove the influence of the lungs on the cardiovascular response to chemoreceptor stimulation. The primary chemoreceptor reflex response involving an increase in sympathetic tone to the resistance vessels is usually masked by the secondary 'lung stretch reflex' (Daly and Scott, 1962) and the procedure used ensures that the responses were not influenced by aortic arch baroreceptors or aortic body chemoreceptors as their afferent fibres run in the vagus nerve in the dog. (Daly and Ungar, 1966).

Selective Initiation of Reflexes

It was necessary in these studies to obtain a selective activation of either baroreceptor or chemoreceptor reflex pathways. Much of the previous work involved the use of a stimulus such as carotid occlusion, a large component of which will involve chemoreceptor stimulation due to the interruption of carotid body blood flow (Von Euler and Liljestrand, 1943; Heymans and Neil, 1958). In the present study much care was taken to ensure that one could produce selective activation of carotid baroreceptors or chemoreceptors.

Baroreceptor Tests

Baroreceptor tests were performed by lowering the perfusion pressure in the carotid circuit. This was achieved by resetting the servo-amplifier (Critchley, 1977) to maintain a lower carotid perfusion pressure

for a given period of time. This did not alter the Pa, O_2 of blood perfusing the bifurcations (Table 1 p82a), and as the animal was ventilated, with an arterial oxygen tension maintained above 13.3 KPa a 'pure' baroreceptor test was performed.

Chemoreceptor Tests

A selective chemoreceptor test was performed by making the blood in the carotid perfusion circuit hypoxic. This was achieved by the infusion of sodium dithionite solution, 1 M at a rate of 150 mg/min, (6 ml/min) into the carotid perfusion circuit before the pump. No reduction in systemic Pa, O_2 was observed showing a localised action of the dithionite. During chemoreceptor tests, the carotid perfusion pressure was held constant at the normal control level.

The Action of Sodium Dithionite as a Hypoxic Agent

One straightforward way of stimulating carotid chemoreceptors in the preparation used would have been to perfuse the bifurcations for a short time period with venous blood taken from the jugular vein but venous P, O_2 was often not low enough or reproducible enough to produce significant cardiovascular responses.

This left one with the alternative of using drugs to directly stimulate the carotid body chemoreceptors or the use of a hypoxic agent. Histotoxic drugs such as KCN have various problems such as recirculation, central toxic actions, difficulty of dosage, and tachyphylaxis. Nicotinic agonists which will stimulate carotid chemoreceptors were excluded as our studies required the use of a nicotinic antagonist and, of course, they do not provide the physiological stimulus, hypoxia.

Critchley and Ungar, 1975 had studied the usage of a reducing agent to lower Pa, O_2. The most satisfactory agent which produced a dose dependent graded hypoxia was sodium dithionite. We can confirm that no recirculation occurs and tachyphylaxis was not observed when infusions of sodium dithionite were used at intervals of five minutes

or less. We confirmed the localised action of sodium dithionite to the carotid chemoreceptors by the absence of reflex responses following sinus nerve denervation. In this way graded stimuli can be applied to the carotid body chemoreceptors without affecting carotid sinus pressure.

One disadvantage of sodium dithionite solution is its instability as oxidation occurs rapidly unless it is stored under liquid paraffin.

Respiration and Heat Control

In all animals the trachea was cannulated and the animals were artifically ventilated, using a Starling 'Ideal' Pump, with a metered oxygen - nitrogen mixture so as to hold Pa, CO_2 at 4 KPa and Pa, O_2 above 13 KPa as measured from frequent carotid arterial blood samples on a Radiometer BMS 3 analyser. A molar solution of sodium bicarbonate was injected intravenously after each arterial sample to hold the arterial pH at 7.4 Body temperature was held at 37^{o}C by a heating pad linked to a rectal thermistor probe. (STD telephones).

Hind-Limb Perfusion

In animals where the sympathetic ganglionic transmission of vasomotor reflexes to resistance vessels was investigated a hind-limb vascular bed was perfused at constant flow. One femoral artery was cannulated (Portex i.v., 8 FG) at the level of the inguinal ligament and its major collaterals ligated. The hind-limb was perfused with blood from the other femoral artery through a Watson Marlow MHRE flow inducer. When the pump was stopped, the perfusion pressure fell to less than 30% of systemic pressure and the pump was set to ensure that the hind-limb perfusion pressure was always higher than systemic blood pressure. One hind-limb was thus perfused at constant flow and changes in perfusion pressure were taken as a measure of neurally mediated changes in the vascular

resistance of that limb.

Saphenous Vein Perfusion

In a number of preparations a Y-piece in the hind-limb perfusion circuit enabled blood from one femoral artery cannulated towards the heart to perfuse independently at constant flow, the hind-limb resistance bed and a medial saphenous vein. Two Watson Marlow MHRE flow inducers ensured a perfusion at constant flow and changes in hind-limb perfusion pressure and saphenous vein perfusion pressure were taken as reflecting changes in hind-limb vascular resistance and saphenous vein diameter, respectively.

Adrenal Venous Blood Collection

In only three out of seven experiments did it prove possible to obtain an adequate adrenal venous collection. The procedure involved cannulation of the left adrenolumbar vein towards the vena cava, with a soft nylon catheter (Portex, Intravenous Catheter, 4FG) to which had been added a short length of silicon tubing. Following cannulation, the adrenal vein was ligated between the left adrenal gland and the vena cava as close to the gland as possible ensuring that the whole output of the left adrenal gland could be collected from the cannula. Samples of blood were collected into tubes on ice for one minute periods before and during baroreceptor and chemoreceptor tests. After centrifugation the separated plasma was stored frozen at - 30°C with sodium metabisulphite (10 mg/ml) prior to catecholamine estimation.

Recording of Blood Pressure

Systemic blood pressure, hind-limb perfusion pressure, saphenous vein perfusion pressure and carotid perfusion pressure were recorded by Bell and Howell L222 pressure tranducers, calibrated against a mercury manometer, and thence to a Honeywell Visicorder 2206 U.V. recorder.

Isolated Preparations

Investigations of Adrenomedullary catecholamine secretion

An 'in vitro' perfusion technique was preferred for the following reasons (1) the number of available glands (2) the alleviation of the need to separate catecholamines from plasma prior to assay thus allowing an 'on-line' semi-automated technique to be developed. (3) the ability to ensure that known concentrations of drugs equilibrate with the receptors (4) the difficulty and instability of 'in situ' preparations.

These seemed important arguments in favour of isolated perfused adrenal glands, but one still has reservations about the technique. In these studies, it has proved necessary, for a number of reasons, to remove adrenal glands from animals at the end of an experimental protocol during which extensive surgery may have occurred and a number of drugs been administered. In all experiments care was taken to ensure that the donor animals had been subjected to similar experimental procedures as far as was possible, though one could not preclude the possibility that the pharmacological response of the adrenal medulla might have been greatly influenced by its prior history and the stress to which its cells were subjected.

Secondly, perfusion with a'physiological salt' solution may alter the secretory response but certainly the results obtained from these studies for resting catecholamine secretion and percentage of adrenaline and noradrenaline correlate closely with those obtained by ourselves and previous groups with blood-perfused in situ preparations. Consistency of agonist response was also obtained but as in any isolated preparation one cannot ensure that the response of the tissue to the agonist mimics that observed in the intact animal. Thirdly, surgical removal and the perfusion method used may have lead to pathological changes in the adrenal gland but a number of studies have noted little anatomical changes under electron microscopy following prolonged perfusion. In approximately 40% of the adrenal glands removed during the

present studies, marked elevations in, or absence of, detectable resting output were observed, this occurring in glands which proved pharmacologically unresponsive and this may indicate structural damage. The reproducibility and sensitivity of the other perfused adrenal glands to stimuli appears to be greatly enhanced if one ensures removal just prior to death of the anaesthetised donor animal, adequate heparinisation or sufficient wash-out of the glands, and if one takes steps to prevent air emboli forming in the gland, from the perfusion apparatus.

For the reasons given one is rather limited to the investigation of pharmacological actions on isolated, perfused adrenal glands where studies can be completed with a high degree of accuracy and reproducibility. It may only be safe to accept the results as secondary or preliminary knowledge as the uncontrollable factors discussed may alter the situation completely from that prevailing normally.

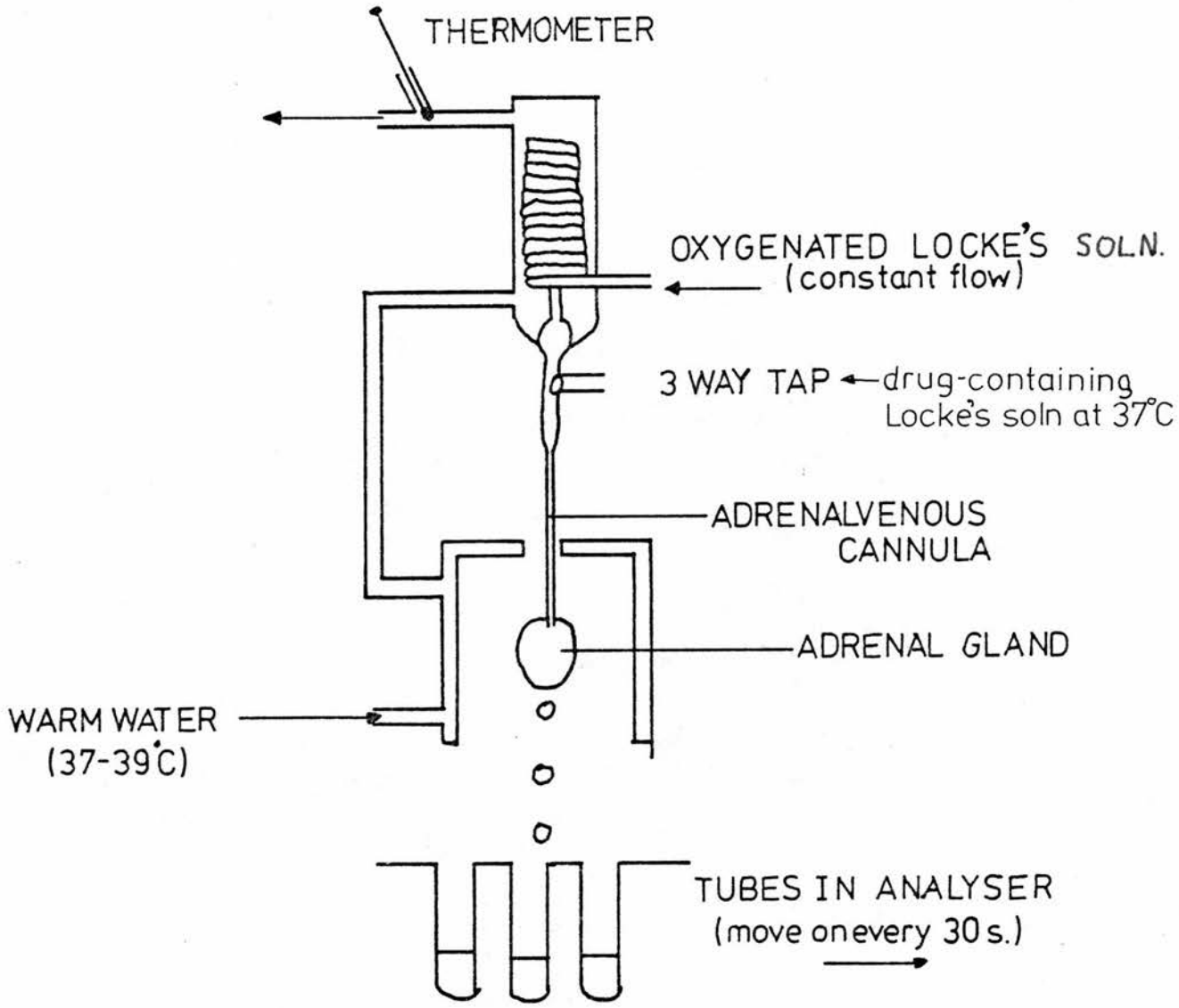
Removal of Adrenal Glands

Adrenal glands were excised from animals by the following procedure which has proved satisfactory in the species studied. In most cases, the animal was under anaesthetic and living during the excision, but occasion demanded that in some instances, removal occurred after death, though glands so removed did not show signs of deterioration.

Following a midline incision, accompanied by a flank incision in larger animals, the abdomen was opened and both adrenal glands exposed. The left and right adrenolumbar veins were cannulated towards the adrenal gland with a special cannula, comprising a length of soft silicon rubber tubing (1·7mm i.d.) pushed over an appropriately sized nylon catheter (5FG). Nylon catheters alone proved unsuitable as they easily penetrated the venous wall, a situation worsened by the angle one had to move the cannula through during cannulation. Following cannulation, the adrenal vein was located and ligated at its junction with the vena cava and the cannula opened to relieve any pressure build-up within the gland. Usually the left adrenal cannulation was

Fig 17

The isolated adrenal gland perfusion apparatus.



completed before excision, then the right adrenal gland was cannulated similarly. This often proved much more difficult due to its very variable anatomy and vasculature. Immediately on excision the glands were flushed with heparinised (100 units/10 ml) saline or heparinised Locke's solution to remove any blood present in them. This procedure proved particularly important where the donor animal was not heparinised. As no differences were observed in the catecholamine output or drug response, in comparison to glands used immediately, some adrenal glands were stored overnight at 4°C. in heparinised saline or Locke's solution, following excision.

In small animals some departures from the normal procedure proved necessary. Usually, in cats, cannulation of the adrenolumbar vein proved possible but occasionally, and consistently in rabbits, it proved necessary to cannulate the renal vein and ligate the adrenolumbar vein and vena cava to produce a small sac communicating with the adrenal gland through the adrenal vein. This was satisfactory as the 'dead-space' produced was very small. Often due to the variable location of the right adrenal gland, often under the vena cava, in most species, cannulation was impossible and regularly the adrenal vein was absent as the gland was in direct vascular contact with the vena cava. Naturally, the cannulation method employed leads to a retrograde perfusion of the adrenal glands but this is necessary in view of the numerous arterial inputs to the adrenal gland (Coupland, 1976).

Perfusion Apparatus

Following excision, the adrenal glands were retrogradely perfused, using a modified Langendorff apparatus (Aimer), with oxygenated Locke's solution which was phosphate-buffered to pH 7.4 (see Fig. 17). The Locke's solution (NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 2.2 mM; Na_2HPO_4, 2.15 mM; NaH_2PO_4, 0.85 mM; Glucose 10 mM; pH 7.4) was maintained at 37°C by the water jacket and perfused the gland at a constant flow of 2 ml /min with

a Watson Marlow MHRE flow inducer. A three channel, two position, glass manifold and tap (Griffin and George) enabled one to switch to a parallel perfusion of Locke's solution containing the required drug. Following perfusion of the gland for a given time with Locke's solution containing a drug, returning the tap to its original position restored the normal perfusion with the control salt solution. The gland itself lay in a funnel which allowed effluent from the gland, out of the arterial vessels, either to go to waste or to enter tubes placed under the funnel in the track of a modified PYE Unicam AC60 Auto Analyser. The track of the analyser moved on every 30 seconds allowing collections to be made accurately for selected time periods during drug infusions. Different time periods of drug infusion were used, being conditioned by onset of action, maintenance of increased secretion and possible problems with tachyphylaxis.

The samples of the adrenal effluent, 1 ml in 30 seconds, were subjected to analysis for noradrenaline and adrenaline concentrations by a differential fluorimetric assay.

ASSAY OF CATECHOLAMINES

Choice of Assay Method

A number of methods have been developed for the assay of catecholamines in tissue and solution, but each method requires application to a specific situation. In the experimental procedure adopted for isolated adrenal gland studies, one required a suitable degree of sensitivity but this need not be particularly high because of the large concentration of catecholamines secreted from the adrenal gland, even at rest. Secondly, a differential analysis of noradrenaline and adrenaline was necessary which could be relied on over a range of differing concentrations. Thirdly, an ideal situation though not entirely essential was the development of an assay procedure which was rapid enough to enable one to obtain the results of one period of drug administration before the next test. This gives one

an 'on-line' assay of the released catecholamine without the need to store samples before assay, thus necessitating completion of experiments 'blind'. Of the techniques available only one, fluorimetry, proved satisfactory for investigations of this sort.

Bioassay techniques though sensitive and providing estimates of both noradrenaline and adrenaline concentrations are wholly dependent on the reproducibility of the assay preparations. Their stability can be altered by many factors notably temperature, pO_2 of the perfusing solution and the influence of drugs in the adrenal effluent. The other problem which has to some measure been overcome by the later assay of Armitage and Vane, 1964 is one of insufficient discrimination between noradrenaline & adrenaline with the preparations used. This makes analysis of the percentages of both catecholamines in an unknown solution inaccurate. Also the process when done with any degree of accuracy is time consuming and does not allow intervals less than the recovery time of the assay preparations and for these reasons bioassay was rejected.

Spectrophotometric methods for the analysis of catecholamines (el Rabbat and Omar, 1978) have been widely used, though the low sensitivity of absorption spectrophotometry has proved unsatisfactory for most studies and fluorimetric techniques are preferred. The two available fluorimetric methods will produce a differential analysis of noradrenaline and adrenaline and the method chosen proved to be adaptable easily to an 'on-line' semi-automated analysis with a degree of sensitivity sufficient for even very low resting catecholamine secretions to be determined reproducibly.

Radioenzymatic methods have provided greatly increased (Peuler and Johnson, 1977) sensitivity over fluorimetry and might allow one to make more detailed studies on release mechanisms as much smaller volumes can be utilised. The methods depend on the chromatographic separation of noradrenaline from adrenaline which preclude their use in a study where time involved in assays is important and where sensitivity is not of prime importance.

As with radioenzymatic methods, no other assay techniques such as mass fragmentography/gas liquid chromatography (Hattox and Murphy, 1978) are satisfactory because of the time taken to prepare samples though these methods may be preferred in assays of peripheral circulating catecholamine levels where the sensitivity of the assay is of paramount importance.

Selection of the Fluorimetric Method

Two similar fluorimetric assay procedures have been developed by numerous workers (For Reviews, Udenfriend, 1962; Callingham, 1967). They both involve oxidation of noradrenaline and adrenaline to their 'chrome' derivatives. At the next stage of the process, the trihydroxyindole (THI) method involves rearrangement of the 'chrome' derivatives to lutines in alkaline conditions. The lutines readily oxidise and this is prevented by a stabilising agent. All THI methods are based on this sequence and only differ in the oxidising and stabilising agents used. The ethylene diamine condensation method (Weil-Malherbe and Bone, 1954) differs, in that following oxidation, a condensation reaction occurs with ethylene diamine to produce the fluorescent quinoxalone products. In the present study the trihydroxyindole method was selected as it is more specific and involves one less stage in the chemical procedure. All THI methods would be sensitive enough for the present studies but we wished to produce a method enabling accurate differentiation between adrenaline and noradrenaline which could be semi-automated to enable 'on-line' measurements. For this reason a two tube method involving oxidation of two halves of the sample at different pHs was rejected and a single tube method developed with a scan over a wavelength range with measurements being made at two known wavelengths. Differential oxidation pH methods, produce lower 'blank' values with better discrimination of noradrenaline from adrenaline without the need for a scan procedure with fluorimetric measurement at two fixed wavelengths. This is advantageous but proved inapplicable to the limited facilities offered by our auto-analyser and the large number of samples involved.

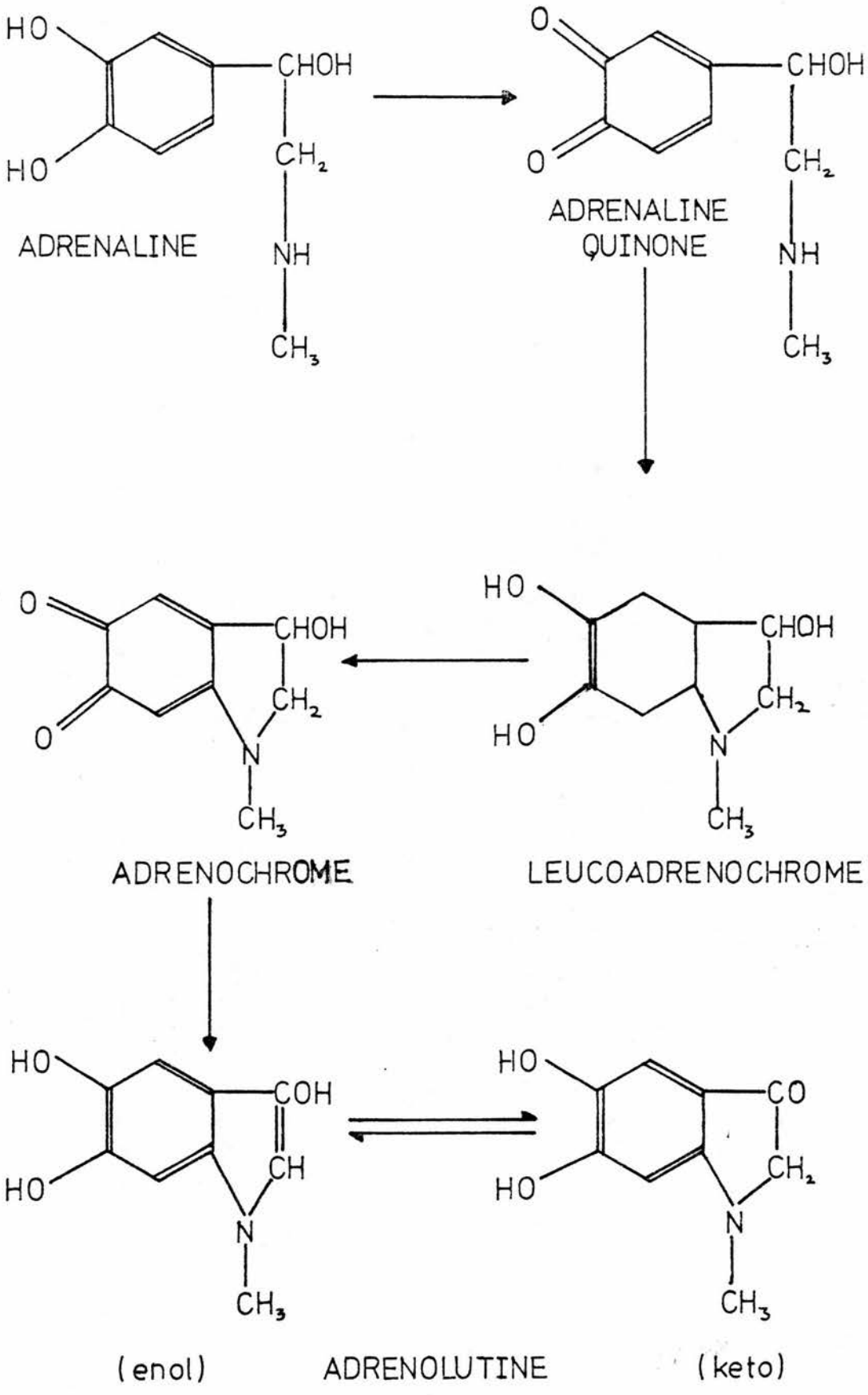


Fig 18

The chemical basis for the trihydroxyindole method of catecholamine analysis.

A Trihydroxyindole Method for Fluorimetric Analysis

The technique used was a development of the trihydroxyindole method of Vendsalu, 1960 and Häggendal, 1963. These methods provided the basis for the present method but various modifications to the procedure succeeded in increasing the level of differentiation between adrenaline and noradrenaline.

Oxidising Agent

A number of oxidising agents have been preferred by different authors; manganese dioxide (Lund, 1951), iodine (Crout, et al 1961), and potassium ferricyanide (Vendsalu, 1960; Anton and Sayre, 1962 and Häggendal, 1963). The last was chosen, as oxidation by iodine can be influenced more readily by the pH of a solution and takes longer to produce the fluorescence. Copper ions were added to the potassium ferricyanide as their use seems to enhance the development of fluorescence (Häggendal, 1963; Valori, Brunori, Renzini and Corea, 1970).

Antioxidant

The antioxidant chosen to stabilise the lutines following their production in alkaline conditions is, in contrast to the choice of oxidant, extremely important if the assay is to be sensitive. The preferred antioxidant was for a long period of time, ascorbic acid (Lund, 1951; Crout, 1961 and Anton and Sayre, 1962) but it was noted that it contributed to the blank value in a time dependent manner (Vendsalu, 1960). Another undesirable feature of its use was the loss of one of the charecteristic fluorescence maxima of the lutines produced on excitation at the shorter wavelengths. This lead to the investigation of the use of sulphur containing compounds of which dimercaptopropanol (B.A.L.) in sodium sulphite gave the most reproducible results with lower blank values. With this agent, two peaks were produced and simultaneous equations used to calculate the catecholamine concentrations. The

separation was still not as great as one would like. B.A.L. when in sodium sulphite solution has a tendency to produce a cloudy solution leading to quenching but this can be minimised by vigorous mixing of the reagents and by using fresh solutions with the minimum necessary B.A.L. concentration. Valori and his colleagues wished to dispense with other antioxidants and described a technique using B.A.L.-formaldehyde solutions. It was found in the present study that their stabilising mixture tended towards cloudiness, gave poor fluorescence, with poor discrimination between catechols and used very large quantities of B.A.L. The initial part of this study used B.A.L.-formaldehyde mixtures but this lead to the development of an assay using B.A.L.-sodium sulphite solutions with an additional acetic acid stage.

Use of Glacial Acetic Acid

When one added glacial acetic acid to a mixture containing B.A.L. formaldehyde, the fluorescence was enhanced but two peaks remained (Valori et al, 1970). In the present study we observed that similar additions of glacial acetic acid to a mixture stabilised with B.A.L. sodium sulphite resulted in loss of the first peak for either amine but with a greater separation of the wavelength for maximum fluorescence for the second peak (see Fig. 19, p77). This separation was maximal between pH 4.5 and pH 5.5 for the final solution. The addition of glacial acetic acid gave a far greater discrimination of the two catecholamines when appropriate wavelengths were read. This had the additional advantage that solutions of this final pH are stable for approximately 1 hour with no change in fluorescence, whereas the alkaline solutions obtained by the Häggendal method were unstable and faded variably over 30 minutes.

From the information obtained using various oxidising and stabilising agents and conditions, the following fluorescent catecholamine assay was designed for use with the auto-analyser available. This technique was used for all experiments where isolated adrenal gland perfusions were assayed. It was found that various general points common to all fluorimetric assays must be considered if the assay is to be accurate and reproducible.

Timing

It is very important to ensure that the time interval between oxidation and production of the lutines and the time between this stage and the final acidification is adequate and reproducible. In this respect using a semi-automated method ensured that accurate time intervals were obtained between addition of reagents.

Blanks

In the literature a number of ways of assessing background fluorescence are documented. In the case of adrenal perfusion studies, the contribution of the reagents to the overall fluorescence of a given sample is very small in comparison to the fluorescence of the high catecholamine concentration present. In these studies, a normal reagent blank was prepared with a 1 ml 'sample' of Locke's solution but in assays of far lower catecholamine content, e.g. peripheral plasma studies, different methods of assessing the 'blank' value have been used.

Delivery of Reagents

Naturally as one measures the final fluorescence produced by a catecholamine concentration, the volume of the sample and the reagents added to it must be reproducible. To obtain an accurate sample volume collections of perfusate lasted 30 seconds before the track of the analyser moved on, and as the perfusion was at constant flow (2 ml/min) a reproducible collection volume of 1 ml was ensured. The reagents were added from 1 ml 'plastipax' syringes installed in the auto-analyser and the given volume set by adjusting the variable syringes drive.

Standards

The fluorimetric assay depends on the measurement of the fluorescence of a sample containing an 'unknown' catecholamine concentration and a comparison of this with the fluorescence produced by known

standard concentrations of noradrenaline and adrenaline. The standards (100µg/ml) were prepared from the 'free base' and dissolved in 0.01 N hydrochloric acid and stored at 4°C. Standards appear to be stable for up to three months in these conditions. As errors can occur in the preparation of these standards and some storage oxidation might occur, the same standard solutions were used during any given experimental series so that values for catecholamine concentrations are calculated assuming the standard solutions to be accurate. In this respect this type of assay is not absolute and depends on the assumption that standard solutions are as one has calculated.

DETAILED FLUORIMETRIC ASSAY PROCEDURE

The following assay procedure was used for the assessment of the catecholamine concentration in samples of perfusate from isolated adrenal glands.

1. Samples of perfusate collected for thirty seconds with perfusion rate of 2 ml/min. hence the volume of sample is 1 ml with pH 7.4
2. At zero time (within five minutes of collection) add 0.5 ml 0.05% Potassium ferricyanide solution with 0.002% copper (ii) chloride (Oxidising Agent)
3. At 3 minutes add 0.5 ml freshly prepared solution of dimercapto-propanol (British Anti Lewisite) 0.4%(v/v) in 10% sodium sulphite solution (Stabilising Agent)
4. At 3 minutes 30 seconds add 0.5 ml 8 N sodium hydroxide and then stir using automatic stirrer with polyethylene rod. (Development of Lutines)
5. At 8 minutes add glacial acetic acid (0.35 ml) to give a final pH of 5.0 - 5.3 .

6. Sample tubes are placed in fluorimeter for measurement within ten minutes.

Blank samples consisted of 1 ml of Locke's solution pH 7.4, treated identically to 'unknowns'. Standards, used in triplicate, consisted of 0.1 ml of a 10 μg/ml noradrenaline or adrenaline solution made up to 1 ml with Locke's solution then treated similarly to 'unknowns'. A small volume of standard must be diluted in this manner as the storage in 0.01M hydrochloric acid leads to unsuitable oxidation pHs if larger volumes are added as the buffering by the phosphate-Locke's solution becomes inadequate.

