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Effects of angiotensin and noradrenaline on discharge in fibres of the cervical sympathetic nerve in cats and dogs

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Summary

1. The effects of angiotensin and noradrenaline on discharge frequency in single and few-fibre preparations of the cervical sympathetic nerve of cats and dogs have been compared.

2. When equal changes in blood pressure were produced by intravenous infusion of these drugs, the changes found in sympathetic discharge were similar both in anaesthetized animals and in a decerebrate one.

3. Angiotensin and noradrenaline also have similar effects on discharge in sympathetic nerve fibres following complete denervation of the baroreceptor and chemoreceptor nerves.

4. It is concluded that when the blood pressure is raised to 200 mmHg by angiotensin, the drug has no central stimulant action on the sympathetic nervous system.

Introduction

There are several reports suggesting that angiotensin stimulates central sympathetic neurones when large doses are administered. Bickerton & Buckley (1961) and Buckley, Bickerton, Halliday & Kato (1963) found that when the vascularly isolated head of one dog was perfused from a donor dog, injection of up to 4 μg/kg angiotensin into the donor resulted in a rise in arterial pressure in both animals. The pressor response in the recipient was abolished by cervical cord section. Laverty (1963) found that infusion of angiotensin into the systemic circulation of rats caused vasoconstriction in the isolated vasculature of the hind limb, and that this response vanished after denervation of the limb. Smookler, Severs, Kinnard & Buckley (1966) and Severs, Daniels & Buckley (1967) performed experiments in cats in which large amounts of angiotensin were injected into the cerebroventricular system. This caused hypertension whereas injection of saline did not ; the angiotensin-induced hypertension was abolished by cord section or sympathetic blockade.

It has recently been reported that low rates of infusion of angiotensin into the vertebral arteries of rabbits and greyhounds can bring about a nervously mediated hypertension (Lowe & Scroop, 1968 ; Rosendorff, Lowe, Laverty & Cranston, 1970). The aim of our experiments was to investigate whether an increase in sympathetic activity contributes to the increase in blood pressure produced by moderate doses

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of angiotensin given intravenously. The effects of angiotensin have been compared with those of noradrenaline, as the nervously mediated responses were said to be specific for angiotensin and not obtainable with the catecholamine.

Methods

The experiments were performed on cats and dogs. The cats, weighing 2·25–3·75 kg were anaesthetized intraperitoneally with sodium pentobarbitone (Nembutal, Abbott) in doses of 40 mg/kg, sometimes after induction with ethyl chloride. Occasionally chloralose (80 mg/kg) was used as anaesthetic agent. The dogs, weighing 7–18 kg were anaesthetized intravenously with sodium pentobarbitone (26·5 mg/kg) without premedication. Drugs were infused through a polythene cannula into the left saphenous vein and femoral arterial pressure was recorded on a Devices single channel recorder, by means of a pressure transducer (Bell & Howell Ltd.). In some experiments intraoesophageal pressure was recorded using a Greer micromanometer (Mercury Electronics) and either this or blood pressure was displayed on the second beam of the oscilloscope. In cats, but not in dogs, a tracheal cannula was inserted.

In dogs the cervical sympathetic nerve was found inside the vago-sympathetic trunk, and usually had 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 dissecting plate and its nerve sheath opened under paraffin oil with a fine sharp knife made from a broken off piece of razor blade. Dissection was continued using the method of Iggo & Vogt (1960) until strands of nerve were obtained containing one or a few active fibres. 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 II asp-β-amide (Hypertensin, Ciba), noradrenaline acid tartrate (Levophed, Bayer) and oestriol di-hemisuccinate (Organon). These were diluted with 0·9% saline and infused at a 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 mmHg to 180–200 mmHg without causing cardiac arrhythmias. In cats the dose range of angiotensin was (36 to 572 ng/min)/cat. In some cats, as little as 143 ng/min was sufficient to raise blood pressure to about 200 mmHg (1 mmHg$\equiv$1·333 mbar). For noradrenaline the range was (0·143–2·3 μg/min)/cat. In dogs the amounts of angiotensin and noradrenaline used were 10 times those used in cats. Rectal temperature was maintained between 35° and 37° C by means of a thermostatically controlled electric blanket (Electrophysiological Instruments Ltd.).

