EFFECTS OF GONADAL HORMONES AND POLYPEPTIDES ON THE NERVES OF THE VASCULAR SYSTEM.

by

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SUMMARY

Recordings have been made of efferent activity in strands of the cervical sympathetic nerves of cats and dogs containing a single or a few spontaneously active fibres. Blood pressure was raised using intravenous or intravertebral infusions of noradrenaline or angiotensin and nerve activity was recorded over periods of 24, 32 or 40 seconds while blood pressure was maintained at a nearly constant level. The effects of intravenous infusions were studied in 143 nerve strands and the spike frequency / blood pressure curves for each strand fell into two distinct groups. In the first group, the relationship was linear and inverse over the full pressure range studied, from around 100 mm Hg to around 200 mm Hg. In the second group, the curve was inverse and linear between 100 and 150 mm Hg, at which point a minimum was usually reached, spike frequency increasing or remaining constant at higher pressures. These two patterns of activity were related to the endocrine state of the animal. Thus, 71 of the 83 fibres studied in normal or castrate females and castrate or oestrogenised males showed the behaviour of the first group; and out of 60 fibres studied in normal males and testosterone treated castrates 50 showed the behaviour of the second group. It was concluded that changes in the spike frequency / blood pressure curve could be induced by sex hormones and that the pattern shown in the second group was dependent on the presence of testosterone.

It is suggested that the absence of continued inhibition at high pressures /

pressures in males is due to chemoreceptor stimulation. Evidence is presented that activation of chemoreceptors occurs at high pressures in male cats and is due to vasoconstriction within the chemoreceptors.

The presence of a chemoreceptor mediated component in males, and its absence in females, may be related to the known action of testosterone to increasing vascular sensitivity to noradrenaline, or possibly to a metabolic action. It is clear from the results that testosterone also affects the responses to angiotensin, but it is not known whether this is related to a change in vascular sensitivity to the peptide.

The effects of noradrenaline and angiotensin on sympathetic discharge have been compared directly in animals with and without functional baroreceptor nerves. The two drugs had similar effects whether infused intravenously or intravertebrally, and in the inverse parts of the spike frequency / blood pressure relationship statistical analysis showed that it was highly unlikely that the effects of the two drugs differed. In animals with baroreceptors denervated, the effects of the two drugs were similar in 18 out of the 19 nerve strands studied. In none of these situations in which the effects of angiotensin and noradrenaline were compared was any difference consistently found which might have been taken as evidence in favour of the hypothesis that angiotensin has a peculiar specific action on central sympathetic neurones. It is concluded that, central stimulation of sympathetic neurones does not contribute to the pressor response found with the doses of angiotensin used in this study.

HISTORICAL REVIEW

The subject of this thesis concerns the interaction of the hormones angiotensin, noradrenaline, testosterone and the oestrogens with the sympathetic nervous system. The work began with a comparison of the effects of angiotensis and noradrenaline on sympathetic discharge, and in the course of these experiments it was found that their effects were dependent on the sex of the animal being studied. The historical review is therefore divided into three sections:

A. Spontaneous activity in the sympathetic nervous system.

B. Inter-relations between angiotensin and the sympathetic nervous system, and,

C. The vascular effects of the sex hormones.

A. SPONTANEOUS ACTIVITY IN THE SYMPATHETIC NERVOUS SYSTEM.

Spontaneous activity is a feature of many cells of the nervous system, for instance the cell network known as the respiratory centre, cortical neurones, and some efferent autonomic fibres. Inherent spontaneous activity such as that found in the cardiac pacemaker is not however the rule. In the respiratory centre and in the cortex, spontaneous activity is largely determined by the afferent input to the cells, and when these inputs are removed, the probability of finding such activity is greatly decreased (Burns 1963, 1968). Deafferented sympathetic neurones also rarely exhibit spontaneous /

spontaneous activity, as found by Alexander (1945) and Polosa (1968). In these preparations which involved the isolation of a block of spinal cord, the tonic activity may have been related to ischaemia. The latter worker also presented evidence that in animals with an intact neuraxis, at least some of the spontaneously discharging sympathetic neurones might have inherent rhythms. He recognised a small group of regularly discharging sympathetic neurones and found that their rhythms could be reset by interpolating an antidromic impulse amongst the regular train. Since this antidromic impulse caused resetting of the rhythm, he argued that this spontaneous activity must originate within the cell, and that the antidromic impulse reinitiated a cyclic process which was responsible for the rhythmic discharge. It should be mentioned however that irregular discharge is a much more common pattern of activity in sympathetic fibres. Much of the spontaneous discharge in sympathetic cells is dependent on excitation from the medulla and other parts of the brainstem, and these higher centres impose rhythmical influences on the sympathetic outflow in phase with the respiratory or cardiac cycles.

Respiratory modulation of sympathetic discharge has been known since Adrian, Bronk and Phillips (1932) first recorded activity in the rabbit cervical sympathetic nerve. Similar patterns were found in the inferior cardiac nerve and the splanchnic nerve of cats by Bronk, Ferguson, Margaria and Soldant (1936) and Gernandt, Liljestrand and /

and Zotterman (1946). Adrian et al (1932) found accentuation of discharge during inspiration in spontaneously breathing rabbits, and believed this to be due to the spread of activity from the respiratory to the vasomotor centre, because it persisted after vagotomy. Bronk et al (1936)however found an expiratory accentuation of discharge in the inferior cardiac nerve of artificially ventilated cats, and interpreted their results as indicating an influence of the pulmonary stretch receptors on the vasomotor neurones, because it disappeared after vagotomy. The inter-relations of respiratory and sympathetic activity were investigated by Tang, Maire and Amassian (1957). They thought that pulmonary stretch fibres influence the sympathetic only indirectly, through their effects on the central respiratory neurones, and that two main direct influences on the vasomotor neurones were responsible for the respiratory modulation of sympathetic discharge. These were

(1) the respiratory variations in blood pressure which affect the vasomotor centre through the baroreceptors, and,

(2) a direct influence of the variations of respiratory centre activity on the vasomotor neurones.

Thus the differences between the results of Adrian et al (1932) and Bronk et al (1936) regarding the phase of respiration in which the increased sympathetic discharge occurred was thought to be related to the mode of ventilation and the different effects of artificial and natural ventilation on blood pressure and respiratory centre activity. Okada and Fox (1967), working on dogs, agreed that these factors /

factors were important, but thought that the pulmonary stretch fibres could influence the vasomotor neurones directly, since hyperventilation, baroreceptor denervation or pneumothorax, and vagotomy were required to obtain cessation of respiratory synchronisation in artificially ventilated animals.

The importance of respiratory changes in blood pressure was also emphasised by Iggo and Vogt (1960). They found that units which were silenced by respiratory increases in blood pressure were also silenced by small pressor doses of adrenaline. Also, stimulation of the pulmonary stretch receptors by clamping the trachea, did not affect the respiratory rhythm of sympathetic discharge. This was in agreement with the results of Adrian et al (1932).

Another possible source of the respiratory rhythm could be the peripheral chemoreceptors. Biscoe and Purves (1967a) have shown that rhythmical changes in carotid chemoreceptor activity can occur during the respiratory cycle, and these are related to cyclic changes in blood gas tensions in the carotid arterial blood. These activities of the chemoreceptors however probably have no importance in the sympathetic rhythms since the change from breathing room air to breathing pure oxygen does not alter the mean level of sympathetic discharge (Millar and Biscoe 1965), and transient periods of hypoxia do not increase sympathetic discharge, at least in the sympathetic nerve to the carotid body (Biscoe and Purves 1967b). However,

some /

some rise in blood pressure did occur during ventilation with these gas mixtures, as far as can be judged from the published records.

Millar and Biscoe (1965) also investigated sympathetic activity during changes in respiratory centre activity produced by inhalation of carbon dioxide. In rabbits, large concentrations of the gas were required to increase the mean level of sympathetic discharge or blood pressure, but the respiratory swings in sympathetic activity were exaggerated at relatively low concentrations of carbon dioxide in the inhaled gas. From this it would seem that moderate increases in respiratory activity affect the distribution of sympathetic impulses in the respiratory cycle, without affecting their total numbers.

In dogs however, Downing, Mitchell and Wallace (1963) also found that high carbon dioxide tensions in the blood perfusing the brain induced hypertension and tachycardia. Similar effects were obtained when the flow rate or the oxygen tension of the blood perfusing the brain were reduced. Other experimental evidence for increased sympathetic activity during brain ischaemia in dogs has been produced by Sagawa, Ross and Guyton (1961) and Sagawa, Carrier and Guyton (1962). Such a cerebral ischaemic response only occurred when blood flow had been reduced to levels of 30 mls / 100 g brain / minute or lower, and it does not therefore play any part in the normal respiratory rhythms seen in the sympathetic nervous system.

The /

The cardiac rhythms of the sympathetic were also first noted by Adrian et al (1932) and have been confirmed by many workers including Bronk et al (1936) and Downing and Siegal (1963). The latter authors showed that if the carotid sinuses of a dog were perfused with blood from a donor animal, the rhythmic discharge in the inferior cardiac nerve of the recipient became synchronous with the heart of the donor. Other evidence that the cardiac rhythm in the sympathetic is determined by the baroreceptors has been presented by Green and Heffron (1968). They found that the latent period between a sudden rise in pressure in the baroreceptors and inhibition of sympathetic discharge in the inferior cardiac nerve was around 200 msecs. Iggo and Vogt (1960) found a similar latent period of 150 - 200 msecs in the cat cervical sympathetic. According to Millhorn (1966) the speed of response of the vascular system following a sudden stimulus to the baroreceptors is quite slow, with a half time of around ten seconds. Millhorn's computer simulation of the phenomenon suggested that the sympathetic controlling mechanisms and the smooth muscle responses were equally responsible for this long half time, but it is difficult to reconcile this view with the short latencies of response that have been reported. Direct experiment by Kezdi and Geller (1968) has shown that the limiting frequencies of the carotid sinus - postganglionic sympathetic loop are faster than predicted by Millhorn, the bandwidth being 0.1 to 2 Hz.

The control of the sympathetic nervous system by the classical baroreceptor /

baroreceptor areas has been reviewed by Heymans and Neil (1958). This is too large a subject to be treated fully here, so only a few points which are of particular relevance to this thesis will be dealt with.

The response of baroreceptor discharge to a change in intrasinus pressure has both rapidly and slowly adapting components. Changes in the rate of rise of pressure during the cardiac cycle result in changes in the rapidly adapting component of discharge, and this in some circumstances reflects changes in cardiac contractility. Sagawa (1967) has studied the role of pulsatile pressures in the carotid sinus in the regulation of blood pressure during haemorrhage. He found that the fall in blood pressure during mild haemorrhage was greater if the baroreceptor regions were only supplied with a static pressure equivalent to the mean systemic blood pressure. The blood pressure settled at a higher level following haemorrhage if the normal pulsations in arterial pressure also reached the baroreceptors. Thus the presence of arterial pulsation in the baroreceptors is of physiological importance for the control of the circulation.

Baroreceptor discharge is known to be modified by changes in the tone of the smooth muscle in the sinus wall. Such changes have been induced by stimulation of the cervical sympathetic (Heymans and Neil 1958; Mills 1968), and by local application of vasoactive drugs such as noradrenaline, adrenaline, vasopressin and nitrites (Landgren, Neil and Zotterman 1952). These changes in tone in the sinus wall during local /

local application of vasoconstrictors can result in quite substantial hypotension. These changes brought about by noradrenaline are not however reproduced by angiotensin. McCubbin and Page (1957) found that injection of the peptide into the adventitia of the sinus did not lower the systemic arterial pressure or decrease the carotid occlusion reflex. However, injection of large amounts into the lumen of the sinus did result in a diminution of this reflex. They concluded that the distribution of receptor sites for angiotensin and noradrenaline was different. More recently, Edmondson and Joels (1969) have studied the effects of angiotensin on the saline-perfused sinus preparation and also found that large concentrations were required to modify baroreceptor discharge. They thought that the increase in baroreceptor discharge that was found was due to the release of noradrenaline from local sympathetic nerve endings, since division of the sympathetic nerve to the carotid body 2½ to 5 hours before administration of angiotensin or reserpine abolished the response to angiotensin. Vasodilators such as nitrites produce the opposite effects, as does topical application of progesterone (Cogni, Rovati, Tusini and Zirondoli 1952). The effects of local administration of vasoactive drugs to the carotid sinus only occur if grossly pharmacological doses of the drugs are used. Iggo and Vogt (1962) found no evidence for such an action during intravenous infusion of moderate doses of noradrenalin or adrenaline.

The peripheral chemoreceptors have different sympathetically mediated /

mediated reflex effects on the circulation, depending on whether their respiratory effects are controlled or not. When ventilation is controlled the responses to chemoreceptor stimulation by local hypoxia are fairly consistent, whether dog or cat, or, aortic or carotid chemoreceptors are studied. These effects are peripheral vasoconstriction in skin, muscle and splanchnic vascular beds, hypertension, bradycardia and diminished cardiac output. The bradycardia is due both to an increase in vagal tone and a reduction in sympathetic tone to the heart. It is clear that the sympathetic innervations of the heart and blood vessels behave differently during chemoreceptor stimulation. (Downing, Remensnyder and Mitchell 1962; Daly and Scott 1963). In contrast, during variations of sinus pressure they change in the same direction. The increase in peripheral resistance during carotid body hypoxia is mediated by the sympathetic nervous system and is abolished by surgical or pharmacological sympathectomy.

When the animal is free to exhibit the full respiratory response to carotid body hypoxia, the primary response described above can be modified considerably. Daly, Hazzledine and Ungar (1967) and Daly and Robinson (1968) demonstrated that the resultant changes in lung inflation can modify sympathetic discharge by activation of pulmonary stretch fibres in the vagus. As a result, in spontaneously breathing animals, tachycardia and vasodilatation may occur instead of bradycardia and vasoconstriction during chemoreceptor stimulation. In the /

the dog the respiratory response to carotid body hypoxia is much more intense than to aortic body stimulation, while in the cat, this difference is not so noticeable. Thus, when the same stimulus is given to the chemoreceptors, the carotid bodies of dogs produce about five times the increase in respiratory minute volume given by cat carotid bodies, and about seven times the response of dog aortic bodies. As a result, in dogs, deviation from the primary response is much more marked with carotid body hypoxia than with aortic body hypoxia (Daly and Ungar 1966).

Bradycardia is the primary response to chemoreceptor hypoxia when ventilation is controlled, but systemic hypoxia always results in tachycardia (Downing and Siegal 1963). This tachycardia is associated with increased discharge in the inferior cardiac nerve and persists after denervation of all known peripheral chemoreceptors. Comroe (1964) thought that the aortic bodies might be partly responsible for the tachycardia of systemic hypoxia, but his views were based on pharmacological experiments that could be interpreted in several ways. The results of Downing and Siegal (1963) however show that the aortic bodies are not necessary for this response, and central receptors responding to blood gas tensions and blood flow have been suggested by Downing et al (1963) to account for hypoxic tachycardia.

This is a convenient point at which to mention that some vasoconstrictors such as noradrenaline, can cause an increase in chemoreceptor discharge during the rise in arterial pressure. In human subjects /

subjects, Cunningham, Hey and Lloyd (1958) found that intravenous infusion of noradrenaline could increase minute ventilation in doses which, in the hands of Scroop, Walsh and Whelan (1965), elevated arterial pressure by 25 - 30 mm Hg. They also showed that the effect was due to an increase in the sensitivity to carbon dioxide, and that in this respect it was similar to the effect of arterial hypoxia. Noradrenaline and other vasoconstrictors also increase the discharge of the peripheral chemoreceptors (Neilaand Joels 1963) and since this activity can be eliminated by an increase in perfusion pressure, it was thought that they might be acting by reducing oxygen flow to the sensory apparatus. That noradrenaline might be producing this effect by a vascular action was also supported by the observation that vasodilators could reduce chemoreceptor discharge, and by the work of Lee, Mayou and Torrance (1964) and Biscoe (1966) which showed that this activity of noradrenaline could be abolished by alpha blocking agents such as phenoxybenzamine. This drug does not interfere with the respiratory response to hypoxia (Heymans, DeSchaepdryver and DeVleeschouwer 1968).

Joels and Neil (1963) stress the importance of the concept that only a small proportion of the total blood flow collected from the carotid body vein traverses the sinusoids, and Purves (1969) has also emphasised that chemoreceptor discharge is poorly related to total blood flow, and better related to oxygen consumption, /

consumption, which in turn may be related to the distribution of blood flow between sinusoids and shunts. The sympathetic nerves to the carotid body play an important role in determining its oxygen consumption (Purves 1968) and stimulation of the cervical sympathetic decreases carotid body blood flow and oxygen consumption, and results in increased chemoreceptor discharge (Mills 1968).

Clearly the localisation of alpha receptors is of importance in determining the distribution of blood flow in response to the catecholamines or to changes in sympathetic activity, and the evidence mentioned suggests that the vessels leading to the sinusoids are sensitive to the transmitter.

Nothing is known about the effects of noradrenaline on the distribution of blood flow in the carotid body, but in the human forearm, Freis and Schnafer (1958) found that the blood flow could be divided into two components, one slow and one fast. Intravenous noradrenaline decreased the total blood flow, but increased the rapid component. If noradrenaline produces similar changes in the carotid body, this would be compatible with a decrease in sinusoidal blood flow.

B. INTER-RELATIONS BETWEEN ANGIOTENSIN AND THE SYMPATHETIC NERVOUS SYSTEM.

In 1956, Braun-Menendez stated that it was universally accepted at that time that the vascular actions of angiotensin were independent of the sympathetic nervous system and sympathomimetic agents. This view /

view was still regarded as the general opinion in 1961 by Page and Bumpus. The conclusion was based on the following evidence:

(1) Interruption of the baroreceptor reflex arc by a variety of procedures increased the pressor responses to angiotensin just as it did for other vasoconstrictors such as adrenaline and noradrenaline. These procedures included cervical spinal cord section and total sympathectomy (Page and McCubbin 1951), spinal anaesthesia (Gregory, Levine and Lindley 1944) and sinus nerve denervation (von Euler and Sjostrand 1941).

(2) Sympathetic blocking agents such as ergotamine and phenoxybenzamine do not affect the blood pressure responses to angiotensin, or increase it.

(3) Drugs which increase the sensitivity of blood vessels to catecholamines, such as cocaine, do not similarly alter the sensitivity to angiotensin (Page and Helmer 1940).

During the decade beginning 1960, many investigators have studied the inter-relations between angiotensin and the sympathetic nervous system. The onset of this work coincided with the availability of larger amounts of synthetic angiotensin and the doses used were often much higher than those necessary for just a rise in blood pressure.