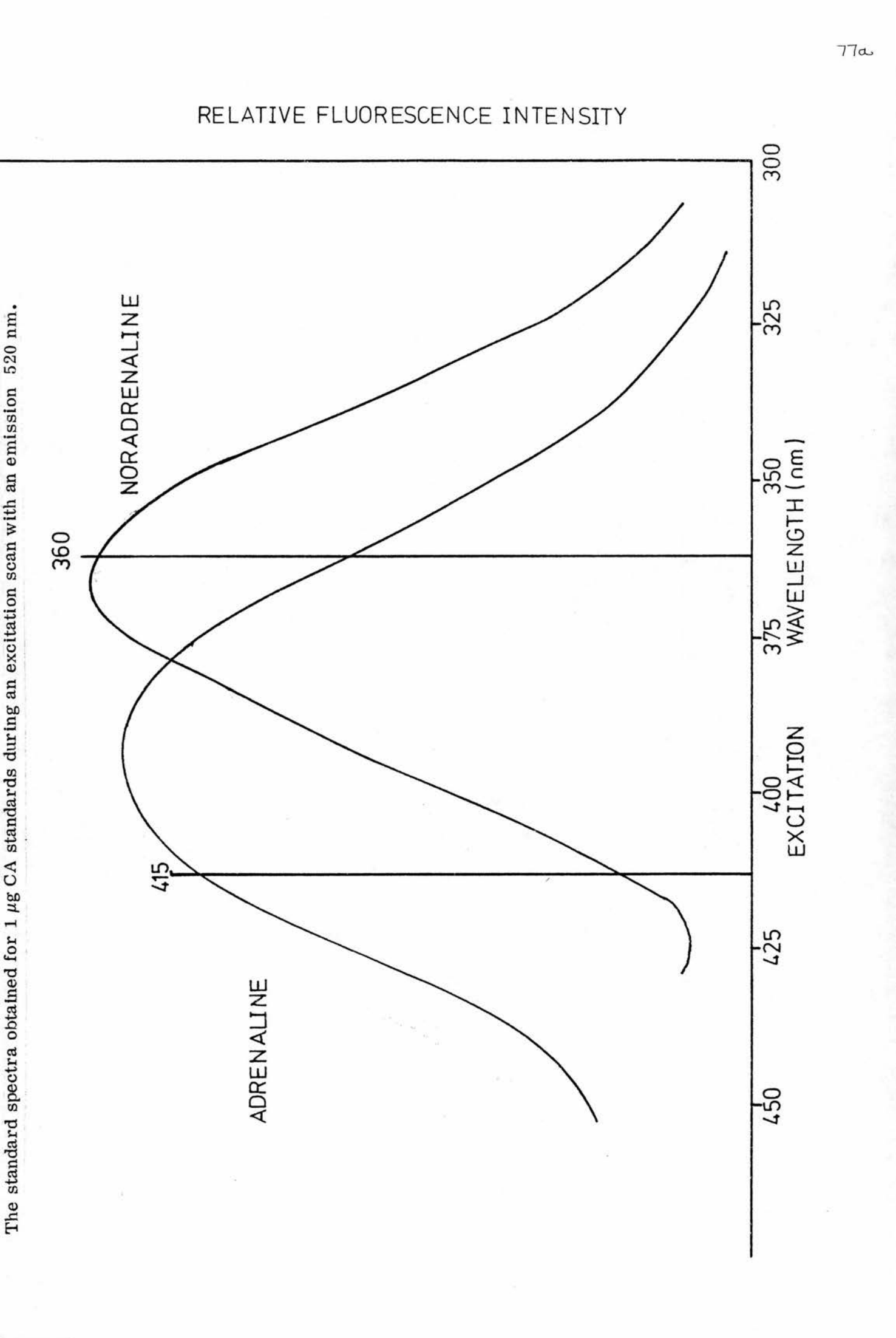
Measurement of Sample Fluorescence

Samples were measured for fluorescence using an Aminco-Bowman Model SPF Spectrophotofluorimeter. The cuvette holder was adapted to hold the test tubes used in the analyser for sample collection as previous investigations showed that these test tubes (Pyrex 7.5 cm x 1 cm) did not appreciably absorb U.V. light over the wavelengthsused. The slit widths were selected as a compromise between sensitivity and reduction of scatter and were as follows:-

Slit A	None	Slit B	2 mm	Slit C	1 mm
Slit D	4 mm	Slit E	(Photomultiplier tube)	5 mm	

Slit A and slit B are on input light-path to cell.
Slit C, slit D and slit E are on emission light-path from cell.

An emission wavelength of 520 nm was selected and an excitation scan performed from 300 nm to scatter (approx. 500 nm). The intensity of fluorescence emitted by the sample was displayed on the scale of the photomultiplier amplifier which has a meter multiplier switch enabling the sensitivity range to be changed from high sensitivity, 0.001 to low sensitivity 1.0 with approximately three-fold differences between positions. The output from the meter produced deflections on a Servoscribe flat bed recorder where the voltage range for full scale deflection could be used to change the overall sensitivity (0.5 V, F.S.D. was normal working range). The



The standard spectra obtained for 1 μg CA standards during an excitation scan with an emission 520 nm.

chart drive of the recorder was synchronised to the scanning switch so that the paper moved with the scan. This enabled the wavelength to be calculated from the distance moved along the chart. Two wavelengths were selected from the superimposed noradrenaline and adrenaline standard curves (see Fig. 19) for the measurement of the fluorescence. The wavelengths chosen were those producing the maximum ratio between the two catecholamines but ensuring a high fluorescence intensity above that for the blank value. They were without interference with the scatter peak near the emission wavelength which can vary greatly from sample to sample. From the fluorescence for the 'unknown' and the noradrenaline and adrenaline standards at the two chosen wavelengths the concentration of the two catecholamines and their relative ratios in a simultaneous sample were calculated, using simultaneous equations via a PDP8 (Digital) computer programme. (See Fig. 20).

Fig. 20

The use of simultaneous equations to calculate the concentration (μg/ml) of noradrenaline and adrenaline and their proportions in an unknown mixture.

$$F_1 = y\,NA_1 + x\,A_1$$

$$F_2 = y\,NA_2 + x\,A_2$$

where x and y are the amounts of adrenaline and noradrenaline respectively.

F_1 and F_2 are fluorescence readings corrected for blank values, at 360/520nm and 415/520nm respectively.

and NA_1, NA_2, A_1 and A_2 are the fluorescence values per μg of noradrenaline and adrenaline at the two excitation wavelengths.

$$y = \frac{F_1\ A_2/A_1 - F_2}{NA_1A_2/A_1 - NA_2}$$

$$x = \frac{F_2 - y\,NA_2}{A_2}$$

FROG RECTUS PREPARATIONS

The frog rectus preparation was chosen for the investigation of affinity constants for "muscarinic" antagonists at nicotinic receptors.

Frogs were stunned and pithed and the rectus abdominus muscle removed and separated into two sections. The muscle was placed in an organ bath, at room temperature, and bathed with Frog-Ringer solution of the following composition NaCl, 108 mM; KCl, 1.8 mM; $CaCl_2$, 1.1 mM; NaH_2PO_4, 0.1 mM; $NaHCO_3$, 2.4 mM; Glucose, 11.1 mM; gassed with air. The muscle was attached at one end to a hook with the free end connected to an isotonic lever system. The movement of an iron slug, attached to the lever, within a transducer coil produced a signal which was amplified, and recorded on a Vitatron flat bed recorder. Following an equilibration period of one hour, during which time the rectus was allowed to stretch against an additional 1 g weight, the required drugs, dissolved in Ringer, were added to the 10 ml organ bath in the following cycle:-

O min	Raise 1 g weight and start recorder
2 min	Add agonist dose
5 min	Stop recorder; Wash rectus twice; Lower 1 g weight to stretch muscle.
10 min	Raise 1 g weight and start recorder

The agonist used was carbachol because of its resistance to acetylcholinesterase, and this dose schedule proved suitable. Following dosage with two different agonist concentrations, repeated twice in a Latin Square, this sequence was repeated again only after equilibration for 1 hour with a known concentration of antagonist. The tissue was always in the presence of the given concentration of antagonist at all times during this stage of the experiment. In a number of preparations a second dose of antagonist was added, usually to give ten times the initial concentration, and a further series of agonist doses administered.

From the results obtained, log. dose/response curves were individually plotted for each preparation and the affinity constant calculated

for the antagonist, from the Gaddum/Schild equation where parallelism occurred between log. dose/response curves.

INDIVIDUAL EXPERIMENTS

AN INVESTIGATION OF VASOMOTOR REFLEX TRANSMISSION THROUGH THE SYMPATHETIC GANGLIA OF THE DOG

Residual transmission of preganglionic stimuli, through sympathetic ganglia, following nicotinic blockade has been attributed to an involvement of muscarinic receptors in transmission (Brown, A.M., 1967; Flacke and Gillis, 1968).

Similarly reflex vascular responses to carotid occlusion and to raised intracranial pressure have components not abolished by nicotinic antagonists but which are atropine-sensitive (Hilton and Steinberg, 1966; Steinberg and Hilton, 1966) though it has been reported that some non-nicotinic transmission may involve a second type of muscarinic receptor not sensitive to atropine but blocked by the amino acid, taurine (Hilton, 1977). Some evidence of an action of atropine alone on sympathetic transmission, has been reported but the results were inconclusive (Chinn and Hilton, 1976).

These experiments have often involved assessment of end organ responses where alterations in other uncontrolled parameters may have influenced the results following drug treatment. In the present study vascular resistance changes were measured in a perfused vascular bed where they indicated changes in sympathetic nerve activity. One was able to stimulate selectively two separate reflex pathways enabling any differences in the pharmacology of sympathetic ganglion transmission to be investigated.

METHODS

Dogs weighing between 9 and 31 kg were anaesthetised with sodium pentobarbitone (30 mg/kg I.V.) and the experiment performed following the methods described in the previous section. Baroreceptor tests were performed four minutes after each chemoreceptor test and a recovery time of two minutes was allowed prior to chemoreceptor tests. Care was taken to match the baroreceptor and chemoreceptor stimuli as closely as possible so as to obtain matched vascular responses to both types of test.

RESULTS

Selectivity of Reflex Stimuli

In two dogs, a local injection of 0.5 ml 2% lignocaine which abolished transmission in the carotid sinus nerve led to a sharp rise in hind-limb perfusion pressure from 165 and 135 mm Hg to 257 and 270 mm Hg respectively, recovering to 200 mm Hg within 10 minutes in both dogs. Similar rises occurred in systemic blood pressure. As one can observe from Fig 21 sinus nerve block completely abolished the vascular responses to both kinds of tests and abolished the reflex changes in systemic blood pressure. In spontaneously breathing dogs the respiratory response to chemoreceptor tests was also abolished. Both reflex responses had recovered after 1 hr.

For the present experiments it was necessary to exclude an action of hyoscine methylbromide on the sensory side of the chemoreceptor reflex. R.J. Docherty and D.S. McQueen (Personal Communication) found that hyoscine methylbromide, at the dosage used in our experiments, did not alter the resting discharge in single afferent fibres from the carotid body, or the increase in impulse frequency in response to cyanide, acetylcholine or nitrogen breathing. In one experiment the reflex effect of chemoreceptor tests on pulmonary ventilation was tested in a spontaneously breathing dog. Neither the resting ventilation, as recorded by an integrated pneumotachogram, nor the increase during chemoreceptor tests, was affected by hyoscine methylbromide. No deterioration of the ventilatory response to chemoreceptor tests was observed during the time course of the experiment.

The Action of Hexamethonium Bromide on Reflex Vasoconstriction

In eight dogs, baroreceptor and chemoreceptor tests were performed before and, between 10 and 30 minutes after, the administration of the nicotinic antagonist, hexamethonium bromide (1 - 2 mg/kg I.V.). The systemic blood pressure tended to fall after hexamethonium but this

Fig. 21

Records from three experiments showing the reflex vasoconstriction following baroreceptor and chemoreceptor tests before and after drug administration. Sections A ,C and E show control vascular responses. Section B shows the abolition of responses 15 min after carotid sinus nerve block. Section D shows the reflex vascular responses to both tests 20 min after hexamethonium bromide (2 mg/kg i.v.). Section F shows the reflex vascular responses to both tests 30 min after hyoscine methylbromide (10 mg/kg i.v.). SBP - mean systemic blood pressure; CPP - carotid perfusion pressure; HLPP - Hind limb perfusion pressure.

Fig. 21

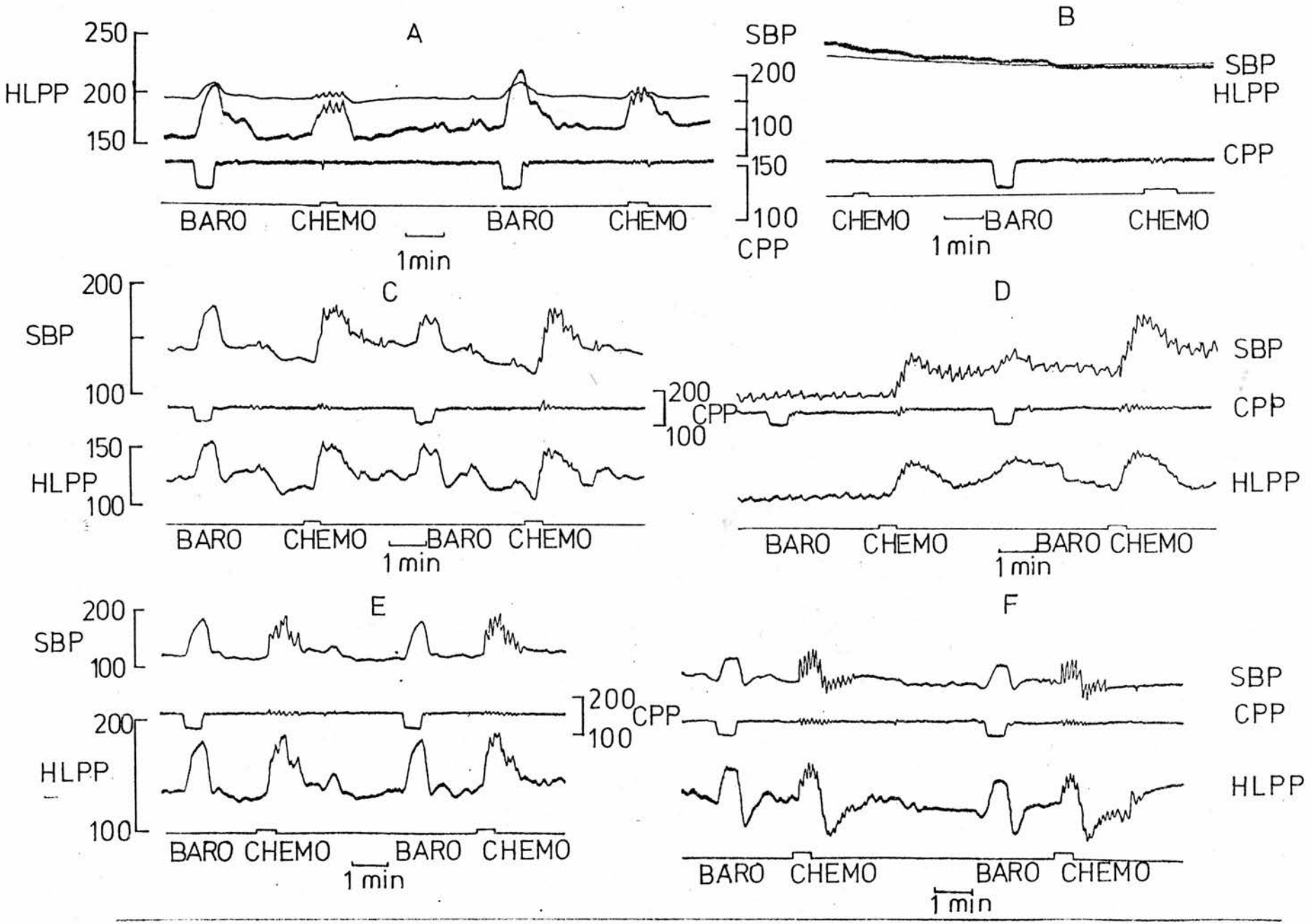

A
250
HLPP 200
150
BARO
CHEMO
BARO
CHEMO
1min
B
SBP
200
100
150
100
CPP
SBP
HLPP
CPP
CHEMO
BARO
CHEMO
1 min
C
200
SBP
100
150
HLPP
100
BARO
CHEMO
BARO
CHEMO
1 min
D
200
CPP
100
SBP
CPP
HLPP
BARO
CHEMO
BARO
CHEMO
1 min
E
200
SBP
100
200
HLPP
100
BARO
CHEMO
BARO
CHEMO
1 min
F
200
CPP
100
SBP
CPP
HLPP
BARO
CHEMO
BARO
CHEMO
1 min

TABLE 1

THE MODIFICATION OF VASOMOTOR REFLEXES BY HEXAMETHONIUM

CPP - Carotid Perfusion Pressure HLPP - Hind-Limb Perfusion Pressure

	BEFORE HEXAMETHONIUM				AFTER HEXAMETHONIUM				INHIBITION %	
	Baroreceptor test		Chemoreceptor test		Baroreceptor test		Chemoreceptor test			
DOG NO.	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (kPa)	HLPP RISE (mmHg)	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (kPa)	HLPP RISE (mmHg)	BARORECEPTOR TESTS	CHEMORECEPTOR TESTS
1	150→120	38	14.2→6	49	150→120	6	14.6→5.5	38	84	22
2	150→100	33	23.5→2.8	12	150→100	4	23.5→3.3	12	88	0
3	130→110	31	14.4→6.4	40	130→110	7	14.4→6.4	40	78	0
4a	130→110	12	14.9→6.1	12	130→110	3	14.5→4.1	11	75	8
4b	130→110	17	14.6→5.2	9	130→110	3	15.7→6.6	9	83	0
5	130→100	29	17.3→4.8	30	130→100	12	14.2→4.4	29	59	3
6	120→100	51	18.4→5.6	78	120→100	4	16.1→5.1	65	92	17
7	150→100	66	15.6→6.1	53	150→100	13	14.1→5.2	50	80	3
8	130→ 80	67	11.6→4.0	53	130→ 80	10	10.2→4.8	57	85	-7
	Means ± S.E.	38 ± 4 (n=20)		37 ± 5 (n=20)		6 ± 1 (n=24)		36 ± 4 (n=24)		

was alleviated by the addition of the drug as a number of divided doses and by injection of 5% dextran in 5% dextrose solution to combat the reduced venous return resulting from venodilatation. There was a transient fall in hind-limb perfusion pressure which was corrected where necessary by increasing the flow. The mean systemic blood pressure was 90 ± 4 mm Hg before and 78 ± 5 mm Hg after hexamethonium bromide. The mean resting hind-limb perfusion pressure was 106 ± 2 mm Hg before and 105 ± 2 mm Hg after hexamethonium. As can be observed from Table 1 and Fig 21 hexamethonium bromide almost completely abolished the responses to baroreceptor tests, but did not significantly alter those to chemoreceptor tests.

The Action of Hyoscine Methylbromide on Reflex Vasoconstriction

In seven dogs, baroreceptor and chemoreceptor tests were performed before, and between 20 and 40 minutes after, the administration of the muscarinic antagonist, hyoscine methylbromide (10 mg/kg I.V.). The mean systemic blood pressure was 107 ± 3 mm Hg before and 104 ± 4 mm Hg after the drug. The mean resting hind-limb perfusion pressure was 117 ± 2 mm Hg before and 113 ± 2 mm Hg after hyoscine methylbromide. The results, as illustrated in Table 2 and Fig 21, indicate that hyoscine methylbromide substantially inhibited the response to chemoreceptor tests ($p < 0.01$), but had little or no effect on those to baroreceptor tests.

The Combined Action of Hexamethonium Bromide and Hyoscine Methylbromide on Reflex Vasoconstriction

In five dogs, as can be observed in Fig 22 , no residual response to baroreceptor tests or chemoreceptor tests was observed in the presence of both hexamethonium bromide (2 mg/kg I.V.) and hyoscine methylbromide (10 mg/kg I.V.). (Fig. 22, p 85a; Table 3, p 84a)

TABLE 2

THE MODIFICATION OF VASOMOTOR REFLEXES BY HYOSCINE METHYLBROMIDE

	BEFORE HYOSCINE METHYLBROMIDE				AFTER HYOSCINE METHYLBROMIDE				INHIBITION %	
	Baroreceptor test		Chemoreceptor test		Baroreceptor test		Chemoreceptor test		BR	CR
Dog No.	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)	TESTS	TESTS
9	150 → 120	55	20.9 → 6.1	47	150 → 120	55	24.5 → 6.2	33	0	30
10	150 → 120	18	19.7 → 5.3	19	150 → 120	21	18.1 → 5.3	7	-17	64
11	150 → 120	47	12.8 → 5.1	57	150 → 120	38	16.5 → 5.7	29	19	49
12	150 → 130	53	24.0 → 8.0	58	150 → 130	46	18.2 → 7.3	28	14	52
13	150 → 130	24	25.0 → 6.4	46	150 → 130	26	24.6 → 6.4	38	-8	17
14	150 → 120	44	19.4 → 5.5	32	150 → 120	41	20.3 → 4.3	20	6	37.5
15	150 → 120	21	14.2 → 4.2	45	150 → 120	23	15.3 → 5.3	21	-9	50
	Means ± S.E.	42 ± 4 (n=20)		44 ± 3 (n=20)		37 ± 3 (n=21)		24 ± 2 (n=21)		

The Action of Taurine on Reflex Vasoconstriction

In three dogs, baroreceptor and chemoreceptor tests were performed before, and between 15 and 30 minutes after, the administration of taurine (30 mg/kg I.V.). No change was observed in hind-limb perfusion pressure or systemic blood pressure following administration. In only 1 of 3 animals did depression of the reflex responses occur following taurine dosage and in this case both reflexes were similarly depressed. It appears from the results in Table 3 that taurine may, only in some instances, inhibit the reflex vasoconstriction, and then during both baroreceptor and chemoreceptor tests.

The Action of Guanethidine Sulphate on Reflex Vasoconstriction

In two dogs, guanethidine sulphate (10 mg/kg I.V.) abolished the changes in hind-limb perfusion pressure in response to baroreceptor and chemoreceptor tests. This confirmed that the mediation of the reflex responses was by increases in sympathetic adrenergic neural activity.

Non-Selectivity of Hexamethonium Bromide

In four additional dogs, hexamethonium bromide (2 mg/kg I.V.) resulted in the abolition of vascular resistance changes to both tests. After a period of thirty minutes the responses to baroreceptor tests became increasingly greater implying a steady decline in the nicotinic antagonism by hexamethonium. The response to chemoreceptor tests did not return and appeared to be permanently impaired. This could result from damage to the sensory pathway or central integration of this reflex and this was only observed in dogs where a very large abrupt fall in systemic blood pressure occurred following hexamethonium administration. This effect was never seen after the administration of the antagonist slowly, together with the addition of a plasma expander. When this technique was used, the action of hexamethonium bromide was always selective.

TABLE 3

THE MODIFICATION OF VASOMOTOR REFLEXES BY HEXAMETHONIUM IN COMBINATION WITH HYOSCINE METHYLBROMIDE (H.M.B.)

	BEFORE HEXAMETHONIUM + H.M.B.				AFTER HEXAMETHONIUM + H.M.B.			
	Baroreceptor Test		Chemoreceptor Test		Baroreceptor Test		Chemoreceptor Test	
Dog. No.	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)
4b	130 → 110	17	14.6 → 5.2	9	130 → 110	0	17.8 → 4.7	0
9	150 → 120	55	20.9 → 6.1	47	150 → 120	1	25.5 → 5.7	2
10	150 → 120	18	19.7 → 5.3	19	150 → 120	0	21.7 → 5.2	0
11	150 → 120	47	12.8 → 5.1	57	150 → 120	0	14.6 → 5.4	0
12	150 → 130	53	24.0 → 8.0	58	150 → 130	3	21.3 → 7.8	1
14	150 → 120	44	19.4 → 5.5	32	150 → 120	5	20.2 → 5.4	5
	MEAN ± S.E.	38 ± 4 (n=14)		34 ± 5 (n=14)		1 ± 0.5 (n=12)		2 ± 1 (n=12)

THE MODIFICATION OF VASOMOTOR REFLEXES BY TAURINE ADMINISTRATION

	BEFORE TAURINE				AFTER TAURINE				% INHIBITION	
	Baroreceptor Test		Chemoreceptor Test		Baroreceptor Test		Chemoreceptor Test		BR	CR
Dog No.	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)	CPP FALL (mmHg)	HLPP RISE (mmHg)	CAROTID pO_2 (KPa)	HLPP RISE (mmHg)	TESTS	TESTS
16	150 → 100	46	13.0 → 6.0	41	150 → 100	61	14.4 → 5.8	50	-32	-10
17	150 → 100	61	14.4 → 5.9	50	150 → 100	66	15.6 → 6.1	53	-8	-18
18	130 → 80	36	11.0 → 4.2	48	130 → 80	19	10.5 → 5.2	33	49	31

DISCUSSION

Baroreceptor Reflex

Our results indicate that the reflex vasoconstriction resulting from inhibition of carotid sinus baroreceptor afferent discharge is wholly abolished by a nicotinic antagonist. It would appear that transmission of this reflex through the sympathetic ganglia involves a purely nicotinic pathway. These results seem to conflict with those of previous workers who have observed a component of the reflex, initiated by carotid occlusion, which was resistant to nicotinic blockade but was atropine sensitive (Steinberg and Hilton, 1966).

Carotid occlusion gives a mixed baroreceptor and chemoreceptor reflex response unless high arterial oxygen tensions are maintained and sinus perfusion pressure is kept above 80 mmHg., since the carotid bodies are sensitive to ischaemia (Heymans and Neil, 1958). This may explain the apparent inconsistencies between the present and previous work.

Chemoreceptor Reflex

The results for chemoreceptor reflex transmission are harder to interpret. Reflex vasoconstriction in response to chemoreceptor stimulation was unaltered by nicotinic blockade but was decreased by hyoscine methylbromide alone with a partial blockade varying from dog to dog between 17% and 64%. As with the baroreceptor reflexes all vascular responses to chemoreceptor reflexes were abolished by the nicotinic and muscarinic antagonists in combination. The simplest explanation of the results is that the main pathway of the chemoreceptor vasomotor reflex is by non-nicotinic transmission to adrenergic post-ganglionic fibres in the sympathetic ganglia, sensitive to high concentrations of muscarinic antagonists, and that a subsidiary nicotinic pathway can sustain partial transmission after muscarinic blockade.

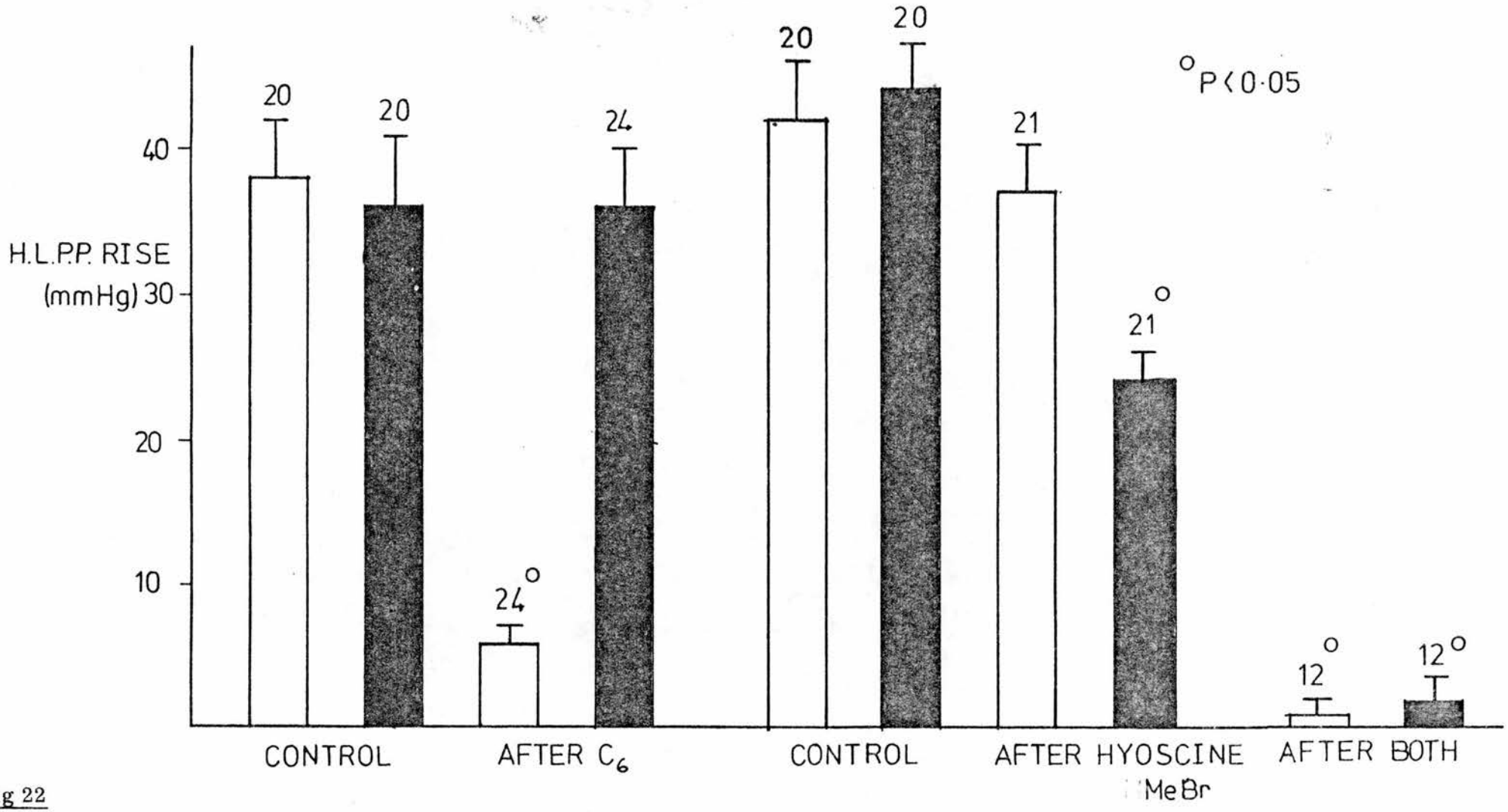


Fig 22

The modification of hind limb responses to baroreceptor and chemoreceptor tests by hexamethonium bromide and hyoscine methylbromide. Open bars baroreceptor reflexes. Closed bars chemoreceptor reflexes.

Pharmacology of Chemoreceptor Reflex Transmission

A problem is the high dose of muscarinic antagonist needed to inhibit the chemoreceptor reflex. The doses used were 100 times greater than those needed to block vagal bradycardia. Hilton, 1978 has postulated the existence, in the canine caudal cervical ganglion, of two muscarinic receptor types of which only one was sensitive to low doses of atropine.

The other receptor could be occupied by taurine or hydralazine (Gomez and Hilton, 1978). We were unable to observe any selective antagonism of the chemoreceptor reflex by taurine and it seems unlikely that it occupies the receptor involved in transmission of the chemoreceptor reflex. A more likely explanation of taurine's action is that a transient inhibition of all ganglionic transmission occurs which can be overcome by a stronger stimulus. This would agree with the electrophysiological data of Bowery and Brown, 1974.

Secondary Vasodilatation and Capacitance Vessels

In most of the preparations a distinct secondary vasodilatation was observed following primary reflex vasoconstriction of the hind-limb resistance vessels. The sensitivity of this vasodilatation to drugs and the vascular responses of a perfused saphenous vein to baroreceptor and chemoreceptor tests, and their modification by drugs, are discussed in Appendix 1.