Experimental routine was as follows: discharge in one or a few fibres of the cervical sympathetic nerve was recorded for a minimum of 24 s at any blood pressure, and often the period was 30 or 40 seconds. Such recordings were made at a series of pressures, the infusion rate of the drug being doubled between each recording. After the highest blood pressure had been reached, a further series of recordings was made as the pressure was lowered in steps. A further record of sympathetic discharge was again obtained when the pressure had settled at resting level and compared with the initial record.

Recordings of nerve activity were made only during periods when blood pressure was steady. Situations in which extrasystoles and other cardiac arrhythmias occurred were avoided, since these can bring about an increase in the sympathetic discharge (Iggo & Vogt, 1960 ; Green & Heffron, 1968). Extrasystoles were not uncommon at very high pressures, and care was taken to disregard records in which they occurred. When supplementary anaesthetic was required during the investigation of a strand of fibres, it was given and the investigation was begun afresh. Photographic recordings were analysed in consecutive periods of 8 s and these readings were then subjected to statistical analysis. The regression coefficient and its significance were calculated. For the purpose of constructing graphs, the discharge has been averaged over three or four such 8 s periods, so that each point represents an average over 24 or 32 seconds.

Results

Animals with intact baroceptors

Observations were made during intravenous infusions of noradrenaline and angiotensin and during intravertebral artery infusions. With both routes of infusion the results fell into two groups. In one (A) there was an inverse linear relationship over the entire range of pressures from 100 to 200 mmHg and this occurred chiefly in female cats and dogs, in castrates of both sexes and in males given large doses of oestrogens. In the other group (B) there was an inverse linear relationship between 100 and approximately 150 mmHg and above that a constant or increasing rate of discharge ; this was found predominantly in male cats and dogs and in testosterone treated castrated cats. The two types of response are thought to relate to the available concentration of testosterone. This substance affects the sensitivity of vascular muscle and there is the possibility that it can affect sympathetic activity reflexly, by modifying the responses of the chemoreceptor blood vessels to pressor drugs. This subject is discussed elsewhere (Morrison & Pickford, 1969, 1970). Despite the two patterns of response of the nerve fibres it will be seen below that, with only two exceptions, noradrenaline and angiotensin did not differentiate between them and exerted identical effects.

Effects of intravenous infusions of angiotensin and noradrenaline

The experimental material consisted of seven female and nine male cats and nine dogs of which three were female. Two of the male cats were treated with 2 mg stilboestrol intramuscularly 24 h before observation and a further two were given 2 mg oestriol dihemisuccinate (ODHS) intravenously at the start of the experiment. Two of the male dogs received 5 mg ODHS intravenously at the beginning of the experiment. One of the female cats was decerebrated under ether and in one of the male cats both vagi were divided in the neck.

A. Preparations showing a linear inverse relationship between blood pressure and spike frequency over the range 100 to approximately 200 mmHg. Twenty-nine satisfactory preparations giving responses of the type shown in Fig. 1 were obtained. Sixteen of the preparations were from female cats and dogs, three from two normal male cats, two from a male dog and eight from the oestrogen treated male cats and dogs. All nerve strands were tested with both drugs. Figure 1 shows that equipressor doses of noradrenaline and angiotensin had similar effects on sympathetic

discharge and, for purposes of statistical analysis, a null hypothesis was adopted that their effects were the same. Regression analysis of all results obtained from one nerve strand, including its resting discharge and blood pressure, showed that the points did not significantly deviate from a straight line ($P<0{\cdot}001$). The decerebrated and, therefore, unanaesthetized female reacted similarly to the others. Thus, from these preparations no evidence was provided that angiotensin stimulated central neurones.

B. Preparations in which the relationship of spike frequency to blood pressure showed an inflexion at pressures around 150 mmHg. Eighteen preparations fell into this group, of which fifteen were from normal male cats and dogs, two were from females and one from an oestrogen treated male cat. All preparations behaved like those in group A up to approximately 150 mmHg mean pressure and thereafter showed an upward gradient or a straight line (Fig. 2). In the pressure range below 150 mmHg the points obtained with angiotensin and noradrenaline again gave regression coefficients that were significant at the 0·1% level. In the higher blood pressure range both angiotensin and noradrenaline were equally effective in bringing about a change in the slope of the line. The vagotomized male behaved like the normal animals. Thus the increase in sympathetic activity at the higher pressures was not specifically evoked by angiotensin but was related rather to the magnitude