In rats, Laverty (1963) found that injection of angiotensin into the systemic circulation caused vasoconstriction in a vascularly isolated innervated hindlimb, if 1 microgram or more were given intravenously. /

intravenously. This vasoconstriction was abolished by section of the nerves to the limb. Smaller doses, in the range 0.1 to 0.5 micrograms i.v., caused a nervously mediated vasodilatation, as did noradrenaline over as equipressor dose range. The effects of small doses of angiotensin were also studied by Gordon and Stephenson (1967) who measured the vasoconstrictor effect of 5 nanograms given i.v. in preparations in which the mesenteric or iliac vascular beds were perfused at constant volume. During pentolinium blockade, the vasoconstrictor response in the mesenteric bed increased, while that in the iliac bed remained unchanged. The arterial pressure response to the angiotensin increased from 12 to 22 mm Hg following blockade with pentolinium. This indicates that i.v. angiotensin in this dose produces a rise in blood pressure which is accompanied by a fall in sympathetic tone.

In cats, Smookler, Severs, Kinnard and Buckley (1966) and Severs, Daniels, Smookler, Kinnard and Buckley (1966) found that injection of 2 or 4 micrograms of angiotensin into the lateral ventricles produced hypertension and tachycardia, whereas injection of saline did not. This response was abolished by sympathetic blockade or cervical cord section. As the response was also absent in cats with cerveau isole section or with midbrain lesions, Severs et al (1966) and Severs, Daniel and Buckley (1967) concluded that angiotensin produced these actions through some centre in the midbrain.

Further evidence for a nervously mediated action of angiotensin in /

in conscious man came from Scroop and Whelan (1966). The rise in blood pressure in these experiments had to be limited for ethical reasons, and as the subjects were also unanaesthetised, the results are regarded as having rather more weight than some of those from animal experiments. They measured hand blood flow during intra-arterial and intravenous infusions of angiotensin. Intravenous infusion resulted in a decrease in hand blood flow, but this decrease was not seen in people with a variety of forms of interference with the sympathetic supply to the hand. These included intra-arterial phenoxybenzamine or bretylium, nerve block with local anaesthetic, and pathological destruction or degeneration of the sympathetic nerves. In contrast, intra-arterial infusion of angiotensin did not decrease hand blood flow.

In the forearm, the situation was different. Scroop, Walsh and Whelan (1965) showed that i.v. angiotensin increased forearm blood flow whereas i.a. angiotensin decreased it. This is compatible with a weak vasoconstrictor action on muscle vessels.

The investigation of Scroop and Whelan (1966) did not pinpoint the site of sympathetic involvement, but it was thought unlikely that the interaction occurred at sympathetic nerve endings, and the authors favoured a central origin for this nervously mediated vasoconstriction, in spite of the differences between hand and forearm blood flows.

In the dog, Daugherty, Scott, Emerson and Haddy (1968) measured forelimb blood flow during i.v. and i.a. infusions of angiotensin, and /

and obtained results similar to those of Scroop et al (1965) in man, viz., that i.a. angiotensin caused vasoconstriction and reduction in blood flow, and i.v. infusions produces no change in blood flow. The dosage was up to 5.1 micrograms / min. i.v. to the dog. Similarly, Geller and Kendrick (1968) who gave 0.05 to 0.1 micrograms / Kg of angiotensin to dogs weighing 20 - 24 Kg, found that the vasoconstrictor responses of the renal circulation of dogs were unaltered by renal denervation or the administration of bretylium.

In contrast, McGiff and Fasy (1965) found that these procedures abolished the renal vascular activity to slightly larger doses of the peptide. These authors, and Geller and Kendrick, both used dogs anaesthetised with morphine and chloralose, yet their results are opposed. McGiff and Fasy found that drugs which affect transmitter release blocked the renal vascular activity of angiotensin, but drugs which blocked the alpha site or ganglionic transmission did not affect it. Tyramine, which releases noradrenaline from nerve endings, restored the response to angiotensin following guanethidine treatment, but was not effective when the stores of catecholamine had been released by reserpine. This work suggests a peripheral interaction rather than a central interaction between angiotensin and the sympathetic.

The first evidence of a central action of angiotensin was produced in dogs by Bickerton and Buckley (1961) and Buckley, Bickerton, /

Bickerton, Halliday and Kato (1963). They found that when the vascularly isolated head of one dog was perfused from a donor, the injection of up to 4 micrograms per Kg of angiotensin into the donor resulted in a rise in blood pressure in both animals. The response in the recipient was abolished by spinal cord section, and this was regarded as indicating a central effect of the peptide. Severs et al (1967) also reported some work on dogs.. Intraventricular injection of 4 micrograms of angiotensin resulted in hypertension and tachycardia, findings also reported in cats by the same authors. Bianchi, DeSchraepdryver, DeVlesschouwer and Preziosi (1960) however found that intracisternal injections of the peptide in dogs usually lowered blood pressure, but the peptide might not have reached the relevant structures in these experiments. Zimmerman (1967) also found no evidence for a central action of angiotensin when 2 or 10 micrograms were administered into the vertebral or carotid arteries of dogs. Flow rates were measured in the hindpaw, gracilis muscle, hindlimb and kidney, and no consistent vasoconstrictor responses were found during these injections.

Greyhounds apparently behave differently. Lowe and Scroop (1968) found that intravertebral infusions of low doses of angiotensin resulted in elevation of blood pressure and heart rate. A large part of this response was found to be due to release of vagal tone, and the pressure elevation was quite small if the vagi were cut. These residual pressor effects could be blocked with sympathetic blocking /

blocking agents, and indicated that some central stimulation could occur.

Similar experiments have been performed on rabbits. Rosendorff, Lowe, Laverty and Cranston (1970) prepared rabbits by tying one vertebral artery and the opposite carotid more than 4 weeks before the acute experiment, in which low doses of angiotensin were infused into the remaining patent vertebral artery. The blood pressure responses to intravertebral and intravenous injections of the same doses were compared, and the response to central administration was higher than that to intravenous infusion, particularly over the lowest range of infusion rates, between 4.5 and 45 nanograms per minute. In more than half the rabbits studied, the intravertebral infusion was accompanied by tachycardia, but the effects of vagotomy were not investigated.

The only work in which attempts have been made to measure sympathetic discharge directly is that of Aars and Akre (1968). They used an electronic integrator to measure the activity in intact cervical sympathetic and renal nerves in the rabbit. In the cervical sympathetic, they found no difference between the effects if i.v. angiotensin and noradrenaline, and in the renal nerves, angiotensin decreased the integrated discharge in the lower range of infusion rates. When the blood pressure was very high due to increased infusion rates of the peptide, a further increase in the infusion rate of angiotensin produced /

produced a transient rise in integrated activity, which was not seen with noradrenaline. The cause of this increase in activity was not further investigated, but the results were taken as indicating a transient centrally mediated action of angiotensin.

Another possible site of interaction between the peptide and the sympathetic is in the sympathetic ganglia. Lewis and Reit (1965) reported that retrograde injection of 0.1 to 1.0 micrograms angiotensin into the cat superior cervical ganglion resulted in a nervously mediated contraction of the nictatating membrane. They believed that the action of angiotensin was a direct one on the postganglionic cells, and not due to the release of acetylcholine, as chronic cervical sympathectomy, hexamethonium and atropine did not affect the response. Also the injection of the peptide was followed by a period of tachyphylaxis, during which the preparation responded to acetylcholine but not to angiotensin. In a later paper (1966) they showed that the response was species specific, being present in the cat, weak in the rabbit and absent in the dog. The response of the cat ganglion was blocked by morphine, but large doses of this drug did not alter the vascular effect of the peptide.

Panisset (1966) agreed that this phenomenon was due to ganglionic stimulation, but found that it could be blocked by hexamethonium and atropine. Very small doses, 0.0001 to 0.01 nanograms administered into the arterial supply of the ganglion in fact inhibited ganglionic transmission, a finding that confirmed earlier work by Haefely, Hurlimann /

Hurlimann and Thoenen (1965). This activity could be blocked by dihydroergotamine, and Panissetspeculated that the depression of ganglionic transmission might be due to release of noradrenaline. In a later paper (1967) he found that angiotensin, in a dose of 0.1 to 100 nanograms injected locally into the eserinised ganglion, induced an increased output of acetylcholine. It also released acetylcholine from strips of guineapig ileum. Khairallah and Page (1961) had previously shown that the contractile response of this tissue to angiotensin could be partly blocked by atropine or morphine and reversed by neostigmine.

Interaction between angiotensin and catecholamine metabolism at sympathetic nerve endings is the other possibility that has received attention in the literature. Distler, Liebau and Wolff (1965) reported that angiotensin, in a concentration of 1 microgram / ml., decreased the amounts of noradrenaline found in pig blood vessels. The same dose of the peptide, when applied to rat aortic strips, caused contractions which decreased in size on repeated application. The initial size of the contraction could be restored by immersing the strip in a solution containing noradrenaline (1 microgram / ml), and the restoration of the response could be prevented by cocaine, which blocks the reuptake of noradrenaline into nerve endings. Palaic and Khairallah (1967) perfused angiotensin through the rat brain at 8 - 800 ng./min., and found that it prevented the uptake of tritiated /

tritiated noradrenaline. Uptake inhibition was also demonstrated by Panisset and Bourdois (1968) and Khairallah (1969). Others have demonstrated increased output of noradrenaline from stimulated sympathetic nerves when angiotensin is present. They include work by Benelli, Della Bella and Gandini (1964) on guinea-pig vas deferens, by Zimmerman (1967) on the dog himdlimb and by Day and Owen (1969) in the intact cat.

One final site of interaction of angiotensin and the catecholamines is at the smooth muscle cells themselves. Hertting and Suko (1966) found that angiotensin did not release tritiated noradrenaline from preparations of the cats spleen that had been loaded with this label, and thought that the effect of angiotensin in potentiating the vascular response to nerve stimulation was due to interaction of the peptide and the catecholamine on the vascular muscle. Panisset and Bourdois (1968) also found that chronically denervated rat mesenteric vessels gave larger responses to the mixture of angiotensin and noradrenaline, than the sum of the individual responses. McGregor (1965) also found that angiotensin administration to a rat isolated mesenteric vessel preparation increased the responses to constant doses of noradrenaline. Similar interactions on the vascular muscle have been demonstrated by Schmitt and Schmitt (1967) and by Zimmerman (1962).

C. THE VASCULAR EFFECTS OF THE SEX HORMONES.

Most of the references to the vascular actions of the sex hormones in the literature concern the oestrogens. Vasodilatation is one of the initial reactions of oestrogen sensitive tissues to the injection of the hormone, (Spaziani and Suddick 1967), and it is followed by a number /

number of other changes including an increase in capillary permeability and the accumulation of water and electrolytes. The endometrial blood vessels normally undergo phasic constrictions in the mature animal (Markee 1932) and oestrogens abolish these contractions and cause further vasodilatation (Holden 1939). The larger uterine blood vessels are also affected and the hyperaemia precedes a rise in oxygen consumption (MacLeod and Reynolds 1938). These reactions can occur in transplanted endometrium and are independent of the myometrial contractions (Kaiser 1948). As a result, uterine blood flow increases considerably, being two or three times the initial level within four hours of the injection in the rat (Spaziani and Suddick 1967). In the sheep, Greiss and Marston (1965) observed a similar significant increase in blood flow through the uterus.

In the sexual skin of the monkey, oestrogens also bring about a rapid vasodilatation, which is independent of the innervation (Zuckerman 1935). Similar changes have been described in the nasal mucosa and the nipple (Mortimer, Wright, Backman and Collip 1936).

Stilboestrol treatment caused an increase in renal plasma flow in the dog (Dance, Lloyd and Pickford 1959), but in women, relatively lower doses of ethinyl oestradiol did not alter glomerular filtration rate or renal plasma flow (Chesley and Tepper 1967). Nassim, Saville and Mulligan (1956) also reported no change in creatinine clearance following stilboestrol treatment in humans.

Oestrogens /

Oestrogens cause dilatation of the small blood vessels of skin in rabbits and in humans. In the rabbit ear, the precapillary arterioles and venules with diameters less than 15 microns dilated following intramuscular injection of oestrin (Reynolds and Forster 1939a). During the 10 to 40 minutes following the injection the skin temperature of the ear decreased steadily from 31 - 33°C to 1 - 2°C above room temperature. The controls which did not receive oestrogen did not show this response. Intramuscular injection of oestrin to men caused an increase in finger volume beginning 3 - 5 minutes after the injection, and the skin temperature either fell or showed no change (Reynolds and Forster 1939b). They interpreted these results as being due to dilatation of the small capacity vessels unaccompanied by a change in total blood flow, and quoted experiments by Carloni which suggested that oestrogens dilated the nail bed capillaries and decreased capillary pressure. The effects of oestrogens on capacity vessels extends to the veins draining the skin, as Goodrich and Wood (1966) have demonstrated that oestradiol 17 beta increased the distensibility of these vessels.

The evidence suggests therefore that oestrogens bring about a redistribution of blood flow and blood volume. In humans, dogs, cats, rats and rabbits these changes occur without any change, or only a slight fall in blood pressure. In the sheep, some oestrogens can induce significant hypotension, and an increase in cardiac output, while /

while other oestrogens seem unable to produce any change in these parameters (Ueland and Parer 1966). The only other species in which cardiac output has been measured following oestrogen administration is the rat. Spaziani and Suddick (1967) found no change in cardiac output, but uterine blood flow increased considerably. In such cases, it is necessary to seek evidence of any compensatory vasoconstriction of resistance and capacitance vessels. Only one such observation is available, and it relates to the responses of the spleen of the dog during natural oestrus. Barcroft and Stevens (1929) and Barcroft (1932) noted that the exteriorised spleen of the conscious dog contracted to half its size at the onset of oestrus, and that this contraction depended on the integrity of the splenic nerves. This can be viewed as a nervously mediated compensatory vasoconstriction. It is not clear whether this extends to the rest of the splanchnic vascular bed. No short term changes in bromosulphthalein clearance have been reported, but a decrease in this parameter has been reported in animals (e.g. Campbell 1957) and women receiving oestrogens or oestrogen-progesterone mixtures over a long period. This change probably reflects metabolic changes in the hepatic cells generally regarded as indicating oestrogen toxicity, and it would therefore be dangerous to interpret this result as a change in hepatic blood flow.

One other observation which may indicate a change in the size of /

of the central reservoir of blood in man is that of Pecora, Putnam and Baum (1963). They found that pulmonary diffusing capacity was reduced significantly 45 minutes following the intravenous injection of conjugated oestrogens. Of the many possible interpretations of this result, one would be that the area of the blood-air interface had decreased, and this could be related to changes in the distribution of the blood volume.

There are many reports of the effects of sex hormones in modifying the sensitivity of the vascular system to the action of drugs, and in altering the susceptibility of the animal to various procedures which result in hypertension.

The female sex seems to be less susceptible to the induction of hypertension than the male. De Muro and Rowinski (1951) treated dogs with deoxycorticosterone acetate (DOCA) for 15 days and found that the blood pressure had increased in males but not in females. No such sex difference was found in immature animals. Stamler (1954) who worked on chickens, and Sturtevant (1956) who worked on rats, found that oestrogens gave some protection against DOCA hypertension. Similarly, the aortas of salt fed male and female rats showed different responses to adrenaline. The aortas of males were more sensitive than those from females, and those from castrate males and females showed sensitivities similar to those from intact males. It would seem therefore that the female hormones were responsible for the decreased /

decreased sensitivity of the aortas of female rats (Vick, Ederstrom and Vergeer 1956).

In rabbits, chronic administration of high doses of noradrenaline resulted in significantly higher increased in blood pressure in males than in females, but the endocrinological basis of this was not studied. (Lorenzen and Headings 1966).

Boxhill and Brown (1955) however found that the pressor response of anaesthetised dogs to the injection of adrenaline was increased following treatment with oestradiol, given in sufficient doses to produce an oestrus vaginal smear. This only occurred in dogs with intact ovaries, and similar treatment in ovariectomised dogs did not increase the pressor response to adrenaline. Males, metoestrus females and spayed females, whether treated with oestradiol or not, all showed similar sensitivities to adrenaline.

Zoster, Revesz and Bandermann (1960) however found no change in the response of limb vessels in the dog to noradrenaline, pitressin or priscoline following ovariectomy, but the absolute level of flow through the leg was said to be rather less following ovariectomy. Insufficient data were given to judge the importance of this statement. Woodbury, Marsh, Ahlquist and Hobensack (1947) also found that large doses of stilboestrol increased the vascular responses of acetylcholine, adrenaline histamine and pitressin. These experiments were however done at a time when the purity of the steroid preparations was in doubt /

doubt, and it is probable that impurities accounted for some of the reported toxicity attributed to the steroid hormones in use at that time. e.g. Grollmann, Harrison and Williams (1940).

The oft reported increase in sensitivity of males as compared with females, to the catecholamines has usually been attributed to a protective action of oestrogens in the latter. The observations on Burn, Finney and Goodwin (1950) however suggested that testosterone may play a part in the increased sensitivity of the vascular system of male cats to the catecholamines. Their experience in the use of Dale's method for the assay of catecholamines was that males were more sensitive than females or castrates of either sex. Bhargava, Dhawan and Saxena (1967) have confirmed these results, and have found that the injection of testosterone propionate increases the sensitivity of these vagotomised spinal cats to noradrenaline. This hormone had greatest effect in the females and least in the intact males.

The oestrogens are also known to produce changes in the sensitivity of the vascular system to the polypeptides vasopressin and oxytocin. Byrom (1938) found that the histological evidence of degeneration in the kidney following the administration of very large doses of pitressin was much more common if the rats had been previously treated with oestrogens. More recently, Lloyd (1959a; 1959b) and Lloyd and Pickford (1961) have shown that during natural oestrus, during the second half of pregnancy and following the injection of oestrogen to the rat, the pressor responses to vasopressin were increased. /

increased. The increased pressor action was also observed during sympathetic blockade with a variety of pharmacological agents and following surgical sympathectomy. The vascular responses to small doses of oxytocin were also examined in these experiments. In male and dioestrus female rats, the doses used had no effect on blood pressure, but in the oestrus female, or during the second half of pregnancy, or following oestrogen treatment or sympathetic blockade, the same doses caused a small rise in blood pressure. In dogs, the results were essentially similar. Lloyd and Pickford (1962) found that hindlimb blood flow in the dog was normally increased by oxytocin, and that following oestrogen administration, lumbar sympathectomy or sympathetic blockade, the same doses of oxytocin decreased the flow. The vessels of the forearm and hand in man also behave in essentially the same manner. (Deis, Kitchin and Pickford 1963; Haigh Kitchin and Pickford 1963). In man, the vasodilatation of limb blood vessels is not accompanied by any change in blood pressure, but there is a transient increase in cardiac output during the vasodilatation (Kitchin, Lloyd and Pickford 1959).