THE POTENCY OF ATROPINE SULPHATE AS AN ANTAGONIST AT GANGLIONIC MUSCARINIC RECEPTORS AND CARDIAC MUSCARINIC RECEPTORS

The necessity to use large doses of a muscarinic antagonist to inhibit the chemoreceptor reflex transmission through the sympathetic ganglia raised the possibility of a heterogeneous muscarinic receptor population. The quantitative investigation of any possible differences in antagonist affinity for different populations of receptors is very difficult as one cannot determine the concentration of antagonist in equilibrium with the receptor sites.

In the present study a comparison was made between the dose of atropine sulphate necessary to produce similar shifts in the frequency-response relationship for vagally induced cardiac slowing, and the frequency-response relationship for changes in hind-limb vascular resistance produced by preganglionic nerve stimulation. As the former action involves a blockade of vagal postganglionic receptors for which an atropine affinity constant has been determined, in vitro, (Barlow, 1971), it was thought, if one assumes similar access to either site, differences in the required dose might provide a guide to differences in receptor type.

METHOD

Dogs were anaesthetised with sodium pentobarbitone (30 mg/kg I.V.) and maintainence doses were administered when required.

Vagal Bradycardia

The peripheral end of the right vagosympathetic trunk was prepared in the neck and inserted through a circular stimulation electrode connected to a Tektronix stimulator. The other trunk was cut. A pulsatile systemic blood pressure was recorded using a fluid filled cannula inserted in the R. common carotid artery to which a pressure transducer (Bell and Howell, L222) was connected via a side-arm. The signal from the

transducer was channelled to an EAL 380 analog computer on which was assembled a ratemeter circuit enabling the heart rate to be measured, beat by beat, in beats/min. Incorporated into the ratemeter was a 'refractory period' to avoid double triggering from a notched pressure trace. Following the initial stage of the experiment the animal was heparinised (1000 units/kg I.V.) following opening of the abdomen, location of the sympathetic trunk and completion of the hind-limb surgery.

Preganglionic Stimulation and Vascular Changes

As described previously, one hind-limb vascular bed was perfused at constant flow. The sympathetic chain was located on the left side, placed on shielded Pt. electrodes at the level L1 - L3 and transected above the electrodes. Stimulation pulses were produced by the Tektronix stimulation unit connected to the electrode via an isolating transformer, the electrodes being bathed in liquid paraffin to prevent local currents.

Experimental Procedure

The R. vagosympathetic trunk was stimulated with supramaximal stimuli (10 V of 10 m sec duration) over the frequency range 0.5 —> 4 c/s (for 30 second periods), every two minutes. The reduction in heart rate was frequency dependent and enabled frequency response curves to be constructed.

Following the administration of atropine sulphate (10 μg/kg —> 20 μg/kg) or (-) hyoscine methylbromide (2 μg/kg —> 4 μg/kg I.V.) ten minutes previous, further frequency response curves were obtained after two different antagonist concentrations.

In the second part of the procedure stimulation of the sympathetic chain supramaximally (15 V, 10 m sec duration) over the range 1 - 8 c/s, for 1 minute periods every five minutes, produced frequency dependent changes in hind-limb perfusion pressure. Once a frequency response sequence had been completed a dose of hexamethonium bromide

TABLE 4

THE MODIFICATION OF VAGAL SLOWING BY ATROPINE SULPHATE. RESULTS SHOWN ARE THE MEANS OF TWO OBSERVATIONS

Dog. No	CONTROL, VAGAL SLOWING OF HEART RATE (b/min) STIMULATION FREQUENCY					VAGAL SLOWING OF HEART RATE AFTER ATROPINE 10 μg/kg I.V. (b/min) STIMULATION FREQUENCY					VAGAL SLOWING OF RATE AFTER ATROPINE 20 μg/kg I.V. (b/min) STIMULATION FREQUENCY				
	0.25 Hz	0.5 Hz	1 Hz	2 Hz	4 Hz	0.5 Hz	1 Hz	2 Hz	4 Hz	8 Hz	1 Hz	2 Hz	4 Hz	8 Hz	16 Hz
A		5	10	17	30		6	10	19	32		7	9	26	35
B		6	8	13	20		4	7	12	22			9	13	24
C			10	15	22			9	13	22			10	16	24
D	7	11	21	30		6	9	17	30		5	7	13	33	
E		6	10	18	24		4	10	16	22		4	12	16	24
						SLOWING AFTER HYOSCINE METHYLBROMIDE (H.M.B.) 2 μg/kg I.V. (b/min)					SLOWING AFTER HMB 4 μg/kg I.V. (b/min)					
F	36	36	59	80			44	47	52	74			28	42	57
G		9	24	41	62		17	26	48	56		14	19	32	39

(2 mg/kg I.V.) was administered to the animals. The residual response to preganglionic nerve stimulation was to be used to obtain frequency-response relationships before, and after, the muscarinic antagonist.

RESULTS

The Action of Atropine Sulphate on Vagal Bradycardia

Frequency/response curves were constructed for vagal bradycardia. There was, in all five dogs, a parallel shift in this relationship following the administration of two doses of atropine sulphate (10 μg/kg and 20 μg/kg I.V.) and from this an equiactive frequency ratio was obtained. As the concentration of antagonist in equilibrium with the receptor population in the heart is unmeasurable, further calculation is not possible. In two dogs, a similar shift was observed after (-) hyoscine methylbromide (2 μg/kg I.V.). These results illustrate that ten minutes exposure to a low intravenous dose of atropine sulphate, in the μg range, produced a substantial reduction in vagally induced bradycardia.

The Action of Antagonists on Sympathetic Ganglionic Transmission

In only three out of seven dogs were residual responses to preganglionic sympathetic stimulation observed following dosage with hexamethonium bromide. As can be seen from Table 5 hexamethonium bromide (2 mg/kg I.V.) almost completely abolished the increase in hind-limb perfusion pressure following different frequencies of pre-ganglionic nerve stimulation in five out of seven animals. In the other two dogs some residual response was observed but the frequency response relationship was very different with a much flattened slope and lower maximum.

Although atropine sulphate (1 mg/kg or 2 mg/kg I.V.), ten minutes previously, produced a shift to the right of the frequency/response

TABLE 5

THE MODIFICATION BY CHOLINERGIC ANTAGONISTS OF HIND-LIMB VASOCONSTRICTION FOLLOWING PREGANGLIONIC SYMPATHETIC NERVE STIMULATION

RISES IN HIND-LIMB PERFUSION PRESSURE (mmHg)

DOG	CONTROL				AFTER C_6 2 mg/kg I.V.				AFTER ATROPINE 1 mg/kg I.V. SUBSEQUENT TO HEXAMETHONIUM (C_6)		
	STIMULATION FREQUENCY				STIMULATION FREQUENCY				STIMULATION FREQUENCY		
	0.5 Hz	1 Hz	2 Hz	4 Hz	0.5 Hz	1 Hz	2 Hz	4 Hz	2 Hz	4 Hz	8 Hz
A		30	32	36		14	15	16	DEATH OF ANIMAL		
B		35	39	43	3	4.5	7		3.5	4.5	7
C		21	28	38		7	9	10		6	8
D	35	60	90		1.5	2	2	2		-	-
E		11	16	19		1.5	3	5		-	-
F		20	24	31		0	0	0			
G			-		DECREASE ONLY, VASODILATION						

TABLE 6

FREQUENCY RATIOS FOR VAGAL SLOWING ANTAGONISED BY MUSCARINIC ANTAGONISTS (Detailed results p 140)

DRUG	MEAN FREQUENCY RATIOS
ATROPINE SULPHATE	2.1
10 μg/kg n = 5	(1.6 → 2.5)
20 μg/kg n = 5	4.4
	(3.2 → 7.0)
HYOSCINE METHYLBROMIDE	3
2 μg/kg n = 2	(1.5 → 5)
4 μg/kg n = 2	9
	(5 → 13)

FREQUENCY RATIOS FOR NEUROGENIC HIND-LIMB VASOCONSTRICTION ANTAGONISED BY ATROPINE SUBSEQUENT TO HEXAMETHONIUM

DRUG	MEAN FREQUENCY RATIOS
ATROPINE SULPHATE	4
1 mg/kg I.V. n = 2	(3 → 5)

In only 3 out of 7 dogs was a residual response observed following nicotinic blockade by hexamethonium.

TABLE 7

THE EFFECT OF HYOSCINE METHYLBROMIDE 10 MG/KG I.V. ON HIND-LIMB VASOCONSTRICTION PRODUCED BY PREGANGLIONIC SYMPATHETIC NERVE STIMULATION (Detailed results p141)

CONTROL RESPONSES (mm Hg)		RISE IN HLPP FOLLOWING HYOSCINE METHYLBROMIDE (mm Hg)	
1 H_z	26	1 H_z	21
2 H_z	37.5	2 H_z	31
4 H_z	59	4 H_z	47

curve only a rough estimate of the "frequency-ratio" was obtainable, and then only in two preparations. In two dogs, atropine sulphate (2 mg/kg, I.V.) or hyoscine methylbromide (10 mg/kg I.V.) administered prior to the addition of hexamethonium bromide were without any action on the responses to preganglionic nerve stimulation (see Table 7).

DISCUSSION

These results indicate that most of the transmission of synchronous preganglionic supramaximal stimuli through the canine lumbar sympathetic ganglia is nicotinic. Some dogs appear to have a fibre population involved in non-nicotinic transmission which seems to be large enough to result in a residual response following nicotinic blockade. This residual transmission, when observed was sensitive to muscarinic antagonists in the mg. range contrasting with the much lower doses administered to produce similar inhibitions of vagal bradycardia. The absence of a residual component of sympathetic ganglionic transmission following nicotinic blockade contrasts with the situation prevailing in other canine sympathetic ganglia where a large component of the response to preganglionic stimulation is not hexamethonium sensitive (Fleisch, Flacke and Gillis, 1969).

This may imply that differences in the relative nicotinic and muscarinic receptor populations on the sympathetic ganglion cells occur between species and different ganglion within the same animal.

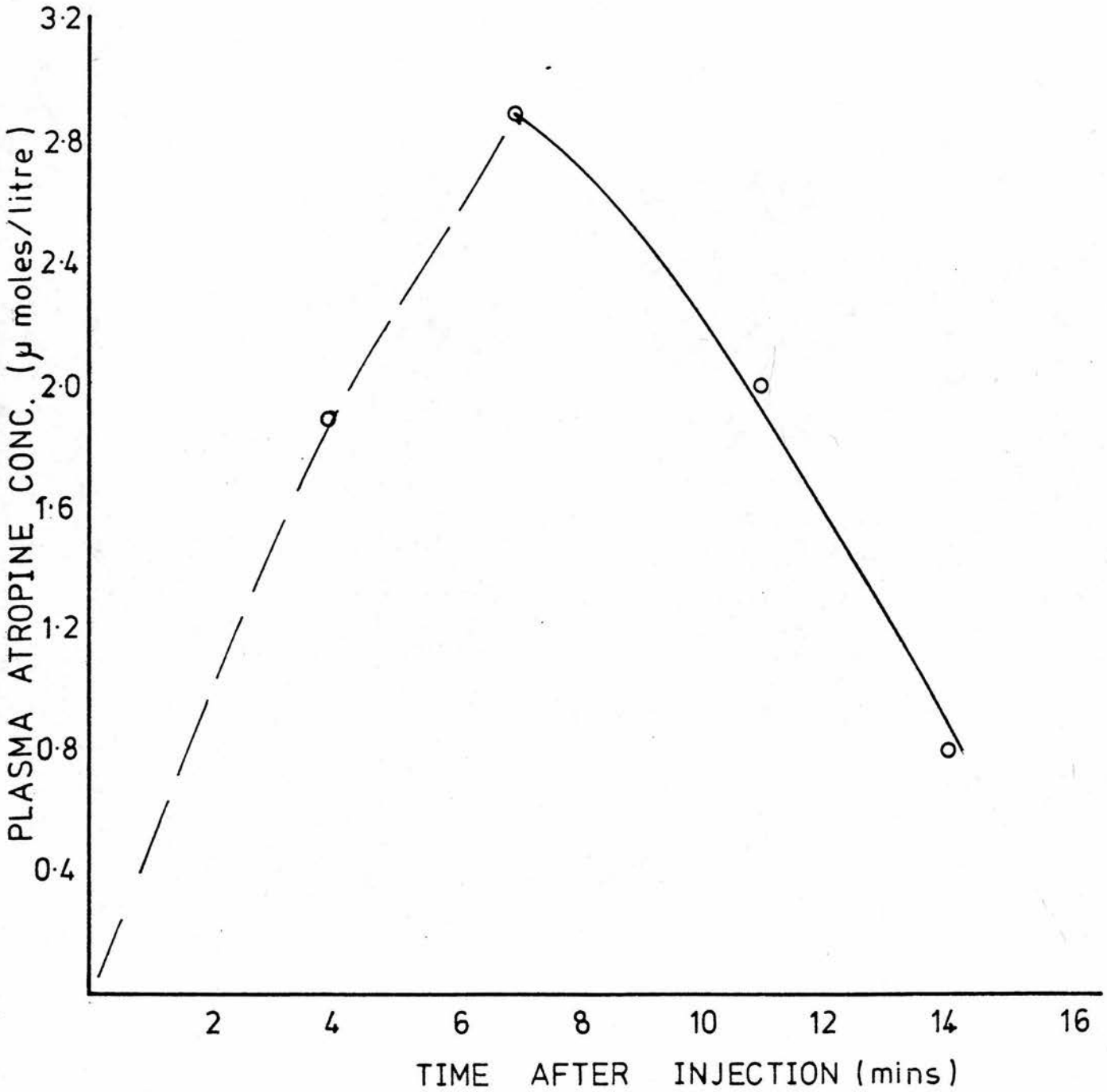
APPENDIX THE CLEARANCE OF ATROPINE SULPHATE IN THE DOG

In the animals which showed atropine-sensitive sympathetic ganglion transmission, following nicotinic blockade, the antagonism by atropine seemed very short-lived with full recovery of the response after 25 - 30 minutes. With this in mind, blood samples were taken from the femoral arteries of two dogs at three minute intervals following atropine dosage (10 mg/kg I.V.). The samples were centrifuged and the atropine concentration within the plasma assayed using guinea-pig ileum preparations with carbachol as the agonist.^ The results shown in Fig 23 indicate that

(Edinburgh Pharmacology staff, 1970)

Fig. 23

The plasma concentration/time curve for atropine sulphate following the administration I.V. of a dose. The calculated $t_{\frac{1}{2}}$ was 3 mins.



over the time period investigated the clearance of atropine from the plasma was very rapid, $t\frac{1}{2}$ approx. 3 minutes. This result has since been confirmed and extended by the precise radioimmunoassay technique (Wurzburger et al, 1977). They have observed a two phase clearance of atropine dependent on plasma concentration. This rapid clearance of high doses of atropine may be of great importance when one compares results obtained by different research groups. Many of the apparent observed differences may result from the differences in equilibration time allowed and hence the prevailing atropine concentration may differ greatly.

AN INVESTIGATION OF THE SELECTIVITY OF MUSCARINIC ANTAGONISTS FOR DIFFERENT CHOLINERGIC RECEPTORS

During two earlier experiments, prior to the present study, it was observed that very large doses of atropine sulphate (50 mg/kg, I.V.) were required to abolish the residual component of the chemoreceptor reflex after nicotinic blockade. This dose of atropine sulphate was sufficient to abolish responses to both reflexes. Although a large component of the action of atropine sulphate may have been centrally mediated, it was felt that some of the antagonist action might be accounted for by nicotinic receptor blocking or local anaesthetic actions. Our aim in these experiments was to obtain a muscarinic antagonist with a greater ratio of muscarinic to nicotinic blocking action than that for atropine sulphate, enabling high doses to be used while still maintaining selectivity.

The muscarinic receptor blocking potency of a number of readily obtainable antagonists, for example at the guinea-pig ileum, is well documented (Abramson, Barlow, Mustafa and Stephenson, 1969). The choice of a suitable tissue containing a nicotinic receptor population proved difficult. Ideally one would obtain a nicotinic antagonist affinity constant for the drugs used where the drugs interact with sympathetic ganglion receptors. As this is not possible except for parasympathetic ganglia, where the anti muscarinic action of the antagonists would interfere with the assay, it was decided to assess the neuromuscular blocking potency of the antagonists. The parallel between neuromuscular blocking potency and ganglionic blocking action appears to hold apart from the few selective agonists and antagonists (Barlow, 1971), though quantitative differences in affinity constant do occur. The preparation used was that of the frog rectus abdominus muscle using carbachol as the agonist to eliminate possible changes in agonist potency resulting from interactions of the antagonists with cholinesterases.

METHODS

The methodology employed is as described in the general methods section (pp 79 - 80).

RESULTS AND DISCUSSION

Antagonist Affinity Constants

The affinity constants of the antagonists tested were calculated from the Gaddum-Schild equation following the construction of log dose/response curves.

Parallel shifts in the log dose/response relationship following antagonist dosage, indicating a competitive inhibition, were obtained with all the antagonists except oxyphenonium bromide where the non-parallelism was very marked. It appears that oxyphenonium bromide is not a competitive antagonist for nicotinic receptors and some irreversible antagonism may account for the marked reduction in the slope of the linear portion of the log dose/response curve.

As can be noted from Table 8 a distinct order of neuromuscular blocking potency was obtained. The equipotent molar ratio was oxyphenonium : atropine : methylhyoscinium : hyoscine were 0.8 : 1 : 3 : 10. When these results are compared with the relative muscarinic antagonist affinity constants of the drugs (see Table 9) it can be seen that hyoscine methylbromide has the greatest ratio of muscarinic to nicotinic blocking potency. It is approximately five times more active than atropine sulphate as a muscarinic antagonist with only one-half the neuromuscular blocking action. This is a greater discrimination of effects than any of the other antagonists tested.

If one correlates the neuromuscular blocking potency with nicotine ganglionic receptor antagonist action it should have meant that a muscarinic antagonist had been found which was selective enough to have

TABLE 8

AFFINITY CONSTANTS FOR MUSCARINIC ANTAGONISTS ON THE NICOTINIC RECEPTORS OF THE FROG RECTUS ABDOMINUS MUSCLE

(Detailed results p142)

DRUG	MEAN DOSE RATIO	MEAN K_B	E.P.M.R. (Relative to atropine)
ATROPINE SULPHATE	8.8	7.8×10^4	1
10^{-4}M n = 4	(4 ⟶ 21)		
HYOSCINE HYDROBROMIDE	1.8	8×10^3	
10^{-4}M n = 3	(1.7 ⟶ 1.9)		10
10^{-3}M n = 4	10	9×10^3	
	(9 ⟶ 11)		
HYOSCINE METHYLBROMIDE	3.4	2.4×10^4	
10^{-4}M n = 4	(2.7 ⟶ 4.5)		2.8
10^{-3}M n = 2	34	3.3×10^4	
	(28 ⟶ 40)		
OXYPHENONIUM BROMIDE*	APPROX. 2	$\triangleq 10^5$	
10^{-5} n = 5			

E.P.M.R. - Equipotent Molar Ratio
K_B - Antagonist affinity constant.
* THIS DRUG PRODUCES A NON-COMPETITIVE INHIBITION

TABLE 9

A COMPARISON OF THE AFFINITY CONSTANTS FOR MUSCARINIC AND NICOTINIC RECEPTORS FOR THE ANTAGONISTS STUDIED.

ANTAGONIST	log K_B ILEUM* (MUSCARINIC)	log K_B RECTUS (NICOTINIC)	$\frac{K_B \text{ ILEUM}}{K_B \text{ RECTUS}}$
Atropine Sulphate	9.00	4.89	13,000
Hyoscine Hydrobromide	8.87	3.92	89,000
Hyoscine Methylbromide	9.70	4.44	182,000
Oxyphenonium Bromide	9.78	$\triangleq$ 5	$\triangleq$ 60,000

* From Barlow (1971)

negligible nicotinic receptor blocking action at the high doses necessary to inhibit the chemoreceptor reflex transmission.

This was proved to be the case as hyoscine methylbromide, though showing some transient non-selective action, solely produced chemoreceptor reflex inhibition without an alteration of the response to baroreceptor tests, at the 10 mg/kg I.V. dose level. In support of this the previous study showed no change in the vascular response to pre-ganglionic nerve stimulation, after hyoscine methylbromide administration (10 mg/kg I.V.).

In addition hyoscine methylbromide has a quaternary structure which should result in little central absorption allowing one to exclude an action of the drug on the central integration of the sensory side of the baroreceptor or chemoreceptor reflex pathway.

STUDIES USING ISOLATED ADRENAL GLAND PERFUSIONS

Evaluation of the Fluorimetric Assay Method

It was necessary to investigate the degree of reproducibility of the fluorimetric assay technique used in the perfused gland studies. Two parameters were investigated, these being the linearity of the assay over a range of catecholamine concentrations and the selectivity of the assay. The differential assay technique used was devised to enable one to distinguish changes in the relative proportions of noradrenaline and adrenaline in an adrenal gland effluent.

METHODS

Standard solutions of known concentration were prepared by the addition of 0.1 ml of a known concentration of catecholamine standard, stored at 4^{o}C in 0.01N, HCl, to 0.9 ml of phosphate-buffered Locke's solution. The samples were then exposed to the required reagents to produce the fluorescent lutine derivatives as described in the general methods (p 76). The final solutions were analysed fluorimetrically with an excitation scan from 300 nm —> 500 nm with an emission wavelength of 520 nm. The slit widths and amplifier gain setting were as have been used throughout this project (see p 77).

RESULTS

The method shows a high degree of linearity for measurements of noradrenaline and adrenaline concentrations from 1 μg/ml down to 50 ng/ml (see Table 11). The latter approximates to the lowest physiological values observed for total catecholamine concentration in a perfusate.

TABLE 10

THE VALIDATION, USING STANDARDS, OF THE TH1 FLUORIMETRIC ASSAY SELECTED (Detailed results p143)

(1) Varying compositions with a total catecholamine concentration of 100 ng/ml.

COMPOSITION OF MIXTURE	% NA	ESTIMATED % NA ± S.D.
100 ng/ml NA	100	97 ± 1 (n = 10)
70 ng/ml NA 30 ng/ml A	70	74 ± 3 (n = 8)
50 ng/ml NA 50 ng/ml A	50	56 ± 1 (n = 10)
30 ng/ml NA 70 ng/ml A	30	35 ± 6 (n = 8)
100 ng/ml A	0	0 (n = 9)

ESTIMATED % A = 101 ± 15

TABLE 11

LINEARITY OF THE ASSAY FOR THE MEASUREMENT OF NORADRENALINE AND ADRENALINE

(Individual estimations p144, p145)

NA CONCENTRATION	MEAN F x S (360 nm)	MEAN F x S (415 nm)	CALCULATED NA CONC. (ng/ml ± S.D.)
1 µg/ml (n = 10)	2.35	1.27	1000 ± 60
500 ng/ml (n = 10)	1.34	0.55	550 ± 60
100 ng/ml (n = 10)	0.27	0.12	115 ± 5
50 ng/ml (n = 10)	0.14	0.05	55 ± 5
BLANKS (n = 6)	0.024 ± 0.002	0.031 ± 0.002	

A CONCENTRATION	MEAN F x S (360 nm)	MEAN F x S (415 nm)	CALCULATED A CONC. (ng/ml ± S.D.)
1 µg/ml (n = 10)	1.27	4.20	1000 ± 60
500 ng/ml (n = 10)	0.58	2.41	510 ± 50
100 ng/ml (n = 10)	0.14	0.50	115 ± 5
50 ng/ml (n = 10)	0.07	0.23	60 ± 10

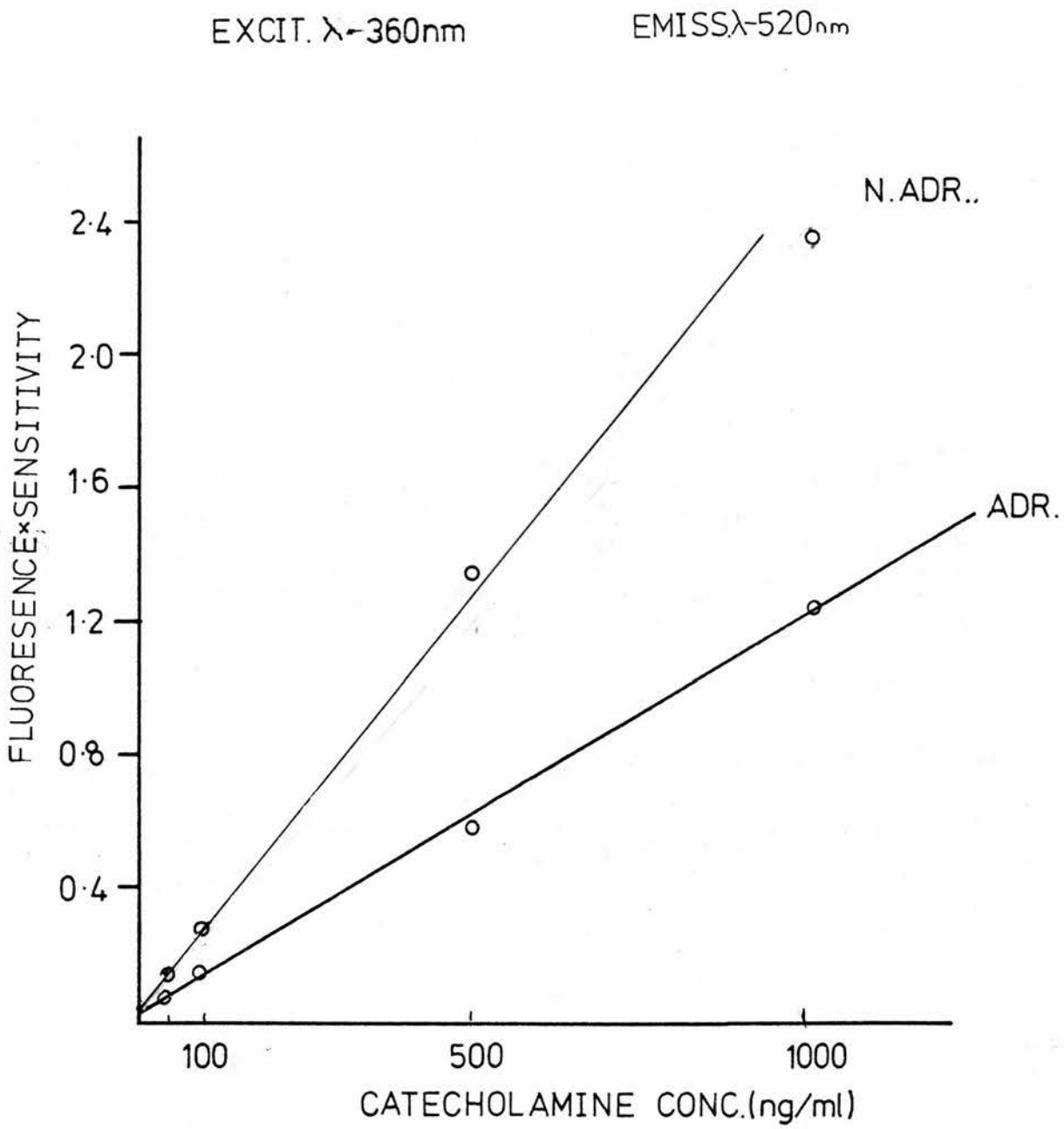
ALL VALUES ARE COMPARED TO THE 1µg/ml STANDARDS USED AS REFERENCE STANDARDS FOR THE CALCULATION

F x S = Fluorescence x Sensitivity setting

The relative contributions of noradrenolutine and adrenolutine at the two excitation wavelengths chosen for measurement (see Figs. 24A and 24B) allow the relative proportions of each catecholamine within a mixture to be measured. The ratio of relative fluorescence at the two measurement wavelengths is such that this assay method offers a differential measurement equal or better than any other reported scanning assay, though differential pH methods may offer greater discrimination (Diamant and Byers, 1976).

Fig. 24A

The relationship between concentration and fluorescence developed for noradrenaline and adrenaline at the two wavelengths selected for measurement.



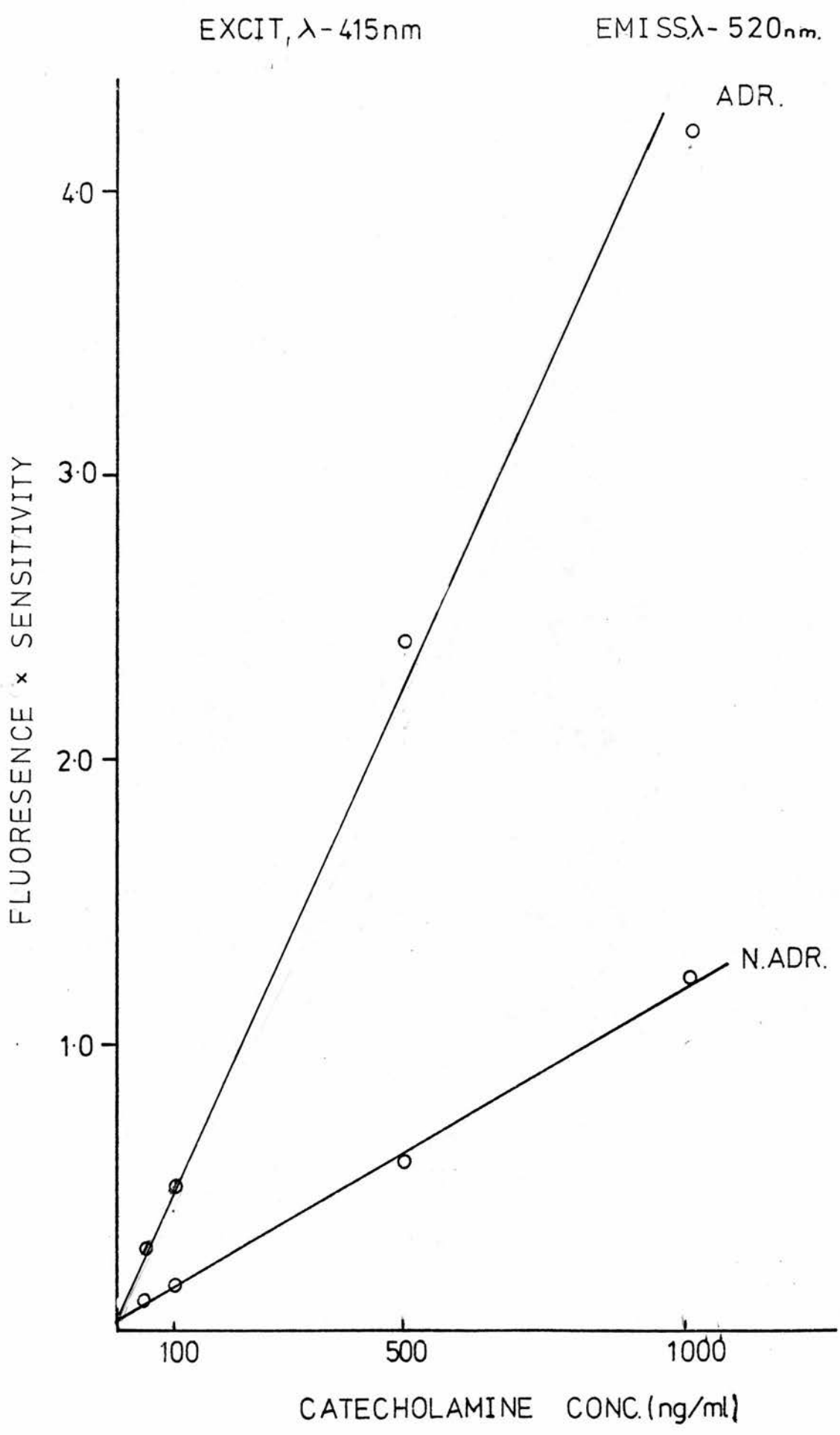


Fig. 24B

The relationship between concentration and fluorescence developed for noradrenaline and adrenaline at the two wavelengths selected for measurement.

