THE SITE OF CARDIOVASCULAR ACTION OF ANGIOTENSIN II IN THE BRAIN

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SUMMARY

1. The site of action of vertebral artery infusions of angiotensin was studied in the chloralose-anaesthetized greyhound.

2. The cardiovascular response to vertebral artery infusion of angiotensin (0.25–2.0 ng kg⁻¹ min⁻¹) was not reduced by clamping the basilar artery between the pons and the pyramidal decussion. There was no response to infusion of angiotensin through a catheter inserted in a rostral direction into the basilar artery above the clamp. The site of action must therefore lie caudal to the pons.

3. Transection of the spinal cord at the first cervical segment did not abolish the response to vertebral artery infusions, which was still mediated by the vagus nerve and abolished by subsequent vagotomy. The site of action must therefore lie rostral to the cervical cord.

4. Local infusions of angiotensin into the small arteries supplying the medulla produced a response similar to that obtained with vertebral artery infusion of angiotensin.

5. These results indicate that the site responsible for these central effects of angiotensin lies in the medulla.

It is well established that angiotensin has a direct effect on the peripheral resistance, but other sites of action have also been described, including the brain (Bickerton & Buckley, 1961; Severs, Daniels, Smookler, Kinnard & Buckley, 1966; Lowe & Scroop, 1969). Bickerton & Buckley (1961) used a cross-perfusion technique in the dog and demonstrated a pressor response to high concentrations of angiotensin which was mediated by the central nervous system and which could be prevented by previous treatment with piperoxane. Responses to lower concentrations have been demonstrated in the rabbit (Dickinson & Yu 1967, Rosendorff, Lowe, Lavery & Cranston, 1970) and in the dog (Scroop & Lowe 1968). The latter authors showed that infusion of angiotensin (0.06–1.0 ng kg⁻¹ min⁻¹) into the vertebral artery of the greyhound...
consistently caused a rise of blood pressure, heart rate and cardiac output, which was brought about predominantly by the vagus nerve. The same rate of infusion had no significant effect when given intravenously or into the carotid artery.

This paper describes the results of experiments to determine the site of this central action of angiotensin; some of these results have been communicated to the Physiological Society (Joy & Lowe, 1970).

METHODS

Experiments were performed on adult male greyhounds weighing 23–34 kg. They were premedicated with morphine (2 mg/kg) and anaesthetized with chloralose (100–130 mg/kg) intravenously; subsequent anaesthesia was maintained as necessary by pentobarbitone sodium (1.0 mg/kg) and the animals were artificially ventilated throughout. Arterial pressure was recorded from a catheter in the femoral artery connected to a Statham pressure transducer; the heart rate was recorded from the ECG by a Grass cardiotachometer connected to a Model 7 Polygraph.

Vertebral artery infusions were given through a thin polythene cannula inserted into one vertebral artery so that the vessel was not significantly obstructed; the opposite vertebral artery was clamped. Carotid artery infusions were given through a similar cannula inserted into one common carotid artery with its external carotid branches ligated. Basilar artery infusions were given through a nylon cannula tied into and occluding the vessel.

To expose the hind-brain, the trachea and oesophagus were retracted to one side, the basi-occiput was exposed and a rectangle of bone removed with a dental drill. Fig. 1 illustrates the main arterial supply to the brain and cervical cord of the greyhound. Each vertebral artery supplies the hind-brain by a number of different channels but these unite to form the basilar artery at the level of the first cervical segment. There is no significant contribution to the blood supply of the hind-brain from the posterior spinal artery. When the basilar artery is ligated, the blood supply to its rostral part is obtained from the carotid artery because there is no significant anastomosis in parallel with the basilar artery. Local infusions of angiotensin rostrally distal to such a ligature or caudally proximal to it are distributed to different areas of the hind-brain. After such infusions and just before the animal was killed, we infused carbon ink for 1–4 min through the same cannula at the same rate as used for the angiotensin solution. The animals were killed by rapid intravenous injection of 5 ml of saturated potassium chloride solution which stopped the heart within about 10 s. The brain was then removed and preserved in 10% neutral formalin.

In each animal a dose of angiotensin was chosen which caused a substantial response during vertebral artery infusion but which had little or no effect on intravenous infusion. Thereafter, this same dose was used for all infusions in the same animal but it differed between animals, ranging from 8 to 64 ng/min. The results were expressed as the integral of the change of blood pressure or heart rate, measured by planimetry.