A constant finding in this work was a similarity between the effects of oestrogens and sympathectomy, whether surgical or pharmacological. Indeed it appeared to be more than just a similarity, since the effects of oestrogens on the vascular response to oxytocin was antagonised and reversed by stimulation of the sympathetic.

Haigh, /

Haigh, Lloyd and Pickford (1965) found that stimulation of the lumbar sympathetic in the oestrogenised dog, caused reversal of the response to oxytocin; from being constrictor because of the presence of oestrogens, it became dilator during the period of stimulation of the lumbar sympathetic. They also found that a similar reversal back to a dilator effect could be produced by small intravenous infusions of adrenaline, but not noradrenaline. Similarly, Pickford and Lloyd (1966) found that adrenaline infusion eliminated the pressor effect of oxytocin in oestrus and oestrogen treated rats. It has been postulated as a result of these experiments (Pickford and Lloyd 1966), that the vascular actions of oestrogen described involve a change in sympathetic activity.

Changes in adrenergic mechanisms following sex hormone treatment have also been noted by other workers. The plasma levels of adrenaline and noradrenaline follow cyclic patterns during the rat oestrus cycle. During oestrus and pregnancy the level of noradrenaline is significantly lower than during dioestrus, and adrenaline levels are lower in males than in females, irrespective of the stage of the cycle (Green and Miller 1966). This sex difference also applies to the level of adrenaline in human plasma and rat adrenals (Weil-Malherbe and Bone 1953; Green and Miller 1966). Rudzik and Miller (1962) also observed that oestrogen treatment and natural oestrus are associated with an increase in the adrenaline content of the rat uterus. /

uterus.

In the rat hypothalamus, Lichtensteiger and Langemann (1969) showed that the noradrenaline content of neurones in the tuberal region followed a well defined sequence of changes during the oestrus cycle. Reciprocal changes in the monoamine oxidase and choline acetylase activity in the rat hypothalamus were also found during the oestrus cycle by Kato and Minaguchi (1964). During pro-oestrus they found an increase in monoamine oxidase activity and a depression in choline acetylase activity. It is doubtful whether this increase in monoamine oxidase activity has any significance, since Wurtmann and Axelrod (1963) found that heart monoamine oxidase activity could be inhibited by 50% without affecting the rate of disappearance of tritiated noradrenaline from the tissue. They also found a sex difference in cardiac monoamine oxidase activity, but this had no effect on the turnover of noradrenaline.

Little is known of the vascular actions of testosterone. Its effect in altering vascular sensitivity to noradrenaline has already been mentioned. Only one report is available on its effects on noradrenaline in peripheral sympathetic nerves. Ryd and Sjostrand (1967) found that the noradrenaline content of the guinea-pig vas deferens and seminal vesicle did not change following castration or testosterone administration, in spite of changes in the size of the muscle and glandular tissue.

Edwards, /

Edwards, Hamilton, Duntley and Hubert (1941) found that androgens have profound effects on the vascularity of the human skin. Their measurements with a recording spectrophotometer showed that castrate males have a smaller quantity of haemoglobin in their skin than normal men, and that a larger proportion of it was in the reduced form. These changes were reversed by testosterone. They suggested that the skin vascular bed of castrates was smaller than normal. Pallor is a well known accompaniment of hypogonadism in the male, and these findings reflect this fact. In addition, Reynolds, Hamilton, di Palma, Hubert and Forster (1942) found that castrate men showed quite large fluctuations in the excitability of the skin blood vessels to mechanical stimuli, and testosterone was said to decrease and stabilise the excitability of these vessels.

Mirand, Johnston, Murphy and Gordon (1966) examined the effects of testosterone propionate on renal haemodynamics and erythropoietin release in dogs. They measured renal blood flow directly, since testosterone can affect the renal handlind of PAH (Gelman and Matthews 1964), and injected testosterone propionate intravenously. Renal blood flow did not change within two hours of this injection.

Progesterone increases PAH clearance and reduces the renal vascular response to angiotensin in women, without altering the size of the blood pressure response (Chesley and Tepper 1967). The amounts of progesterone used were those required to increase plasma levels of the /

the hormone to the concentration normally found in late pregnancy. During pregnancy the pressor response to 5 micrograms of angiotensin is reduced (Abdul-Karim and Assali 1961). The renal responses to angiotensin during pregnancy were investigated by Chesley, Wynn and Silvermann (1963). They measured urine flow, sodium excretion and the clearances of inulin and PAH,and found that the renal clearances and urine flow showed diminished responses to angiotensin during pregnancy. Hettiarachi and Pickford (1968) investigated the effects of angiotensin on the blood pressure of the female rat during the oestrus cycle, pregnancy and pseudopregnancy, and following the administration of oestrogens and progesterone. Oestrogens had no effect on the pressor response of angiotensin, but pregnancy, pseudopregnancy and progesterone administration decreased the hypertensive effect of the peptide. The effects of oestrogens and progesterone on the pressor response to angiotensin contrast with their effects on the vascular responses of oxytocin. Lloyd (1958) and Fullerton and Morrison (1965) found that pseudopregnancy or treatment with progesterone induced a pressor response to oxytocin, as did oestrogen, or testosterone (Honore 1964). In the rat, eserine causes, among its many actions, an increase in blood pressure which is mediated by the sympathetic nervous system (Varagic 1955). During this increase in sympathetic activity the pressor response of oxytocin in oestrogen-treated rats is abolished, but that occurring in pseudopregnant and progesterone /

progesterone treated rats is unchanged. Thus the antagonism between oestrogen action and sympathetic activity mentioned earlier does not extend to the action of progesterone.

One final point might be made about the interaction of sex hormones and the adrenergic mechanisms. Marshall (1969) has presented evidence which suggests that oestrogens and progesterone may affect the numbers of alpha and beta receptors in the rabbit myometrium. The effect of stimulation of the hypogastric nerve on the activity of the oestrogen dominated uterus is excitation, which is blocked by phentolamine. The progesterone dominated uterus however was inhibited by hypogastric nerve stimulation, and this activity was blocked by propanalol. The noradrenaline content of the uteri in these two cases did not differ, and essentially similar results were obtained when the above experiments were repeated using noradrenaline administration instead of hypogastric nerve stimulation.

AIMS OF THE PRESENT INVESTIGATION.

The aims were twofold:

1. To record activity in sympathetic nerve fibres during the infusion of angiotensin. The effects of angiotensin were compared with those of noradrenaline, since the central actions attributed to angiotensin were not found with the catecholamine. The aim was to find out whether such a central action contributed to the rise in arterial pressure when moderate doses of angiotensin were infused, as suggested by Scroop and Whelan (1966). The methods used avoided the administration of the drug directly into the arterial supply of the brain, as this was thought to be undesirable because of the possibility of producing cerebral vasoconstriction and ischaemia.

2. To study the effects of oestrogens on sympathetic activity. The possibility that oestrogens might modify sympathetic activity had been considered by Pickford and Lloyd (1966); However the finding that a sex difference existed in the behaviour of sympathetic discharge when blood pressure was raised led to a fuller endocrinological and neurological investigation of this phenomenon. The observations on the action of oestrogen are confined to its effect on this phenomenon.

METHODS

The experiments were performed on cats and dogs. The cats, weighing 2.25 to 3.75 Kg were anaesthetised with sodium pentobarbitone (Nembutal, Abbott) intraperitoneally in doses of 40 mg/kg, sometimes after induction with ethyl chloride. Occasionally chloralose was used in a dose of 80 mg/Kg. The dogs, weighing 7 to 18 Kg were anaesthetised with sodium pentobarbitone (26.5 mg/Kg) intravenously without premedication. Drugs were infused through a polythene cannula into the left saphenous vein, and femoral arterial pressure was recorded by means of a pressure transducer (Bell and Howell Ltd.) on a Devices single channel recorder and, usually, on one channel of the oscilloscope. On the occasions when blood pressure was not displayed on the oscilloscope, this channel was used for recording intra-oesophageal pressure, the transducer being a Greer micromanometer (Mercury Electronics). A tracheal cannula was inserted in cats but not in dogs, and when artificial ventilation was desired it was given by means of a Palmer Respiration Pump.

The cervical sympathetic nerve was dissected free of the common carotid artery and vagus nerve. In dogs, it was found inside the vagosympathetic trunk, and usually possessed its own nerve sheath. Its position was often indicated by a small blood vessel running along the sheath of the vago-sympathetic trunk. The sympathetic nerve was mounted on a small black perspex dissecting plate and its nerve sheath opened under liquid paraffin with a fine sharp knife made from a broken /

broken off piece of razor blade. Dissection was continued using the method of Iggo and Vogt (1960) until strands of nerve were obtained containing one or a few active fibres, which could be consistently identified. Action potentials were recorded using silver electrodes, amplified with a Tektronix Type 122 preamplifier, and displayed on one beam of a Tektronix Type 502A dual beam oscilloscope, the tube of which was photographed on moving film for analysis at a later date.

The drugs used were angiotensin (Hypertensin, Ciba), noradrenaline (Levophed, Bayer), oestriol dihemisuccinate (Organon), phentolamine (Rogitine, Ciba), phenoxybenzamine (Dibenyline, S.K. & F.), heparin (Pularin, Evans Medical) and testosterone propionate (B.D.H.). The angiotensin and noradrenaline were diluted with 0.9% NaCl and infused at constant rate by means of a Palmer Infusion Pump. The amounts infused were those found to be sufficient for each individual animal to raise blood pressure from a resting level of around 100 - 110 mm Hg to 180 - 200 mm Hg, without causing cardiac arrhythmias. In cats, the dose range of angiotensin was 36 to 572 ng/min per cat. For noradrenaline the range was 0.143 to 2.3 micrograms per minute per cat. In dogs the amounts used were ten times those used in cats. Rectal temperature was maintained between 36 and 38°C by means of a thermostatically controlled electric blanket (Electrophysiological Instruments Ltd). Experimental routine was as follows: discharge in /

in the nerve strand was recorded for a minimum of 24 seconds at any blood pressure, and often the period was 30 to 40 seconds. These recordings were made at a series of pressures, the infusion rate being changed between each recording. Generally the pressure was raised to a maximum and then lowered in a series of about 5 or 6 steps. A further record of sympathetic discharge was again obtained when the pressure had settled at resting level and compared with the initial record.

Drugs were given by infusion and not by single injections to avoid rapid changes in blood pressure. Situations in which extrasystoles or other cardiac arrhythmias occurred were avoided, since these affect sympathetic discharge, and when arrhythmias did occur, such as at high rates of infusion of the drugs, these records were disregarded. When supplementary anaesthetic was required during the investigation of a strand of fibres, it was given and the investigation begun afresh. Photographic recordings were analysed in consecutive periods of eight seconds and these readings were subjected to statistical analysis. For the purpose of constructing graphs, the discharge has been averaged over three or four such eight second periods.

Carotid chemoreceptor recordings. These were made from filaments of the sinus nerve. The sinus region was exposed, the hypoglossal nerve sectioned and the cervical sympathetic nerve was divided below the superior /

superior cervical ganglion. The sinus nerve was identified and dissected free of the surrounding connective tissue. Care was taken not to injure the carotid body or its venous drainage. The glossopharyngeal nerve was cut as far centrally as possible and the sinus nerve, together with a section of the glossopharyngeal nerve, was dissected as described previously.

Injection of drugs into the carotid body arterial supply.

A fine polythene cannula was introduced in a retrograde direction into the lingual artery and advanced until its tip was just above the origin of the thyroid artery, on both sides. During intra-arterial injection of phentolamine or phenoxybenzamine the large branches of the carotid bifurcation were occluded so as to aid localisation of the adrenergic blockade to the carotid bodies. In these preparations the depressor, cervical sympathetic and vagus nerves were also divided below the superior cervical ganglion.

Measurement of total carotid body blood flow.

The venous drainage of the carotid body was identified. The segment of the internal jugular or transverse posterior pharyngeal veins into which the carotid body vein ran was cannulated with a fine polythene tube after all other tributaries to the segment had been tied off, and the animal heparinised. Total carotid body blood flow was measured by timing the flow along a Hamilton 50 microlitre syringe held horizontal at the level of the cat's heart.

RESULTS

Section 1.

In this section, cats and dogs in various endocrine states have been studied. Intravenous infusions of noradrenaline or angiotensin have been used to raise blood pressure, and the relationship between sympathetic discharge frequency and mean arterial pressure was obtained using either or both of these vasoconstrictors. 176 strands of sympathetic nerve fibres have been studied, and a comparison of the effects of angiotensin and noradrenaline has been made in 94 of these. The results from the two species will be presented separately.

A. NERVE FIBRE PREPARATIONS FROM ANIMALS WITH INTACT BARORECEPTOR NERVES.

(a) Female cats.

20 nerve strands from 7 female cats, one of which was an unanaesthetised decerebrate, were studied. 17 of these gave responses similar to those shown in Fig. 1. An inverse linear relation exists between spike frequency and mean blood pressure over the full range of pressures studied, from 100 to 200 mm Hg. In the 3 remaining strands, derived from 2 cats which also gave 6 'typical' responses, elevation of arterial pressure above 150 mm Hg. was associated with an increase or no change in sympathetic discharge. In the former instance, sympathetic discharge reached a minimum between 140 and 160 mm Hg.

In 13 of these nerve strands the effects of angiotensin and noradrenaline /

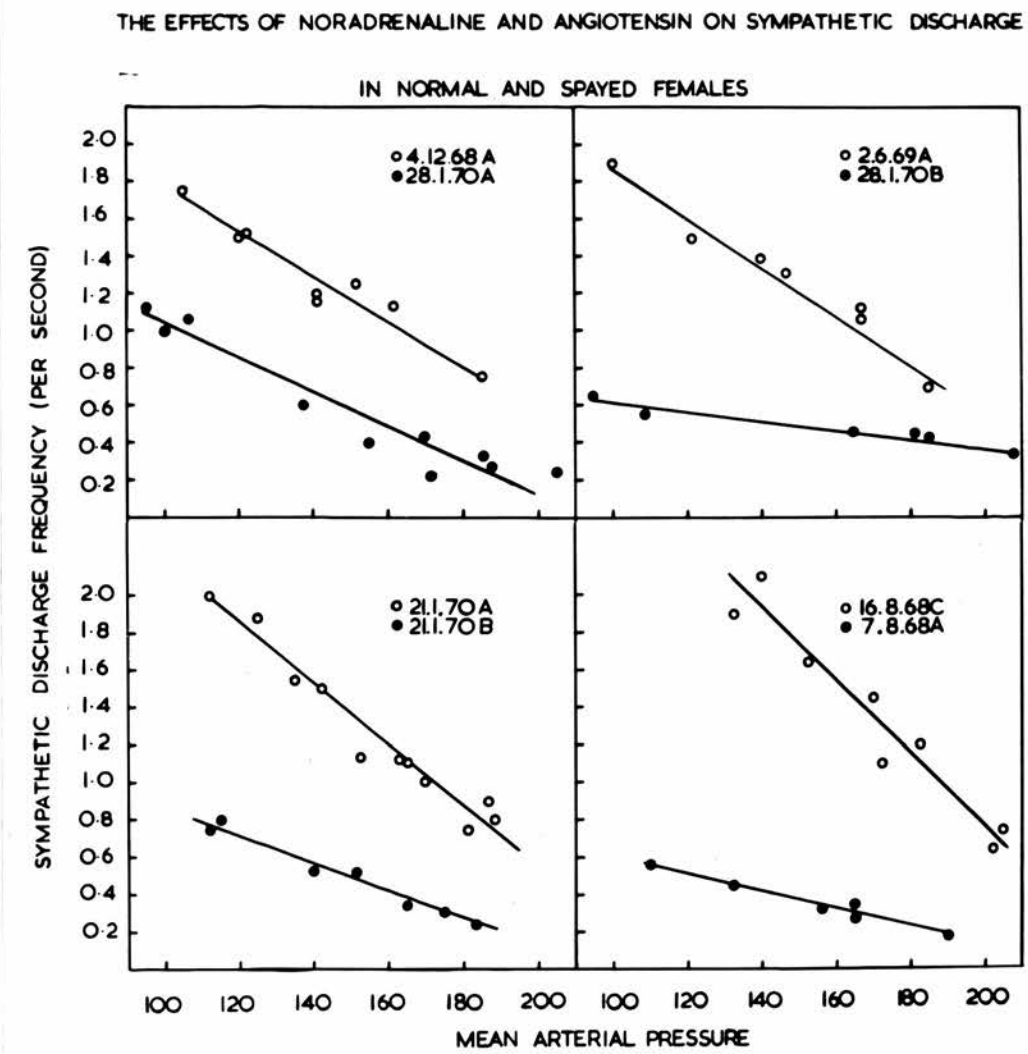


Figure 1. The effects of noradrenaline and angiotensin on sympathetic discharge in normal female cats and dogs, and in spayed female cats.

Ordinate: Mean Spike Frequency.
Abscissa: Mean Blood Pressure. (m.m. Hg.)
Intact Female Cats: 7.8.68; 16.8.68; 21.1.70.
Intact Female Dog : 4.12.68.
Castrate Female Cats: 2.6.69; 28.1.70.

In this and subsequent figures the capital letters following dates refer to the particular nerve strand under study.

noradrenaline were compared and Fig. 2 shows some examples of the results. Inhibition of sympathetic discharge was dependent on blood pressure, and was not determined by the nature of the vasoconstrictor used. At any level of blood pressure, the dimunution of sympathetic discharge frequency was similar, whether angiotensin or noradrenaline were being infused. Angiotensin and noradrenaline appear to be equally effective in reducing resting sympathetic discharge. The hypothesis that equipressor doses of noradrenaline and angiotensin have quantitatively similar effects on sympathetic discharge has been tested statistically by calculating the significance of the regression coefficients of the spike frequency/blood pressure relationship of all the points on the graph, whether obtained at resting blood pressure or during the infusion of these drugs. If the regression coefficient had a low P value, this was interpreted as indicating that the two drugs had similar effects. The null hypothesis that equipressor doses of the two vasoconstrictors have similar effects on sympathetic discharge was upheld, since the regression coefficients always had P values less than 0.01 and often less than 0.001. There was therefore no evidence for the hypothesis that an increase in central sympathetic activity contributed to the pressor action of angiotensin, but not to the pressor action of noradrenaline. Any significant selective stimulation of central sympathetic neurones by angiotensin is therefore unlikely.