THE RELEASE OF CATECHOLAMINES FROM ADRENAL GLANDS, PERFUSED IN VITRO, BY HYDROCORTISONE

There is conclusive evidence that corticosteroids, released into the adrenal portal system from the adrenal cortex, can regulate the biosynthesis of catecholamines within the chromaffin cells (see p 31). In addition to this regulatory role it is conceivable that corticosteroids might have a direct secretory stimulant action on the chromaffin cells though this action has been sparsely investigated.

Reductions in the catecholamine content of dog adrenal glands had been observed following treatment with an anterior pituitary extract (Houssay, Biasotti, Mayzocco and Sammartino, 1933) and similar depletions of catecholamines within rat adrenal glands were observed following corticotrophin dosage (Hökfelt, 1951; Eränkö, 1955). One interpretation of the results of Wurtman, Casper, Poherecky and Bartler, 1968 is that the reduction in canine adrenomedullary resting catecholamine output observed after hypophysectomy, and reversible by corticotrophin administration, indicates an involvement of the adrenocortical-pituitary axis in the control of adrenomedullary secretion. Confirmation for this concept comes from Critchley and Ungar, 1974 who studied the release of adrenomedullary catecholamines in response to prolonged chemoreceptor stimulation, in dogs. This increased secretion was abolished by cycloheximide an inhibitor of steroidogenesis and it was noted that the intravenous administration of a synthetic corticotrophin raised the output of adrenomedullary catecholamines over 50 minutes to 4 - 5 times resting levels in innervated or denervated glands. These findings suggested that the rapid and sustained rise of 11-hydroxycorticosteroid adrenal venous levels observed following hypoxia (Marotta, 1972) would be associated with a marked stimulatory action on the adrenomedullary chromaffin cells. A direct action of hydrocortisone on sheep adrenal glands was reported by Critchley, 1977 and this also may be the situation in the rat (Roffi, et al, 1966).

In this present study it was decided to further extend these observations by ascertaining whether hydrocortisone did in fact release catecholamines from the canine adrenal medulla in a dose dependent manner and whether this action of hydrocortisone might be paralleled in other species.

METHODS

As previously described (p68), adrenal glands were removed surgically from animals under anaesthetic prior to perfusion with Locke's solution. The glands were perfused at 2 ml/min at constant flow. Drug administration following preliminary experiments was by a five minute infusion of Locke's solution containing the appropriate drug concentration. The protocol for drug administration and sample collection was as shown in Table 12.

In experiments where an antagonist drug was used the appropriate concentration of the drug was allowed to equilibrate with the adrenomedullary receptors over 90 minutes and following antagonist administration the adrenal gland was always being perfused by this concentration of antagonist.

The catecholamine content of samples of perfusion effluent was assayed fluorimetrically as previously described and the total catecholamine concentration and the relative percentages of noradrenaline and adrenaline in the sample computed. The incremental release of adrenomedullary catecholamines is expressed as percentages of control adrenomedullary secretion rate with the percentage of noradrenaline in the increment compared with that of the resting output.

During the course of these experiments differences in the sensitivity and time-course of adrenomedullary response to drugs were observed and this is discussed.

TABLE 12

SCHEDULE FOR INFUSION OF HYDROCORTISONE
TO CANINE ADRENAL GLANDS

TIME (MINS)	
-2	Collect control adrenal gland effluent for 30 sec.
-1	Collect control adrenal gland effluent for 30 sec.
0	Switch on infusion
1	Collect consecutive samples of effluent for 30 sec.
2	
3	
4	
5	Collect consecutive samples of effluent for 30 sec. Switch off infusion.
6	Collect consecutive samples of effluent for 30 sec.
7	
8	

RESULTS

CANINE ADRENAL GLANDS

Variations in Sensitivity to Hydrocortisone Succinate

A large variation in the sensitivity of the secretory response of isolated, perfused, adrenal glands to hydrocortisone succinate was observed between glands removed from cross-bred collies or fox-hounds (see Fig. 25A and Fig. 25B). The evoked response in fox-hound glands was much less pronounced with a slower time course of secretion. This appears to concur with differences observed by Critchley and Ungar (Critchley, 1977) in the relative response of cross-bred collies and greyhounds to Adrenocorticotrophic hormone (A.C.T.H.) administration. The catecholamine release evoked by A.C.T.H. administration or prolonged chemoreceptor stimulation in the collies was of greater magnitude and shorter onset and duration, than that observed in greyhounds. It would appear that this difference in response may be a genuine difference in adrenal sensitivity between different breeds of dog though whether this applies for other secretagogues has not yet been investigated.

One must be aware before considering the results presented below that these represent data obtained from adrenal glands which responded to hydrocortisone and as such only represent approximately 50% of the adrenal glands removed during this study. Unresponsive glands (the other 50%) fell into two categories, the majority being unresponsive to all agonists whereas a number of glands were obtained where responses to agonists other than hydrocortisone were observed. It seems that the large variation in responsiveness depends largely on the previous experimental protocol and hence the stress to which the donor animal was subjected. One possibility is that maintained stress conditions may have resulted in depletion of the adrenomedullary catecholamines, or exposure to high concentrations of corticosteroid, thus making the gland insensitive to further steroid dosage. Attempts to overcome these problems by adrenal

CROSS-BRED COLLIES

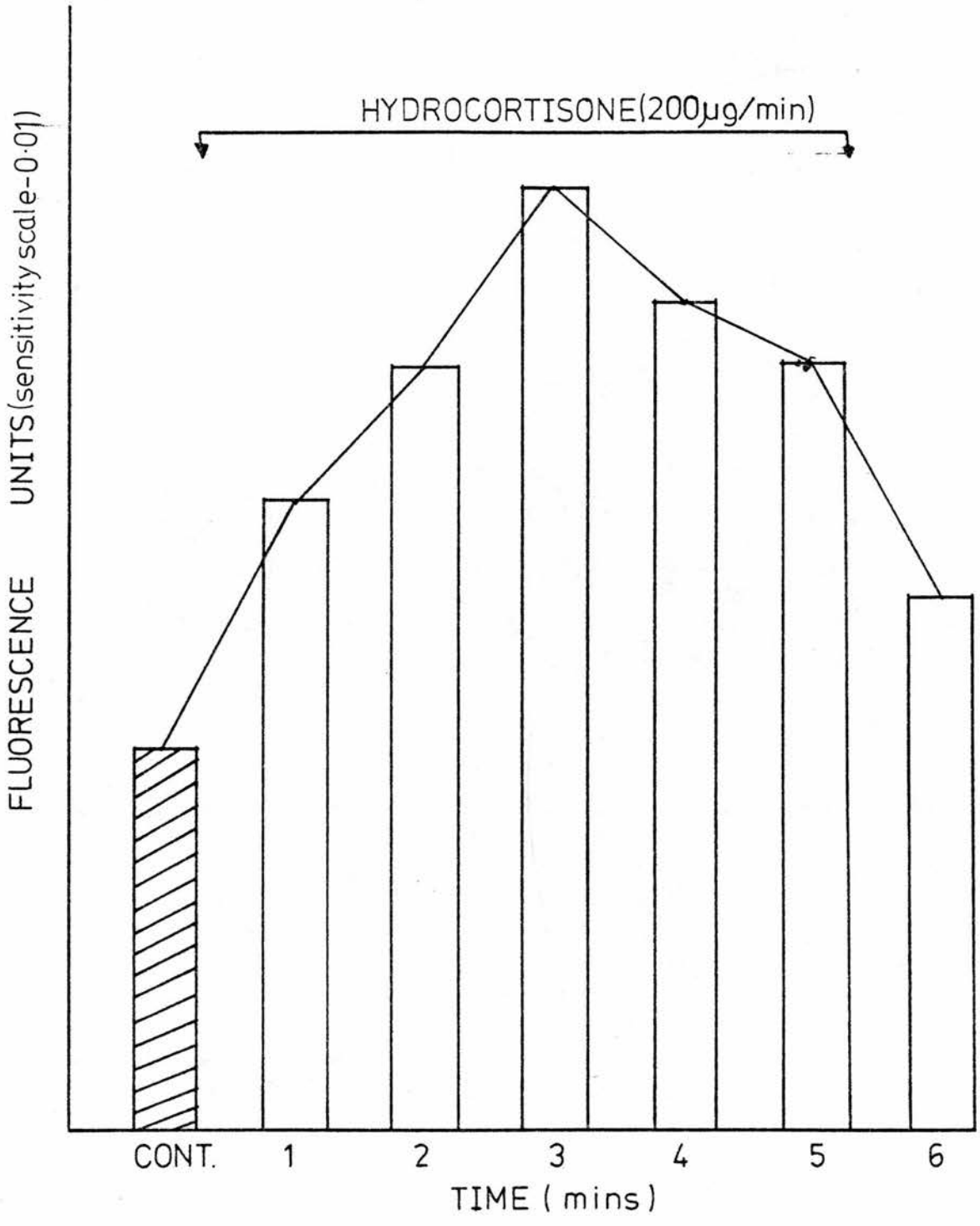


Fig. 25A

The time course of catecholamine secretion, evoked by hydrocortisone administration, from the adrenal glands of cross-bred collies and fox-hounds.

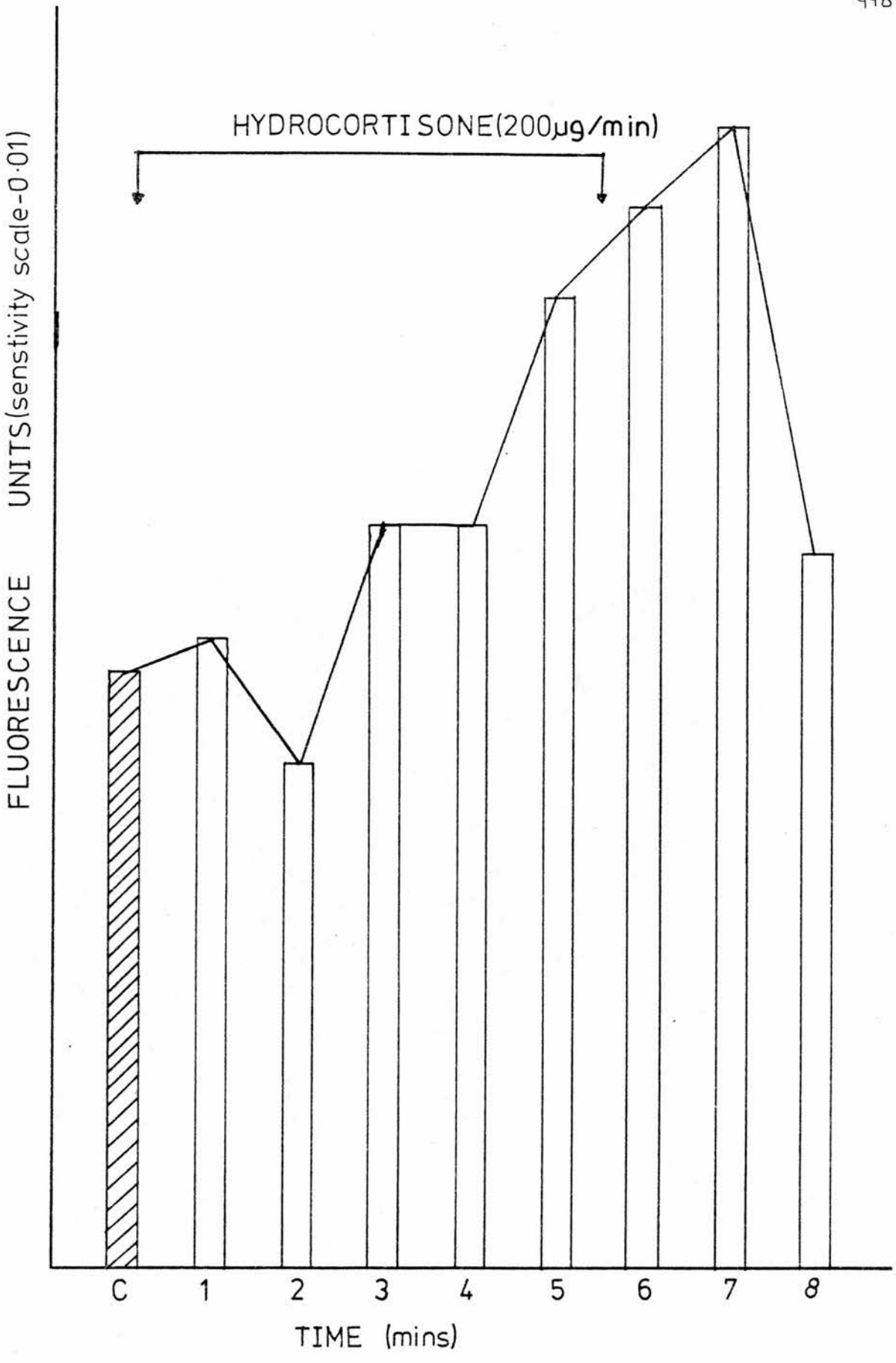


Fig. 25B

The time course of catecholamine secretion, evoked by hydrocortisone administration, from the adrenal glands of cross-bred collies and fox-hounds.

gland denervation or removal of glands without major surgery when animals were to be sacrificed did not alter the proportion of adrenal glands which were unresponsive to either all the tested secretagogues or hydrocortisone alone.

The Evoked Release of Adrenomedullary Catecholamine By Hydrocortisone Succinate

A dose dependent increase in adrenomedullary catecholamine secretion from canine adrenal glands was evoked by a five minute infusion of hydrocortisone succinate in the dose range (20 μg/min —> 400 μg/min).

The percentage of noradrenaline present in the incremental secretion was not altered from that in the resting output (see Table 13). As can be observed from Fig25 there was a rapid onset of drug action with a stimulation of secretion rate occurring within minutes of the start of the infusion and declining rapidly following the end of the infusion.

The Action of Cholinergic Antagonists on the Steroid Induced Secretion

The addition of atropine sulphate (10^{-6}M) reduced the response to hydrocortisone infusions by a variable amount (see Table 14) whereas the response was unaltered by hexamethonium bromide (10^{-4}M). Although this might indicate that the action of hydrocortisone was perhaps mediated via muscarinic receptors or by a release of acetylcholine from nerve endings associated with muscarinic receptors further evidence from chronic denervation studies was inconclusive (see p 112). The addition of an anticholinesterase,physostigmine, did not alter the resting secretion or the response to hydrocortisone so a non-specific action of atropine cannot be excluded. The high proportion of fox-hound adrenal glands perfused latterly, which proved insensitive to hydrocortisone precluded making more than one observation with hyoscine methylbromide, as an alternative antagonist. More information would have been obtained if other non-cholinergic agonists had been tested before and after the

TABLE 13

THE RESPONSE OF CANINE AND FELINE ADRENAL GLANDS TO INFUSIONS OF HYDROCORTISONE SUCCINATE

(Individual results p 146)

CROSS-BRED COLLIES:

HYDROCORTISONE DOSE (5 min Infusion)	TOTAL EVOKED CA RELEASE (% of control)	% NA (control & test)
20 $\mu g\ min^{-1}$ (n = 7)	153 ± 8	21 ± 4 → 27 ± 3
60 $\mu g\ min^{-1}$ (n = 9)	221 ± 33	16 ± 4 → 22 ± 5
200 $\mu g\ min^{-1}$ (n = 24)	328 ± 50	19 ± 3 → 24 ± 2
400 $\mu g\ min^{-1}$ (n = 11)	805 ± 99	15 ± 4 → 18 ± 4

FOX HOUNDS:

HYDROCORTISONE DOSE (5 min Infusion)	TOTAL EVOKED CA RELEASE (% of control)	% NA (control & test)
200 $\mu g\ min^{-1}$ (n = 16)	167 ± 16	8 ± 3 → 9 ± 3
400 $\mu g\ min^{-1}$ (n = 18)	248 ± 35	7 ± 2 → 10 ± 3

CATS:

HYDROCORTISONE DOSE (5 min Infusion)	TOTAL EVOKED CA RELEASE (% of control)	% NA (control & test)
400 $\mu g\ min^{-1}$ (n = 10)	96 ± 7	39 ± 3 → 38 ± 3

TABLE 14

THE MODIFICATION OF THE HYDROCORTISONE EVOKED CA SECRETION BY CHOLINERGIC ANTAGONISTS (Individual results p149)

CROSS-BRED COLLIES

		EVOKED CA SECRETION (% OF CONTROL)		
		BEFORE DRUGS	AFTER ATROPINE (10^{-6}M)	AFTER C_6 (10^{-4}M)
Hydrocortisone Succinate 200 $\mu g/min^{-1}$	n = 5	264 ± 50	143 ± 14 (n = 3)	275 (n = 2)
400 $\mu g/min^{-1}$	n = 6	814 ± 141	411 ± 153 (n = 6)	755 ± 250 (n = 3)

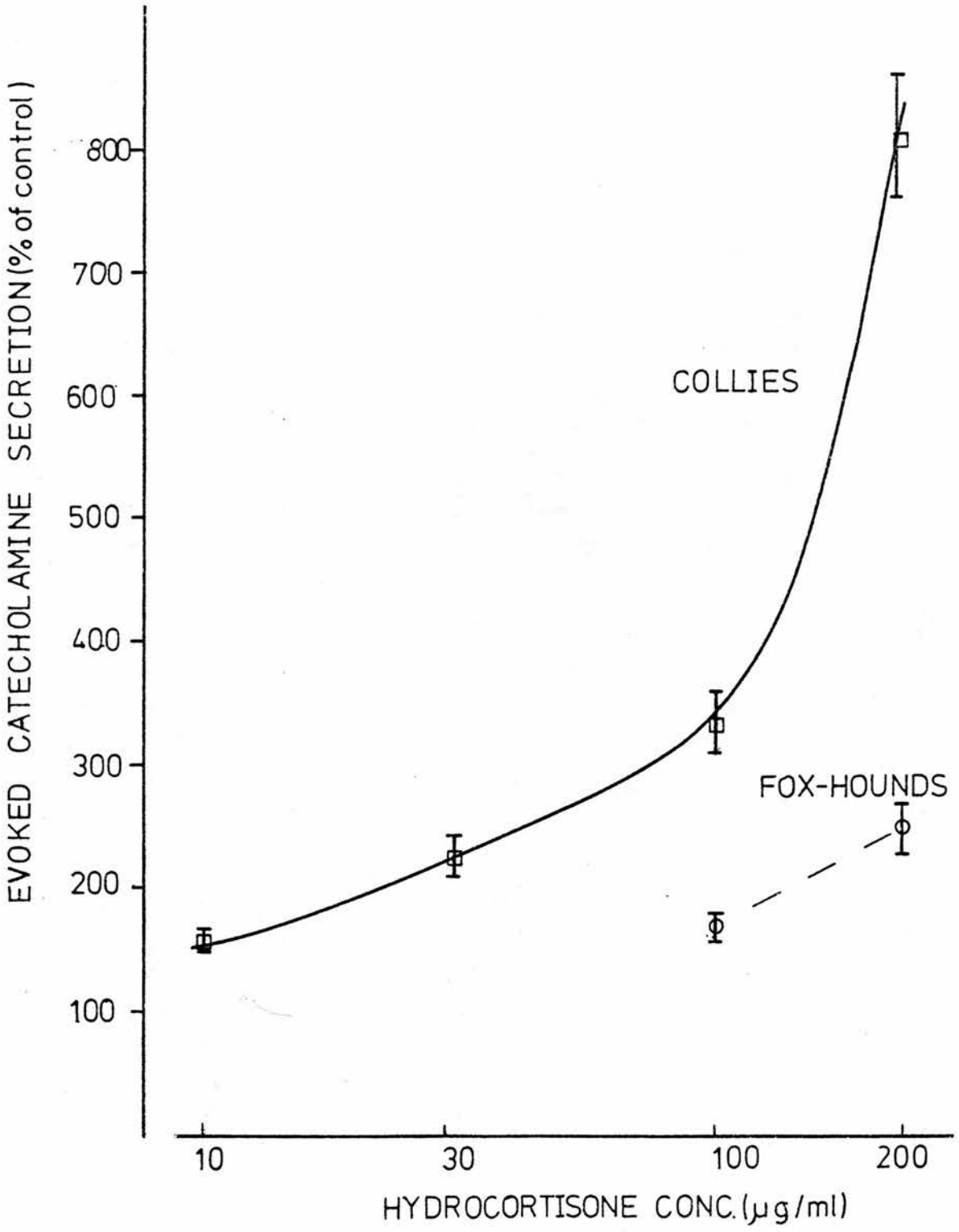
FOX-HOUNDS

		EVOKED CA SECRETION (% OF CONTROL)		
		BEFORE DRUGS	AFTER ATROPINE (10^{-6}M)	AFTER C_6 (10^{-4}M)
Hydrocortisone Succinate 200 $\mu g/min^{-1}$	n = 3	173	100 (n = 2)	250 (n = 2)
400 $\mu g/min^{-1}$	n = 2	281	117 (n = 2)	366 (n = 2)

C_6 - hexamethonium

Fig 26

The dose dependent release of catecholamines from the canine adrenal medulla by hydrocortisone succinate as an infusion over five mins.



addition of atropine but this was an oversight on the part of the investigator.

Feline Adrenal Glands

In this study of isolated, perfused, cat adrenal glands about 70% of the glands perfused responded to secretagogues, although the fineness of the vasculature made the numbers of glands able to be removed surgically much smaller.

The Action of Hydrocortisone Succinate on Isolated, Perfused, Feline Adrenal Glands.

As can be noted from Table 13 the infusion of hydrocortisone succinate for a five minute period, in a dose producing a large increment of catecholamine secretion from canine adrenal glands, was without effect on feline glands. On four occasions larger doses of hydrocortisone (> 1mg/min) were infused and no enhancement of secretion was observed even over a 30 minute collection period. These adrenal glands which were insensitive to hydrocortisone responded reproducibly to at least one other secretagogue suggesting that the normal secretory mechanism was functional and that hydrocortisone is not an adrenomedullary secretagogue in the cat.

DISCUSSION

The present study confirms that hydrocortisone is a potent adrenomedullary stimulant in the dog. The release of catecholamines evoked by hydrocortisone is dose dependent and non-selective similar to the action of other secretagogues. The rapid time course of this response seems to separate this action of hydrocortisone from those observed in other tissues where the postulated action involves de novo protein synthesis and may modulate responses over a long time period. (Wurtman et al., 1972.) This selective action of only glucocorticosteroids in releasing catecholamines (Critchley, 1977) raises the possibility of alternative hypotheses for the role of glucocorticoids

in controlling catecholamine biosynthesis and the secretory response of the chromaffin cells to a number of stimuli. These points and the importance which may need to be attached to species variation is discussed in the context of the adrenomedullary secretory mechanism in the general discussion (p 125).

The absence of any response of cat adrenal glands to corticoids appears to be yet another difference between species in their adrenomedullary pharmacology and together with the evidence of Bloom et al., 1977 , using bovine glands, may rule out the involvement of corticosteroids in a secretory role in all species with evidence for their action only existing for the dog and the rat (Roffi et al., 1966).

THE ACTION OF CHOLINERGIC AGONISTS AND K^+ IONS AS ADRENOMEDULLARY SECRETAGOGUES

The secretion of catecholamines from the adrenal medulla produced by nicotinic and muscarinic agonists appears to vary between species. In the cat both categories of agonist produce a large increase in adrenomedullary secretion but the composition of this secretion differs.

Nicotinic agonists may evoke a secretion containing predominantly noradrenaline and muscarinic agonists a secretion containing predominantly adrenaline though both these observations are controversial when one reviews the literature (see p 40). Much of the assessment of selective release of either catecholamine depends on comparison with resting catecholamine output for which widely varying estimates have been made

In the dog the situation is as controversial with non-selective release apparently occurring in response to muscarinic agonist action but different groups of workers report both non-preferential and preferential release evoked by nicotinic agonists.

In the present study using a more selective fluorimetric technique it was decided to compare the resting secretion and drug evoked secretion of catecholamines from isolated, perfused, adrenal glands.

METHODS

Adrenal glands were removed surgically from both cats and dogs under anaesthesia or following death. The isolated glands were retrogradely perfused with oxygenated Locke's solution as previously described (see Methods p 68). A constant flow rate of 2 ml/min was maintained. The concentration of catecholamines and the relative proportions of noradrenaline and adrenaline were obtained by a differential fluorimetric assay for each perfusion effluent

sample. The differential assay procedure is explained in p 77 . The protocol of drug administration was similar in both cat and dog adrenal glands and this together with the sample collection procedure is illustrated in Table 15.

Calculation of Results

As described earlier the data were obtained following computation by simultaneous equations of the fluorescence produced by samples at two wavelengths during an excitation scan.

To compare relative proportions of the catecholamines in resting secretions and evoked secretions many workers have chosen one of two ways of measurement. The most common method is that used by Douglas and Poisner, 1965 and others, which is merely to compare the composition of the drug - evoked secretion in response to one drug with that produced by another. The contribution produced by the resting secretion is ignored and often experimental values are not given thus making any assessment of preferential secretion meaningless, being merely a comparison of the composition of secretions evoked by different drugs. The second more usual method of calculation is merely to compare the concentration and composition of drug-evoked catecholamine secretion with that present in the normal basal output. Where the magnitude of a drug-evoked release is large the resting secretion becomes a small proportion of the catecholamine output and this method produces accurate estimations of the secretion produced. In the present study only small changes in the catecholamine output were observed in response to drugs and for one to assess a preferential secretion of either catecholamine one must compare the incremental secretion with that of the basal secretion for an accurate measurement to be made. When this calculation is applied quite large differences in the calculated composition are obtained from those, using the other method.

TABLE 15

SCHEDULE FOR INFUSION OF CHOLINERGIC AGONISTS TO ISOLATED ADRENAL GLANDS

TIME (MIN)	
-2	Collect control adrenal gland effluent for 30 sec.
-1	Collect control adrenal gland effluent for 30 sec.
0	Switch on infusion
$\frac{1}{2}$	Collect effluent for 30 secs.
1	
$1\frac{1}{2}$	
2	
	Switch off infusion
$2\frac{1}{2}$	Collect samples for 30 sec.
3	
4	

RESULTS

Resting Secretion

The resting catecholamine secretion rate from isolated perfused feline adrenal glands was 154 —> 203 ng/min which is similar to that found as the basal release from similarly perfused canine glands. There was a significant difference in the percentage of the output which was noradrenaline between the two species with the cat having a much higher noradrenaline percentage in its basal output. These results are contained in Table 16.

NICOTINIC AGONISTS

Nicotine

Following a two minute infusion of nicotine 10^{-5} M both cat and dog adrenal glands showed a marked increase in adrenomedullary secretion. The dose of nicotine used did not alter the relative catecholamine composition in either species from that observed at rest.

In contrast the more ganglion-selective nicotinic agonist, H.P.P.T.M.A. (Fig 5, p13a) (Critchley et al., 1976) released a similar increment of catecholamine but with a much higher percentage of noradrenaline when administered to cat adrenal glands. Similar doses administered to dog adrenal glands perfused similarly have previously been shown to produce a non-selective secretion of catecholamines. The results to both these agonists are illustrated in Table 16.

Muscarinic Agents

The addition of acetyl-β-methylcholine (10^{-6} M) to cat adrenal glands produced a similar increase in adrenomedullary secretion to that

observed after nicotine administration. This drug induced incremental secretion contained a much lower percentage of noradrenaline than that of the resting secretion. The same dose of the drug when administered to canine adrenal glands, for the same time period, produced a similar increment of secretion but with no alteration in the composition of this secretion. The results for this section are contained within Table 16.

Potassium Chloride (KCl)

56 mM KCl when administered for three minutes to cat adrenal glands produced a large increase in the catecholamine secretion rate of the order of six-fold. There was accompanying this a significant increase in the noradrenaline percentage within this evoked secretion.

These results are discussed in the light of their implications on adrenal medullary pharmacology and physiology in the general discussion (p 129).