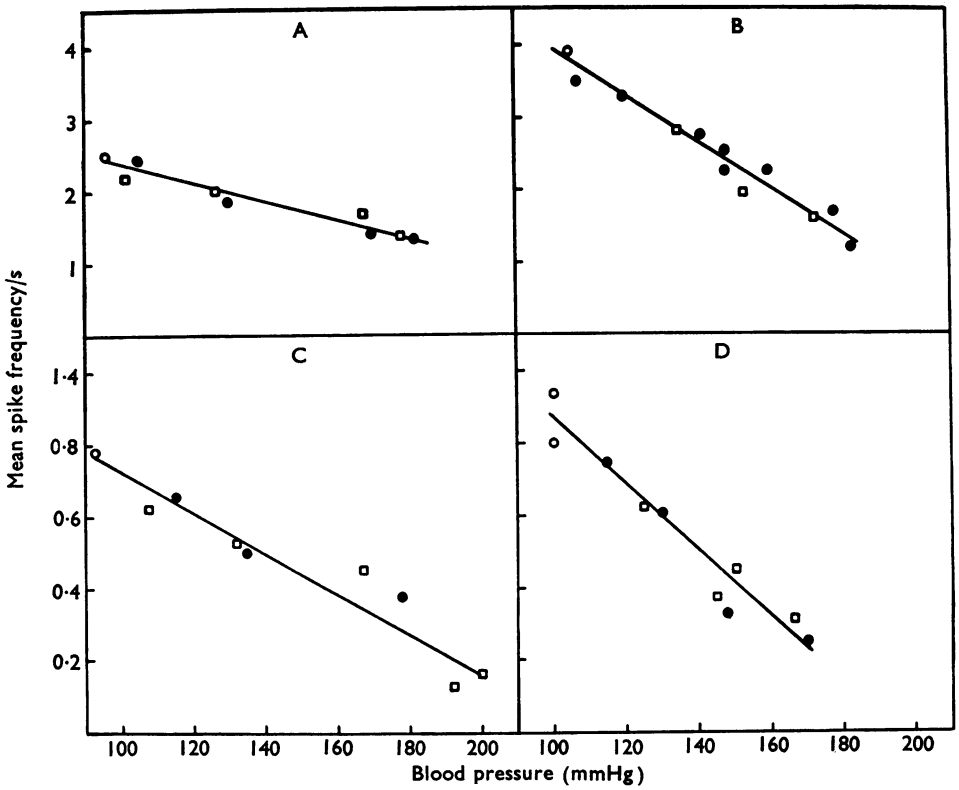

FIG. 1. Effects of intravenous angiotensin, (□) and noradrenaline (●) on sympathetic discharge. A and B, normal female cat; C, oestrogen treated male cat; D, castrated male cat. ○, Resting blood pressure and sympathetic discharge. Ordinate, mean spike frequency/s; abscissa, mean blood pressure (mmHg).

of the pressor effect of the drugs. The only exception was one fibre in a female dog in which noradrenaline decreased the discharge rate linearly over the range 110–163 mmHg, but angiotensin increased the rate as blood pressure was elevated from 148–193 mmHg. It is unfortunate that information was not obtained about the effect on this fibre of higher doses of noradrenaline. In this group this was the only strand in which the effects of angiotensin and noradrenaline differed.

Effects of intra-vertebral artery infusions of angiotensin and noradrenaline

Fourteen nerve strands from three cats were studied. Seven strands were from a normal male cat and seven from a female and a castrated male. The effects of angiotensin and noradrenaline were similar. The relationship between spike frequency and blood pressure in the female and the castrated male were entirely linear. In the normal male both drugs induced a change of gradient at about 150 mmHg, exactly as was found with intravenous infusions. The angiotensin was infused in the same dose both intravenously and intravertebrally and had similar effects on blood pressure and sympathetic discharge by both routes of administration. Figure 3 shows the relationship between spike frequency and blood pressure in one unit from the castrated male and that activity was silenced by raising the pressure to about 160 mmHg by angiotensin. Iggo & Vogt (1962) have previously described