(b) /

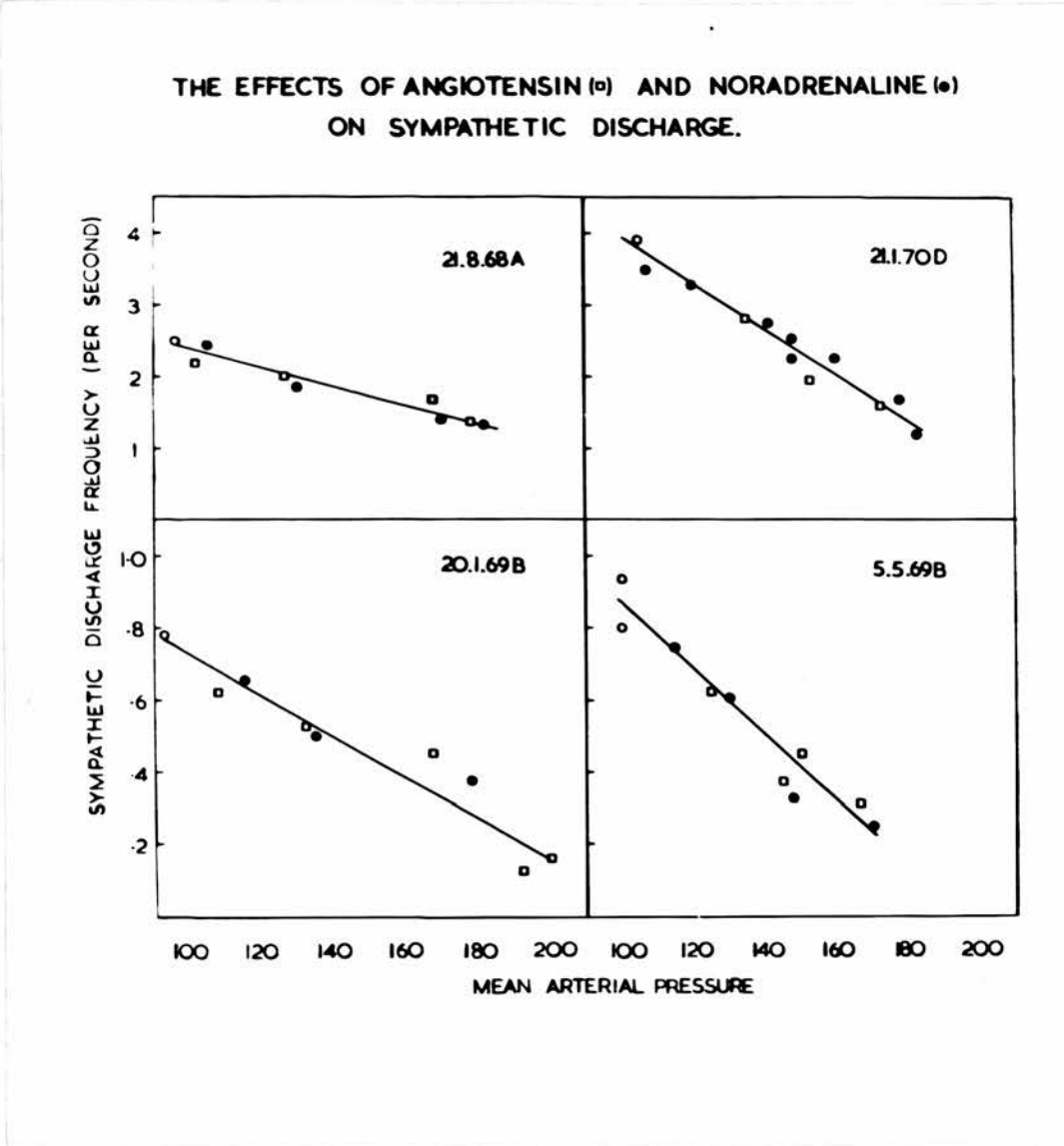


Figure 2. The effects of angiotensin and noradrenaline on sympathetic discharge in four nerve strands from female and castrate and oestrogen treated male cats.

Ordinate: Mean Spike Frequency.
Abscissa: Mean Blood Pressure (m.m. Hg.)
Female Cats: 21.8.68 and 21.1.70.
Oestrogen treated male cat: 20.1.69.
Castrate male cat: 5.5.69.

(b) Male cats.

21 nerve strands from 7 male cats were studied. One of the cats had a bilateral vagotomy, but the depressor nerve was spared. 18 of the nerve strands gave a relationship between spike frequency and mean arterial pressure similar to those shown in Figure 3. At blood pressures below 150 mm Hg there is a linear inverse relationship, but between 140 and 160 mm Hg a minimum is reached, and at higher pressures the sympathetic discharge increases or remains constant. Three other strands from 2 cats behaved as shown in Figure 1. Both of these cats also produced strands with the characteristic male response described above. In the pressure range less than 150 mm Hg, 13 strands which were studied during angiotensin and noradrenaline infusions gave regression coefficients that were usually significant at the 0.1% level. In the higher pressure range, both angiotensin and noradrenaline were effective in bringing about a change in the slope of the spike frequency/arterial pressure relationship, and the increase in sympathetic activity usually recorded was not specifically evoked by angiotensin, but seemed to be related rather to the magnitude of the pressor response to both these agents as shown in Figure 4.

(c) Male cats treated with oestrogens.

2 male cats were treated with 2 mgs stilboestrol 24 hours before the acute experiment, and 2 more were treated with 2 mgs oestriol dihemisuccinate /

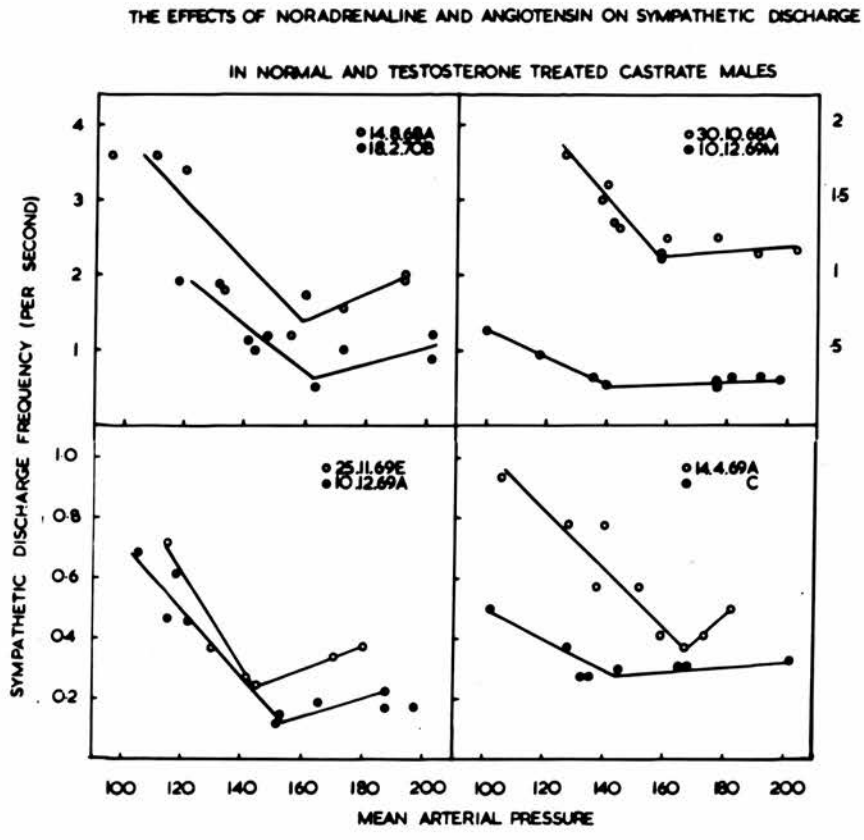


Figure 3. The effects of angiotensin and noradrenaline on sympathetic discharge in eight nerve strands from normal and testosterone treated castrate male cats.

Ordinate: Mean Spike Frequency.
Abscissa: Mean Blood Pressure. (m.m. Hg.)
Male cats: 14.8.68; 14.4.69; 10.12.69.
Male dog: 30.10.68.
Testosterone treated castrate male cats: 25.11.69; 18.2.70
The coordinates of the bottom two graphs are the same.

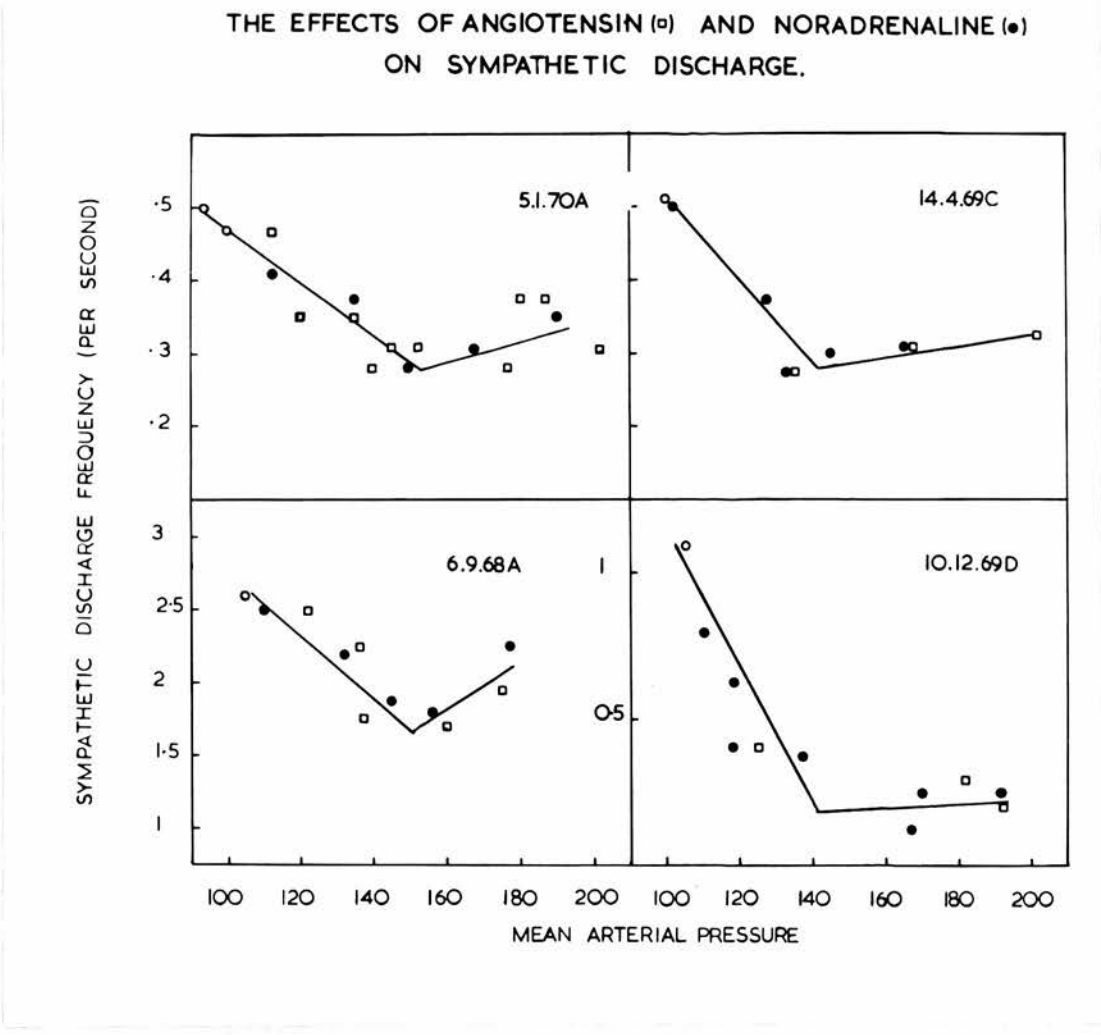


Figure 4. The effects of angiotensin and noradrenaline on discharge in four strands of the cervical sympathetic nerves of four male cats.

Ordinate: Mean spike frequency.
Abscissa: Mean blood pressure. (m.m. Hg.)

dihemisuccinate intraveously at the start of the acute experiment. 14 strands from these animals were studied. 11 of the 14 gave discharge rate/blood pressure relations that could be described by an inverse linear relationship as shown in Figure 5, and in the remaining 3 there was an inflexion in the relationship occurring around 150 mm Hg. 6 of the strands were studied during both angiotensin and noradrenaline infusions, and the distribution of points for the two drugs was very similar, the inverse parts of the relationship not departing significantly from a straight line.

(d) Female dogs.

6 satisfactory strands were obtained from 3 dogs, and the effects of angiotensin and noradrenaline were examined in each case. In 5 of these, angiotensin and noradrenaline had similar effects on sympathetic discharge, depending on the extent of the blood pressure elevation, and in 4 of the 5 the relationship between discharge frequency and arterial pressure could be described by a straight line as shown in Figure 1, while the other showed an inflexion around 150 mm Hg.

The remaining single unit in this group behaved as follows: noradrenaline decreased the discharge linearly over the range 110 to 163 mm Hg., and angiotensin also decreased the discharge rate initially, but spike frequency increased dramatically as increasing infusion rates of the peptide raised arterial pressure from 148 to 193 /

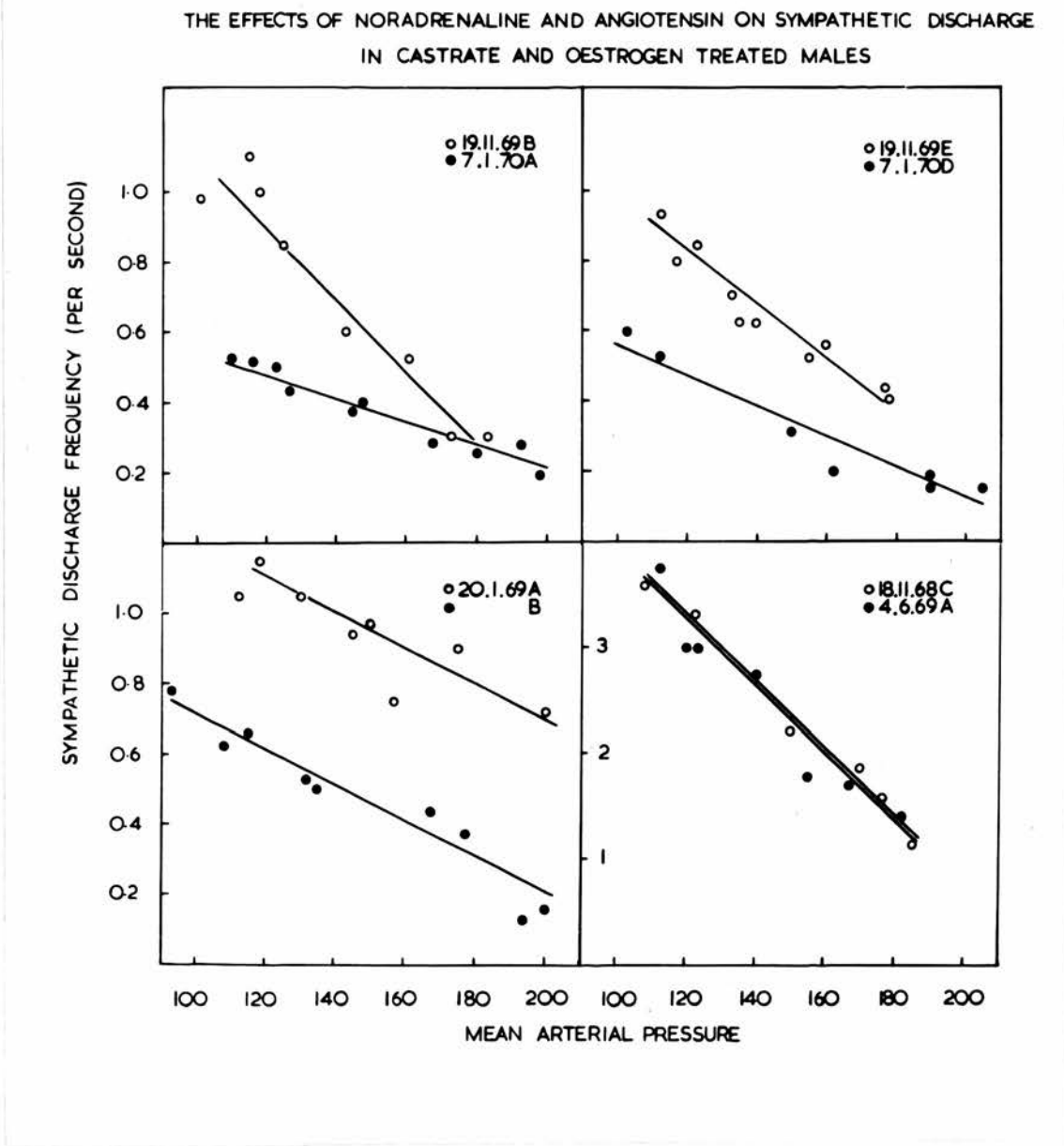


Figure 5. The effects of noradrenaline and angiotensin on sympathetic discharge in eight nerve strands from castrate male cats and oestrogen treated male cats and dogs.

Ordinate: Mean Spike Frequency.
Abscissa: Mean Blood Pressure. (m.m. Hg.).
Castrate male cats: 19.11.69; 7.1.70.
Oestrogen treated male cat: 20.1.69.
Oestrogen treated male dogs: 18.11.68; 4.6.69.

193 mm Hg. Unfortunately the changes in pressure induced by the two drugs were in this case dissimilar and information is lacking on the effects of higher doses of noradrenaline because of the occurrence of cardiac arrhythmias at these pressures with the catecholamine.

(e) Male dogs.

7 successful nerve preparations from 4 male dogs behaved as follows. Angiotensin and noradrenaline affected sympathetic discharge identically, the extent of the inhibition being related to the arterial pressure change. 5 of the 7 strands increased their discharge rate at pressures above 150 mm Hg as shown in Figure 3 and in the two remaining strands sympathetic discharge continued to fall at higher pressures so that the whole relationship could be described by a straight line.

(f) Male dogs treated with oestrogens.

4 male dogs were treated with 5 mgs oestriol dihemisuccinate intravenously at the start of the acute experiments. 9 strands of fibres were investigated and 8 of these gave inverse straight line relationships between impulse frequency and arterial pressure as shown in Figure 5, and one showed an inflexion around 150 mm Hg. In three of these preparations a comparison of the effects of angiotensin and noradrenaline was made and no significant differences found /

found between the effects of the two pressor agents using the statistical methods described earlier.

(g) The effect of castration on response of cervical sympathetic nerve fibres of male cats to the infusion of the pressor agents.