TABLE 16

THE RELEASE OF CA FROM CANINE AND FELINE ADRENAL GLANDS FOLLOWING THE ADMINISTRATION OF CHOLINERGIC AGONISTS AND POTASSIUM CHLORIDE

(Results of individual experiments p150)

DOGS

DRUG	RESTING SECRETION ng min^{-1} ± S.E.	%NA ± S.E.	EVOKED SECRETION ng min^{-1} ± S.E.	%NA ± S.E.
Nicotine, 10^{-5}M (n = 6)	190 ± 40	23 ± 5	+630 ± 200	18 ± 6
Acetyl B Methylcholine, 10^{-6}M (n = 10)	280 ± 40	24 ± 2	+680 ± 130	28 ± 2

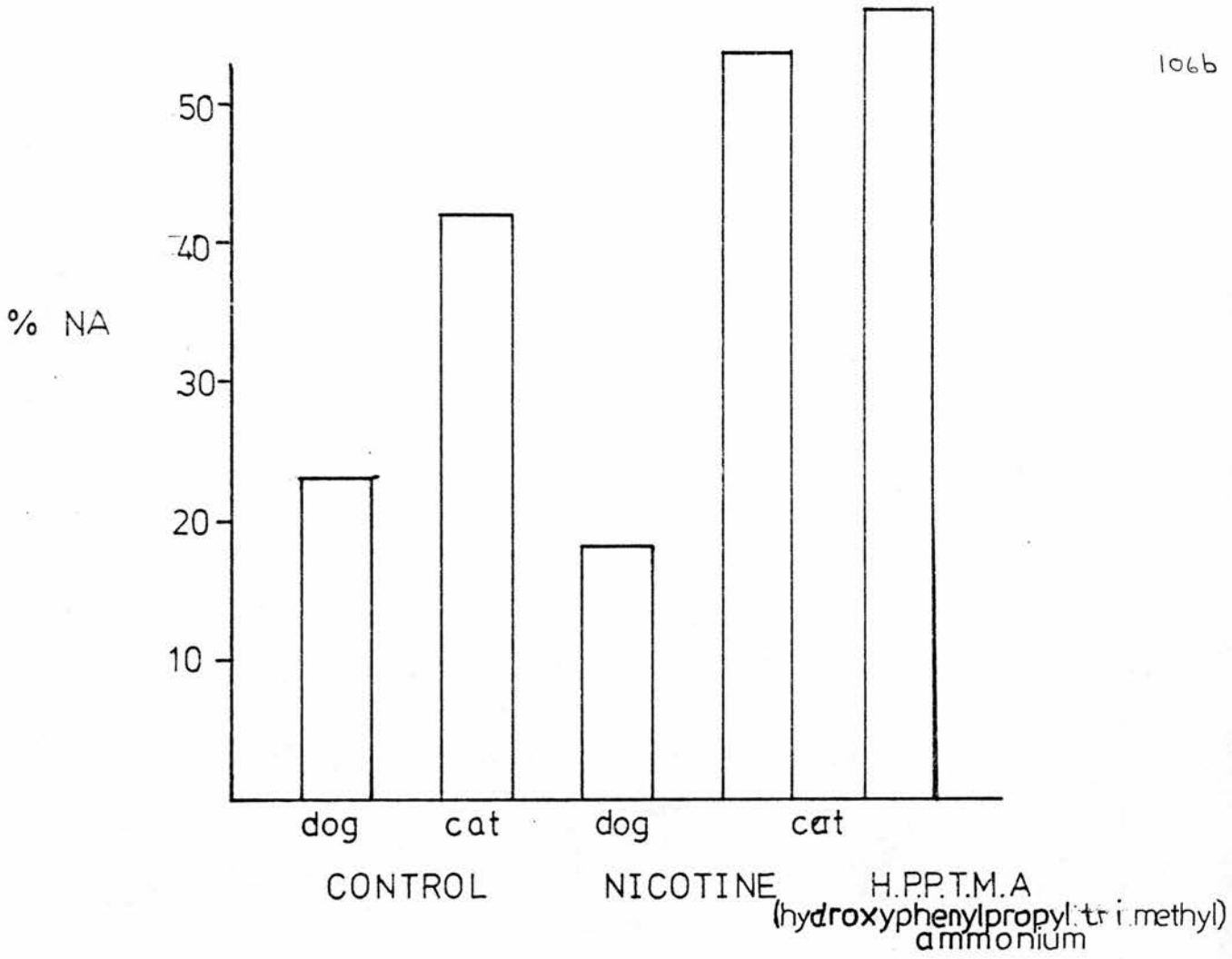
CATS

DRUG	RESTING SECRETION ng min^{-1} ± S.E.	%NA ± S.E.	EVOKED SECRETION ng min^{-1} ± S.E.	%NA ± S.E.
Nicotine, 10^{-5}M (n = 8)	160 ± 30	42 ± 4	*470 ± 70	55 ± 9
Acetyl B. Methylcholine, 10^{-6}M (n = 6)	150 ± 30	41 ± 4	*350 ± 40	*16 ± 3
Potassium Chloride, 56 mM (n = 6)	200 ± 20	38 ± 4	+1260 ± 200	*66 ± 3
mHPPTMA, 10^{-6}M (n = 12)	170	37	840	57

From Critchley, 1977

*Significantly different: $p \leq 0.05$ by paired t test

\+ t test not applied due to altered variance



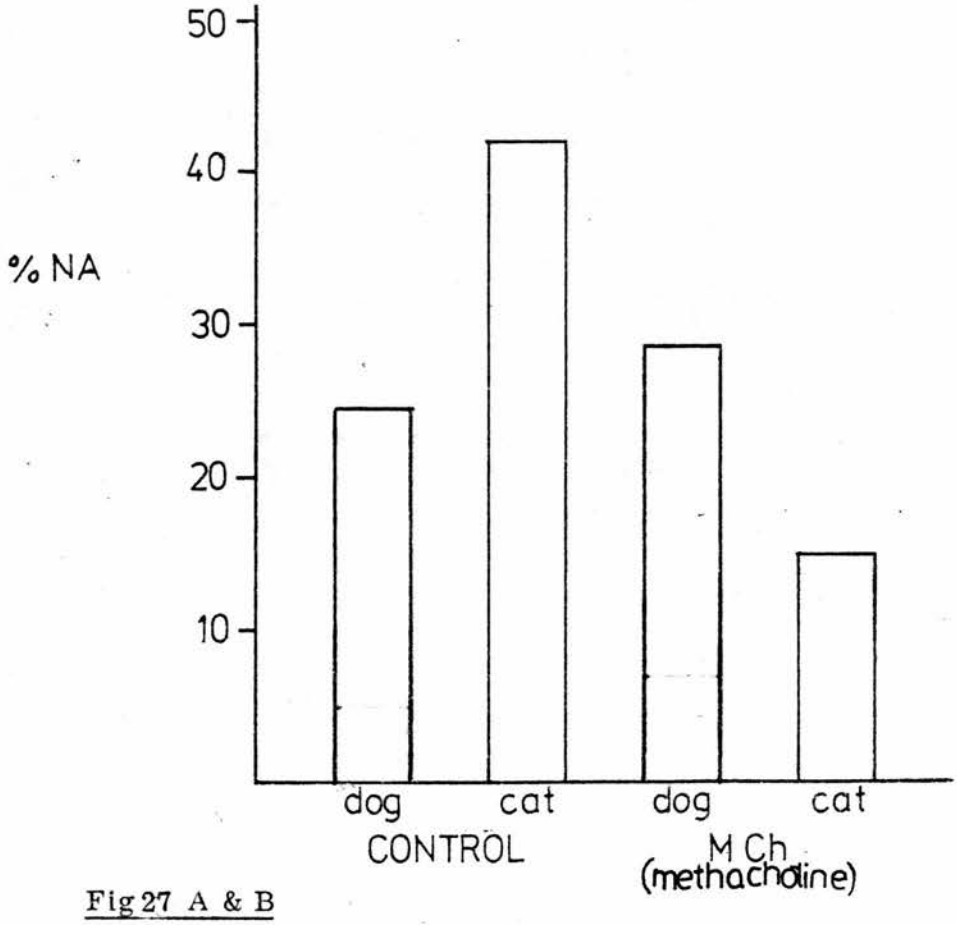


Fig 27 A & B

The comparison of the relative proportion of noradrenaline in the evoked secretion from feline and canine adrenal glands following the administration of cholinergic agonists.

ANTAGONIST AFFINITY CONSTANTS FOR ADRENOMEDULLARY MUSCARINIC RECEPTORS

In some preliminary studies and in a number of experiments where a muscarinic agonist was seen to stimulate catecholamine release from the adrenal medulla, this release could be inhibited by muscarinic antagonists such as atropine (Critchley, Tibenham, Ungar, Waite and West, 1975). In view of the high concentrations of antagonist required to block the response, it was decided to investigate the affinity constants of a number of antagonists for the adrenomedullary receptors.

METHODS

Canine adrenal glands were excised and perfused retrogradely with oxygenated Locke's solution at 37^{o}C through the adrenolumbar vein at a constant flow of 2 ml/min. The effluent was collected and the catecholamine content assayed by the trihydroxyindole method. The glands were stimulated by two low and two high doses of the specific muscarinic agonist, acetyl β methylcholine, prior to a 90 min equilibration with perfusate containing the antagonist. A typical dose cycle and sampling procedure is shown in Fig. 28. The response to the agonist was defined as the increment of the catecholamine output expressed as a percentage of the resting output. Further higher doses of agonist, in the presence of antagonist, were administered to produce similar responses to those previously obtained enabling the affinity constant of the antagonist to be calculated from the Gaddum/Schild equation.

Fig. 28 THE DOSE CYCLE AND SAMPLING PROCEDURE FOR THE MEASUREMENT OF ADRENOMEDULLARY MUSCARINIC AFFINITY CONSTANTS

DOSE CYCLE

Time (mins)	
0	Add low dose agonist (Concentration between 10^{-7}M and 10^{-6}M acetyl-β-methylcholine) (Two minute infusion)
10	Add high dose agonist (Within range 10^{-6}M → 10^{-5}M) (Two minute infusion)
20	Low dose agonist
30	High dose agonist
40	Switch to Locke's solution containing antagonist
120	Low dose agonist (in presence of antagonist)
130	High dose agonist
140	Low dose agonist
150	High dose agonist

Contd.

SAMPLING PROCEDURE

Time (min)	
-2	Collect control adrenal gland effluent for 30 sec.
-1	Collect control adrenal gland effluent for 30 sec.
0	Switch on infusion
$\frac{1}{2}$	Collect consecutive samples of effluent for 30 sec. periods
1	
$1\frac{1}{2}$	
2	
	Switch off infusion
$2\frac{1}{2}$	Collect consecutive samples of effluent for 30 sec. periods
3	
4	

RESULTS

Antagonist Affinity Constants

Table 17 shows a comparison of the affinity constants for the muscarinic receptors of the adrenal medulla with those previously reported for the guinea pig ileum. The adrenomedullary affinity constants are consistently two orders of magnitude lower than those obtained for the guinea pig ileum. On four occasions hexamethonium bromide 10^{-4}M was without action on the stimulating action of Acetyl β methylcholine.

DISCUSSION

It would appear from the results obtained that there exists in the canine adrenal medulla a population of muscarinic receptors for which the muscarinic antagonists tested showed an affinity two orders lower than previously reported for the guinea pig ileum. This implies that the muscarinic receptors differ in structure from those associated with parasympathetic postganglionic neuroeffector junctions which normally show similar affinity constants. See Table 17.

TABLE 17

A COMPARISON OF THE ANTAGONIST AFFINITY CONSTANTS FOR the muscarinic receptors of THE ADRENAL MEDULLA AND THOSE VALUES REPORTED FOR THE GUINEA-PIG ILEUM

ANTAGONIST	MEAN DOSE RATIO	ADRENOMEDULLARY AFFINITY CONSTANT (K_B MEDULLA)	REPORTED ILEUM AFFINITY CONSTANT (K_B ILEUM) (Abramson et al, 1969)	$\frac{K_B \text{ ILEUM}}{K_B \text{ MEDULLA}}$
Atropine Sulphate	7.4			
10^{-6}M, n = 3	(7 ⟶ 7.8)	6.4×10^6	10^9	160
Oxyphenonium Bromide	18			
2×10^{-7}M, n = 3	(9 ⟶ 34)	8.7×10^7	6×10^9	70
Oxyphenonium Bromide	77			
2×10^{-6}M, n = 3	(55 ⟶ 117)	3.8×10^7	6×10^9	160
Hyoscine Methyliodide	10.4			
2×10^{-7}M, n = 3	(5.2 ⟶ 18)	4.7×10^7	5×10^9	110

Results for individual experiments p152

THE EVOKED SECRETION OF ADRENOMEDULLARY CATECHOLAMINES BY HISTAMINE, IN THE DOG

The direct action of histamine on the chromaffin cells of the cat adrenal medulla has been confirmed by a number of workers (p 45). No direct evidence has been obtained for its action as a secretagogue on canine adrenomedullary cells. Staszewska-Barczak and Vane, 1965 noted that predominantly adrenaline was released into the adrenal venous blood following the intravenous administration of histamine to a dog. This increase in secretion was reduced after administration of nicotinic antagonists. De Schaepdryver, 1959; Robinson and Jochim, 1960 reported a release of catecholamines into the adrenal venous blood following the intravenous administration of histamine (50 - 100 μg/kg). Ganglion-blocking agents reduced this response. In the former study the noradrenaline percentage increased and in the latter study the percentage of adrenaline in the adrenal venous catecholamine was increased, when analysed fluorimetrically.

METHOD

The adrenal glands were removed surgically as previously described and perfused at a constant flow of 2 ml/min with the usual fluorimetric catecholamine detection. Histamine acid phosphate (20 —> 200 μg/min) was administered as a 2 minute infusion with the drug protocol and sample procedure as illustrated in Table 18.

RESULTS

A dose dependent evoked secretion was observed in response to doses of histamine with no significant difference in the noradrenaline percentage in the increment when compared to the basal secretion (Fig 29, Table 19)

TABLE 18

SCHEDULE FOR INFUSION OF HISTAMINE TO ISOLATED ADRENAL GLANDS

TIME (MIN)	
-2	Collect control adrenal gland effluent for 30 sec.
-1	Collect control adrenal gland effluent for 30 sec.
0	Switch on infusion
$\frac{1}{2}$	Collect effluent for 30 sec. periods
1	
$1\frac{1}{2}$	
2	
	Switch off infusion
$2\frac{1}{2}$	Collect samples for 30 sec. periods
3	
4	

TABLE 19

THE RELEASE OF CATECHOLAMINES FROM CANINE ADRENAL GLANDS FOLLOWING HISTAMINE ADMINISTRATION

HISTAMINE DOSE (Infused for 2 min)	EVOKED CA RELEASE (% of control)	% NA (control → increment)
20 μg/min (n = 10)	139 ± 13	32 ± 4 → 34 ± 4
60 μg/min (n = 6)	189 ± 10	29 ± 3 → 31 ± 2
200 μg/min (n = 7)	333 ± 75	31 ± 6 → 32 ± 7

Results of individual experiments; p153

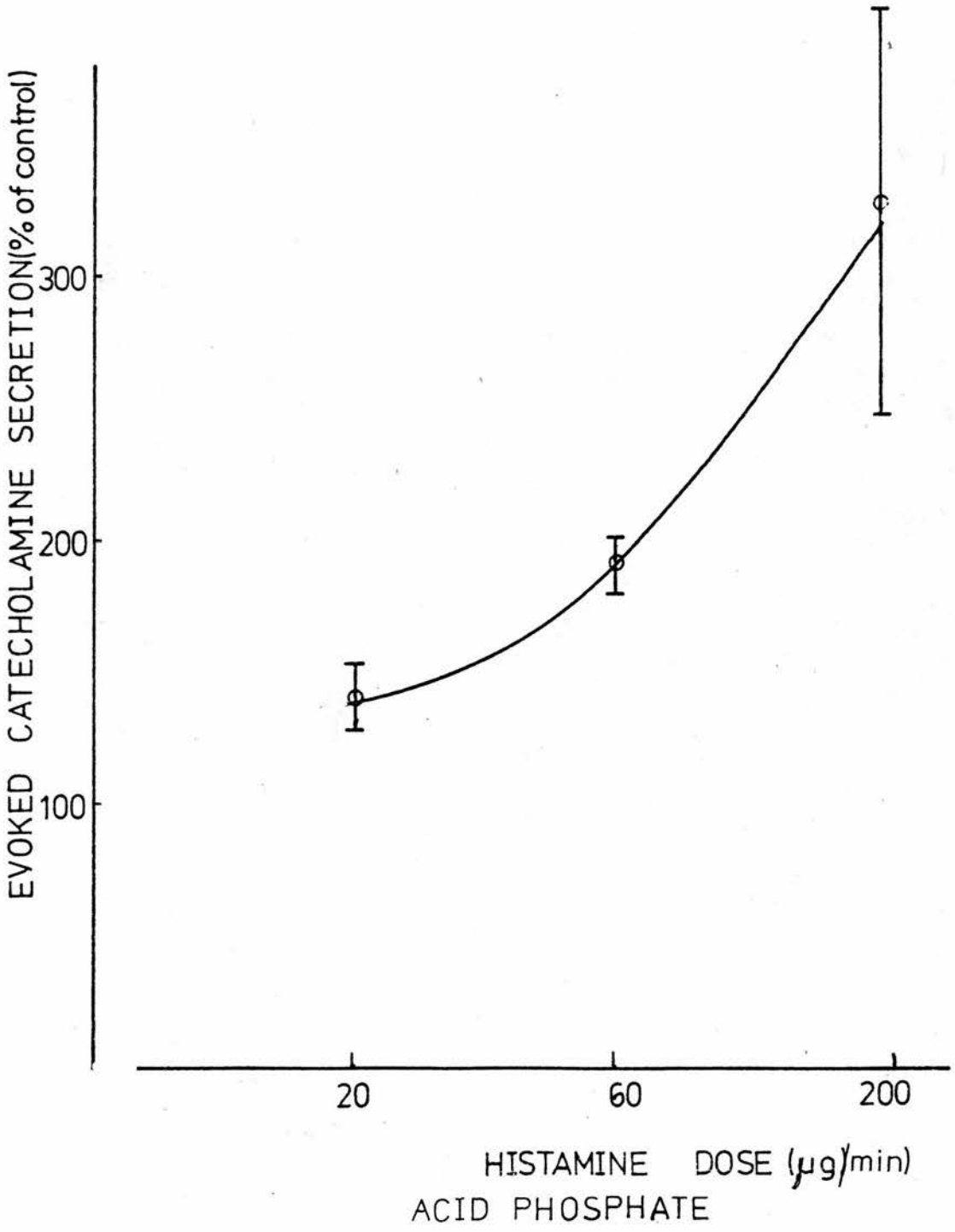
DISCUSSION

These results confirm that histamine is a secretagogue for canine adrenomedullary chromaffin cells. The sensitivity of these responses, in whole animal experiments, to nicotinic antagonists may well be accounted for by failure to denervate the adrenal glands. Much of the increased secretion rate may arise from the baroreceptor reflex increase in sympathetic tone initiated by the decrease in blood pressure characteristic of histamine injection. This increase in splanchnic tone will of course to a large part be sensitive to nicotinic blockade.

The non-selective release of catecholamines in the present study contrasts with the situation in the cat where a preferential release of adrenaline occurs and also contradicts earlier reports obtained in dogs. The difference between results from experiments on the canine adrenal medulla might be accounted for by the more selective assay used and the isolated perfusion where assay of the perfusate avoids many of the difficulties present in the differential assay of adrenal venous catecholamine levels.

Fig 29

The dose-dependent release of catecholamines from the canine adrenal medulla following the administration of histamine acid phosphate.



Vertical bars = standard errors of the means.

PRELIMINARY OBSERVATIONS

In this section a number of experiments are described where due to experimental difficulties only preliminary results were obtained.

The response of the adrenal medulla to baroreceptor and chemoreceptor tests

In this experiment an attempt was made to parallel the investigation of vasomotor reflex transmission through the sympathetic ganglia with a study at the splanchnic-adrenal junction. The purpose of the procedure was to use antagonists to expand the results obtained previously (Critchley, 1977). In only 3 out of 7 experiments did the surgery involved allow an adequate adrenal venous blood collection to be established (see p 66) and only preliminary observations were obtained.

Samples of adrenal venous blood were collected for one minute time periods before and during baroreceptor and chemoreceptor tests, as previously described. Following column separation by the method of Renzini, Valori, Brunori, 1970 the catecholamine content was assayed by a fluorimetric trihydroxyindole method but only using the higher oxidation pH (Laverty and Taylor, 1968; Peyrin and Peqiquent, 1972) and measurement at a single excitation wavelength as solely the total catecholamine was measured.

It is apparent from the results (see Table 20) that only some information was obtained on resting adrenal venous CA concentration under pento-barbitone anaesthesia as well as some information about reflex sensitivity.

TABLE 20

THE RESPONSE OF THE CANINE ADRENAL MEDULLA TO BARORECEPTOR AND CHEMORECEPTOR TESTS AND ITS MODIFICATION BY CHOLINERGIC ANTAGONISTS (Detailed results; p 154)

PRIOR TO DRUG ADMINISTRATION		AFTER HEXAMETHONIUM BROMIDE 2mg/kg	
Adrenal venous plasma CA concentration (ng/ml)			
Control (n=3)	212 (73 → 470)	Control (n=1)	130
Baroreceptor test (BR test) (n=3)	250 (91 → 590)	Baroreceptor test (n=1)	200
Control (n=3)	200 (93 → 390)	Control (n=1)	155
Chemoreceptor test (CR test) (n=3)	265 (85 → 550)	Chemoreceptor test (n=1)	200
After H.M.B. 10 mg/kg I.V.			
Control (n=2)	110 (92 → 127)		
BR test (n=2)	296 (202 → 390)		
Control (n=2)	127 (92 → 162)		
CR test (n=2)	164 (98 → 230)		

n values represent number of experiments performed (i.e. number of animals).
H.M.B. - hyoscine methylbromide

An Investigation of the possible indirect cholinergic releasing action of hydrocortisone as an adrenomedullary secretagogue.

Following observations in isolated canine adrenal glands that hydrocortisone's action, as an adrenomedullary secretagogue, could be partially inhibited by atropine, it was decided to investigate whether this release might be mediated by an action on cholinergic nerve endings.

Five dogs were unilaterally sympathectomised, (T2 - L3) following a laparotomy and allowed to recover. After three weeks, during which time degeneration of the splanchnic nerve endings should have been complete, the animals were sacrificed and both adrenal glands removed. The response of the denervated gland was compared to the contralateral gland with an intact nerve supply but in only two animals from five were any changes noted. (see Table 21)

In all cases poor responses were obtained from the control innervated glands thus making comparison impossible. Most dogs did show reduced responses to hydrocortisone in the gland taken from the denervated side but this may just reflect the poor reproducibility of response from adrenal glands removed following cannulation. Further investigations may be useful but neither removal of glands for isolated perfusion, or collection of adrenal venous blood seem suitable techniques unless large numbers of experiments are envisaged.

The Action of Muscarinic Agonists as Ganglionic Stimulants and the Action of Antagonists.

The high doses of muscarinic antagonists required to inhibit the chemoreceptor reflex transmission seemed irreconcilable with the low antagonist doses needed to inhibit the ganglion stimulant action of muscarinic agonists (see p 17). With this in mind an attempt was made to investigate the intravenous dose of hyoscine methylbromide necessary to inhibit the ganglion stimulant action of the muscarinic agonists McNA343 and Acetyl β methylcholine.

TABLE 21

A COMPARISON OF THE HYDROCORTISONE-EVOKED CA RELEASE FROM CHRONICALLY DENERVATED CANINE ADRENAL GLANDS TO THAT FROM THE CONTROL INNERVATED GLAND IN THE SAME ANIMAL

DOG NO.	HYDROCORTISONE EVOKED CA SECRETION (% OF CONTROL)			
	INNERVATED GLAND		DENERVATED GLAND	
C1	200 μg/min	110		142
	400 μg/min	100		117
C2	200 μg/min	124		100
	400 μg/min	140		100
C3	400 μg/min	146		100
	1 mg/min	155		100
C4	200 μg/min	140		100
			1 mg/min	100
C5	200 μg/min	115		
	400 μg/min	145		100

Only two dogs were used in this study as no further donor dogs became available for the cross perfusion. The dogs were anaesthetised and one hind limb of the recipient dog was vascularly isolated and perfused at constant flow with carotid arterial blood from a donor animal. The nerve supply to the hind-limb remained intact. Blood entered the limb via a cannula in the femoral artery and returned down a pressure gradient to the jugular vein of the donor animal via a cannula in the femoral vein of the perfused limb. Changes in perfusion pressure were taken to reflect changes in the vascular resistance of the limb mediated neurogenically. The muscarinic agonists were administered into the aorta of the recipient by way of an umbilical catheter passed down from the left common carotid artery. Both sinus nerves were blocked by the administration of 5% lignocaine applied locally to prevent any baroreceptor reflexes. The vagi were cut to remove any influence from aortic arch baroreceptors or volume receptors.

In both dogs triphasic changes in hind-limb perfusion pressure were observed in response to McNA343. An initial rise was followed by a fall then a maintained rise in pressure. Acetyl β methylcholine purely produced a decrease in hind-limb perfusion pressure. Both these responses to muscarinic agonists were antagonised by hyoscine methylbromide administered fifteen minutes earlier as a dose of 1 mg/kg i.v. (see Table 22). Though these preliminary observations imply that a much lower dose of antagonist is required to block the response to exogenously applied agonist than is required to inhibit chemoreceptor reflex neurotransmission, the difference may arise from changes in equilibration, antagonist clearance (see p 90) and extrapolation to other species is not really justified.

<u>The Evaluation of an Incubation Method as an Alternative to Adrenal Gland Perfusion, and an Investigation of the Action of Hydrocortisone on the Rabbit and Guinea-Pig Adrenal Medulla</u>.

In a number of species vessel size prevents the cannulation and removal of the adrenal glands for isolated perfusion. An alternative

TABLE 22

THE ACTION OF MUSCARINIC AGONISTS AS GANGLION STIMULANTS AND ITS MODIFICATION BY HYOSCINE METHYLBROMIDE (Detailed results; p157)

AGONIST	CONTROL sustained H.L.P.P. RISE (mm Hg)	sustained H.L.P.P. RISE AFTER HYOSC. METHYLBROMIDE 1 mg/kg i.v.
M^CNA 343, 200 µg i.a. (Fig6, p16a) (n = 4)	18 (15 → 25)	3.5 (0 → 6)
Acetyl-B-Methylcholine 100 µg i.a. (n = 4)	-38 (-33 → -47)	-19.5 (-13 → -24)

i.a. - intra-aortic

TABLE 23

THE RESTING AND DRUG EVOKED OUTPUT OF CA FROM ISOLATED RABBIT AND GUINEA-PIG ADRENALS INCUBATED, IN VITRO (Detailed results; p158)

TOTAL CA SECRETION ($ng\ mg^{-1}\ 10\ min^{-1}$)

		CONTROL	+ HYDROCORTISONE (500 μg/ml)
RABBIT			
(n = 11)	Mean ± S.E.	560 ± 154	800 ± 734
GUINEA-PIG			
(n = 11)	Mean ± S.E.	345 ± 57	561 ± 161

In all cases only adrenaline was secreted.

The secretion in the presence of hydrocortisone was not significantly different (paired t test) from the control in either set of experiments. ($p > 0.5$)

technique employed by Boonyaviroj and Gutman, 1977 was to incubate glands in a physiological salt solution in the absence and presence of a suspected secretagogue. In this study we employed a similar approach with glands removed from rabbits and guinea-pigs.

Adrenal glands were removed and sectioned in two. The wet weights were recorded then the glands placed in a known volume of Locke's solution (composition as previously described) containing an anti-oxidant sodium metabisulphite (10 mg/100 ml). The paired halves were incubated at 37°C for ten minutes one in the presence of the desired agonist concentration and the other as a control. The catecholamine concentration in the medium was compared between test and control to assess whether any secretory activity had occurred. A 1 ml sample of the medium was assayed in triplicate by the trihydroxy indole fluorimetric method (see p 77) with no sample preparation proving necessary.

A very widely varying control catecholamine concentration was observed and duplication was poor (Table 23). Although an evoked secretion appears to have occurred when one solely considers the catecholamine concentration in the medium, no significant release occurred when a paired t-test is applied. In fact in only 50% of the glands did any evoked secretion arise and one must conclude that the technique is too variable to allow one to comment with any confidence as to whether hydrocortisone succinate can increase adrenomedullary catecholamine secretion in the rabbit and the guinea-pig.

DISCUSSION OF RESULTS

SYMPATHETIC GANGLIA

Synaptic Transmission of Vasomotor Reflexes

From our experimental results the synaptic transmission of a baroreceptor reflex through the canine lumbar sympathetic ganglia involves a purely nicotinic pathway. This parallels the observed transmission following preganglionic synchronous stimulation which in 5 out of 7 cases was totally hexamethonium sensitive. The small residual response observed contrasts with the large component of non-nicotinic transmission observed under similar conditions in the caudal cervical and stellate ganglia of the same species (Fleisch et al, 1969) or in a number of sympathetic ganglia in other species (Trendelenburg, 1966). This again supports the view that extrapolation of results from different preparations in terms of an overall impression of sympathetic ganglionic transmission, may be difficult.

In marked contrast, the synaptic ganglionic transmission of a chemoreceptor reflex has a large non-nicotinic component with a marked inhibition of transmission being observed following the addition of a muscarinic antagonist alone. Once again this contrasts with the observations of previous groups of workers, where muscarinic antagonist administration was only effective, on the residual response, following full nicotinic blockade, when increases in sympathetic firing were induced by preganglionic stimulation or a physiological stimulus (Hilton and Steinberg, 1966; Brown, A.M., 1967). When one considers the apparent sensitivity of the chemoreceptor reflex transmission to muscarinic antagonists in relation to the commonly observed lack of residual response to preganglionic stimulation following nicotinic blockade, an inconsistency is apparent.

One simple explanation of this would be that, if following a carotid chemoreceptor stimulus, only a small proportion of the

sympathetic preganglionic fibres is activated and that these fibres are predominantly associated with muscarinic receptors on the postganglionic cells. This might explain the large inhibition of a chemoreceptor reflex by a muscarinic antagonist. When all the preganglionic fibres are stimulated, as with a supramaximal preganglionic stimulus, one would have to postulate that a much larger fibre population was activated and that the overwhelming transmission within the ganglia was by nicotinic receptor activation. In order to investigate this hypothesis one would have to ascertain the number of preganglionic fibres within the population which are activated by a chemoreceptor reflex which is, as yet, uninvestigated. Evidence from studies on somato-sensory sympathetic reflexes (Sato and Schmidt, 1973) has suggested a selective activation of different preganglionic sympathetic fibre groups, associated with the types of afferent fibre stimulated, but the pharmacology of this ganglionic transmission is still uninvestigated.

If one is to base the understanding of selective pharmacology of sympathetic ganglionic transmission at the level of selective activation of different fibre groups one must account for two observations.

The first is that large differences occur in the degree of residual response observed following nicotinic blockade between ganglia of the same and other species. These differences would have to be accounted for by changes in fibre group activation and in the association of pre-ganglionic fibres with post-junctional receptors. It may be hard to explain the differences in antagonist sensitivity between the present and other studies in these terms. Secondly, we have noticed that the vascular response to preganglionic nerve stimulation both before and after nicotinic blockade is, in the small number of preparations studied, much less than that to a physiological stimulus. Carotid chemoreceptor stimulation produces a large increase in hind-limb resistance even though one postulates only activation of a small fibre population. This would only be explained by a very large facilitatory effect, initiated by a physiological, rather than a synchronous, stimulation of the preganglionic fibres.

An alternative hypothesis is that the pattern of sympathetic discharge initiated by different physiological stimuli may account for the pharmacological differences in reflex transmission. The baroreceptor reflex involves a reduction in baroreceptor inhibitory tone from the carotid sinus stretch receptors, resulting in a change in sympathetic tone which is synchronous with the carotid pulse. In this way a situation analogous to synchronous nerve stimulation prevails with trains of stimuli of higher frequency than before being transmitted to the ganglion cells during baroreceptor tests. As the carotid body chemoreceptors produce an asynchronous discharge in response to an hypoxic stimulus this would appear to be mimicked by an asynchronous sympathetic firing pattern. Differences in the pharmacology of the ganglion transmission may reflect the inhibition by a muscarinic antagonist of a modulating mechanism necessary to provide sufficient summation of ganglion cell sensitivity to allow transmission of an asynchronous random input.