Angiotensin was dissolved in physiological saline (0.9% sodium chloride w/v) and infused for 5 min; all infusions were preceded and followed by control infusions of saline. The following drugs were used: angiotensin II (Val^-angiotensin asp-β-amide; Hypertensin CIBA), lignocaine hydrochloride, chloralose (C₆H₁₁O₆Cl₃, B.D.H.), pentobarbitone sodium (Abbott), morphine sulphate (Macarthy's Ltd).
RESULTS

Effects of basilar artery ligature and local infusion

In nine dogs angiotensin was infused into the vertebral artery before and after ligating the basilar artery between the pons and the pyramidal decussation; a sample experiment is shown in Fig. 2 and the averaged results in Fig. 3. Ligation of the basilar artery did not reduce either the blood pressure or heart rate response to vertebral artery infusions. The mean blood pressure response was actually greater after the basilar artery had been ligated, but this can probably be explained in terms of the blood concentration of angiotensin which would have been higher.
when the vertebral blood flow had been reduced. These results suggest that the site of action lies within the area supplied by the vertebral and basilar arteries caudal to the ligature. To verify this the basilar artery was cannulated either rostrally, distal to the ligature or caudally, proximal to it.

In eight dogs, infusion of angiotensin in a rostral direction distal to the ligature had no significant effects, which confirmed that its site of action was not in the area supplied by the basilar artery immediately above the ligature. However, it seemed possible that the site of action might lie further rostrally, where, although normally supplied by the vertebral artery, this part of the brain stem would be supplied by the carotid when the basilar artery had been ligated and local infusion into the latter artery might not reach such an area. This possibility was excluded by infusing angiotensin into the carotid artery in five dogs both before and after the basilar artery had been ligated. It was confirmed that the response in both circumstances was less than the response to intravenous infusion.

In eight dogs angiotensin was infused in a caudal direction proximal to the basilar ligature. In four of these animals the cannula was placed directly in the basilar artery, and in the remaining four into one of its tributaries. In seven of the animals this local infusion reproduced the pattern
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of response to a vertebral artery infusion (the eighth is discussed below). The magnitude of the response was not always the same, sometimes being larger and sometimes smaller than the response to a vertebral artery infusion. This variation seems to be adequately explained in terms of concentration and area of perfusion; the concentration achieved with a fixed rate of infusion into a small vessel would be higher than that achieved during a vertebral artery infusion, but the area of perfusion might include only part of the site of action of angiotensin. Fig. 4 illustrates a typical experiment.

Fig. 3. Pooled data from nine dogs showing the effect of ligation of the basilar artery, on the response of blood pressure (to left) and heart rate (to right) to infusions of angiotensin into the vertebral artery (VERT); intravenously (VEN); the carotid (CAR); and into the basilar artery (BAS). The basilar infusions were administered in a cephalad direction through a catheter inserted rostral to the ligature. Intravertebral angiotensin caused a pressor response and tachycardia which was not reduced by ligating the basilar artery. The bars show one standard error either side of the mean and the mean blood pressure response was significantly greater after ligation of the basilar artery ($P<0.05$).

The approximate area of distribution of the infused angiotensin was ascertained by infusion of carbon ink just before the animal was killed. After removal and fixation the brain was sectioned and the cut surfaces inspected for deposits of carbon ink; Fig. 5 shows the correlation between the areas filled with ink and the presence or absence of a response to angiotensin solution infused at the same rate through the same cannula. These results confirm that the site of action of angiotensin lies caudal to the pons and suggest that it lies within the caudal half of the medulla. They also explained why in one animal infusion directly into the basilar artery caudal to a ligature caused no response; in this animal only the posterior cerebellum had been perfused.

Effect of cord section at the level of $C_1$

To confirm that the site of action of angiotensin lies in the medulla, we studied the effect of cord section. The response to angiotensin in the greyhound is largely mediated by the vagus
nerve (Scroop & Lowe, 1969); therefore if the site of action and its connecting pathways to the vagal nuclei are rostral to the first cervical segment, section of the cord at this level should not abolish the response to angiotensin. Fig. 6 illustrates a typical result of such an experiment. Cord section initially abolished the response to vertebral artery infusion but this returned gradually over the next few hours although not to its previous value. At this stage the response was still mediated by the vagus nerve because it was abolished by subsequent vagotomy.

![Graphs showing blood pressure and heart rate responses](image)

**Fig. 4.** A sample experiment showing the effects of infusion of angiotensin into the basilar artery, which was ligated in the lower medulla, a catheter then being inserted proximal to the ligature, in a caudal direction. The left hand panels show the pressor response and tachycardia produced by infusion of angiotensin (32 ng/min) into the vertebral artery, before (1) and after (3) the ligation. This response is also produced by infusion of the same dose into the basilar artery (4) whereas intravenously it has almost no effect (2).