3 castrate male cats were studied. Out of 22 nerve fibre preparations which changed their discharge rate when the vasoconstrictors were infused, 19 showed relationships between spike frequency and mean blood pressure which were inverse and linear in their entirety and similar to those found in normal female cats shown in Figure 5. The remaining three nerve strands showed inflexions in this relationship, with either an increasing or a constant discharge frequency as blood pressure was raised above 150 mm Hg.

(h) The effects of the pressor agents on cervical sympathetic discharge in spayed female cats.

Four female cats were ovariectomised at least four weeks before being used in acute experiments. The activity of 12 strands of their cervical sympathetic nerve fibres was studied during the infusion of either noradrenaline or agiontensin or sometimes both of these. Eleven of these nerve strands gave inverse straight line relationships between impulse frequency and blood pressure as previously found in normal females and castrate males. Examples are shown in Figure 1. In the remaining nerve preparation the response consisted of inhibition of discharge below 150 mm Hg and an increase /

increase in discharge at higher pressures.

(j) The effect of testosterone propionate on the response of cervical sympathetic nerve fibres of castrate male cats to the infusion of the pressor agents.

Three cats were studied. A single injection of 10 mgs testosterone propionate was given intramuscularly on either the second or third day before the acute experiment to two of the castrate male cats, while the other animal received 5mgs intramuscularly on the day preceding the acute experiment, and a further 5 mgs at the start of the experiment. A total of 22 nerve strands were studied, and 17 gave responses that were typical of intact adult male cats as shown in Figure 3. The remaining 5 fibres did not show any deviation from the inverse linear relation characteristic of castrates and normal females.

B. THE EFFECTS OF INTRAVERTEBRAL INFUSIONS OF ANGIOTENSIN AND NORADRENALINE ON SYMPATHETIC DISCHARGE.

Intravertebral infusions of the drugs were given in one female, one male and one castrate male cat. Seven strands from the female and castrate male decreased their activity in proportion to the rise in blood pressure produced by the drugs. The extent of the blood pressure change during intravertebral infusions was similar to the change found on intravenous administration, in contrast to the results of Lowe and Scroop (1968) who worked on chloralose anaesthetised greyhounds. /

greyhounds. Another seven strands from one intact male cat gave results similar to those described earlier for intravenous infusions. Six of the strands decreased their activity during the infusions in the pressure range below 150 mm Hg, and their activity increased or remained constant above this pressure. In one strand spike frequency was inversely related to blood pressure. The effects of angiotensin and noradrenaline were again very similar, and in only two cases did the discharge during the drug infusions exceed the spontaneous level. This occurred with angiotensin in one case and with noradrenaline in the other. Figure 6 shows one example of a unit which was silenced by intravenous administration of angiotensin at about 165 mm Hg., and which was also silenced by intravertebral infusion of the same drug at 158 mm Hg.

C. THE EFFECTS OF BARORECEPTOR DENERVATION.

The operative procedure in these animals included section of the vagus, depressor and carotid sinus nerves on both sides, as well as stripping the adventitia of the carotid arteries and denervation of the common carotid baroreceptor regions. These animals no longer showed a rise in blood pressure when the common carotid arteries were occluded low down in the neck. Nineteen nerve preparations were examined from four cats and two dogs of both sexes and in all cases but one the responses to angiotensin and noradrenaline were indistinguishable. /

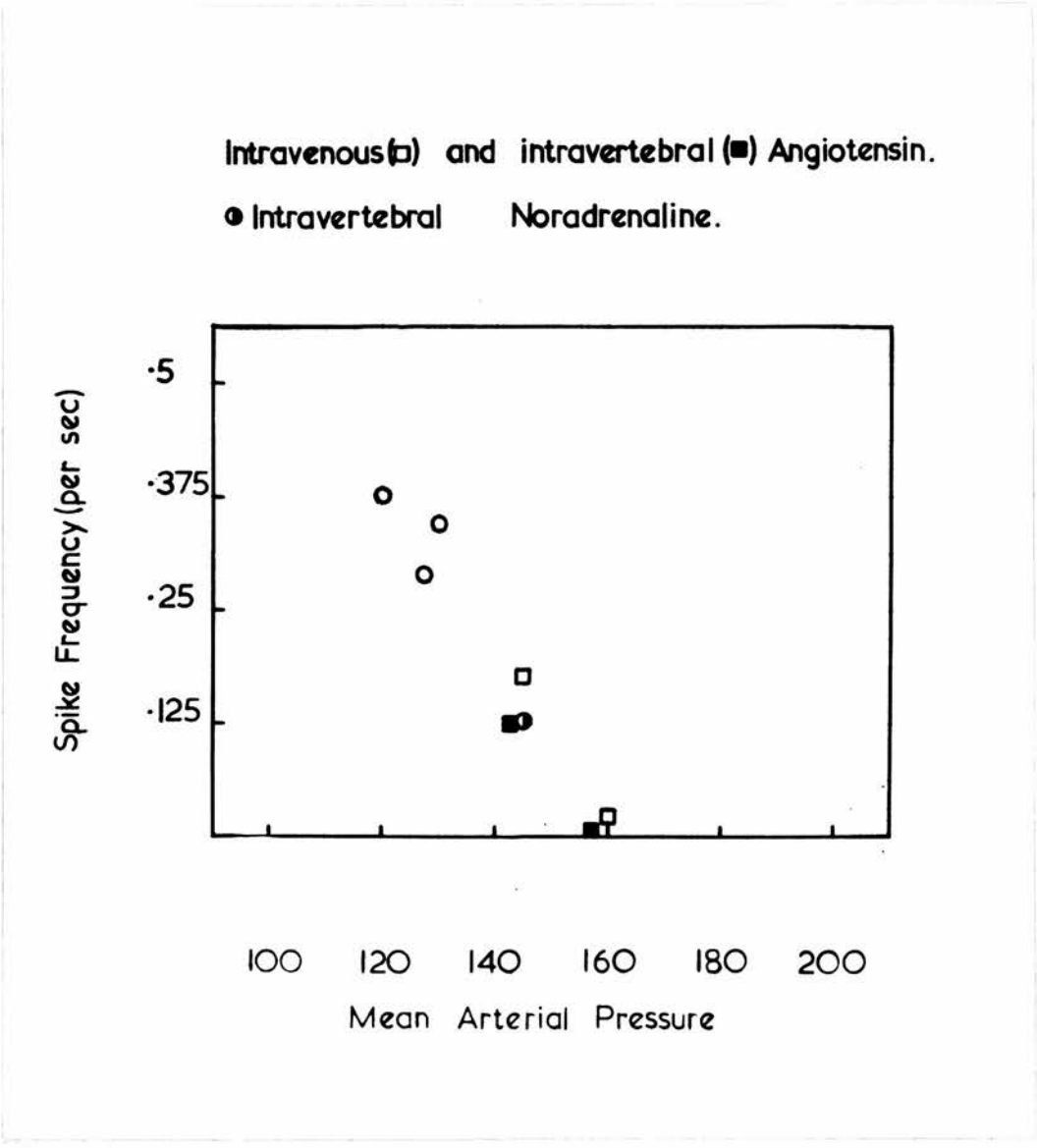


Figure 6. Ordinate: Mean Spike Frequency
Abscissa: Mean Blood Pressure. (m.m. Hg.)

The effects of intravenous and intravertebral infusions of angiotensin and noradrenaline on sympathetic discharge in one unit from a castrate male cat. $Y = 1.59 - 0.01X$ P less than 0.001

indistinguishable.

11 of the 19 strands did not change their rate of discharge when the drugs were infused, as shown in Figure 7. Four preparations decreased, and three others increased their discharge slightly when the pressor agents were infused. In the one remaining nerve strand, noradrenaline did not alter the rate of discharge but angiotensin infusion was accompanied by an increase, as shown in Figure 7D.

Section 2.

In this section, the neurological basis of the sex difference in the spike frequency/blood pressure relation is investigated in cats. Attention was focussed around the chemoreceptors because high doses of the drugs did not usually increase sympathetic discharge in male cats with denervated baroreceptors. The sex difference appeared to lie in this region and the aim of the experiments then was to eliminate the increase in sympathetic discharge above 150 mm Hg in male cats by interfering with the effects of the vasoconstrictors on the chemoreceptors, and to record directly the activity of the chemoreceptors during infusions of the drugs.

A. EXPERIMENTS ON MALE CATS.

(a) The effects of ventilation with pure oxygen on sympathetic discharge.

In three male cats ventilated artificially to avoid changes in tidal /

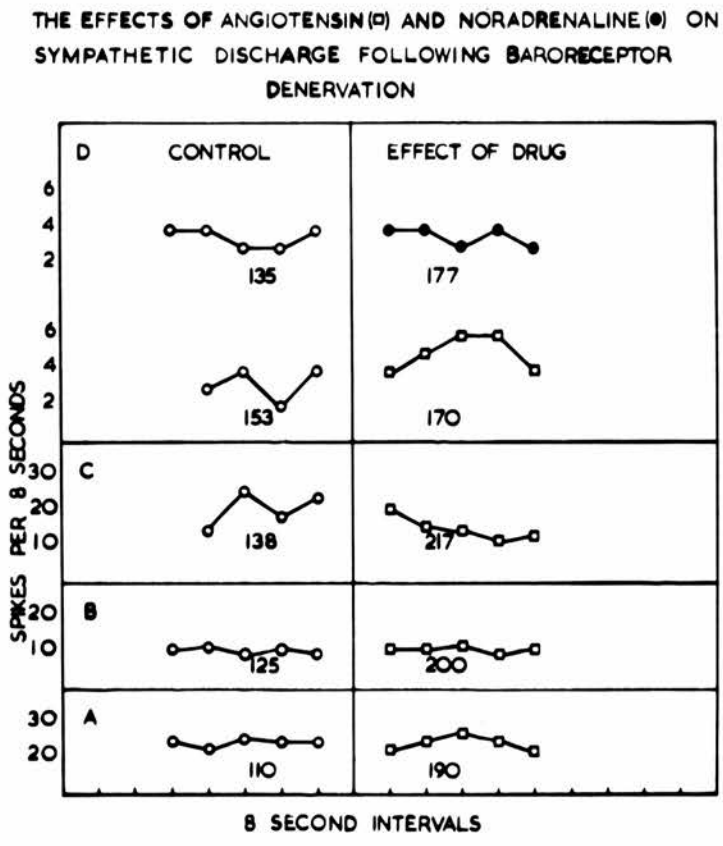


Figure 7. The effects of baroreceptor denervation. The numbers below each plot represent the mean blood pressure at the time of the recording.

tidal volume when gas mixtures were changed, sympathetic discharge was recorded during ventilation with air or with pure oxygen. During ventilation with air, the vasoconstrictors produced the same relationship between spike frequency and mean blood pressure as shown in Figure 3. During ventilation with pure oxygen, the same fibres failed to show the increase in discharge above 150 mm Hg., and instead the discharge continued to fall along the same line that was obtained at pressures below 150 mm Hg. during ventilation with air or oxygen. A total of ten strands have been examined and all but one was affected in the manner described during ventilation with oxygen. Figure 8 shows the results from four of these strands.

(b) The effects of localised alpha receptor blockade of the carotid chemoreceptors on sympathetic discharge after aortic chemoreceptor denervation.

In two male cats, the vagus, depressor and cervical sympathetic nerves were sectioned below the superior cervical ganglion, and the lingual artery was cannulated in a retrograde direction on either side, the polythene cannula being advanced to just above the origin of the thyroid artery. Both of these cats gave sympathetic discharge frequency/arterial pressure relationships that were typical of their sex and similar to Figure 3, following these procedures. Local injection of either 1 mg phenoxybenzamine or phentolamine through each of these cannulae during occlusion of the major branches of the common carotid /

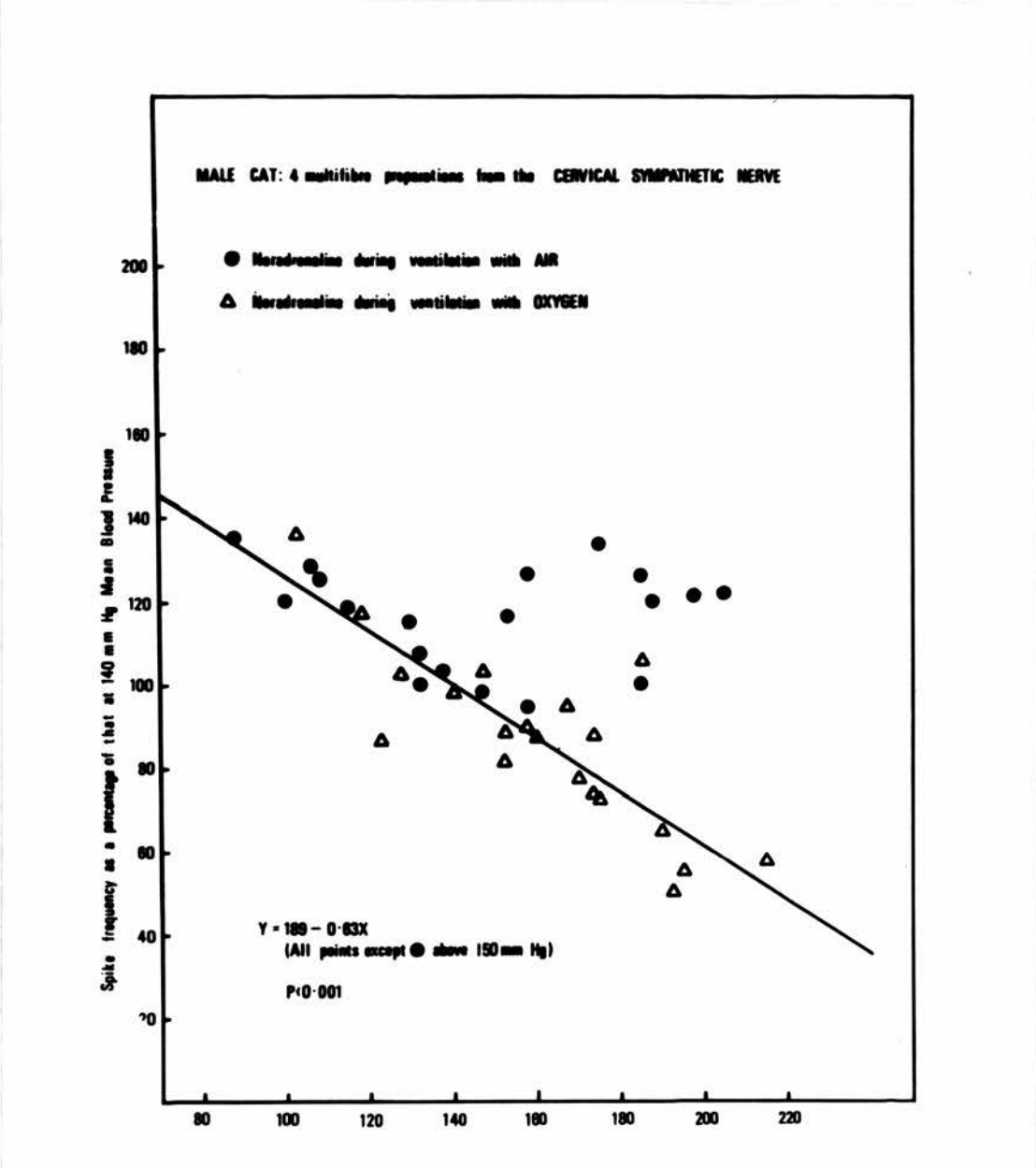


Figure 8. The effects of oxygen on sympathetic discharge in male cats.

Ordinate: Spike Frequency as a percentage of that at 140 mm Hg.

Abscissa: Mean Blood Pressure (m.m. Hg.)

carotid artery abolished the increase in sympathetic discharge which previously had accompanied the intravenous infusion of high doses of noradrenaline. However the response to intravenous infusions of angiotensin was unaffected by these localised injections of blocking agents, as shown in Figure 9. For a short period following the injection of the blocking agents, there was intense sympathetic activity which reverted to its original level after a few minutes.

(c) The effects of the pressor agents on chemoreceptor discharge.

The effects of angiotensin and noradrenaline on carotid chemoreceptor discharge were studied in four male cats. 13 chemoreceptor units of the carotid sinus nerve were studied during spontaneous or artificial ventilation with air, and following section of the cervical sympathetic nerve. Noradrenaline usually caused an initial decrease in discharge frequency, and this was followed by an increase in discharge at higher infusion rates, the inflexion occurring at blood pressures less than 150 mm Hg. in all units studied. Angiotensin usually induced similar behaviour, and Figure 10 shows the discharge in four fibres during pressure elevation with noradrenaline or angiotensin. In 2 of the fibres in which noradrenaline caused an increase in activity, angiotensin either left discharge frequency unaffected or caused a further decrease at pressures in excess of 180 mm Hg. Sometimes, when both angiotensin and noradrenaline caused an increase in chemoreceptor discharge at high pressures, the magnitude of the increase was greater with noradrenaline than with angiotensin,as shown in /

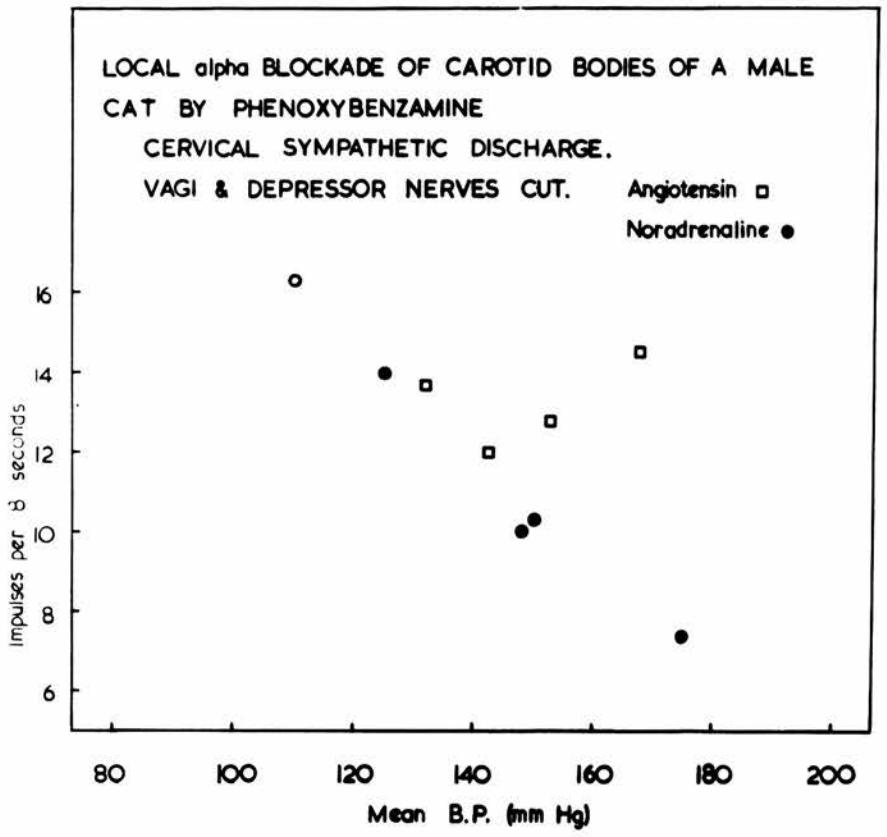


Figure 9. The effects of blockade of chemoreceptor alpha receptors with phenoxybenzamine.