Pharmacology of Sympathetic Ganglionic Transmission via Muscarinic Receptors

In the present studies, a number of pieces of information suggest that the sensitivity of the muscarinic receptors involved in the transmission of a chemoreceptor reflex may be abnormally low. An extremely high dose of the muscarinic antagonist, hyoscine methylbromide, was needed to block the transmission of vasomotor reflexes following nicotinic blockade. In a small number of experiments, where this non-nicotinic transmission was observed, a high dose of either atropine sulphate or hyoscine methylbromide was necessary to block the residual vascular response to preganglionic synchronous stimulation following nicotinic blockade. These doses are approximately two orders of magnitude higher than those necessary to produce similar blockade of vagally mediated bradycardia in the dog. Although one cannot assess the affinity constant of the antagonists for the ganglion cell receptors, this information together with the observations on adrenomedullary muscarinic receptors (p. 107).

might indicate a difference in receptor type between these and cardiac muscarinic receptors. These observations are not supported by previous workers in the cat, rabbit and rat SCG and dog stellate ganglion where far lower doses of muscarinic antagonist, atropine sulphate being the only antagonist used, are sufficient to block similar residual transmission or the response to muscarinic agonists.

Although species differences may account for the apparent discrepancies a factor which may be of great importance is the equilibration time allowed for antagonist binding. As was explained earlier (see p 90). the clearance of atropine sulphate from plasma is much more rapid than previously anticipated and though data is not available for other species, it may indicate in the dog at least that the difference in required antagonist dosage among authors, may be explained in terms of the equilibration time allowed prior to retesting of the response. Similar data for hyoscine methyl-bromide are not available as no previous studies have used this drug.

Another explanation of the difference in the dog may arise from the fact that the primary chemoreceptor reflex is to slow the heart and no physiological pathway involving sympathetic fibres associated with a chemo-receptor reflex would be necessary in the stellate ganglion. The muscarinic mediated ganglionic transmission of a chemoreceptor reflex would occur in the lumbar sympathetic ganglia but not in the stellate ganglia where withdrawal of sympathetic tone and increased vagal tone mediate bradycardia during a primary chemoreceptor reflex.

In using this argument we still have some reservations about the apparent dependance on equilibration time. Lower doses of atropine sulphate (1 mg/kg I.V.) when administered just before the assessment of a chemoreceptor reflex, produced no inhibition. The preliminary observations from only two experiments with close arterial dosage of muscarinic agonists (pp 112 - 113) raises the possibility that two populations of muscarinic receptors may exist in the dog lumbar chain ganglia. With the present techniques we are unable to extend the investigation of sympathetic ganglionic muscarinic receptor pharma-cology in a more quantitative manner and without information on ganglionic affinity constants with different muscarinic antagonists one cannot at this stage distinguish heterogeneity amongst receptor populations.

Structural Implications of Pharmacological Selectivity

The selectivity of synaptic transmission of the baroreceptor and chemoreceptor reflexes may occur at two levels within the sympathetic ganglia (see Fig. 30). Two populations of postganglionic cell may exist, one containing solely cholinergic receptors of the nicotinic type and one containing muscarinic receptors. This would produce a situation where a 'final common path' would not be present but separate groups of pharmacologically distinct postganglionic fibres would be associated with groups of preganglionic fibres. An alternative hypothesis is that each postganglionic neurone contains more than one receptor type on its cell soma and that groups of preganglionic fibres converge on the same postganglionic neurone but are associated with different receptor groups within it. Further complications of the relationship between muscarinic and nicotinic receptors might also explain the observed results. For example it is clear that synaptic transmission of the chemoreceptor reflex involves muscarinic receptor activation but a large component of the reflex transmission remains after muscarinic blockade. One could explain this by assuming that the major transmission was nicotinic and that the sensitivity to muscarinic antagonists is an indication that a modulation is essential for chemoreceptor reflex transmission, which can carry the required impulses following nicotinic blockade. With a hypothesis of this type the reflex pathways would show selective pharmacology not in terms of activation of morphologically distinct fibres but by altering the controlling influences necessary to allow transmission of differing inputs.

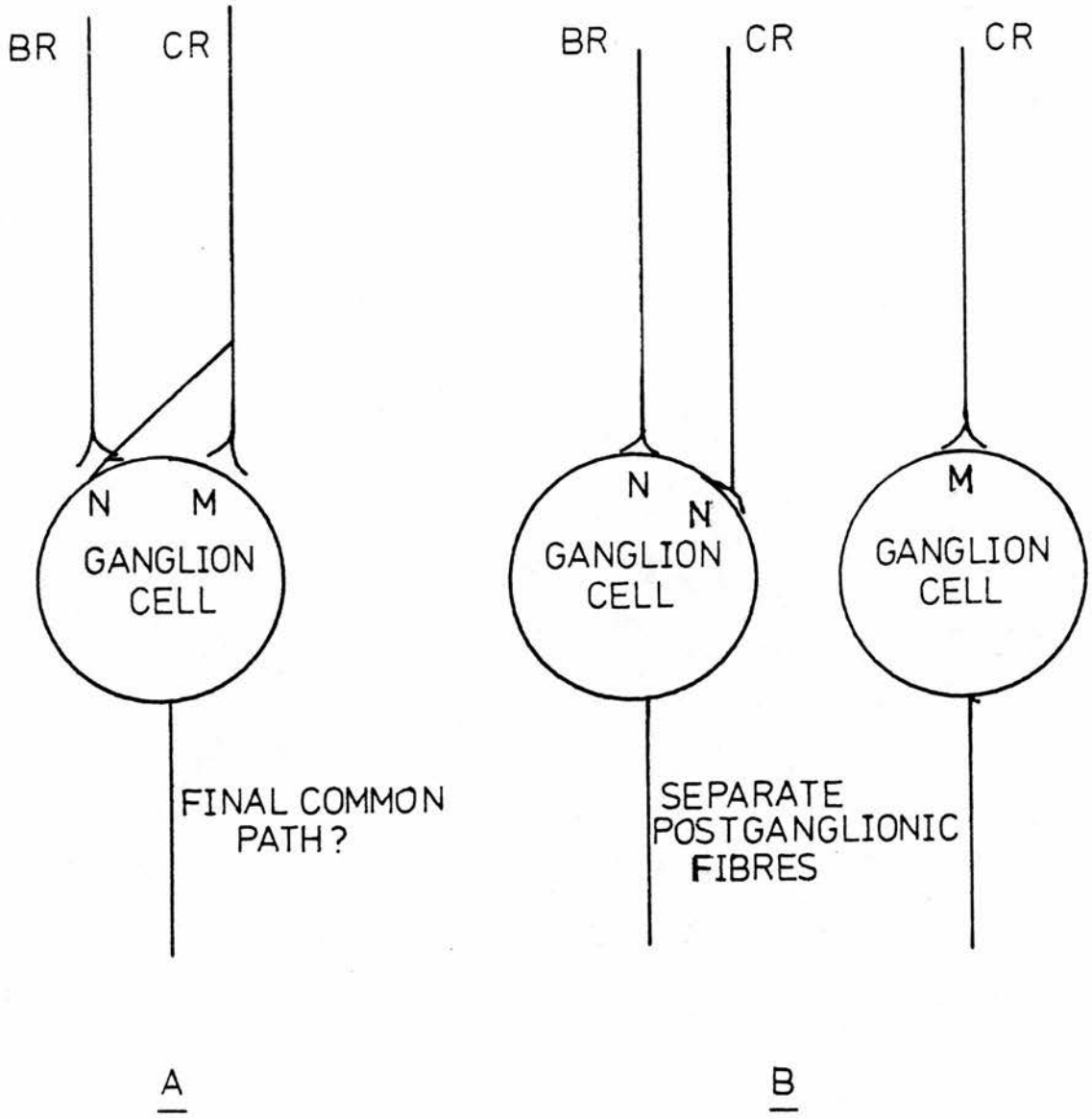
A further interesting suggestion is that the pharmacology of transmission using different fibre groups in the cat SCG depends on the end structure innervated (Koss and Rieger, 1977). This, when allied to the knowledge that distinct groups of preganglionic fibres and postganglionic cells are discernable both anatomically and electrophysiologically, suggests this concept requires further investigation.

The distinct pharmacology of vasomotor reflexes may depend on the groups of fibres activated and a similar reflex involving a different

Legend to Fig. 30

Alternative hypotheses for the involvement of cholinergic receptors in sympathetic ganglionic transmission. Model A assumes that all ganglion cells contain both receptor types and a final common path prevails. In Model B separate ganglion cells should be found with only one cholinergic receptor type either nicotinic or muscarinic. Preganglionic fibres will be associated with separate cells. BR=fibres activated during a baroreceptor reflex. CR=fibres activated during a chemoreceptor reflex.

Fig. 30


BR
CR
N
M
GANGLION CELL
FINAL COMMON PATH?
A
BR
CR
CR
N
N
M
GANGLION CELL
GANGLION CELL
SEPARATE POSTGANGLIONIC FIBRES
B

end structure, for example a pilomotor response, may exhibit different pharmacology.

Although with the use of cardiovascular responses as indicators of sympathetic activity these problems seem rather elusive, one starting point might be the application of single fibre recording techniques. Both recording preganglionically and post-ganglionically during physiological stimuli would distinguish the level at which pharmacological differences occur, and whether overlap of fibre stimulation occurs during sympathetic firing induced by different sensory inputs. Initial detailed studies involving somato-sympathetic reflexes (Sato and Schmidt, 1973), have shown groups of sympathetic fibres which are reflexly stimulated following electrical stimulation of one or more particular afferent sensory fibres. Little modification of the impulse pattern is observed when the recording is from post- rather than pre-ganglionic fibres but no pharmacological study has been performed and this area of selective transmission by different reflexes remains to be investigated.

Summary

Much of the previous evidence presented regarding the characteristics of sympathetic ganglion transmission has involved investigations in particular species and extrapolation of data to other sympathetic ganglia within or between species may be invalid. There is a large volume of evidence for the generation of slow synaptic potentials by ganglion cells but their role still awaits evaluation. Our view is that the muscarinic actions of acetylcholine, in addition to nicotinic transmission, may provide a mechanism for controlling the level of ganglion cell excitability readily influenced by the previous impulse pattern to which the cell was subjected. This would explain the way in which certain drug and physiological pretreatments can alter the muscarinic actions of exogenous drugs and acetylcholine. In view of the large subliminal fringe present in most mammalian ganglia, a mechanism for the regulation of ganglion excitability to allow for cell recruitment may be extremely important for normal transmission. From our results we would postulate in addition to this process or arising from

the slow E.P.S.P., a normal transmission of impulses during a chemoreceptor reflex involving an interaction of acetylcholine with muscarinic receptors. Whether this is observed in other than canine lumbar sympathetic ganglia awaits further investigation as does the possibility that this muscarinic receptor population, which is apparently relatively insensitive to antagonists, is involved in the transmission of other reflexes. One possibility is that these receptors mediate a modulatory response essential for the integration of non-synchronous inputs to the cells but whether these postulated heterogenous muscarinic receptors are solely present or are present in addition to a receptor population more accessible to exogenous agonists and of a more 'conventional' structure remains to be confirmed or disproved.

THE ADRENAL MEDULLA

Basal Secretion

The basal secretion rate of catecholamines from isolated, perfused adrenal glands was similar in magnitude to that reported by 'in situ' studies, but with a large variation among groups of glands. It is most likely that, in the absence of neural and humoral influences, the major component of the resting output of catecholamines from an isolated adrenal gland will arise from the spontaneous breakdown of the granules. In this respect, the variability of basal outputs among different groups of glands may reflect the level of deterioration prior to and during perfusion, and the degree of adrenomedullary hormone depletion produced by the surgical procedure to which the glands were subjected.

With the long equilibration time allowed prior to drug testing, one hour, usually one observed little decline in resting output over the time course of an experiment. This contrasts with other reported results where a steady decline in resting catecholamine output and drug responsiveness have been observed when shorter equilibration times have been used. In an isolated, perfused preparation one can exclude any influence on basal catecholamine secretion via the release of acetylcholine from splanchnic nerve endings still present following removal of the gland. This is apparent from the lack of any change in basal secretion following administration of physostigmine, hexamethonium or muscarinic antagonists. Also the retrograde nature of the perfusion excludes any influence of spontaneously released adrenocortical hormones on the adrenomedullary basal secretion.

There is a measure of controversy regarding the factors involved in the regulation of the resting adrenomedullary secretion. The predominant influence is believed to be the level of splanchnic tone (Vogt, 1965) and even allowing for the widely varying assessments of adrenal venous catecholamines among studies, a consistent reduction in adrenal catecholamine outflow is observed following acute or chronic denervation.

In addition to neural influences the present study suggests that in species where corticosteroids have a role as adrenomedullary secretagogues, they may provide an important controlling influence on the adrenomedullary resting output, via the steroid rich blood present in the adrenal portal system. In addition to a secretory role at high concentrations it seems likely that corticosteroids may have an important modulating role on the sensitivity of chromaffin cells to splanchnic nerve stimulation or circulating hormones.

In summary, the basal secretion of catecholamines from the adrenomedullary chromaffin cells appears to depend on the level of splanchnic nerve activity, but in some species the level of corticosteroids in the portal system may be important in the regulation of catecholamine output. Other factors such as the level of circulating hormones and the possibility of a large contribution derived from spontaneous granular breakdown may make varying contributions to the basal adrenomedullary secretion.

One factor which may be of experimental rather than physiological importance is the observation that mechanical stimuli, such as an air embolism, can cause a large release of catecholamines (10 —> 100 fold). Similarly on occasions brief interruptions of the flow of salt solution perfusing the adrenal gland could cause similar rises in secretion rate. This raises the possibility that the adrenal medulla may be extremely sensitive to mechanical stimuli or changes in adrenal flow or hypoxia. This might make one question how much the effects of locally administered drugs commonly used in 'in situ' experiments, may arise from their actions on adrenal blood flow rather than a direct secretory action on the chromaffin cells. This may also be important in situations where physiological stimuli may cause mechanical disturbance of the adrenal venous outflow, for example during hyperventilation with intense chemoreceptor stimulation.

Selectivity of Basal Catecholamine Secretion

The present study confirms the wide differences in the proportions of noradrenaline and adrenaline present in the resting adrenomedullary output of different species.

In the cat our results confirm the previous findings of Critchley, 1976 that the normal output of noradrenaline represents 40% of the usual resting catecholamine secretion. This figure though similar to that reported in the adrenal venous blood of cats (Critchley, Ungar and Welburn, 1973) contradicts the findings of other workers (Klevans and Gebber, 1970; Feuerstein and Gutman, 1971). These differences may reflect differences in analytical technique but more likely reflect the physiological influences on an adrenal gland perfused in vitro, rather than in situ.

Unlike other species the latter technique may lead to preferential secretion of one of the amines in response to physiological stimuli which might account for the variability in the reported content of the basal secretion from cat adrenal glands.

In the dog a much lower percentage of noradrenaline was observed (20 - 25% NA in collies; 10% in fox-hounds) and these percentages are very similar to those reported by other authors both in the perfusate from isolated glands (Vogt, 1965; Robinson, 1967; Critchley, Tibenham and Ungar, 1975) and in adrenal venous blood.

Incubated rabbit and guinea-pig glands showed a resting adrenomedullary secretion composed purely of adrenaline which agrees with the indirect conclusions of earlier studies (Shepherd and West, 1951; Coupland, 1976; Da Prada, et al, 1978).

When one compares the composition of the basal catecholamine secretion from an adrenal gland and the reported composition of stored catecholamine in the adrenal medulla of the same species, it becomes

clear that conclusions about evoked secretions, on the basis of changes in stored catecholamine levels, are tenuous. Even 'at rest' some selective release occurs and much of the catecholamine detected following adrenal gland homogenisation may not be immediately available for secretion. Until a comparative study is attempted relating catecholamine storage to secretion in a number of species one remains unsure of how much differences in experimental technique may account for the inconsistencies between different authors' published data.

Drug Evoked Adrenomedullary Secretion

Hydrocortisone Succinate

Hydrocortisone succinate was found to be a potent adrenomedullary secretatogue in the dog. Although the dose required for an action on isolated perfused adrenal glands seems high in relation to circulating corticosteroid levels it is of a similar order of magnitude to the concentrations reported in canine adrenal venous blood under physiological conditions (Marrota, 1972). This reflects the high concentration of corticosteroids present in the portal system which interconnects the adrenal cortex and medulla. The adrenomedullary secretagogue action has been distinguished as a glucocorticoid action (Critchley, 1977) and may be of physiological importance.

Our own and other studies have only established this action in two species, the dog and the rat (Roffi et al., 1966). Hydrocortisone we found to be without action on isolated perfused cat adrenal glands and it seems to be without action on bovine glands also, if one extrapolates from the adrenal venous levels reported by Bloom et al, 1977. With this selectivity apparent in only two species it is of interest to note that the enzyme induction observed by the action of glucocorticoids on P.N.M.T. and tyrosine hydroxylase (Wurtmann et al, 1972) may in fact involve withdrawal of end product inhibition. Coincidentally the species investigated during studies on P.N.M.T. induction by corticosteroids were the same as those reported to show a secretagogue action of steroids, namely the rat and the dog. This allows for an alternative explanation of enzyme induction in the adrenal medulla in response to corticosteroid as resulting from a maintained stimulation of secretion rather than a direct action of steroid on the protein synthesis mechanism itself. The possible induction of catecholamine synthetic enzymes following maintained stimulation by other directly acting secretagogues, as opposed to neurogenic transynaptic induction (see pp 30 - 31) has been overlooked.

The mechanism of action of hydrocortisone on the chromaffin cell seems rather complex. Unlike the other reported actions of corticosteroids a rapid onset of action occurs with a response commencing with a similar time-course to the action of other secretagogues. The evoked secretion of catecholamines from both breeds of dog tested was dose-dependent and large in magnitude compared to the response to other agonists and as no selective release of either catecholamine was observed one assumes that both chromaffin cell types are sensitive to hydrocortisone. Preliminary observations with cholinergic antagonists suggested that hydrocortisone's action on isolated, perfused adrenal glands might be sensitive to muscarinic but not nicotinic antagonists. In order to investigate whether this action did involve the release of acetylcholine from certain splanchnic nerve endings a number of chronic denervation experiments were performed but no conclusive results were obtained.

Finally, the response to hydrocortisone varied greatly between the breeds of dog used and a number of adrenal glands proved totally insensitive to hydrocortisone. The former observation parallels the response of different breeds of dog to A.C.T.H. (Critchley, 1977) and no explanation can be offered at present for this sensitivity difference between breeds and the marked insensitivity of some glands to hydrocortisone.

In summary, it seems that only in some species might glucocorticoid concentrations in the adrenal portal system subserve a secretory role on the adrenal medulla. This action may be important in the mediation of catecholamine secretion in response to a number of physiological stimuli e.g. stress, hypoxia, but there is evidence against this action of steroids in other than the dog. The mechanism of action of glucocorticoids on canine adrenomedullary chromaffin cells is still unknown but the possibility of an indirect rather than a direct secretory action cannot be excluded.

Cholinergic Drugs

Both nicotinic and muscarinic agonists were investigated for actions on the adrenal medullae of cats and dogs. A small number of previous studies have provoked much controversy over the types of cholinergic receptor present on chromaffin cells in different species though most previous studies have merely used an assay for total catecholamines. The use of a standardised catecholamine assay technique was viewed as a means of excluding the difficulty of comparing results of studies where widely varying assay methods and experimental designs had been employed. The improved differential analysis, together with an assay of the incremental secretion (p 104), should one hoped give a much truer picture of the mode of action of cholinergic agonists.

From the results obtained a species difference in receptor populations becomes apparent between the cat and the dog. The latter did not show any selective release of catecholamines to either selective nicotinic or muscarinic agonists and one must assume that both noradrenaline and adrenaline - containing cells possess both receptor types. This contrasts with the observations of two groups of workers (Robinson, 1967; Tsujimoto et al, 1972) but the former presents very little data and in the latter only in the first fifteen seconds of drug infusion were any significant differences observed.

In the cat, a non-selective release was obtained after nicotine (10^{-5}M) which agrees with the observations of Douglas and Poisner, 1965 and Rubin and Miele, 1968. A quantitavely similar evoked secretion produced by the ganglion-selective nicotinic agonist HPPTMA shows a very similar increased percentage of noradrenaline. (Critchley, Tibenham and Ungar, 1975).

With regard to muscarinic drugs our present results show that the selective muscarinic agonist, acetyl β methylcholine causes a preferential secretion of adrenaline from isolated cat glands. We must conclude from these observations that within the cat adrenal medulla, in contrast to the dog, the nicotinic receptors are located predominantly on

noradrenaline-containing chromaffin cells and the muscarinic receptors predominantly on the adrenaline-containing cells.

The role of muscarinic receptors in the adrenal medulla of both these species remains to be established and for example, little residual transmission from splanchnic nerve endings to chromaffin cells occurs following nicotinic blockade (Lee and Trendelenburg, 1967). The presence of muscarinic receptors on chromaffin cells combined with the knowledge that detailed changes in drug sensitivity can occur under various similar conditions, suggests that the chromaffin cells of the adrenal medulla may share a common muscarinic receptor development with postganglionic sympathetic nerve cells and this is discussed in greater detail later (p 131).

We have also investigated the detailed pharmacology of the adrenomedullary muscarinic receptor in the dog, though attempts to extend this approach to a more detailed study of structure-activity relationships, were unsuccessful. The muscarinic receptor on canine chromaffin cells with its low antagonist sensitivity may be a true case of muscarinic receptor heterogeneity, though whether this feature extends to other species or to the sympathetic ganglia (as discussed in pp 131 - 133) remains to be seen.

Potassium Chloride

Our results confirm those of Rubin and Miele, 1968 as in both cases one observed an increased percentage of NA in the CA output from the cat adrenal medulla in response to 56 mM KCl. This implies that the NA cells are more sensitive to a depolarising stimulus than the A cells. This unusual response requires further investigation as it may reflect a genuine physiological difference between the two types of chromaffin cell.

Histamine

In canine adrenal glands a dose-dependent histamine evoked catecholamine secretion was observed and this evoked secretion was non-selective, the composition not varying from that in the basal secretion. This appears to be the first study investigating a direct action of histamine on the canine chromaffin cells. Staszewska-Barczak and Vane, 1967 felt that much of the response of dog adrenal glands to histamine was indirectly mediated by increases in splanchnic tone and thus they did not exclude the possibility that the observed response to histamine may have been amplified by a baroreceptor reflex which could be initiated by the vasodilator action of the drug.

From our present study one can conclude that canine chromaffin cells possess histamine receptors of a similar sensitivity to those present within the feline adrenal medulla (Smith and Robinson, 1970). One major difference between the two species is that histamine receptors may be located solely on adrenaline-containing cells in the cat, whereas both cell types seem to possess histamine receptors in the dog.

Comparison of the Sympathetic Ganglia and the Adrenal Medulla

When one reads the literature there are obvious similarities in the physiological function of sympathetic postganglionic neurones and adrenomedullary chromaffin cells. The present study has confirmed a number of specific examples where the two cell types are pharmacologically and physiologically similar.

The synaptic transmission of a baroreceptor reflex through the canine sympathetic ganglia seems to involve a pathway containing predominantly nicotinic receptors and this closely parallels the activation of solely nicotinic receptors in the cat adrenal medulla during the same reflex (Critchley, 1977). We are still unable to confirm whether this parallelism applies for nicotinic receptors on the canine chromaffin cells as, unlike the cat, identification of receptor activation by selective preferential catecholamine secretion is not possible. No selective secretion of either catecholamine was observed with studies on canine adrenal glands. The baroreceptor reflex induced by lowering carotid sinus pressure may involve activation of solely nicotinic receptors on both the sympathetic postganglionic neurones and the adrenomedullary chromaffin cells but this has not been proven conclusively.

When one considers the carotid chemoreceptor reflex one sees a synaptic transmission through the sympathetic ganglia in which a large non-nicotinic component predominates (p 85). One explanation is that this involves,in the main, activation of muscarinic receptors on the postganglionic cells. Again we are only able to extrapolate these findings to the obvious role of muscarinic receptors in mediating the responses of cat chromaffin cells during carotid chemoreceptor reflexes. The parallel study in the dog is still at the preliminary stage. We have, however, much evidence that the muscarinic receptors within the canine adrenal medulla may differ in structure from those associated with postganglionic parasympathetic neurones. A number of our observations may indicate the

presence of a similar receptor type within the canine sympathetic ganglia studied. This could prove to be a very important case of muscarinic receptor heterogeneity and may again illustrate a close parallel in the pharmacological characteristics of postganglionic sympathetic neurones and chromaffin cells.

Arising from our work a number of questions remain unanswered. Firstly, are these different populations of cholinergic receptors present on the same or different postganglionic cells and is there in different species a parallelism between chromaffin cell receptor populations and the receptors present on sympathetic postganglionic cells? One approach to this problem must be an investigation of sympathetic ganglionic transmission by a more exact method, for example single fibre recording, combined with more detailed studies of adrenomedullary catecholamine release during distinct physiological situations with an emphasis on the determination of the receptors involved in the synaptic transmission to the chromaffin cells during vasomotor reflexes.

Any further investigation of the pharmacology of the heterogeneous muscarinic receptors postulated depends on the development or identification of a receptor antagonist with high affinity for these receptors rather than being able solely to draw conclusions from studies showing reduced affinity for "traditional" antagonists. The existence of a third type of muscarinic receptor present within the sympathetic ganglia which is distinguished by differences in agonist potency alone is not beyond the realms of possibility but one needs to compare receptor populations in different species to resolve the obvious discrepancies in the literature (p 16). Perhaps a more fruitful approach is by receptor binding studies rather than the more complex pharmacological approach.

An additional discovery has been the observed sensitivity of canine adrenomedullary cells to hydrocortisone, at dose levels similar to

corticosteroid concentrations known to be present in the adrenal portal system in a number of physiological situations. At present this secretagogue action of corticosteroids seems only to apply to the dog, of the species studied, but this may still indicate an important role for adrenocortical humoral responses during vasomotor reflexes (Critchley and Ungar, 1974). The relative importance of increased catecholamine output from the adrenal medulla in relation to increased sympathetic drive to the vasculature is still not really well defined and may vary greatly in different species. When we look closely at the degree of carotid stimulation required during our experiments to produce changes in vascular resistance, venoconstriction or adrenomedullary catecholamine secretions, we find the latter requires a much greater stimulation. All these reflex responses can only provide one with information about anaesthetised preparations and may differ greatly from those in a conscious animal. Even still, it can be noted that the relative sensitivity of vasomotor reflexes seems to differ depending on the area of the sympathetic nervous system stimulated.

It is not until one attempts a detailed study of what is known about sympathetic function does one realise how little is known about the organisation of sympathetic neurones. We know little about the importance of morphologically distinct fibre populations, their selective activation and much is left to be understood about the role of the sympathetic ganglia, let alone the complexities of the neuroeffector junction.

We know much about the biochemical organisation and cellular functioning of the chromaffin cell but its role in the autonomic regulation of the cardiovascular system and the mechanism it has developed to fulfil this role, remain to a large extent uninvestigated. In fact, much remains to be discovered about the comparative physiology of a sympathetic ganglion and the adrenal medulla. Many similarities probably arise as a result of a common embryological development but it is not surprising that common features are seen as both cells when considered as functional units are presented with the same problems. Both chromaffin and sympathetic

ganglion cells have needed to develop specific mechanisms to 'decode' the complex neural firing patterns impinging on them and to integrate this to a form where the required message is passed on by a neural or a humoral mechanism to the target tissue. Therefore it is not surprising that one finds and probably will continue to find more similarities in the electrophysiological, biochemical and pharmacological mechanisms present in the two cell types.

APPENDIX NO. 1

Vasodilatatory responses and saphenous vein reflex vascular responses

VASODILATATORY RESPONSES AND SAPHENOUS VEIN REFLEX VASCULAR RESPONSES

When we examined the changes in the hind-limb perfusion pressure during both baroreceptor and chemoreceptor tests a biphasic response was observed in most cases. The marked vasoconstriction was followed by a secondary vasodilatation in a large proportion of the animals investigated. A number of factors may account for this secondary effect and these are discussed. In addition to this "secondary vasodilatation" following vasomotor reflex induced rises in limb resistance, in a number of experiments secondary vasodilatation was observed following changes in vascular resistance produced by preganglionic sympathetic nerve stimulation. Following the abolition of vasoconstriction responses a residual vasodilatation was observed on some occasions.

Additionally the vascular response of a capacitance vessel, the canine medial saphenous vein, to baroreceptor and chemoreceptor reflexes was investigated and these preliminary observations are reported in this appendix.

METHODS

Secondary Vasodilatation

Two series of experiments were performed. In the first series involving 17 dogs the data was obtained from the results of the study on vasomotor reflex transmission and the method employed has been described previously (p 81).

The second series of experiments share the same experimental protocol employed in the study of vascular responses to preganglionic sympathetic nerve stimulation. These experiments were performed using 7 dogs anaesthetised with sodium pentobarbitone and with the mode of stimulation and hind-limb perfusion performed as before (p 87).

Saphenous Vein Perfusion

These were performed on 7 dogs anaesthetised either with sodium pentobarbitone (30 mg/kg I.V.) or, in an attempt to increase venous tone, Chloralose-Urethane (2.5%/25%; 0.2 ml/kg I.V.). The experimental procedure has previously been illustrated (p 66) and merely involved a small degree of additional surgery.

RESULTS

Vasomotor reflexes

As can be observed from Fig. 31 Table 24 most preparations studied showed a secondary fall in hind-limb vascular resistance following the primary vasoconstriction during a chemoreceptor reflex. This fall in perfusion pressure was not related to the magnitude of the initial rise. The incidence of a secondary vasodilatation during baroreceptor tests was much less, 53% against 73% and on a number of occasions secondary rises in pressure prevailed. These vasodilatatory responses were unaltered by the administration of propranolol, hyoscine methylbromide, hexamethonium bromide but were reduced by α-Flupenthixol, a dopamine antagonist, and by a combination of nicotinic and muscarinic antagonists or guanethidine sulphate. In animals where preganglionic sympathetic stimulation was used secondary vasodilatations were observed following neurally induced vasoconstrictions in 2 out of 7 dogs and these vasodilatatory responses were still observed in preparations where the initial constriction was abolished by cholinergic blockade. These residual vasodilatations were reduced by α-flupenthixol and not by propranolol.

Saphenous Vein Perfusion

In all except one animal the response of a perfused saphenous vein to baroreceptor and chemoreceptor tests was a small venoconstriction. This rise in saphenous vein perfusion pressure was resistant, during either reflex, to blockade by hexamethonium bromide and was much

TABLE 24

THE INCIDENCE OF AND SENSITIVITY TO ANTAGONISTS OF THE OBSERVED SECONDARY VASODILATATION OF THE DOG HIND-LIMB VASCULAR BED DURING BARORECEPTOR AND CHEMORECEPTOR TESTS

These results represent data from 17 dogs where the observed incidence of this vasodilatation was 53% during baroreceptor tests and 73% during chemoreceptor tests.