Fig. 7 illustrates the combined results from seven dogs and we can conclude from these that at least part of the site of action of angiotensin lies above the level of the first cervical segment of the cord. It is not clear why cord section should temporarily abolish the response, but this seems to be a non-specific effect, previously noticed by Scroop & Lowe (1969).

**DISCUSSION**

These results show that the site of central action of blood-borne angiotensin in low concentrations lies in the medulla.
FIG. 5. Correlation between the response to angiotensin and the area of distribution of carbon ink infused through the same catheter at the same rate (sixteen animals). On the left, positive correlations—proximal to a basilar artery ligature; on the right, negative correlations—catheter inserted distal to a basilar artery ligature, except for the lower right hand sample (see text). There was a response to angiotensin in every animal in which the caudal half of the medulla became stained with ink.

FIG. 6. A sample experiment showing the effect of cervical cord section at C1 on the response to intravertebral infusions of angiotensin (left hand panels). After C1 section there is still a tachycardia and pressor response which is mediated by the vagus nerve; the former is reduced and the latter abolished by subsequent vagotomy. The residual pressor effect after vagotomy is adequately explained by recirculation of the infused angiotensin, because intravenous infusion of the same dose has a greater effect (right hand panels).
If the distribution of carbon ink accurately represents the distribution of the preceding infusion of angiotensin our results indicate that the site of action lies within the caudal half of the medulla, but there are sound theoretical reasons for doubting whether carbon ink is a reliable marker. Although its particle size averages 40 μm it flocculates and therefore blocks some of the vessels through which it flows. Once a substantial number of vessels have been blocked the local resistance will have increased and the area of distribution may therefore have changed. However, this factor will always tend to overestimate the normal distribution of an infusate, and it is unlikely to invalidate our main conclusions based on a positive correlation between an effect and the apparent area of perfusion.

![Graph showing effect of cervical cord section at C1 on response to infusions of angiotensin](image)

**Fig. 7.** Pooled data from seven dogs showing the effect of cervical cord section at C1 on the response to infusions of angiotensin. Intravertebral infusion (VERT) causes the usual tachycardia and pressor response before cervical cord section. 30-120 min after cord section, it still causes a response although this is significantly reduced ($P<0.02$ for heart rate and $P<0.05$ for blood pressure). This residual response is still mediated by the vagus nerve, because it is abolished by subsequent vagotomy. Control infusions of angiotensin were given intravenously (VEN) and into the carotid artery (CAR). The carotid and venous blood pressure histograms after C1 section and after vagotomy are the means of values obtained in three animals only.

There are two sites in the medulla which demand particularly close consideration; the cardio-regulatory centres (including the vagal nuclei and reticular formation) and the area postrema. Since the predominant effect of angiotensin is to cause inhibition of vagal tone, it is possible that it may act directly on the vagal nuclei. However, all the other known actions of angiotensin are excitatory rather than inhibitory and its central effects are not mediated by the vagus.
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alone but also by the sympathetic nervous system (Scroop & Lowe, 1969). It therefore seems likely that angiotensin acts at some site other than the vagal nuclei.

The area postrema, which lies within the caudal half of the medulla, is very close to the vagal nuclei and has some interesting properties which are particularly relevant to the central action of a large molecule such as angiotensin. In the area postrema the blood-brain barrier seems deficient (Wislocki & Putnam 1920, 1924) and non-medullated nerve fibres pass from it towards the nucleus of the tractus solitarius (Borison & Brizzee, 1951; Brizzee & Neal, 1954; Gwyn & Wolstencroft, 1968). Its function is obscure although part of it appears to be involved in the vomiting response to cardiac glycosides (Borison & Brizzee, 1951), an action which is mediated by the vagus.

It seems unlikely that angiotensin brings about its central effects via the cerebrospinal fluid, because the blood/CSF barrier is relatively impermeable to polypeptides of comparable size such as vasopressin (Vorherr, Bradbury, Hoghoughi & Kleeman, 1968). In addition, the time relationships are inappropriate to this route of action in that angiotensin infusions have very short-lived after-effects (see Fig. 2), but injections into the CSF have long-lasting effects (Rosendorff et al., 1970). The effects of the latter therefore seem of doubtful relevance to the response to blood-borne angiotensin, and the supra-pontine site of action suggested by Severs, et al. (1966) and Severs, Daniels & Buckley (1967) does not conflict with our results, because they were studying the effect of high concentrations perfused through the cerebral ventricles.

Our experiments suggest that the medulla is the only part of the brain in which blood-borne angiotensin in low concentrations has cardiovascular effects. We chose these concentrations because they permitted a very simple experimental design which minimized the effect of re-circulating angiotensin. It may be that higher concentrations can also affect other sites, but these are unlikely to be important in the cardiovascular response to angiotensin at physiological concentrations in the blood.

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REFERENCES