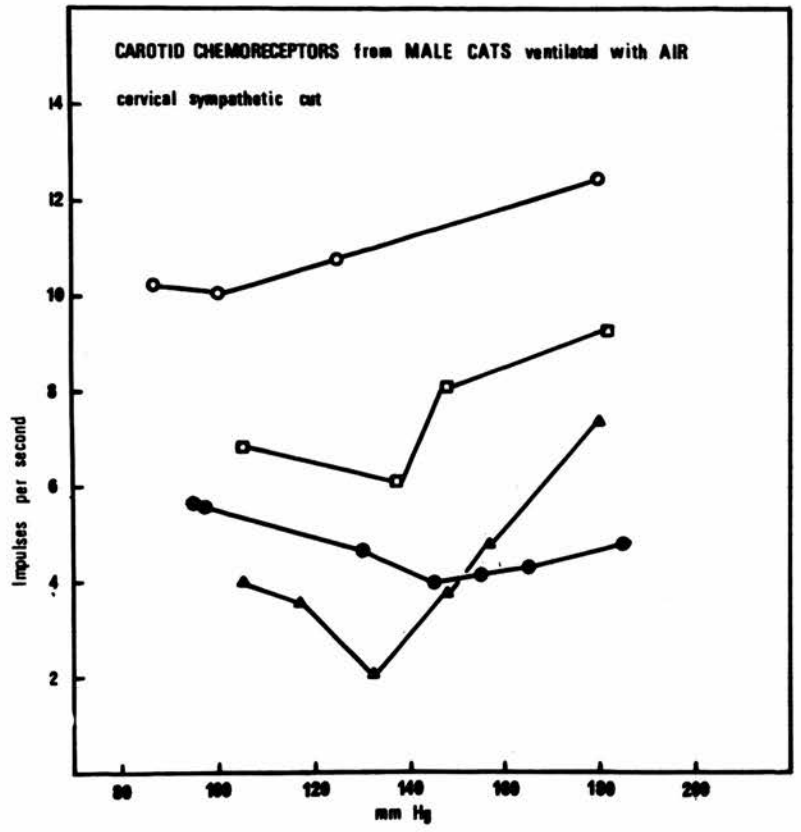


Figure 10. The effects of infusions of noradrenaline or angiotensin on discharge in four chemoreceptor units from male cats. The four symbols refer to the unit under study and not to the drug being infused.

in Figure 11. The increased chemoreceptor discharge could always be abolished by ventilation with 100% oxygen.

(d) The effects of angiotensin and noradrenaline on total carotid body blood flow.

Figure 12 shows the relation between total carotid body blood flow and mean arterial pressure. No distinction has been made between the points obtained with angiotensin and noradrenaline as the effects of the drugs were identical. Blood flow increased as the perfusion pressure was raised, and there was no indication of any change in the slope of the relationship around 140 - 150 mm Hg. Following cervical sympathectomy and sinus nerve denervation, the flow increased at all pressures.

(e) The effects of noradrenaline infusions on arterial oxygen tensions in male cats.

Arterial pO_2 was measured using a Bishop oxygen electrode on samples of arterial blood during the infusion of noradrenaline at different rates. The volume of each blood sample was replaced with dextran. Three male cats, one of which was a castrate, were used and the vasoconstrictor infusions had little effect on arterial pO_2. There was no evidence of a fall in arterial pO_2 above 150 mm Hg in the intact males, which might have accounted for the increase in chemoreceptor discharge in this pressure range. The results are shown in Figure 13.

B. /

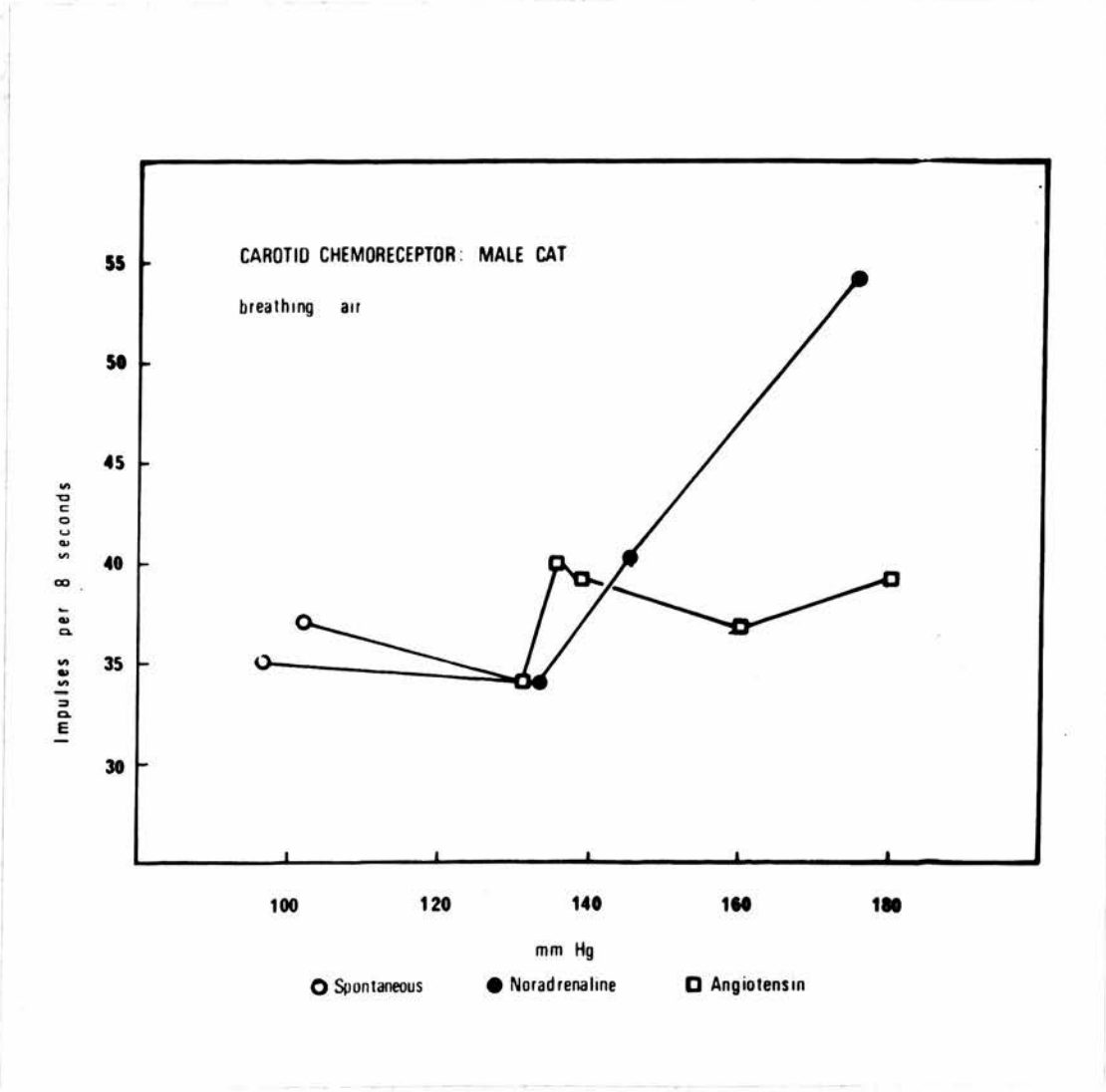


Figure 11. The effects of noradrenaline (closed circles) and angiotensin (open squares) on discharge in one chemoreceptor fibre from a male cat.

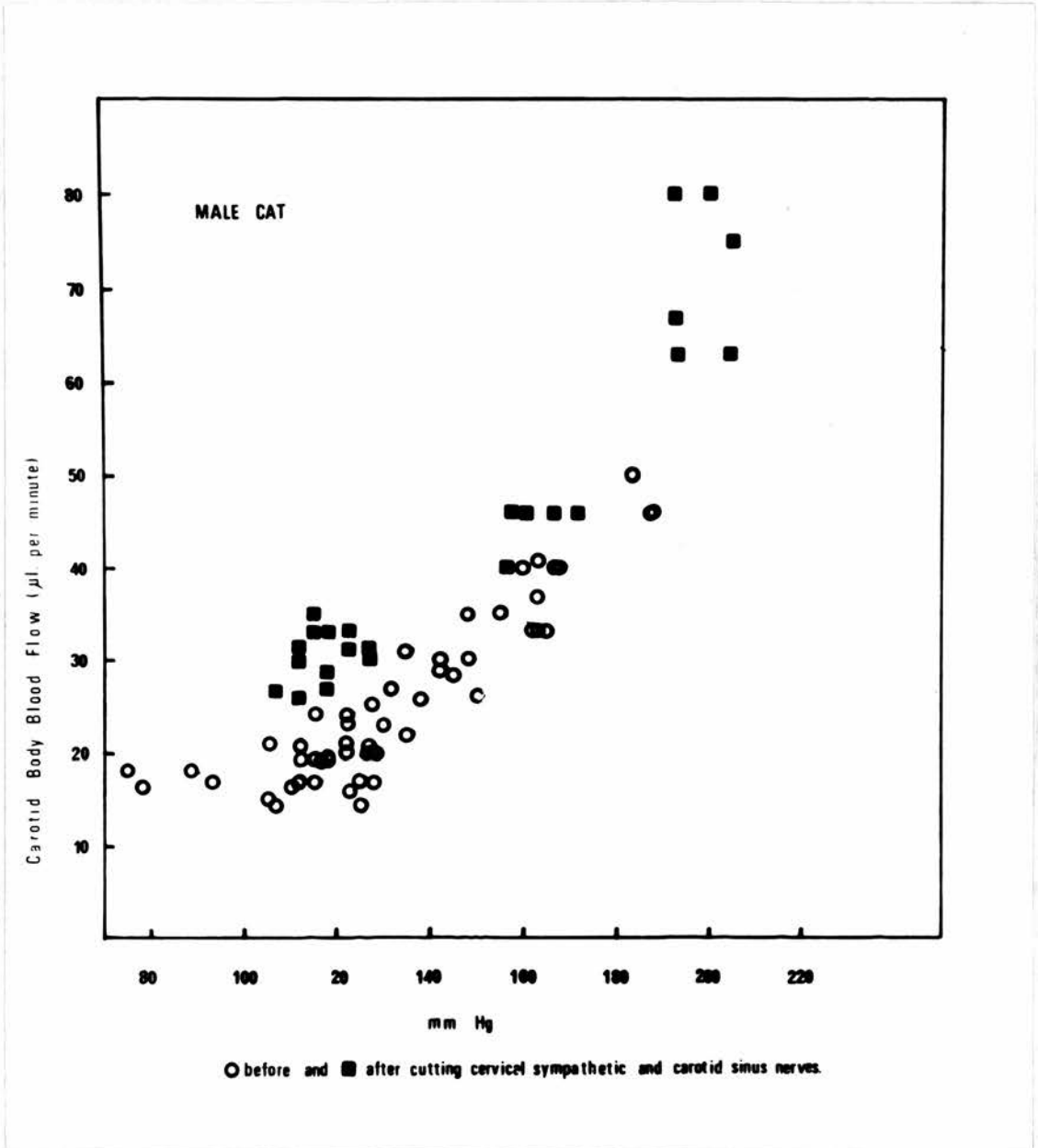


Figure 12. The effects of raising blood pressure with infusions of noradrenaline or angiotensin on total carotid body blood flow in a male cat.

Ordinate: Total Carotid Body Blood Flow.

Abscissa: Mean Blood Pressure. (mm. Hg.)

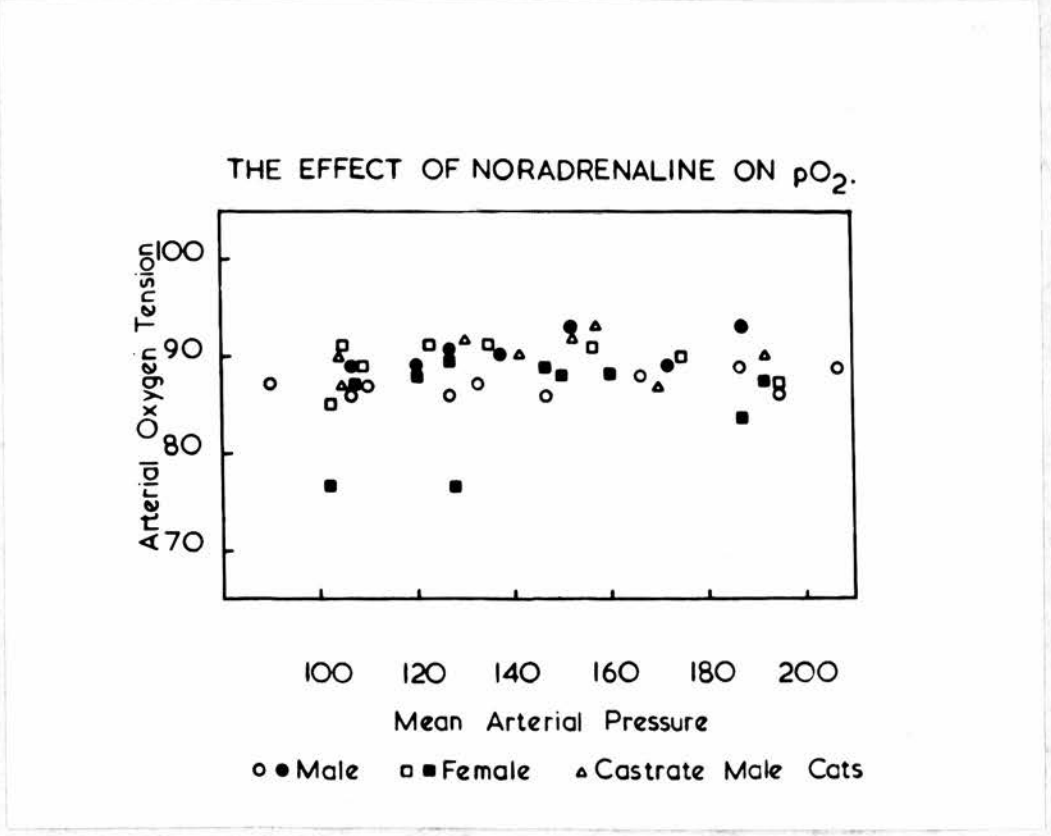


Figure 13. The effect of noradrenaline infusions on the oxygen tension of arterial blood from male, female and castrate male cats.

Ordinate: Arterial Oxygen Tension (mm Hg.)

Abscissa: Mean Arterial Pressure (mm Hg.)

B. EXPERIMENTS ON FEMALE CATS.

(a) The effects of the pressor agents on chemoreceptor discharge.

Four female cats have been used, and eleven chemoreceptor fibres of the carotid sinus nerve have been studied. Figure 14 shows the typical response which was found in eight fibres. As blood pressure was increased with the drugs, chemoreceptor discharge fell and no inflexion occurred in the discharge rate/blood pressure relationship. Usually, the two pressor agents had similar effects as shown by the fibre illustrated in Figure 14. Figure 15 shows results from 5 such fibres, and the points obtained with the individual drugs have not been distinguished. In one other fibre, noradrenaline, produced some increase in discharge between 170 and 180 mm Hg, but raising the blood pressure to this level with angiotensin produced a further fall in discharge. In the two remaining fibres an inflexion occurred between 140 and 160 mm Hg, and at higher pressures the chemoreceptor discharge began to rise.

(b) The effects of the drugs on total carotid body blood flow.

Figure 16 shows the relationship between mean arterial pressure and total carotid body blood flow in one of two female cats studied. Flow increased as arterial pressure increased both in the innervated organ, and following cervical sympathectomy, and sinus nerve section.

(c) The effects of noradrenaline on arterial oxygen tensions in female cats.

Arterial /

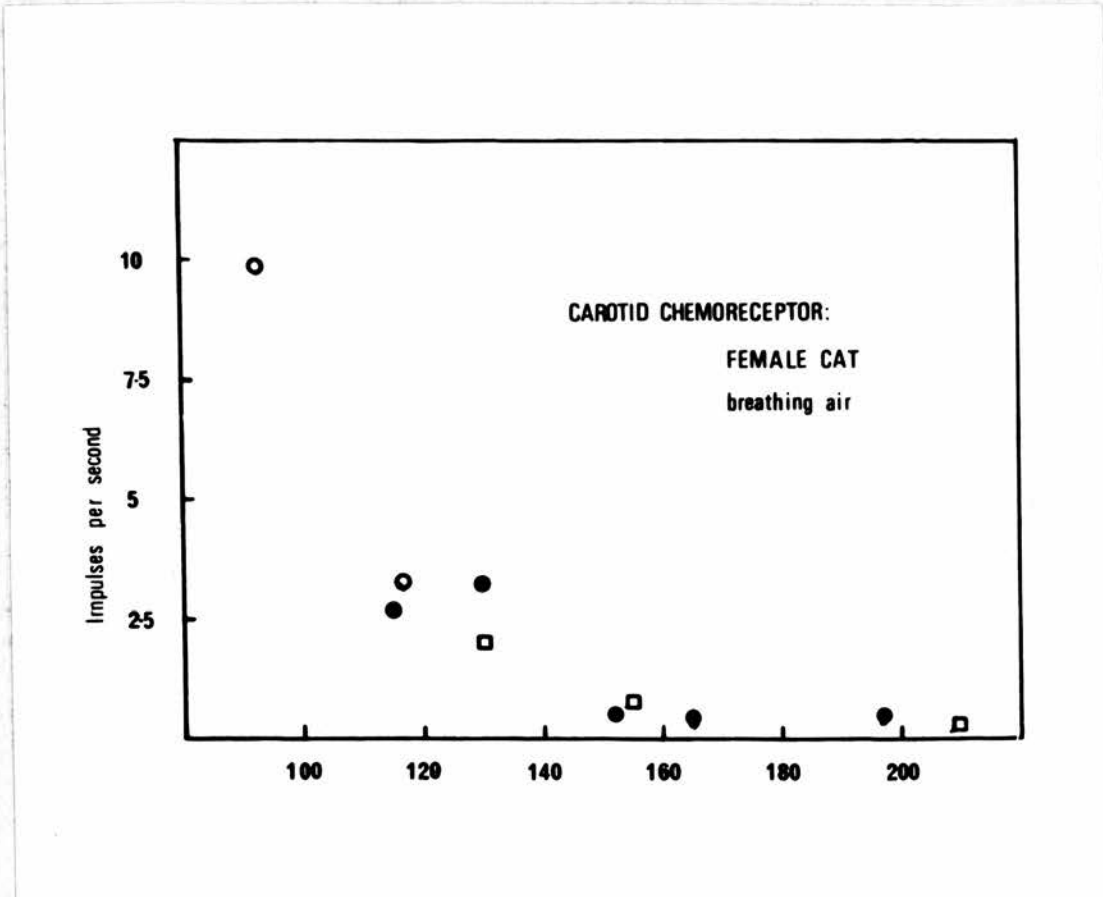


Figure 14. The effects of noradrenaline and angiotensin on discharge in a carotid chemoreceptor unit from a female cat breathing air.