DRUG	Hind-limb perfusion pressure (HLPP) FALL BEFORE DRUG (mm Hg)	Hind-limb perfusion pressure (HLPP) FALL AFTER DRUG (mm Hg)
	Baroreceptor test (BR test) 5 (n = 24)	BR test 4 (n = 19)
Hexamethonium Bromide 2 mg/kg i.v.	Chemoreceptor test (CR test) 15 (n = 20)	CR test 15 (n = 22)
	BR test 10 (n = 19)	BR test 15 (n = 25)
Hyoscine Methylbromide 10 mg/kg i.v.	CR test 20 (n = 17)	CR test 19 (n = 24)
	BR test 7 (n = 3)	BR test 6 (n = 3)
Propranolol 1 mg/kg c.a.	CR test 21 (n = 4)	CR test 20 (n = 4)
	BR test 10 (n = 6)	BR test 5 (n = 6)
α-Flupenthixol 100 μg/kg c.a.	CR test 20 (n = 6)	CR test 2 (n = 6)

c.a. - drugs injected into hind-limb perfusion circuit before pump. (ie close arterially)

TABLE 25

THE MODIFICATION OF HIND-LIMB VASOCONSTRICTION BY α FLUPENTHIXOL 100 μg/kg c.a.

REFLEX VASOCONSTRICTION

Rise in HLPP before α Flupenthixol (mm Hg)		Rise in HLPP after α Flupenthixol (mm Hg)	
BR test (n = 6)	36	BR test (n = 6)	20
CR test (n = 6)	22	CR test (n = 6)	23

VASOCONSTRICTION INDUCED BY SYMPATHETIC NERVE STIMULATION

Rise in HLPP before α Flupenthixol (mm Hg)		Rise in HLPP after α Flupenthixol (mm Hg)	
1 H_z (n = 3)	32.5	1 H_z (n = 3)	13.5
2 H_z (n = 3)	41	2 H_z (n = 3)	22.5
4 H_z (n = 3)	47	4 H_z (n = 3)	27

DRUG INDUCED VASOCONSTRICTION

Rise in HLPP before α Flupenthixol (mm Hg)			Rise in HLPP after α Flupenthixol (mm Hg)		
NA	3 μg c.a. (n = 2)	16	NA	3 μg c.a. (n = 2)	3.5
	10 μg c.a. (n = 2)	34		10 μg c.a. (n = 2)	12.5
ISOP	3 μg c.a. (n = 2)	-32	ISOP	3 μg c.a. (n = 2)	-23

BR test - Baroreceptor test CR test - Chemoreceptor test

H.L.P.P. - Hind-limb perfusion pressure ISOP. - Isoprenaline sulphate

Legend to
Fig. 31
p.136c

A typical record from a vasomotor reflex transmission experiment. This illustrates the characteristic small rise in S.V.P.P. produced by chemoreceptor stimulation, (B), very little change being seen during a baroreceptor reflex (A). One should also note the marked secondary fall in H.L.P.P. following both baroreceptor and chemo-receptor reflexes. S.V.P.P.-Saphenous vein perfusion pressure H.L.P.P. - Hind limb perfusion pressure C.P.P. - Carotid perfusion pressure S.B.P. - Systemic blood pressure.

Fig. 31

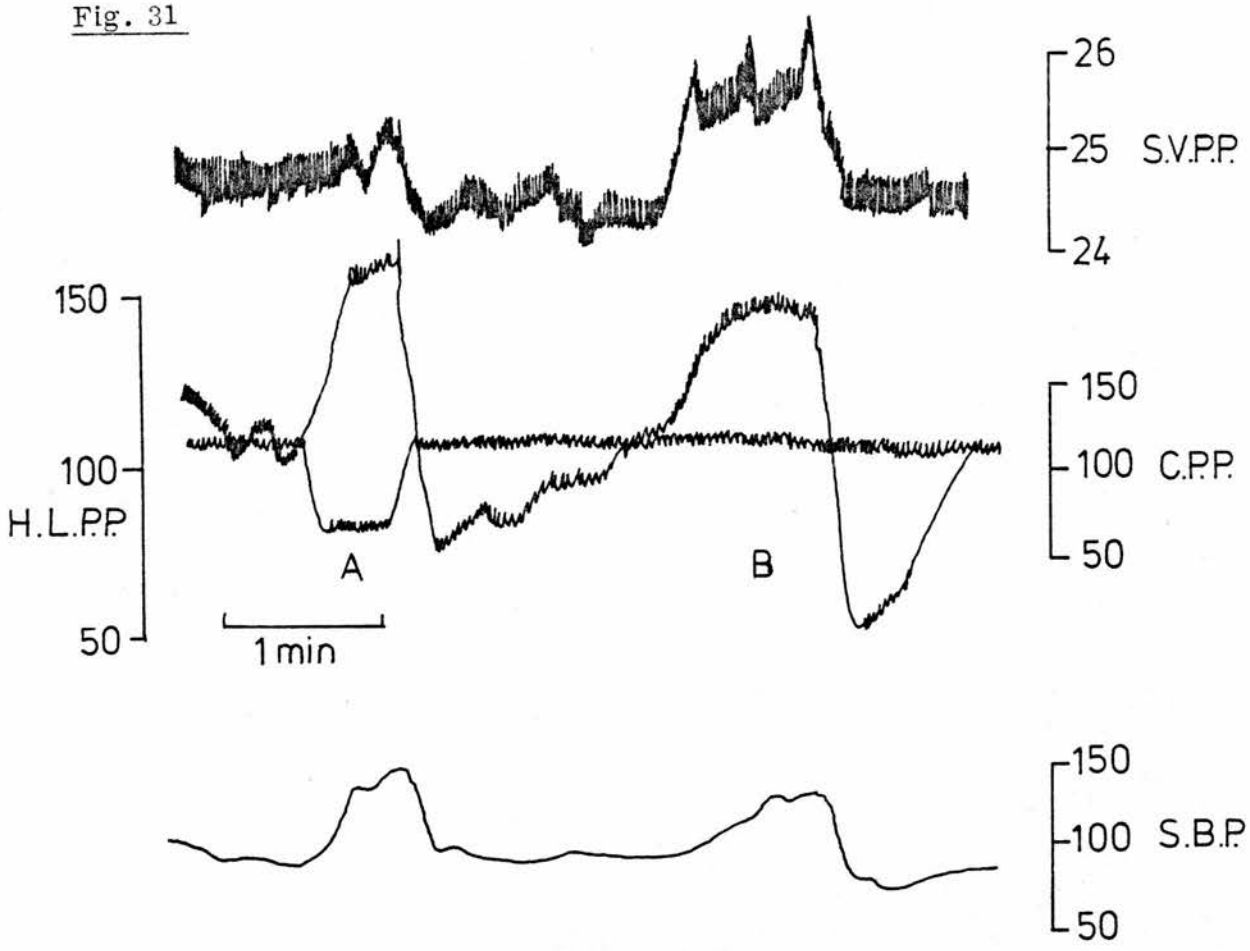

26
25
S.V.P.P.
24
150
100
H.L.P.P.
50
A
B
1 min
150
100
C.P.P.
50
150
100
S.B.P.
50

TABLE 26

THE MODIFICATION OF THE REFLEX MEDIATED SAPHENOUS VEIN RESPONSES FOLLOWING INTRAVENOUS ADMINISTRATION OF HEXAMETHONIUM BROMIDE OR A MUSCARINIC ANTAGONIST RESPECTIVELY

	BEFORE HEXAMETHONIUM				AFTER HEXAMETHONIUM (2mg/kg I.V.)				% INHIBITION	
	Baroreceptor Test (BR test)		Chemoreceptor Test (CR test)		Baroreceptor Test		Chemoreceptor Test		BR Test	CR Test
Dog. No.	CPP FALL (mmHg)	SVPP RISE (mmHg)	CAROTID PO_2 (KPa)	SVPP RISE (mmHg)	CPP FALL (mmHg)	SVPP RISE (mmHg)	CAROTID PO_2 (KPa)	SVPP RISE (mmHg)		
1	150→100	1.5	15.6→6.1	2.7	150→100	1.9	14.1→5.2	3.0	-26	-11
2	130→ 80	4.0	11.6→4.0	4.2	1.30→80	1.5	10.2→4.8	6.3	62.5	-50
3	150→100	-1.0	17.8→0.7	2.0	150→100	0	17.8→0.7	2.0	0	0
	BEFORE HYOSCINE METHYLBROMIDE				AFTER HYOSCINE METHYLBROMIDE (10mg/Kg I.V.)					
4	150→100	1.0	16.2 3.1	1.6	150→100	1.8	16.4 3.0	2.7	-80	-68
5	120→ 90	1.0	17.8→0.7	2.3	110→ 80	1.0	14.5→0.4	1.0	0	57
6	150→120	2.5	14.2 4.2	6.0	150→120	3.0	15.3 5.3	4.0	-20	33
	BEFORE α FLUPENTHIXOL				AFTER α FLUPENTHIXOL (100μg/Kg)*					
7	120→ 90	-3	16.5→4.6	-12	120→ 90	-1	21.0→3.7	2		

C.P.P. - carotid perfusion pressure S.V.P.P.- saphenous vein perfusion pressure

* α Flupenthixol was injected into the saphenous vein perfusion circuit before the pump.

(see p62a)

TABLE 27

THE MODIFICATION OF HIND-LIMB SECONDARY VASODILATATION BY α FLUPENTHIXOL

CONTROL

STIM FREQ.	H.L.P.P. FALL (mmHg)
8 Hz n = 5	19
16 Hz n = 5	31

After α Flupenthixol 100 μg/kg c.a.

8 Hz n = 4	4
16 Hz n = 5	8

c.a.- drugs injected into hind-limb perfusion circuit before pump (ie close arterially).

STIM. FREQ. - stimulation frequency H.L.P.P. - hind-limb perfusion pressure

larger in magnitude in the case of chemoreceptor test responses when stimuli producing matched resistance changes to either test were employed. In the remaining dog a marked venodilatation was noted in response to a chemoreceptor test whereas the baroreceptor test response was, as before, a small vasoconstriction. (Table 26).

DISCUSSION

A number of mechanisms might be expected to account for a secondary vasodilatation in a hind-limb vascular bed. The most likely candidate, reactive hyperaemia, can be excluded when one considers the conditions of perfusion, constant flow, and the independence of the vasodilatation from the magnitude of the preceeding vasoconstriction. A number of agonists can act to produce vasodilatation and the existence of cholinergic sympathetic vasodilation fibres is well documented.

In the present study we found that nicotinic, muscarinic and β receptor antagonists did not reduce the vasodilatation making it improbable that activation of sympathetic vasodilator fibres or circulating catecholamines accounted for the response. Indeed the secondary vasodilatation was observed much less frequently during baroreceptor tests and this appeared to arise from a masking of the response by a slow onset vasoconstriction. This constriction may well reflect the response to circulating catecholamine from the adrenal medulla where the threshold to baroreceptor induced secretion seems much lower than that to carotid chemoreceptor stimulation.

To summarise the observed vascular response is resistant to muscarinic, nicotinic and β blockade and remained, in some instances, following external preganglionic nerve stimulation even when a combination of the above drugs was employed. The vasodilatation in response to vasomotor reflexes was, however, abolished when full cholinergic blockade was produced. This leaves us with the problem of differentiating between an active vasodilatatory response mediated via a distinct receptor population on the vascular smooth muscle or the response resulting from

a reduction in sympathetic tone. Any action which reduces or abolishes sympathetic tone will appear to reduce the degree of vasodilatation possible by purely changing the resting vascular resistance in the perfused bed. This is less of a problem on the arterial side where some degree of myogenic arteriolar tone can compensate but is of extreme importance when one considers venomotor tone.

When we observed a reduction in the vasodilatation following α flupenthixol one might assume that this was an active dopamine mediated vasodilatation. It has been shown that peripheral vasodilatation can be produced by dopamine but that this may involve a reduction in noradrenaline overflow (Enero and Langer, 1975; Lokhandwala and Buckley, 1978). This action of dopamine has been shown in perfused dog hind-limbs (Bell, Conway, Lang and Padanyl, (1975) and Buylaert, Willems and Bogaert, 1978) but may not be present in the cat (Hausler, 1976). Similar evidence with regard to the action of histamine has been obtained (Heitz and Brody, 1975). In the present studies we were dubious about the selectivity of α-flupenthixol, as a dopamine antagonist and we can confirm that at the dosage used it depresses the vascular response of both resistance and capacitance vessels to a close arterial injection of noradrenaline (see Table 25). A further assay for α blocking action using isolated rabbit aorta preparations has produced a log affinity constant for α receptors for α flupenthixol of 6.78 and 5.44 for β flupenthixol. This may not markedly differ from their affinity constants at peripheral dopamine receptors though lack of a suitable tissue is impeding investigation of this surmise. Isolated spirally cut canine femoral arteries and saphenous veins did not relax to dopamine or apomorphine thus reinforcing the idea of an indirect action as the mechanism producing vasodilatations. In addition to α blocking actions α Flupenthixol may exhibit marked antihistamine properties as we cannot exclude the possibility that a histamine mediated vasodilatation was observed. In summary, the secondary vasodilatation we noted in most of our experiments appears to be sensitive to abolition of sympathetic tone by complete ganglion blockade or guanethidine treatment and is sensitive to α flupenthixol. Until a more selective dopamine antagonist is obtained,

with sufficient information about its selectivity, we cannot distinguish by which mechanism, decrease in sympathetic tone, dopamine or histamine mediated vasodilatation, either direct or indirect, the secondary vasodilatation during vasomotor reflexes and following preganglionic nerve stimulation is initiated.

The saphenous vein response to both baroreceptor and chemoreceptor reflexes was a venoconstriction. This contradicts the findings of Pelletier and Shepherd, 1972 and ourselves in one preparation where a large venodilatation was observed during chemoreceptor tests. One factor governing the venous response to chemoreceptor stimulation is the level of venous tone present at any given time. Venous tone was much greater in this preparation, as witnessed by the low flow setting needed to maintain the correct perfusion pressure, and in agreement with the above authors we found that pentobarbitone or chloralose-urethane anaesthesia greatly depressed the venous tone. This isolated observation raises the possibility that the normal primary chemoreceptor reflex response is a venodilatation if one was able to use a non-depressant anaesthetic. Marshall, 1977 has suggested that, in cats, vasodilatations rather than vasoconstrictions are observed in peripheral vascular beds during carotid chemoreceptor stimulation when one uses a steroid anaesthetic said to produce little hypothalamic depression. The use of such an anaesthetic in dogs is not possible and the mechanism of any venodilatation awaits further investigation with different anaesthetics.

APPENDIX II

Detailed results for individual experiments

FREQUENCY RATIOS IN THE PRESENCE OF ANTAGONISTS

(see Table 6)
p.89b

VAGAL SLOWING

ATROPINE SULPHATE 10 μg/kg i.v.

DOG	A	1.6	B	2.0	C	1.9	D	2.5	E 2.3

MEAN = 2.1

20 μg/kg i.v.

DOG	A	3.2	B	3.6	C	3.6	D	7	E 4.5

MEAN = 4.4

HYOSCINE METHYLBROMIDE 2 μg/kg i.v.

DOG	F	5	G	1.5

MEAN = 3

4 μg/kg i.v.

DOG	F	13	G	5

MEAN = 9

HING-LIMB RESISTANCE

ATROPINE SULPHATE 1 mg/kg i.v.

DOG	B	3	C	5

MEAN = 4

THE EFFECT OF H.M.B. ON HIND-LIMB VASOCONSTRICTION (See Table 7) p 89c

Stimulation Freq. (Hz)	H.L.P.P. rise (mm Hg)		
CONTROL			
1	91 → 140	49	
	100 → 130	30	26
	82 → 95	13	
	82 → 94	12	
2	91 → 140	49	
	100 → 145	45	37.5
	82 → 111	29	
	82 → 109	27	
4	91 → 145	54	
	100 → 155	55	59
	82 → 145	63	
	82 → 145	63	
After H.M.B. (10 mg/kg i.v.)			
1	64 → 93	29	
	91 → 127	36	21
	82 → 94	12	
	82 → 87	5	
2	64 → 100	36	
	91 → 136	45	31
	82 → 104	22	
	82 → 103	21	
4	64 → 109	45	
	100 → 149	49	47
	82 → 129	47	
	82 → 130	48	

H.M.B. - hyoscine methylbromide H.L.P.P. - hind-limb perfusion pressure

AFFINITY CONSTANTS FOR MUSCARINIC ANTAGONISTS ON NICOTINIC RECEPTORS OF FROG-RECTUS ABDOMINUS MUSCLE (See Table 8) p93a

ANTAGONIST	DOSE RATIO	log K_B
Atropine sulphate	22, 5.0, 3.7, 4.5	5.32, 4.60, 4.43, 4.54
10^{-4}M, n = 4	MEAN = 8.8	MEAN = 4.89
Hyoscine hydrobromide	1.7, 1.9, 1.75	3.85, 3.94, 3.88
10^{-4}M, n = 3	MEAN = 1.8	MEAN = 3.89
10^{-3}M, n = 4	9,11,9,10	3.88, 4.01, 3.90, 3.95
	MEAN = 10	MEAN = 3.95
Hyoscine methylbromide	2.7, 2.9, 4.5, 3.6	4.22, 4.27, 4.54, 4.41
10^{-4}M, n = 4	MEAN = 3.4	MEAN = 4.38
10^{-3}M, n = 2	28, 40	4.44, 4.59
	MEAN = 34	MEAN = 4.51
Oxyphenonium bromide	Non-competitive but dose ratio $\simeq$ 2 at 10^{-5}M	
10^{-5}M $\rightarrow$ 10^{-4}M, n = 6		

n = number of determinations

VALIDATION OF FLUORIMETRIC ASSAY (Table 10) p.95a

MIXTURE COMPOSITION	% NA	CALC. % NA
100 ng/ml NA	100	97, 98, 98, 96, 98, 97, 98, 96, 96, 96 MEAN = 97 ± 1 ± S.D.
70 ng/ml NA 30 ng/ml A	70	71, 71, 71, 75, 77, 77, 77, 77 MEAN = 74 ± 3
50 ng/ml NA 50 ng/ml A	50	58, 56, 56, 56, 57, 55, 57, 57, 56, 55 MEAN = 56 ± 1
30 ng/ml NA 70 ng/ml A	30	31, 31, 37, 34, 43, 27, 41, 41 MEAN = 35 ± 6
100 ng/ml A	0 (100)	115, 115, 115, 108, 87, 87, 87, 87, 102 MEAN = (101 ± 15)

LINEARITY OF ASSAY [CONC. VS FLUORESC.] (Table 11) p.95b

A Conc.	F x S (360 nm)	F x S (415 nm)	Calc. [A] (ng/ml) ± S.D.
1 μg/ml (n = 10)	0.93, 1.2, 1.2, 1.21 1.21, 1.29, 1.29, 1.29 1.27, 1.23	3.6, 3.9, 4.2 4.5, 4.4, 4.4 4.4, 4.4, 4.4, 4.2	
	1.27	4.20	1000 ± 60
500 ng/ml (n = 10)	0.55, 0.55, 0.55, 0.57, 0.57, 0.58, 0.58, 0.63, 0.58, 0.63	2.25, 2.25, 2.25, 2.40, 2.41, 2.42, 2.52, 2.52, 2.52, 2.55	
	0.58	2.41	510 ± 50
100 ng/ml (n = 10)	0.12, 0.12, 0.12, 0.14, 0.14, 0.12, 0.17, 0.17, 0.17, 0.17	0.53, 0.53, 0.53, 0.56, 0.50, 0.50, 0.47, 0.47, 0.47, 0.47	
	0.14	0.50	115 ± 5
50 ng/ml (n = 10)	0.06, 0.06, 0.06, 0.07, 0.08, 0.08, 0.07, 0.08, 0.08, 0.07	0.22, 0.22, 0.22, 0.23, 0.24, 0.24, 0.24, 0.23, 0.23, 0.23	
	0.07	0.23	58 ± 7

n = number of determinations

LINEARITY OF ASSAY [CONC. VS FLOURESC.] (Table 11) p.95b

NA CONC.	CALC. NA CONC. ± S.D.
1 μg/ml (n = 10)	940, 960, 1060, 960, 960, 980, 1050, 1050, 1130, 1020
	1000 ± 60
500 ng/ml (n = 10)	450, 540, 540, 530, 548 531, 511, 608, 528, 547
	547 ± 60
100 ng/ml (n = 10)	110, 110, 110, 115, 114 115, 114, 120, 120, 120
	115 ± 6
50 ng/ml (n = 10)	51, 50, 50, 60, 58 61, 62, 62, 62, 50
	55 ± 5

n = number of determinations

THE RESPONSE OF CANINE AND FELINE ADRENAL GLANDS TO HYDROCORTISONE SUCCINATE (Table 13) p100a

CROSS-BRED COLLIES

Hydrocortisone dose	Evoked CA release (% of control) ± S.E.
20 $\mu g\ min^{-1}$ (n = 7)	114, 171, 142, 164, 163, 138, 164 153 ± 8
60 $\mu g\ min^{-1}$ (n = 9)	386, 172, 115, 213, 156, 325, 173, 117, 333 221 ± 33
200 $\mu g\ min^{-1}$ (n = 24)	1197, 218, 311, 308, 233, 115, 219, 900, 332, 243, 261, 247, 280, 263, 209, 382, 333, 236, 203, 180, 390, 335, 152. 328 ± 50
400 $\mu g\ min^{-1}$ (n = 11)	1100, 1140, 351, 1031, 1160, 559, 490, 903, 509, 452, 1158. 805 ± 99

FOX-HOUNDS

Hydrocortisone dose	Evoked CA release (% of control)
200 $\mu g\ min^{-1}$ (n = 16)	124, 114, 300, 100, 133, 160, 138, 142, 200, 330, 175, 166, 200, 138, 124, 125 167 ± 16
400 $\mu g\ min^{-1}$ (n = 18)	140, 142, 150, 150, 135, 500, 150, 162, 140, 225, 400, 366, 600, 460, 266, 138, 156, 180, 248 ± 35

CATS

Hydrocortisone dose	Evoked CA release (% of control)
400 $\mu g\ min^{-1}$ (n = 10)	60, 100, 100, 78, 100, 100, 150, 100, 90, 80. 100

n = number of determinations

THE RESPONSE OF CANINE AND FELINE ADRENAL ADRENAL GLANDS TO HYDROCORTISONE (Table 13) p100a

% OF NORADRENALINE

CROSS-BRED COLLIES

Hydrocortisone dose	% NA ± S.E. Control ⟶ (Test)
20 $\mu g\ min^{-1}$, n = 7	14(21), 36(40), 23(32), 3(11), 27(30), 22(28), 20(29) 21 ± 4 ⟶ 27 ± 3
60 $\mu g\ min^{-1}$, n = 9	2(45), 9(18), 28(30), 32(33), 26(28), 0(2), 11(10), 13(12), 15(18). 16 ± 4 ⟶ 22 ± 5
200 $\mu g\ min^{-1}$, n = 24	2(26), 33(33), 19(23), 24(33), 27(34), 35(34), 11(0), 19(22), 16(15), 20(29), 57(41), 37(27), 32(37), 32(46), 9(9), 2(10), 13(20), 12(19), 6(26), 10(22), 10(14), 15(9), 16(16), 13(17), 19 ± 3 ⟶ 24 ± 2
400 $\mu g\ min^{-1}$, n = 11	9(8), 21(27), 20(9), 50(44), 0(18), 6(8), 21(31), 3(8), 15(15), 11(22), 14(4). 15 ± 4 ⟶ 18 ± 4

FOX-HOUNDS

200 $\mu g\ min^{-1}$, n = 16	20(32), 20(0), 0(0), 0(5), 0(0), 11(7), 0(10), 15(0), 9(4), 24(15), 5(31), 38(32) 0(0), 0(0), 0(0), 0(0). 8 ± 3 ⟶ 9 ± 3

n = number of determinations

400 $\mu g\ min^{-1}$, n = 18	0(0), 0(0), 4(0), 0(0), 20(0), 4(14), 5(18), 3(0), 10(28), 16(12), 22(33), 38(17), 11(26), 10(16), 9(2), 0(8), 0(7), 0(0).
	$7 \pm 2 \longrightarrow 10 \pm 3$

CATS

400 $\mu g\ min^{-1}$, n = 10	39(37), 40(40), 42(42), 31(43), 41(41), 29(42), 42(42), 42(20), 37(27), 45(49).
	$39 \pm 3 \longrightarrow 38 \pm 3$

n=number of determinations

MODIFICATION OF RESPONSES TO HYDROCORTISONE BY CHOLINERGIC ANTAGONISTS (Table 14) p100b

CROSS BRED COLLIES

HYDROCORTISONE	EVOKED CA SECRETION (% OF CONTROL) ± S.E.		
	BEFORE DRUGS	AFTER ATROPINE (10^{-6}M)	AFTER C_6 (10^{-4}M)
200 μg min^{-1}	333; 204; 179; 426; 177	136; 123; 170	242; 307
	MEAN 264 ± 50 (n = 5)	143 ± 14 (n = 3)	275 (n = 2)
400 μg min^{-1}	1140; 351; 1031; 1160; 503; 700.	415; 186; 450; 1125; 123; 170.	1185; 325; 755.
	MEAN 814 ± 141 (n = 6)	411 ± 153 (n = 6)	755 ± 250 (n = 3)

FOX-HOUNDS

HYDROCORTISONE	EVOKED CA SECRETION (% OF CONTROL)		
	BEFORE DRUGS	AFTER ATROPINE (10^{-6}M)	AFTER C_6 (10^{-4}M)
200 μg min^{-1}	173 (n = 3)	100; 96	305; 200
		98 (n = 2)	250 (n = 2)
400 μg min^{-1}	151; 400	125; 109	400; 331
	281 (n = 2)	117 (n = 2)	366 (n=2)

THE RELEASE OF CA BY CHOLINERGIC AGONISTS AND KCL (Table 16) p.106a

DOGS	RESTING SECRETION (ng min^{-1})	EVOKED SECRETION (ng min^{-1})
Nicotine, 10^{-5}M (n = 6)	360; 180; 100; 200; 60; 280.	580; 640; 300; 620; 180; 460.
	MEAN = 190 ± 40	630 ± 200
Acetyl-β-Methylcholine (n = 10) 10^{-6}M	220; 240; 210; 130; 170; 180; 420; 400; 410; 460.	310; 1400; 860; 280; 380 320; 1400; 560; 590; 700.
	MEAN = 280 ± 40	680 ± 130

CATS	RESTING SECRETION (ng min^{-1})	EVOKED SECRETION (ng min^{-1})
Nicotine, 10^{-5}M (n = 8)	180; 360; 120; 180; 200; 60; 60; 160.	380; 920; 360; 320; 280; 380; 580; 540.
	MEAN = 160 ± 30	470 ± 70
Acetyl-β-Methylcholine (n = 6) 10^{-6}M	240; 200; 200; 60; 60; 160.	300; 240; 260; 480; 340; 500.
	MEAN = 150 ± 30	350 ± 40
Potassium Chloride, 56mM (n = 6)	180; 300; 240; 180; 160; 160.	1620; 1440; 1420; 1360; 300; 1440.
	MEAN = 200 ± 20	1260 ± 200

% OF NORADRENALINE

DOGS	% OF NA CONTROL (INCREMENT)
Nicotine, 10^{-5}M (n = 6)	16(24); 11(15); 46(44); 22(16); 29(5); 15(2).
	MEAN = 23 ± 5 → (18 ± 6)

All means are ± S.E.
n = number of determinations

Acetyl-β-Methylcholine, 10^{-6}M (n = 10)	36(37); 24(25); 24(31); 24(5); 20(28); 14(21); 23(33); 33(26); 24(25); 20(26).
	MEAN = 24 ± 2⟶(28 ± 2)

CATS

Nicotine, 10^{-5}M	47(44); 52(100); 58(58); 23(78); 36(47); 45(17); 41(54); 39(49);
	MEAN = 42 ± 4⟶(55 ± 9)
MCh, 10^{-6}M	55(0); 27(8); 30(26); 40(22); 33(13); 48(32).
	MEAN = 41 ± 4⟶(16 ± 3)
KCl, 56 mM	47(74); 40(65); 40(68); 43(71); 20(50); 40(68).
	MEAN = 38 ± 4⟶(66 ± 3)

All means are ± S.E.
n = number of determinations

AFFINITY CONSTANTS FOR MUSCARINIC ANTAGONISTS ON CANINE ADRENAL MEDULLA (TABLE 17) p108a

DRUG	DOSE RATIO	K_B
Atropine SO_4. n = 3 (10^{-6}M)	7.8; 7.3; 7.0 Mean = 7.4 ± 0.2	6.4×10^6
Oxyphenonium Bromide. n = 3 2×10^{-7}M	9.2; 12.1; 34 Mean = 18.4 ± 8	8.7×10^7
Oxyphenonium Bromide. n = 3 2×10^{-6}M	60; 55; 117 Mean = 77 ± 20	3.8×10^7
Hyoscine Methylbromide. n = 3 2×10^{-7}M	8.4; 5.2; 17.7 Mean = 10.4 ± 3.7	4.7×10^7

n=number of determinations

THE ACTION OF HISTAMINE AS AN ADRENOMEDULLARY SECRETAGOGUE IN THE DOG (Table 19) p.1096

HISTAMINE DOSE	EVOKED CA RELEASE (% OF CONTROL) ± S.E.
20 $\mu g\ min^{-1}$ (n = 10)	150; 123; 119; 106; 103; 118; 121; 171; 242; 137.
	MEAN = 139 ± 13
60 $\mu g\ min^{-1}$ (n = 6)	189; 196; 193; 168; 214; 181.
	MEAN = 189 ± 10
200 $\mu g\ min^{-1}$ (n = 7)	324; 168; 134; 399; 180; 700; 425.
	MEAN = 333 ± 75

HISTAMINE DOSE	% NA CONTROL → (% NA IN EVOKED SECRETION)
20 $\mu g\ min^{-1}$ (n = 10)	28; 28; 41; 33; 9; 37 29; 51; 32; 31.
	MEAN = 32 ± 4
	(25); (21); (40); (38); (20); (42); (27); (60); (32); (35).
	MEAN = 34 ± 4.
60 $\mu g\ min^{-1}$ (n = 6)	35(26); 34(33); 18(34); 32(27); 30(37); 23(31)
	MEAN = 23 ± 3 (31 ± 2)
200 $\mu g\ min^{-1}$ (n = 7)	31(39); 28(34); 62(65); 31(27); 25(27); 31(26); 8(4).
	MEAN = 31 ± 6 (32 ± 7)

All means are ± S.E.
n = number of determinations

THE RESPONSE OF THE CANINE ADRENAL MEDULLA TO BARORECEPTOR AND CHEMORECEPTOR TESTS (Table 20) p111a

ADRENAL VENOUS PLASMA CA CONC. (ng/ml)

DOG No	CONTROL (n = 3)		AFTER C_6 2 mg/kg		
			CONTROL		
AC1	34; 49; 137	(73)	AC3	90; 170	(130)
AC2	115; 86	(93)			
AC3	850; 360	(470)			

BARORECEPTOR TEST (n = 3) C.P.P. fall (mmHg) [150 → 100]

BR TEST (n = 1) C.P.P fall (mmHg) [150 → 100]

DOG No.			DOG No.		
AC1	53; 69; 151	(91)	AC3	125; 260	(200)
AC2	140; 161	(150)			
AC3	460; 563	(510)			

DOG No.	CONTROL (n = 3)		DOG No.	CONTROL (n = 1)	
AC1	34; 182; 138	(118)	AC3	95; 215	(155)
AC2	87; 99	(93)			
AC3	370; 410	(390)			

CHEMORECEPTOR TEST (n = 3)

CR TEST (n = 1)

DOG No.	Carotid P_aO_2 (KPa)		DOG No.	Carotid P_aO_2 (KPa)	
AC1	[14.2 → 4.2]		AC3	[15.0 → 3.1]	
	120; 182; 174	(159)		120; 275	(200)
AC2	[15.2 → 6.3]				
	83; 87	(85)			
AC3	[14.5 → 4.1]				
	510; 590	(550)			

C_6 - hexamethonium C.P.P. - carotid perfusion pressure

contd.