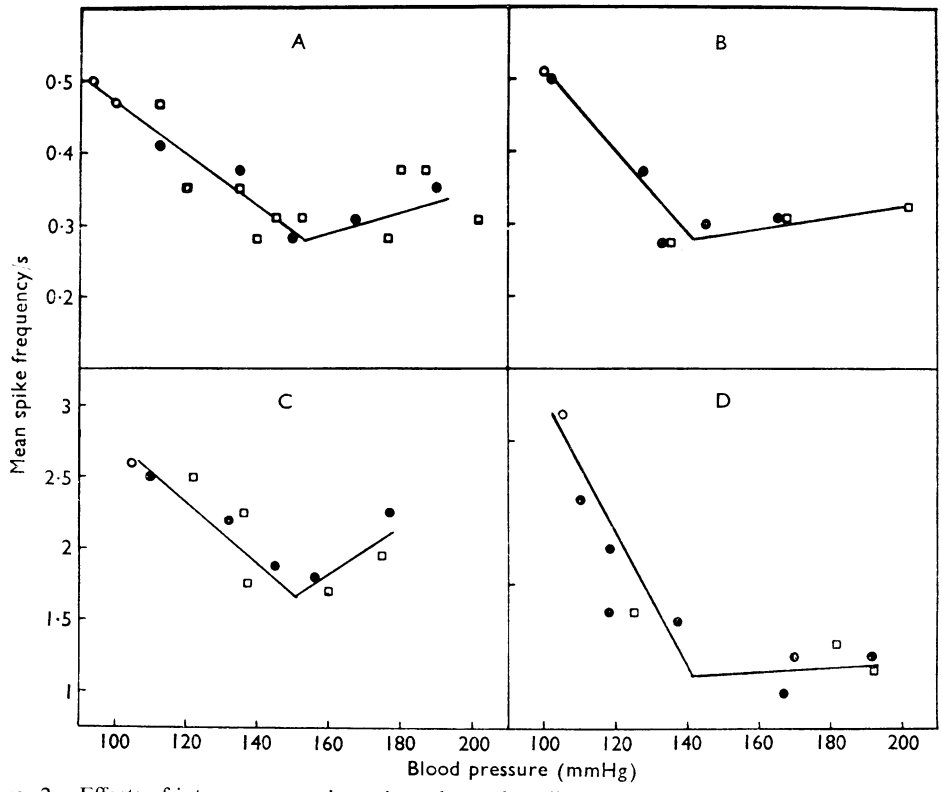

FIG. 2. Effects of intravenous angiotensin and noradrenaline on discharge in strands of the cervical sympathetic nerve in four male cats. □, Angiotensin; ●, noradrenaline, ○, resting blood pressure and sympathetic discharge. Ordinate, mean spike frequency/s; abscissa, mean blood pressure (mmHg).

this type of behaviour in sympathetic fibres. Thus, again, both drugs affected alike the types of response seen under A and B and there was no suggestion of a central action of angiotensin.

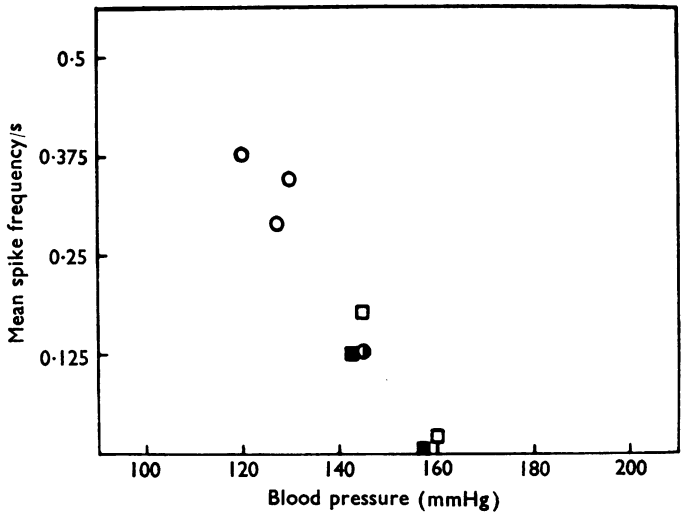

FIG. 3. Effects of intravertebral artery and intravenous infusions of angiotensin and noradrenaline on sympathetic discharge in one nerve strand from a castrated male cat. □, Intravenous angiotensin; ■, intravertebral artery angiotensin; ○, resting blood pressure and sympathetic discharge; ◑, intravertebral artery noradrenaline. Ordinate, mean spike frequency/s; abscissa, mean blood pressure (mmHg).