Ordinate: Impulses per second; Abscissa: Mean Blood Pressure (mm. Hg.)

Open Circles: Spontaneous activity. Closed circles: Noradrenaline.

Open squares: Angiotensin.

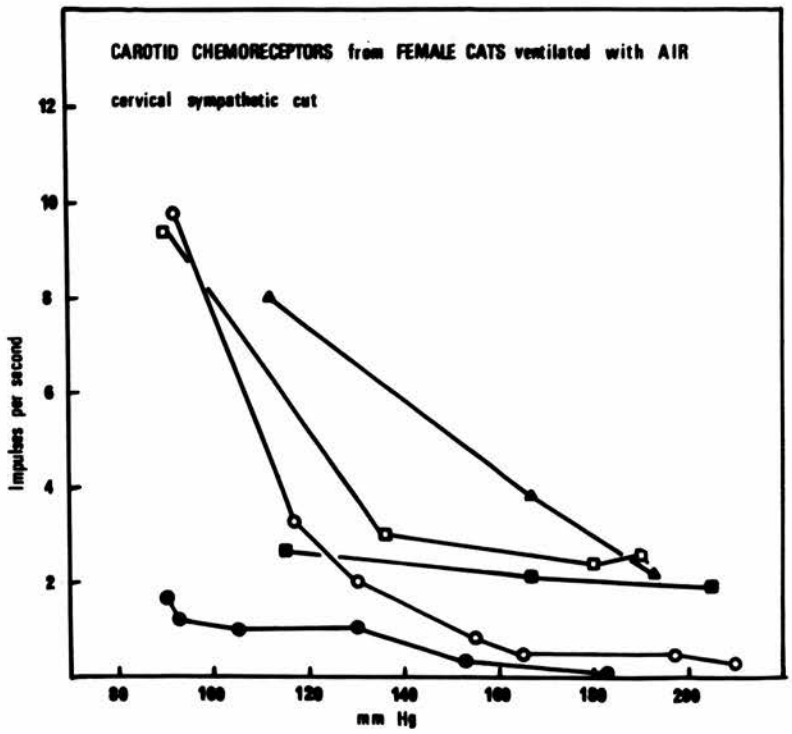


Figure 15. The effects of infusions of angiotensin or noradrenaline on discharge in five chemoreceptor units from the carotid sinus nerves of four female cats.

Ordinate: Impulses per second; Abscissa: Mean Blood Pressure (mm. Hg.)

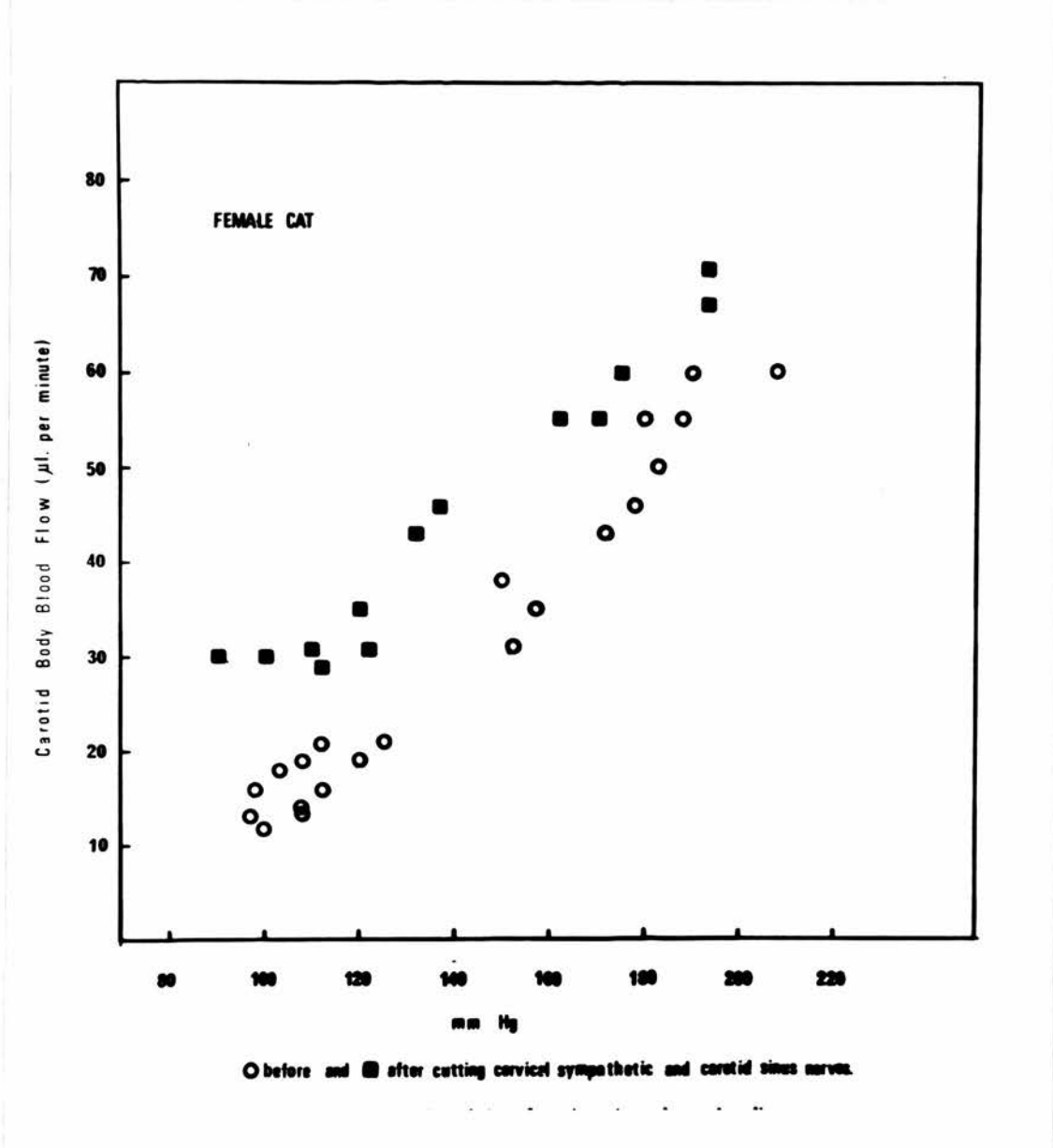


Figure 16. The effects of angiotensin or noradrenaline on total carotid body blood flow in a female cat.

Ordinate: Carotid body blood flow.

Abscissa: Mean Blood Pressure (mm. Hg.)

Arterial pO_2 was measured in two female cats and the results are shown in Figure 13. One female was slightly hypoxic at resting blood pressure, but the oxygen tension rose during the noradrenaline infusions, and did not return to the low level following them. In the other female there was little change in pO_2 during the periods of hypertension.

Figure 17 shows a statistical analysis of the incidence of the inflexions in discharge rate which occur in the two sexes in cervical sympathetic and carotid chemoreceptor fibres between 140 and 160 mm Hg. or less (for the chemoreceptor discharge). When the animals are breathing air the incidence of the phenomenon is similar in chemoreceptors and in the sympathetic, in both sexes, but when the incidence in the chemoreceptors or in the sympathetic is compared in males and females, it is clear that the probability of there being a sex difference in the chemoreceptors and in the sympathetic is high. Figures 18 and 19 are photographs of experimental records of activity recorded from the cervical sympathetic and carotid sinus nerves of cats.

INCIDENCE OF INFLEXION IN DISCHARGE RATE AT 140-160 mm Hg IN CAT CERVICAL SYMPATHETIC & CAROTID CHEMORECEPTOR FIBRES

analysed by Chi-square

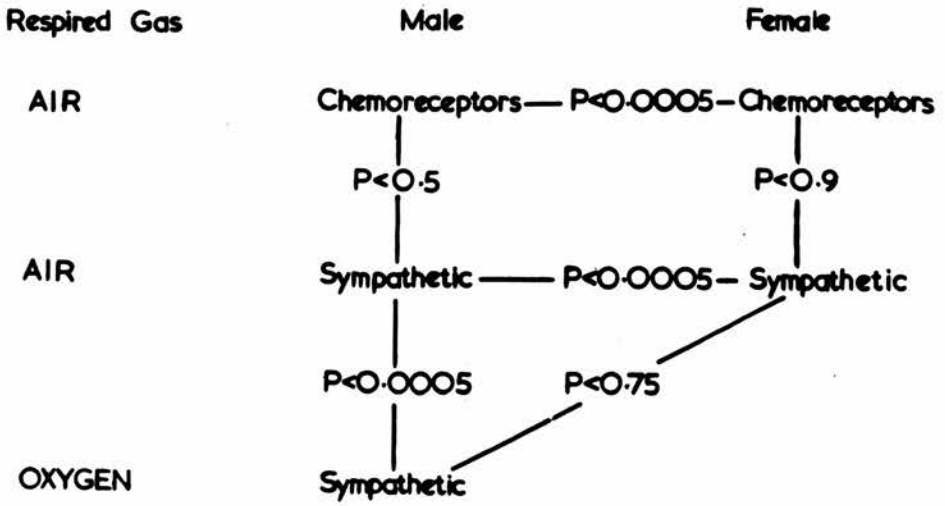
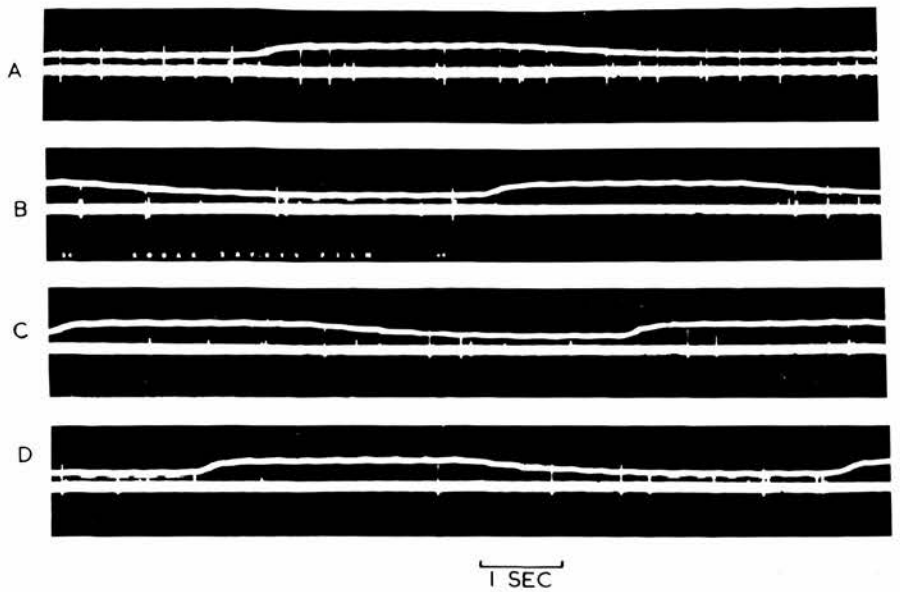


Figure 17.

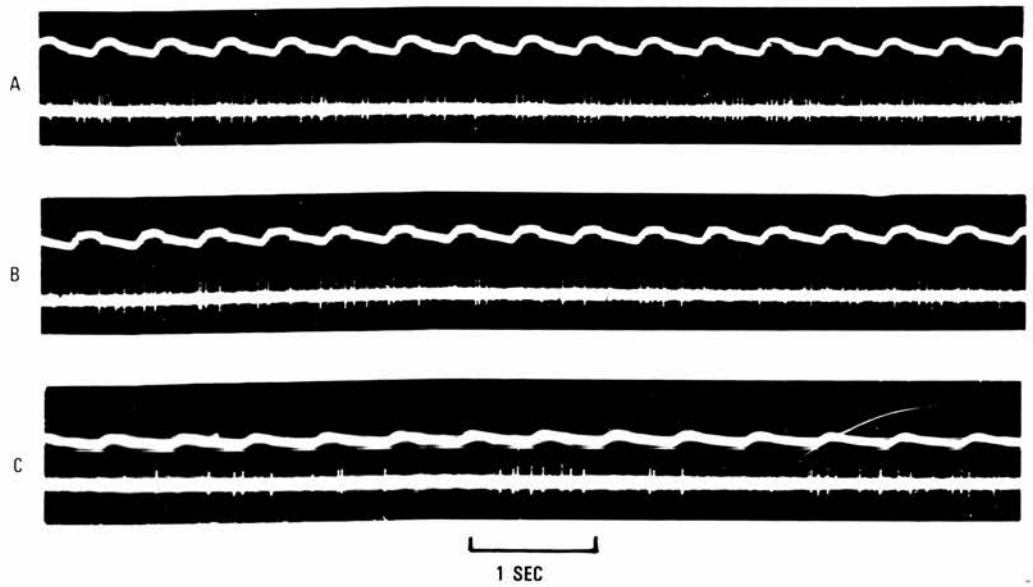


THE EFFECT OF NORADRENALINE ON SYMPATHETIC DISCHARGE IN A MALE CAT

UPPER TRACE OESOPHAGEAL PRESSURE, INSPIRATION ↓

BLOOD PRESSURES, A 102, B 142, C 155, D 180 mm Hg.

Figure 18.



CAROTID CHEMORECEPTOR ACTIVITY IN A MALE CAT VENTILATED WITH AIR

A. ANGIOTENSIN INFUSION: B.P. 172 mm Hg

B. NORADRENALINE INFUSION: B.P. 158 mm Hg

C. RESTING B.P. 98 mm Hg

Figure 19.

DISCUSSION

The first point for discussion is the relationship between the sympathetic nerve fibres studied and the innervation of the blood vessels. One cannot be sure of the ultimate destination of any particular efferent fibre, but one can study its reflex activities. The cervical sympathetic nerve is not a homogenous group of fibres since it supplies efferents to a number of functionally distinct end organs, only some of which receive a tonic resting discharge. These include the blood vessels and some smooth muscle in the orbit, while other effectors such as the erector pili muscles are not subject to continuous influence from the sympathetic (Langley 1900). In anaesthetised cats only some 20% of the efferent nerve fibre population is spontaneously active (Polosa 1968), and this may be purely a reflection of the fact that they are motor fibres. In the somatic motor system and in the motor control of the intrinsic laryngeal muscles (Eyzaguirre, Sampson and Taylor 1966) recruitment of inactive fibres is one means of increasing effector activity, and it seems quite likely that this method of control could extend to the sympathetic division of the autonomic nervous system. If this is so, the inactive sympathetic fibres might innervate any of the end organs mentioned.

The greater part of the spontaneous activity in the cervical sympathetic is inhibited by an acute rise in blood pressure (Adrian et al 1932; Iggo and Vogt 1960, 1962). However the latter workers also /

also found some fibres which did not show the responses of the majority, and other investigators have confirmed and extended these observations (Millar and Biscoe 1965; Widdicombe 1966.) The function of these fibres is unknown.

The fibres studied in this work have been divided into groups depending on their responses to drug induced hypertension, and this stimulus was found to cause a decrease, a decrease followed by an increase, or no change in activity. The majority of fibres showed some inhibition of activity during hypertension, and this was probably entirely due to increased baroreceptor activity, since animals with sectioned baroreceptor nerves showed little change in sympathetic discharge frequency during hypertension. Sympathetic nerve fibres which do not alter their activity during hypertension have previously been reported by Widdicombe (1966). His group IIc included fibres which did not respond to adrenaline or carotid arterial occlusion. These fibres showed respiratory variations in their discharge and were excited by asphyxia. Millar and Biscoe (1965) also described fibres in the rabbit cervical sympathetic and splanchnic nerves which gave high frequency bursts of action potentials and were unaffected by adrenaline hypertension.

In the sympathetic nerve to the carotid body, Biscoe and Purves (1967b) found fibres whose discharge rate was unaffected by transient periods of hypoxia or by the resultant hypertension (as far as can be /

be judged from their published records). The possibility that there are two groups of sympathetic fibres, one adrenergic and one cholinergic, in this nerve has been reviewed by Biscoe and Silver (1966). One of the characteristics of sympathetic cholinergic fibres elsewhere is that their discharge, if indeed they are spontaneously active, is not affected by blood pressure (Uvnas 1961). It would be pure speculation at the moment to suggest the function of the fibres in the cervical sympathetic which are unaffected by hypertension, but two distinct possibilities seem clear, viz., that they could be on the cholinergic pathway, or that they could be concerned with the adrenergic innervation of the smooth muscles of the eye.

Sympathetic fibres which increase their activity during adrenaline hypertension have also been described previously by Millar and Biscoe (1965) and Widdicombe (1966). In both reports these fibres had large action currents. Widdicombe found that his sympathetic group III fibres were activated by adrenaline and also by chemoreceptor stimulation, laryngeal irritation and lung inflation, and the time course of the discharge was unrelated to the time course of the blood pressure change. In addition, bilateral carotid occlusion during adrenaline hypertension did not affect the response of the one fibre tested. Similar behaviour was found in the non sympathetic efferent fibres of the sinus nerve by Biscoe and Sampson (1968). Brody (1966) has suggested that fibres showing increased activity /

activity during hypertension may be connected with histaminergic vasodilatation. The activities of the proposed histaminergic vasodilators has recently been reviewed by Campbell (1970).

In the present experiments, the strands which showed graded responses to changes in arterial pressure could be divided into two groups:

Group I - those strands whose activity was inversely related to arterial pressure from 100 to 200 mm Hg; this type of behaviour occurred predominantly in female or castrate cats.