AFTER HYOSCINE METHYLBROMIDE 10 mg/kg I.V.

CONTROL (n = 2)

DOG No.

AC1 220; 82; 117 (127)

AC2 90; 94 (92)

C.P.P. fall (mmHg)

BR TEST [150 → 100] n = 2

DOG No.

AC1 215; 129; 262 (202)

AC2 360; 420 (390)

CONTROL (n = 2)

DOG No.

AC1 116; 209 (162)

AC2 98; 86 (92)

CHEMORECEPTOR TEST (n = 2)

DOG No.

AC1 92; 103 (98)

Carotid P_aO_2 (kPa) [15.0 → 5.5]

AC2 210; 250 (230)

Carotid P_aO_2 (kPa) [15.6 → 5.9]

A COMPARISON OF THE HYDROCORTISONE EVOKED CA RELEASE IN DENERVATED AS COMPARED TO INNERVATED ADRENALS (Table 21) p.112a

DOG		EVOKED CA SECRETION (% CONTROL) INNERVATED	DENERVATED
C1	200 $\mu g\ min^{-1}$ H/C	106; 116 [110]	139; 145 [142]
	400 $\mu g\ min^{-1}$ H/C	100; 100 [100]	110; 124 [117]
C2	200 $\mu g\ min^{-1}$ H/C	120; 128 [124]	100; 100 [100]
	400 $\mu g\ min^{-1}$ H/C	160; 120 [140]	100; 100 [100]
C3	400 $\mu g\ min^{-1}$ H/C	142; 150 [146]	100; 100 [100]
	1 mg $\mu g\ min^{-1}$ H/C	160; 150 [155]	100; 100 [100]
C4	200 $\mu g\ min^{-1}$ H/C	160; 120 [140]	100; 100 [100]
	1 mg min^{-1} H/C		100
C5	200 $\mu g\ min^{-1}$ H/C	114; 113 [115]	
	400 $\mu g\ min^{-1}$ H/C	155; 135 [145]	100; 100 [100]

H/C- hydrocortisone succinate
Mean values in parenthesis.

THE ACTION OF MUSCARINIC AGONISTS AS GANGLION STIMULANTS (Table 22) p113a

AGONIST	H.L.P.P. RISE (mmHg) CONTROL	AFTER H.M.B. 1 mg/kg I.V.
M^CNA343, 200 µg i.a. (n = 4)	Dog K1 16; 25	6; 3
	Dog K2 15; 17	5; 0
	MEAN = 18	MEAN = 3.5
Acetyl-β-Methylcholine 100 µg i.a. (n = 4)	Dog K1 -34; -47	-24; -21
	Dog K2 -33; -37	-13; -20
	MEAN = -38	- 19.5

H.L.P.P.- hind-limb perfusion pressure H.M.B.- hyoscine methylbromide
n = number of determinations. i.a.- intra-aortic

THE RESTING AND DRUG-EVOKED OUTPUT OF CA FROM ISOLATED RABBIT AND GUINEA-PIG ADRENALS INCUBATED IN VITRO (Table 23) p113b

CA SECRETION (ng/mg/wet weight tissue)

RABBIT	CONTROL	HYDROCORTISONE
(n = 11)	118; 188; 605; 480; 840; 270; 140; 1730; 720; 283.	315; 102; 58; 848; 2400; 790; 170; 880; 800; 1640.
MEAN ± S.E.	560 ± 154	800 ± 734

GUINEA-PIG	CONTROL	HYDROCORTISONE
(n = 11)	440; 590; 150; 106; 466; 71; 393; 36; 623; 141; 432; 390.	1770; 1290; 280; 818; 151; 200; 163; 568; 83; -68; -480.
MEAN ± S.E.	345 ± 57	561 ± 161

Neither result is significant by paired t-test
n = number of determinations

APPENDIX III

List of drugs and chemicals

LIST OF DRUGS AND CHEMICALS

Acetic acid, glacial (B.D.H. 'Aristar')

Acetyl-B-methylcholine ('Methacholine', B.D.H.)

Adrenaline, free base ('L-Epinephrine', Sigma)

Amberlite CG 50 Na^+ 100 - 200 mesh (B.D.H.)

Ascorbic acid (B.D.H.)

Atropine sulphate (B.D.H.)

Boric Acid (B.D.H. 'Analar')

British Anti-Lewisite (2-3 Dimercaptopropanol, B.D.H.)

Calcium chloride (1M; B.D.H. 'Analar')

Carbamylcholine ('Carbachol', B.D.H.)

α (cis) Flupenthixol (Lündbeck)

β (trans) Flupenthixol (Lündbeck)

Glucose (B.D.H. 'Analar')

Guanethidine sulphate (B.D.H.)

Heparin (mucous) ('Pularin' Evans 5000 units/ml and 1000 units/ml)

Hexamethonium bromide (Koch-Light then purified by recrystallisation)

Histamine acid phosphate (B.D.H.)

Hydrocortisone succinate (Soluble inj., Glaxo)

m Hydroxyphenlypropyl T.M.A. (Dr. R.B. Barlow)

Hyoscine hydrobromide (B.D.H.)

Hyoscine methylbromide (Racemic mixture, Upjohn; (-) Scopolamine methylbromide, Sigma)

Hyoscine methyliodide (Dr. R.B. Barlow)

Iodine, resublimed (B.D.H.)

Lignocaine (2% xylocaine, Astra)

M^{c}NA 343 (M^{c}Neil Corp.)

Nicotine hydrogen tartrate (B.D.H.)

Noradrenaline, free-base ('L-arterenol', Sigma)

Oxyphenonium bromide (Ciba-Geigy)

Potassium chloride (B.D.H., 'Analar')

Potassium ferricyanide (B.D.H., 'Analar')

Propranolol ('Inderal', I.C.I.)

Sodium bicarbonate (B.D.H. 'Analar')

Sodium chloride (B.D.H. 'Analar')

Sodium dithionite (B.D.H.)

Sodium dihydrogenorthophosphate 2 $H_2 0$. (B.D.H.)

Sodium E.D.T.A. (Sigma)

Disodium hydrogenorthophosphate 2 $H_2 0$ (B.D.H.)

Sodium hydroxide (B.D.H. 'Analar')

Sodium iodide (B.D.H.)

Sodium metabisulphite (B.D.H. 'Analar)

Sodium pentobarbitone ('Nembutal' Abbott; or 'Sagatal', May and Baker)

Sodium sulphite, anhydrous (B.D.H. 'Analar')

Taurine (Sigma)

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The release of catecholamines from perfused canine adrenal glands by corticosteroids

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We have shown (Critchley & Ungar, 1974) that intravenous administration of corticotrophin in dogs causes the release of adrenaline and noradrenaline from the adrenal medulla. This release is inhibited by cycloheximide, a drug that blocks the secretion of corticosteroids from the adrenal cortex. This observation raises the possibility that the output of corticosteroids from the cortex may regulate that of catecholamines from the medulla in parallel with neural influences. We have now studied the influence of hydrocortisone on the output of catecholamines from isolated perfused adrenal glands.

Adrenal glands were excised from dogs under pentobarbitone anaesthesia, and perfused retrogradely through the adrenolumbar vein with Locke's solution at a constant flow of 2 ml./min. The effluent from the cut ends of the arteries was collected in 30 sec fractions by an Unicam AC60 analyser, which made estimations of catecholomine concentrations by the trihydroxyindole fluorimetric method.

After collection of samples from the resting gland hydrocortisone was infused for 8 min at one of three rates. The results are shown in Table 1. In three glands similar responses were obtained with deoxycorticosterone.

Table 1. Results from 8 adrenal glands having a mean resting output of 202 ± 24 ng/min

	Hydrocortisone sodium succinate infused at		
	30 μg/ml.	50 μg/ml.	100 μg/ml.
Incremental output of catecholamines (ng/min)	48 ± 7	253 ± 54	610 ± 117

These results support the theory that corticosteroids may play a part in regulating the release of adrenal catecholamines as well as the synthesis of adrenaline.

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tagonist affinity constants for renomedullary muscarinic receptors

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scarinic agonists have been shown to stimulate echolamine release from the adrenal medulla (Critchley, Tibenham, Ungar, Waite & West, 1975). The action of these secretagogues is inhibited by muscarinic antagonists such as atropine. In view of the high concentrations of antagonist required to block the response, it was decided to investigate the affinity constants of a number of antagonists for the adrenal medullary receptors.

Canine adrenal glands were excised and perfused retrogradely with oxygenated Locke's solution at 37°C through the adrenolumbar vein at a constant flow of 2 ml/minute. The effluent was collected for 30 s periods and the catecholamine content assayed by the trihydroxyindole method (Vendsalü, 1960). The glands were stimulated by two low and two high doses of the specific muscarinic agonist acetyl beta methylcholine ($10^{-8} \rightarrow 10^{-6}$ M) prior to a 90 min equilibration with perfusate containing the antagonist. The response to the agonist was defined as the increment of catecholamine output expressed as a percentage of the resting output. Further higher doses of agonist, in the presence of antagonist, were administered to produce similar responses to those previously obtained enabling the affinity constant of the antagonist to be calculated from the Gaddum/Schild equation (Table 1).

Table 1 A comparison of the antagonist affinity constants for the adrenal medulla and those values reported for the guinea pig ileum

Antagonist	*Mean dose ratio*	*Adrenomedullary affinity constant (K_B medulla)*	*Reported ileum affinity constant (K_B ileum)*	$\frac{K_B \text{ ileum}}{K_B \text{ medulla}}$
Atropine sulphate, 10^{-6} m, $n=3$	7.36 (6.99–7.80)	6.36×10^8	10^{9*}	157
Oxyphenonium bromide, 2×10^{-7} m, $n=3$	18.38 (9.17–34.20)	8.69×10^7	$5.98 \times 10^{9*}$	69
Oxyphenonium bromide, 2×10^{-6} m, $n=3$	77.06 (54.7–116.6)	3.80×10^7	$5.98 \times 10^{9*}$	157
Methylhyoscinium iodide, 2×10^{-7} m, $n=3$	10.42 (5.18–17.69)	4.71×10^7	5.03×10^{9}†	107

* Abramson, Barlow, Mustafa & Stephenson, 1969.
† Barlow, Franks & Pearson, 1973.

We conclude that in the canine adrenal medulla there exists a population of muscarinic receptors for which the muscarinic antagonists tested showed an affinity two orders lower than that reported for the guinea-pig ileum receptors and this may prove to be a true case of muscarinic receptor heterogeneity.

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EFFECT OF CHOLINERGIC ANTAGONISTS ON SYMPATHETIC GANGLIONIC TRANSMISSION OF VASOMOTOR REFLEXES FROM THE CAROTID BARORECEPTORS AND CHEMORECEPTORS OF THE DOG

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SUMMARY

1. In anaesthetized dogs the reflex vascular resistance changes in a perfused hind limb were studied following carotid baroreceptor or chemoreceptor stimulation.

2. The observed rises in resistance were sympathetically mediated and thus provided a means of studying the action of the cholinergic antagonists on the sympathetic ganglion transmission.

3. The reflex response to carotid baroreceptor stimulation produced by lowering the pressure in the carotid sinuses was abolished by hexamethonium bromide but not reduced by hyoscine methyl bromide.

4. The reflex response to carotid body chemoreceptor stimulation, by hypoxia, was not altered by hexamethonium bromide but was greatly reduced by the hyoscine methyl bromide. No reflex response was seen when both antagonists were present.

5. These results indicate that sympathetic ganglion synaptic transmission during the baroreceptor reflex is mediated by nicotinic receptor activation. The transmission evoked by chemoreceptor stimulation involves muscarinic receptors with a subsidiary nicotinic pathway. High doses of an antagonist were necessary to block the muscarinic component of transmission and this is discussed in relation to previous work.

6. No non-cholinergic transmission of the reflex responses was observed.

INTRODUCTION

The existence of muscarinic receptors in sympathetic ganglia is well established. Residual responses to electrical stimulation of preganglionic sympathetic nerves, after the administration of nicotinic antagonist drugs, have been attributed to muscarinic transmission (Brown, 1967; Flacke & Gillis, 1968; Trendelenburg, 1966). Similarly the reflex responses to carotid occlusion (Steinberg & Hilton, 1966) and to raised intracranial pressure (Hilton & Steinberg, 1966) have components that resist blockade by nicotinic antagonists, the residue being blocked by atropine-like drugs. Chinn & Hilton (1976) stated that the cardiac responses to electrical stimulation of the sympathetic rami can be partly blocked by atropine and partly by chlorisondamine.

These observations are compatible with the theory that there are two populations of ganglionic synapses, having nicotinic and muscarinic post-junctional receptors respectively. An alternative explanation is that muscarinic receptors are present but do not have a direct role in transmission unless the main nicotinic receptors are blocked.

This project was provoked by the chance observation that hexamethonium bromide, at a dose that abolished the reflex rise in blood pressure in response to lowering the carotid sinus pressure, did not even diminish the rise in blood pressure to carotid body hypoxia. Both responses were abolished by guanethidine sulphate. These observations made a prima facie case for non-nicotinic transmission in the sympathetic ganglia of the chemoreceptor, but not of the baroreceptor, vasomotor reflex. We have therefore investigated this difference.

METHODS

Anaesthesia

Dogs weighing between 9 and 31 kg were anaesthetized by an I.V. injection of sodium pentobarbitone (30 mg/kg). Anaesthesia was maintained by further doses as necessary.

Respiration and heat control

The trachea was cannulated and connected to a Starling 'Ideal' pump. The lungs were ventilated with a metered oxygen-nitrogen mixture so as to hold P_{a,CO_2} at 4 KPa and P_{a,O_2} above 13 KPa, measured from frequent arterial blood samples on a Radiometer BMS3 analyser. A molar solution of sodium bicarbonate was injected I.V. after each arterial sample to hold the arterial pH at 7·4. Body temperature was held near to 37 °C by a heating pad linked to a rectal thermistor probe.

Hind-limb perfusion

One femoral artery was cannulated at the level of the inguinal ligament, and its major collaterals were ligated. The hind limb was perfused with blood from the other femoral artery through a Watson Marlow MHRE pump. When the pump was stopped the perfusion pressure always fell to below 30% of the systemic pressure. The pump was set so that hind-limb perfusion pressure was always higher than systemic blood pressure. One hind limb was thus perfused at constant flow, and changes in perfusion pressure were taken as a measure of changes in vascular resistance in that limb.

Carotid perfusion

Both common carotid arteries were cannulated both ways, and blood from one of them was perfused into both, towards the head, by a Watson Marlow MHRE pump. Both superior thyroid, internal carotid and external carotid arteries were ligated, and any other branches between the point of cannulation and the origins of the lingual arteries. Only the lingual arteries were left open, to maintain an adequate flow through the perfusion system.

A pressure transducer was connected to the perfusion circuit. The signal was passed through a servo amplifier to the perfusion pump, so that perfusion pressure could be set at will and held constant.

Recording of blood pressure

Systemic blood pressure, hind limb and carotid perfusion pressures were recorded by means of Bell and Howell L222 pressure transducers, calibrated against a mercury manometer, on a Honeywell Visicorder 2206.

Stimulation of reflexes

Two kinds of tests were performed. Baroreceptor tests consisted of a lowering of carotid perfusion pressure, from a constant resting level, while the P_{a,O_2} of the perfusing blood was held high. Chemoreceptor tests consisted of a lowering of the P_{a,O_2} of the perfusing blood by infusing into it a 1 M solution of sodium dithionite, at a rate of about 150 mg/min (Critchley & Ungar, 1974), while holding the perfusion pressure constant. The systemic arterial blood gas tensions did not change whilst sodium dithionite was infused into the carotid circuit. In each experiment we adjusted the stimuli in the two kinds of tests to match the amplitudes of the reflex responses. The duration of each test was 30 sec.

Both vagosympathetic trunks were cut in the neck to abolish compensatory reflexes from thoracic receptors.

Drugs

Drugs used were sodium pentobarbitone (Nembutal, Abbot or Sagatal, May and Baker), hexamethonium bromide (Koch-light, then recrystallized), (±) hyoscine methyl bromide (Upjohn), guanethidine sulphate (B.D.H.), sodium dithionite (B.D.H.) dextran with dextrose (Fisons and B.D.H.), and lignocaine (Astra Chemicals).

RESULTS

Evaluation of baroreceptor and chemoreceptor tests by carotid sinus nerve block

In two dogs baroreceptor and chemoreceptor tests were performed before and after the application of 0·5 ml. 2% lignocaine solution to each carotid sinus nerve. The hind-limb perfusion pressure immediately rose from 165 and 135 mmHg to 257 and 270 mmHg respectively, and recovered within 10 min to 200 mmHg in both dogs. Similar rises occurred in the systemic blood pressure. The results are illustrated in Fig. 1*A* and *B*. Sinus nerve block totally abolished the vascular responses to both kinds of tests and also abolished the reflex changes in systemic blood pressure. In spontaneously breathing dogs the respiratory response to chemoreceptor tests was also abolished.

Both reflex responses had recovered after 1 hr.

The action of hexamethonium bromide on reflex vasoconstriction

In six dogs baroreceptor and chemoreceptor tests were performed before, and between 10 and 30 min after, the I.V. administration of the nicotinic antagonist hexamethonium bromide (1–2 mg/kg). The systemic blood pressure tended to fall after hexamethonium bromide but was restored by the injection of 5% dextran in 5% dextrose solution if necessary. There was a transient fall in hind-limb perfusion pressure which was corrected if necessary by increasing the flow. The mean systemic blood pressure was 90 ± 4 before and 78 ± 5 mmHg after hexamethonium bromide. The mean resting hind-limb perfusion pressure was 106 ± 2 before and 105 ± 2 mmHg after hexamethonium bromide.

The results are set out in Table 1(*a*) and illustrated in Fig. 1.

Hexamethonium bromide almost completely abolished the responses to baroreceptor tests, but did not significantly affect those to chemoreceptor tests.

The action of hyoscine methyl bromide on reflex vasoconstriction

In six dogs baroreceptor and chemoreceptor tests were performed before, and between 20 and 40 min after, the I.V. administration of the muscarinic antagonist

hyoscine methyl bromide (10 mg/kg). The mean systemic pressure was 107 ± 3 before and 104 ± 4 mmHg after the drug. The mean resting hind-limb perfusion pressure was 117 ± 2 before and 113 ± 2 mmHg after hyoscine methyl bromide.

The results are set out in Table 1(*b*) and illustrated in Fig. 1. Hyoscine methyl bromide substantially inhibited the responses to chemoreceptor tests ($P < 0{\cdot}01$), but had little or no effect on those to baroreceptor tests.

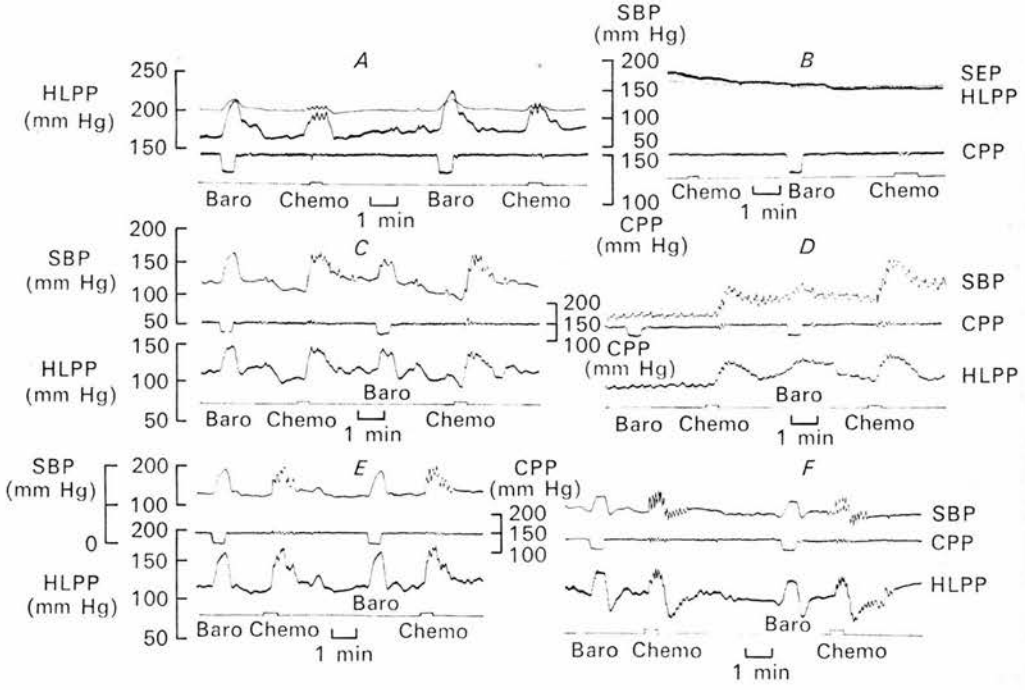

Fig. 1. Records from three experiments showing the reflex vasoconstriction following baroreceptor and chemoreceptor tests before and after drug administration. Sections *A*, *C* and *E* show the control vascular responses to both tests before drug administration. Section *B* shows the abolition of responses 15 min after carotid sinus nerve block. Section *D* shows the reflex vascular responses to both tests 20 min after I.V. administration of hexamethonium bromide (2 mg/kg). Section *F* shows the reflex vascular responses to both tests 30 min after I.V. administration of hyoscine methyl bromide (10 mg/kg). SBP, mean systemic blood pressure; CPP, carotid perfusion pressure; HLPP, hind-limb perfusion pressure.

Combined action of hexamethonium bromide and hyoscine methyl bromide on reflex vasoconstriction

In five dogs there was no residual response to baroreceptor tests or to chemoreceptor tests in the presence of both hexamethonium bromide (2 mg/kg) and hyoscine methyl bromide (10 mg/kg).

The action of guanethidine sulphate on reflex vasoconstriction

In two dogs the intravenous administration of guanethidine sulphate (10 mg/kg) abolished the changes in hind-limb perfusion pressure in response to both baroreceptor and chemoreceptor tests. In two further dogs the close arterial injection of

TABLE 1. The modification of the reflex vasoconstriction following intravenous administration of hexamethonium bromide or hyoscine methyl bromide respectively

CPP, carotid perfusion pressure; HLPP, hind-limb perfusion pressure

(*a*) Hexamethonium bromide

	Before hexamethonium				After hexamethonium				Inhibition (%)	
	Baroreceptor test		Chemoreceptor test		Baroreceptor test		Chemoreceptor test			
Dog no.	CPP fall (mmHg)	HLPP rise (mmHg)	Carotid P_{O_2} (kPa)	HLPP rise (mmHg)	CPP fall (mmHg)	HLPP rise (mmHg)	Carotid P_{O_2} (kPa)	HLPP rise (mmHg)	Baro-receptor tests	Chemo-receptor tests
1	150 → 120	38	14·2 → 6·0	49	150 → 120	6	14·6 → 5·5	38	84	22
2	150 → 100	33	23·5 → 2·8	12	150 → 100	4	23·5 → 3·3	12	88	0
3	130 → 110	31	14·4 → 6·4	40	130 → 110	7	14·4 → 6·4	40	78	0
4*a*	130 → 110	12	14·9 → 6·1	12	130 → 110	3	14·5 → 4·1	11	75	8
4*b*	130 → 110	17	14·6 → 5·2	9	130 → 110	3	15·7 → 6·6	9	83	0
5	130 → 100	29	17·3 → 4·8	30	130 → 100	12	14·2 → 4·4	29	59	3
6	120 → 100	51	18·4 → 5·6	78	120 → 100	4	16·1 → 5·1	65	92	17
	Means ± S.E.	30 ± 3 (n = 17)		34 ± 6 (n = 15)		6 ± 1 (n = 19)		31 ± 4 (n = 16)		

(*b*) Hyoscine methyl bromide

	Before hyoscine Me Br				After hyoscine Me Br				Inhibition (%)	
	Baroreceptor test		Chemoreceptor test		Baroreceptor test		Chemoreceptor test			
Dog no.	CPP fall (mmHg)	HLPP rise (mmHg)	Carotid P_{O_2} (kPa)	HLPP rise (mmHg)	CPP fall (mmHg)	HLPP rise (mmHg)	Carotid P_{O_2} (kPa)	HLPP rise (mmHg)	Baro-receptor tests	Chemo-receptor tests
7	150 → 120	55	20·9 → 6·1	47	150 → 120	55	24·5 → 6·2	33	0	30
8	150 → 120	18	19·7 → 5·3	19	150 → 120	21	18·1 → 5·3	7	−17	64
9	150 → 120	47	12·8 → 5·1	57	150 → 120	38	16·5 → 5·7	29	19	49
10	150 → 130	53	24·0 → 8·0	58	150 → 130	46	18·2 → 7·3	28	14	52
11	150 → 130	24	25·0 → 6·4	46	150 → 130	26	24·6 → 6·4	38	−8	17
12	150 → 120	44	19·4 → 5·5	32	150 → 120	41	20·3 → 4·3	20	6	37·5
	Means ± S.E.	45 ± 3 (n = 21)		46 ± 3 (n = 21)		39 ± 3 (n = 23)		25 ± 2 (n = 25)		

guanethidine sulphate (1·75 mg/kg) to the perfused hind limb abolished the hind-limb response to chemoreceptor stimulation without affecting the systemic response.

These experiments show that both reflex responses are mediated solely by the activation of sympathetic adrenergic fibres. The absence of a dilator response to chemoreceptor stimulation after guanethidine administration is consistent with the conclusion of Marshall (1977) that chemoreceptor-induced cholinergic vasodilatation, seen in the decerebrate animal, is not seen under barbiturate anaesthesia.

DISCUSSION

Baroreceptor reflex

As far as the carotid sinus baroreceptors are concerned, our results indicate that reflex vasoconstriction in the canine hind limb involves nicotinic transmission in the sympathetic ganglia to adrenergic vasomotor fibres. The reflex is abolished by moderate doses of hexamethonium bromide, and is unaffected by doses of hyoscine methyl bromide that lack nicotinic antagonist activity.

These results seem to conflict with those of previous workers, who have shown that the reflex response to carotid occlusion has a component resistant to blockade by nicotinic antagonists but sensitive to atropine (Steinberg & Hilton, 1966).

Carotid occlusion, however, gives a mixed baroreceptor and chemoreceptor reflex response unless high arterial oxygen tensions are maintained, since the carotid bodies are sensitive to ischaemia (Heymans & Neil, 1958).

Chemoreceptor reflex

Our results for the carotid body chemoreceptors are harder to interpret. Reflex vasoconstriction in response to chemoreceptor stimulation is unaffected by doses of hexamethonium bromide that abolish a reflex response, of similar amplitude and time course, to lowering of carotid sinus pressure. In some experiments we gave doses of hexamethonium bromide five times those sufficient to block the baro-receptor response without impairing that to chemoreceptor stimulation. In the presence of hexamethonium bromide the chemoreceptor response is abolished by hyoscine methyl bromide. In the absence of nicotinic antagonism, however, we only obtained a partial blockade varying from dog to dog between 17 and 64%.

The interaction of the antagonists on the chemoreceptor reflex implies that the pathways of the reflex must involve a complex interaction of muscarinic and nicotinic transmission. The simplest explanation of our results is that the main pathway of the chemoreceptor vasomotor reflex is by non-nicotinic transmission to adrenergic post-ganglionic fibres, in the sympathetic ganglia, sensitive to high concentrations of muscarinic antagonists, and that a subsidiary nicotinic pathway can sustain partial transmission after muscarinic blockade.

A problem is the high dose of muscarinic antagonist needed to inhibit the chemo-receptor reflex. The doses that we used are 100 times greater than those needed to block vagal bradycardia. In preliminary experiments with atropine we were concerned about the low ratio of muscarinic to nicotinic blocking potency, and indeed whether we were dealing with a population of nicotinic receptors relatively resistant to blockade. With hyoscine methyl bromide we are on stronger ground since the ratio of muscarinic to nicotinic blocking potency is about 20 times that for atropine.

Experiments on isolated tissues showed hyoscine methyl bromide to be about five times more potent than atropine as an antagonist at the parasympathetic post-ganglionic junction but about four times less potent than atropine as a nicotinic antagonist. Additionally, being a quaternary amine, hyoscine methyl bromide lacks the actions of atropine or hyoscine on the central nervous system.

For the present experiment it was necessary to exclude an action of hyoscine methyl bromide on the sensory side of the chemoreceptor reflex. R. J. Docherty & D. S. McQueen (personal communication) found that hyoscine methyl bromide at the dosage used in our experiments did not alter the resting discharge in single afferent fibres from the carotid body or the increase in impulse frequency in response to cyanide, to acetylcholine, or to nitrogen breathing.

In one of our own experiments we tested the reflex effect of chemoreceptor tests on pulmonary ventilation in a spontaneously breathing dog. Neither the resting ventilation, as recorded by an integrated pneumotachogram, nor the increase during chemoreceptor tests, was affected by hyoscine methyl bromide. No deterioration in the ventilatory response to chemoreceptor tests was seen during the time course of the experiment.

The high doses of antagonist needed raises the possibility of heterogeneous muscarinic receptors. This would be compatible with our findings on the homologous muscarinic receptors of the canine adrenal medulla (Henderson & Ungar, 1977). We found that the affinity constants for three muscarinic antagonists, to the release of catecholamines by acetyl β methylcholine from perfused adrenal glands, were of the order of 100 times lower than their corresponding affinity constants at parasympathetic post-ganglionic endings.

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