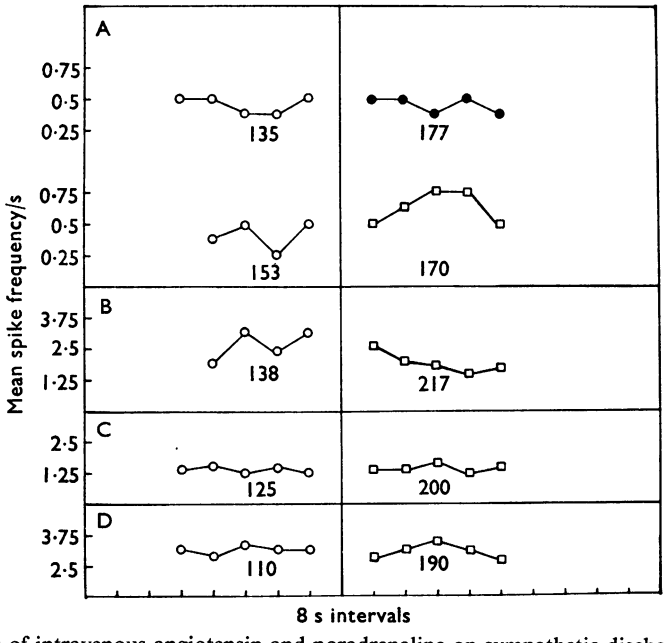

FIG. 4. Effects of intravenous angiotensin and noradrenaline on sympathetic discharge in cats with denervated baroreceptors. □, Angiotensin; ●, noradrenaline; ○, resting blood pressure and sympathetic discharge. Ordinate, mean spike frequency/s; abscissa, 8 s intervals. The figures below each set of readings indicate the blood pressure in mmHg at the time the recordings were made.

Animals with denervated baroreceptors

The operative procedure in these animals included section of the vagus, depressor and carotid sinus nerves as well as stripping the adventitia of the carotid arteries and denervation of the common baroreceptor region. These animals no longer showed a rise in blood pressure when the carotid arteries were occluded low down in the neck. Nineteen preparations were examined from four cats and two dogs. There were four types of response and in all cases except one the response to angiotensin and noradrenaline were indistinguishable on any one fibre. Eleven of the nineteen preparations did not change their rate of discharge when one or other of the drugs was infused and examples are shown in Fig. 4 (C and D). In four preparations the discharge rate decreased slightly as the pressure was raised (Fig. 4B). In three fibres both drugs caused an increase in discharge rate. The results from the remaining preparation were the only ones in this group which suggested a possible difference in the effect of the two drugs on discharge rate; noradrenaline did not alter the rate (Fig. 4A, top curve on right) but angiotensin produced a transient increase (Fig. 4A, second curve from top, on right).

Discussion

The cervical sympathetic is not a homogeneous group of fibres, since it supplies efferents to a number of functionally distinct end organs, only some of which are tonically activated, such as the blood vessels and the smooth muscles in and around the eye (Langley, 1900). Polosa (1968) has recently shown that only about 20% of fibres in the cervical sympathetic are spontaneously active in anaesthetized cats; it would seem likely that these fibres supply structures such as the blood vessels which show paralytic effects when the nerve is sectioned. The erector pili muscles are not subject to continuous influence from the cervical sympathetic (Langley, 1900) and are not likely to be innervated by fibres showing a spontaneous level of discharge. Most fibres in the cervical sympathetic are inhibited by an acute rise in arterial pressure (Adrian, Bronk & Phillips, 1932). Our work is concerned with those fibres which showed spontaneous activity and which, from the characteristics of their rhythm, in all probability innervated vascular muscle. The fact that these fibres showed two patterns of response to a rise in blood pressure is irrelevant in the present context. The only important point to consider is how the fibres, whatever their pattern of discharge, behaved towards infusions of noradrenaline and angiotensin.