Group II - those strands whose activity was inversely related to arterial pressure from 100 to 150 mm Hg and stayed constant or increased at pressures above 150 mm Hg; this type of behaviour occurred predominantly in male or testosterone treated castrate cats.

These results show that angiotensin and noradrenaline caused changes in sympathetic discharge which were related to the absolute level of arterial pressure, and when the two drugs were compared, there was little evidence of an excess of sympathetic activity that might be attributed to a central action of the peptide. In fact the two populations of points, representing sympathetic discharge during infusion of angiotensin and noradrenaline infusion respectively, do not differ significantly. Between 100 and 200 mm Hg in Group I and between 100 and 150 mm Hg in Group II, regression studies on the combined /

combined populations of points gave gradients which were statistically highly significant (P less than 0.001), and this indicates that the probability of angiotensin and noradrenaline having differing effects on sympathetic discharge is quite small.

This failure to find consistent differences in sympathetic discharge when blood pressure is raised to similar levels with noradrenaline or angiotensin was in some respects surprising. In addition to the possible existence of central stimulation by angiotensin, small differences in sympathetic activity might have been expected, since noradrenaline has a positive inotropic action not found with the peptide. This activity makes a considerable difference to baroreceptor discharge, and mean frequency of discharge in single baroreceptor units is higher with noradrenaline than with angiotensin at any mean pressure. However, mean frequency of discharge might not be highly significant, since the results of Kezdi and Geller (1968) suggests that the high frequency components are filtered out by the sympathetic controlling centres. Thus, when the baroreceptor - renal nerve loop is considered, it would seem that the main effects produced by the baroreceptors can be described in terms of a DC component and a phasic component with a bandwidth of 0.1 to 2 Hz. If this is so, the respiratory changes in arterial pressure and the heart rate would be of importance in determining sympathetic discharge, but the higher harmonics of the cardiac frequency would seem to be of less importance, in /

in contrast to their effects on baroreceptor discharge.

Cardiac frequencies in these experiments were quite fast,usually above 110/minute, and probably attributable to the predominant use of pentobarbitone anaesthesia, and the lack of cardiac rhythms was probably related to the fact that the heart rate was usually around the limiting frequency of the baroreceptor - sympathetic nerve loop found by Kezdi and Geller (1968). The behaviour of the heart rate was not controlled,and in dogs, noradrenaline hypertension was usually associated with a bradycardia not seen with angiotensin. In cats this difference was rarely seen.

The decrease in sympathetic activity during hypertension in these experiments was due to the increased baroreceptor discharge. There was little evidence following baroreceptor denervation of either decreased or increased sympathetic activity during infusion of noradrenaline or angiotensin. The result with noradrenaline confirms earlier observations by Iggo and Vogt (1962). The main inhibitory influence on sympathetic discharge is removed in these experiments and the results support the conclusion already reached that an increase in preganglionic sympathetic activity does not accompany the infusion of angiotensin. Thus the behaviour of sympathetic discharge during infusion of these drugs seems to be determined reflexely, and central actions of the drugs or changes in cerebral blood flow do not seem to be involved. The results using this range of doses of the peptide therefore /

therefore do not support the hypothesis that central sympathetic stimulation contributes to the pressor response of angiotensin, as suggested by Scroop and Whelan (1966). They however do not give any information on the possible effects of intravertebral angiotensin, and this will be discussed later.

The central actions proposed by Scroop and Whelan (1966) only affected the skin circulation, and one possible explanation of their results involves the peripheral interaction of angiotensin with the sympathetic nerve endings, by blockade of noradrenaline reuptake. The effect of blockade of the reuptake process might be expected to depend on the amount of noradrenaline released from nerve endings. Thus, when the postganglionic neurones are destroyed or conduction is stopped by local anaesthetic, or when the release of noradrenaline is blocked by bretylium, or its action on the vascular muscle interfered with by phenoxybenzamine, one might expect that any action of angiotensin attributable to blockade of the noradrenaline reuptake process would be less effective in causing a reduction in blood flow. Apart from the last instance, this would be due to less accumulation of noradrenaline in the tissues, and in the case of phenoxybenzamine, the action of any noradrenaline present would be blocked anyway. One possible reason for the difference between the hand and the forearm in the experiments of Scroop et al (1965) and Scroop and Whelan (1966) could be differences in the behaviour of discharge in the sympathetic nerves /

nerves to skin and muscle or to differences in the metabolism of the transmitter in these tissues. During intravenous infusions of angiotensin or noradrenaline, the blood pressure is raised, so inhibition of sympathetic discharge to muscle is to be expected (Barcroft 1963), whereas the innervation of the hand is said to be little affected by the baroreceptor reflex (Greenfield 1963). The effects of a reuptake-blocking action of angiotensin during intravenous infusion of the drug might be expected to be greater in tissues showing less inhibition of sympathetic discharge, namely the skin in this case. A possible difference in the metabolism of the transmitter in these tissues is suggested by the results of Zimmerman (1967) who found that noradrenaline output in the venous effluent from the dog paw was increased by angiotensin or by imipramine (a potent antagonist of the reuptake process) and that the effect was potentiated by sympathetic nerve stimulation. No such increase occurred in the effluent from the dog gracilis muscle.

The results described here were recordings from nerve fibres that were predominantly preganglionic, so their discharge reflects the activity of the central control mechanisms, unaffected by peripheral integration in sympathetic ganglia. In the postganglionic fibres of the inferior cardiac nerve, angiotensin is known to decrease activity, although its action was not compared directly with any other procedure causing hypertension (Downing and Siegal 1963; Green and Heffron 1968). /

1968).

Aars and Akre (1968) studied the effects of intravenous infusions of noradrenaline and angiotensin on the integrated activity of intact renal and cervical sympathetic nerves in the rabbit. In the cervical sympathetic, no differences were found between the drugs, but in the renal nerves they found a short lived difference which was interpreted as being due to a transient central action of the peptide. It is not possible to assess the fibres which showed a transient increase in activity with high doses of angiotensin were the same fibres which showed profound inhibition during the initial rise in arterial pressure. As already stated, the sympathetic is a rather heterogeneous collection of fibres, and fibres increasing their activity with adrenaline are known. In some of their published records, Aars and Akre (1968) raised arterial pressure higher with angiotensin than with noradrenaline, and whether this could go some way towards explaining the difference between the two drugs is not known.

The main direct evidence for central actions of angiotensin has been produced by Cranston's group in London. In the dog and the rabbit, they have found that intravertebral infusion of small doses of angiotensin caused hypertension and tachycardia. In the dog, Lowe and Scroop (1968) found that these changes were largely mediated by release of vagal tone, which indicates that these results would not explain the findings of Scroop and Whelan (1966) in the human hand.

The /

The effects of intravertebral infusions of angiotensin (Lowe and Scroop 1966; Rosendorff et al 1970) are similar to the effects of cerebral ischaemia (Downing et al 1963). One of the possibilities is that angiotensin causes vasoconstriction in the brainstem areas affected by blood gas tensions, and that in the absence of a rise in perfusion pressure, a state of stagnant hypoxia is produced. Angiotensin is known to produce vasoconstriction of the retinal vessels in man, (Dollery, Hill and Hodge 1963), but this may have been due to autoregulation in response to increased arterial pressures. Even if this explanation is true for the moderate infusion rates used, it does not follow that further vasoconstriction cannot be superimposed on the autoregulation when large doses are involved, as has been the case in many of the experiments on central actions of the peptide.

In the present study, infusions of angiotensin or noradrenaline were given intravenously or intravertebrally at 36 - 284 ng / min and 0.143 - 1.14 micrograms / min respectively in two experiments. There was no consistent difference between the effects on blood pressure of intravenous or intravertebral infusions of the drugs. In Figure 6 the peptide were given intravenously and intravertebrally at 142 and 284 ng/min., and there was little difference between the two routes of administration in blood pressure or in sympathetic discharge. In no case was sympathetic discharge increased above the resting level during infusions of the drugs.

The /

The intravenous infusions used in this work and in that of Aars and Akre (1968) avoid the difficulties associated with possible cerebral ischaemia, and the methods used allow a direct estimate of the activity in sympathetic nerve fibres. One difference however is that transient changes in sympathetic activity were not followed in the present work, since the blood pressure was allowed to come to a steady state before the filmed records were made. A more important difference is that spikes in single fibres or a few fibres were counted in these experiments, and this allowed the activities of these nerve fibres to be followed in more detail than the complex integrated activities from intact sympathetic nerves, as used by Aars and Akre (1968).

The conclusion from the present work is that increased sympathetic activity does not play a part in the pressor response to intravenous infusions of angiotensin.

The most surprising result of these experiments was the unexpected sex difference in the behaviour of discharge in the cervical sympathetic fibres when vasoconstrictors were infused. The results of section 1 indicate that the rise in sympathetic discharge at pressures above 150 mm Hg is related to the presence of endogenous or exogenous testosterone and that this can be effectively antagonised by a large dose of oestrogens. The rise in sympathetic discharge occurred in the same fibres that showed a decrease in activity at lower /

lower pressures. This was confirmed in the single fibre studies, and care was always taken in the analysis of multifibre records to be sure that the rise in activity had not occurred in previously inactive fibres. It would seem most unlikely that fibres such as those of Widdicombe's group III were responsible for the increased activity at high pressures, since the discharge in such fibres is not closely related to blood pressure, as was the case with the activity described, and the group III fibres also all had large action currents that could not have been easily overlooked. There are no previous reports of a sex difference in the response of sympathetic discharge to pressor drugs. Few investigators however have studied quantitatively the responses of single units or few fibre preparations of sympathetic nerves. Iggo and Vogt (1962) compared the effects of different drugs on sympathetic discharge in 6 cats. They used bolus injections rather than infusions in the greater part of their work, so the samples of sympathetic activity at any blood pressure level were usually smaller than the present ones, and their results indicate that the relationship between impulse frequency and blood pressure was not always linear. In their Figure 3 for instance, a rise in pressure of 50 mm Hg caused greater inhibition of sympathetic activity than a rise of 100 mm Hg, when adrenaline was investigated. This response is similar to the response of the male and testosterone treated castrate cats in the present work. Aars and Arke (1969) found /

found a linear relation between sympathetic activity and blood pressure during noradrenaline infusion in rabbits. It is not clear whether single units were studied however, and sympathetic discharge was completely abolished around 120 mm Hg. A similar linear relation between sympathetic discharge and blood pressure during haemorrhage in rabbits was also found by Millar and Biscoe (1965). The evidence presented suggests that testosterone is somehow involved in the difference between the two types of response and that this hormone is responsible for the sex difference described. A small preliminery examination of the behaviour of baroreceptor units during vasoconstrictor infusion did not reveal any change in the spike frequency - blood pressure relationship that might have been responsible for the sex difference described. It appeared therefore that the absence of an inverse relation between sympathetic discharge frequency and blood pressure above 150 mm Hg in males occurred in spite of increasing baroreceptor discharge. The suggestion is made that the arterial chemoreceptors are involved in the sex difference in behavour of sympathetic discharge. In section 2 of the results, evidence is presented that the increased sympathetic activity occurring at pressures above 150 mm Hg in males is related to chemoreceptor stimulation by the vasoconstrictors. The evidence for the presence of a chemoreceptor influence at these pressures in males is as follows:

1. Following baroreceptor and chemoreceptor denervation in male cats, /

cats, the infusion of high doses of vasoconstrictor generally caused neither an increase nor a decrease in sympathetic discharge, and this suggested that the increase in activity arose reflexly from the sino-aortic areas.

2. The effect of breathing oxygen instead of air was to prevent the increased activity in the sympathetic and chemoreceptor nerves during infusion of high doses of vasoconstrictors. The oxygen could have been preventing a possible initial hypoxia, but there is no evidence that there was any sex difference in initial pO_2 or in the effects of noradrenaline on it. Alternatively, the effect of high pO_2 during ventilation with oxygen might have been related to inhibition of activity in some other chemoreceptors, other than the peripheral ones, which were also sensitive to changes in vascular resistance.

3. The effects of noradrenaline in increasing sympathetic activity are eliminated by cutting the vagi and depressor nerves, and at the same time producing a localised alpha receptor blockade of the carotid bodies by phenoxybenzamine or phentolamine. Following these procedures, angiotensin still caused an increase in sympathetic activity above 150 mm Hg. This experiment points to the importance of vasoconstriction in the arterial chemoreceptors. However it is unlikely that the alpha blocking agent was confined solely to the arterial chemoreceptors, and could conceivably have acted via some other site. This /

This is thought unlikely since the systemic circulation still responded satisfactorily to noradrenaline following the local administration of blocking agents.

4. The response of chemoreceptor discharge to infusion of the vasoconstrictors was an increased discharge which began at pressures less than 150 mm Hg, and the incidence of this was much less common in females (Figure 14).

The actions of noradrenaline are thought to be mediated by the blood vessels to the chemoreceptors, and there is little reason to suggest that angiotensin should act in any other manner in this tissue. Joels and White (1968) have previously investigated the effects of catecholamines on respiration and carotid chemoreceptor activity. In order to get a respiratory response they sometimes, but not always had to use higher doses of noradrenaline than used in the present experiments, and an arterial pressure compensator was also used which minimised the rise in perfusion pressure, and presumeably also the increase in total carotid body blood flow. It was not clear whether they had sexed their animals, and it could be that the variations in sensitivity of the chemoreceptors in their experiments was related to sex.

Lee, Mayou and Torrance (1964) found that chemoreceptor discharge during noradrenaline hypertension was always greater than that found when blood pressure was raised to the same pressure by mechanical means. /

means. In considering the absolute level of discharge however they found that noradrenaline sometimes decreased it, while on other occasions it could increase it or leave it unaffected. They thought that this was related to the balance between chemoreceptor vasoconstriction and the rise in perfusion pressure by affecting chemoreceptor blood flow. Mills (1968) similarly found that when the stellate ganglion was stimulated, the ipsilateral aortic chemoreceptors increased their activity, but on the opposite side a fall in chemoreceptor discharge occurred as blood pressure increased during the sympathetic stimulation. In these experiments, stellate ganglion stimulation could activate fibres going to the aortic and carotid chemoreceptors since the cervical sympathetic was not cut, and although the ipsilateral depressor nerve was divided, reflex respiratory stimulation and increase in oxygen tension could have been induced through the carotid body.

There is some doubt as to the cause of the decreased chemoreceptor activity reported during hypertension by Lee et al (1964) and in the aortic body contralateral to the stellate ganglion stimulated by Mills (1968), and also in the work presented here. It was considered by Neil and Joels (1963), Lee, Mayou and Torrance (1964) and Mills (1968) that this effect was due to increased blood flow through the chemoreceptor sinusoids, but the recent results of Biscoe, Bradley and Purves (1969) suggest that this might not be true. They found that carotid chemoreceptor discharge was independent of blood pressure, and total /

total carotid body blood flow, over a wide range of both of these parameters. Throughout these experiments, blood gas tensions were monitored and controlled, and one possibility is that these were not adequately controlled in previous work. If the carbon dioxide tension was low in the earlier experiments it is possible that autoregulation failed to occur, since this gas seems to be necessary for autoregulation to occur (McCloskey 1968).

No information is available about pCO_2 in these experiments, but measurements of arterial pO_2 were made on 5 animals. Elevation of blood pressure with noradrenaline might have two effects on ventilation and therefore on pO_2. Firstly, hypertension per se inhibits ventilation by activation of baroreceptor afferents (Heymans and Neil 1958), and secondly chemoreceptor stimulation which might be induced by the catecholamine could increase ventilation. The effects of noradrenaline on arterial, pO_2 will depend on both these effects and probably also on the initial level of pO_2. In the present work, resting pO_2 in four of the cats was 85 - 91 mm Hg and 76 in the other. Noradrenaline hypertension caused little change in arterial oxygen tension except in the one cat with a low initial pO_2, in which the oxygen tension increased to the level found in the other animals.

If the action of noradrenaline on the chemoreceptors is mediated by their blood vessels, it is not surprising that the catecholamine had different effects in the two sexes. As mentioned earlier, sex differences/

differences in the vascular responses of various vaso-active agents are well known, and in cats the evidence suggests that testosterone is responsible for the increased vascular sensitivity of males to the catecholamines. The results in section 1 show the association between the presence of endogenous or exogenous testosterone and the increased sympathetic discharge at high blood pressures when noradrenaline or angiotensin are infused. Reasons were given earlier for believing that this reflex response arose in the chemoreceptors, and one explanation of the sex difference is that in the presence of testosterone the glomeral vessels are more sensitive to the pressor agents. The results of section 2A (b) show that alpha receptor sites are necessary for the male type of response, and this probably means that vaso constriction by the catecholamine is an intrinsic part of the mechanism.

It was found in these experiments that treatment of castrate cats with testosterone propionate for 1 - 3 days was sufficient to induce the response characteristic of male cats. Bhargava et al (1967) however found that the vascular response to noradrenaline was depressed at 24 hours and was maximal 7 days after the injection of testosterone.

A metabolic action of testosterone on the carotid body has also to be considered. The hormone might bring about changes in metabolism which would lower pO_2 locally, provided the blood flow were kept constant. An interesting parallel series of results exists in the action /

action of testosterone on erythropoietin production by the kidneys. Erythropoietin is released from the kidney during hypoxia, apparently by a direct action on renal tissue (Fisher and Langston 1968). It is also released when renal blood flow is decreased by intra-arterial infusion of angiotensin or noradrenaline, and these are thought to be acting by lowering renal pO_2 levels (Fisher, Samuels and Langston 1968). Testosterone itself can increase erythropoietin production (Fried and Gurney 1968), but this may be related to the renotrophic action of testosterone. The erythropoietic response to hypoxia is increased in the presence of testosterone, (Mirand, Gordon and Wenig 1965; Naets and Wittek 1968) but no results are available as far as is known, on the effect of testosterone on the erythropoietic response to noradrenaline and angiotensin. It could be that the metabolic actions of testosterone are involved in this response, and also in the phenomenon described here.

The work presented reinforces earlier evidence that the sex hormones have effects on the vascular system in amounts present in physiological conditions. This study suggests that the presence of testosterone in male cats plays an important part in determining the behaviour of sympathetic discharge when blood pressure is raised with noradrenaline or angiotensin. The precise mode of action of testosterone is obscure, but evidence is presented that the characteristic behavour of sympathetic discharge in male cats may be altered by interfering /

interfering with the vasoconstrictor effect of noradrenaline in the arterial chemoreceptors, or by inhibiting chemoreceptor activity with oxygen. The experiments cast no light on whether testosterone is acting between the alpha site and the contraction mechanism, or on the density of alpha receptors in vascular muscle, or on some site remote from the vascular bed, such as the metabolic activity of the glomus cells.

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