Much of the early evidence for a central sympathetic action of angiotensin came from experiments in which large doses of the peptide were used in cross-perfusion experiments (Bickerton & Buckley, 1961), in isolated innervated hind limb preparations (Laverty, 1963) and by direct injection into the cerebroventricular system (Smookler *et al.*, 1966; Severs *et al.*, 1967). On the other hand, some workers found that intracisternal injections of angiotensin caused a fall in blood pressure (Bianchi, DeSchaepdryver, Devleeschouwer & Preziosi, 1960). Again, Zimmerman (1967) could find no consistent vasoconstrictor response in hind limb or kidney vessels which could be attributed to a central action of angiotensin. It is questionable whether the injection of large amounts of the drug, particularly when given directly into the cerebral ventricles, is relevant to the problem of whether the quantities normally required to raise blood pressure are also capable of central sympathetic stimulation.

Scroop & Whelan (1966) believed that intravenous administration of low doses of angiotensin could exert a central effect. They found that intravenous infusions decreased hand blood flow in conscious subjects and that this failed to occur following a variety of forms of interference with the sympathetic supply to the part. It was thought unlikely that the action was peripheral and the authors suggested that angiotensin had a central effect. On the other hand, Scroop, Walsh & Whelan (1965) using identical techniques had previously found no evidence for a central stimulant action of angiotensin when forearm blood flow was measured. Thus, if angiotensin has a central action it does not manifest itself on muscle blood vessels. The opposing findings on hand and forearm vessels may depend on peripheral events and be explained by differences between skin and muscle metabolism of the catecholamines (Zimmerman & Whitmore, 1967).

Other workers have used small amounts of angiotensin given by infusion into the vertebral artery of rabbits which had previously had the opposite vertebral and the homolateral internal and external carotid arteries tied (Rosendorff *et al.*, 1970). The infusions led to an increase in blood pressure which was thought to be due to sympathetic stimulation in the central nervous system. In greyhounds, however, the pressor response to intravertebral artery infusion of angiotensin appeared to be mediated largely through a vagally induced increase in cardiac output (Lowe & Scroop, 1968). Using this route of injection may not be informative since it is not known whether angiotensin constricts some or many of the cerebral blood vessels and could, for this reason, induce secondary rather than specific neuronal effects.

Not many workers have made direct observations of the activity in sympathetic nerves when angiotensin is administered. Aars & Akre (1968) studied the effect of angiotensin and noradrenaline on the integrated response of intact cervical sympathetic nerves of rabbits and found no difference between the effects of the drugs. In the renal nerves, however, they found a short lived difference between the actions of the drugs and interpreted this as being due to a transient central effect of angiotensin. Downing & Siegal (1963) and Green & Heffron (1968) recorded from strands of the inferior cardiac nerves and found that impulse frequency fell as blood pressure was raised by a number of agents, including angiotensin. Its action was not compared with that of other drugs.

The results described here are from recordings of nerve fibres that are predominantly preganglionic so that the discharge rate reflects the activity of the central control mechanism unaffected by peripheral integration in the sympathetic ganglia. The design of the experiments required averaging sympathetic discharge over 30–40 s at constant pressure. This made it impossible to say whether there were any transient stimulatory effects of angiotensin such as those noted by Aars & Akre (1968) in the renal nerves. Apart from two fibres, no difference was found between the effects of angiotensin and noradrenaline on the spike frequency/blood pressure relationship. This was true with both chloralose and pentobarbitone anaesthesia and in the decerebrate animal. In the two exceptional fibres, one was from a female in which the pressure rise induced by noradrenaline caused a steady decrease in discharge activity, whereas that due to angiotensin increased activity above 148 mmHg. The other fibre was in a preparation in which the baroreceptors were denervated and angiotensin caused a non-maintained increase in spike frequency.

Despite doubts as to the usefulness of the procedure, three animals were given intra-vertebral artery infusions of angiotensin and noradrenaline because other

workers using this route believed they found evidence for a central action of angiotensin (Rosendorff *et al.*, 1970). In our work no difference was seen between the effects of angiotensin and noradrenaline nor any sign that either exerted a central pressure raising effect.

The indirect evidence for a possible central site of action of angiotensin is contradictory. The more direct evidence obtained by other workers recording activity on intact and multifibre preparations of sympathetic nerves does not support the idea of a maintained central stimulant action of this substance. In this work, almost the entire weight of evidence from single and few-fibre preparations, including those from animals with denervated chemo- and baroreceptors, is against any maintained stimulant action of angiotensin when it is used in low and near-physiological concentrations.

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