CHRONIC HYPERTENSION ELICITED BY INFUSION OF ANGIOTENSIN INTO VERTEBRAL ARTERIES OF UNANAESTHETIZED DOGS

K. FUKIYAMA, J. W. MCCUBBIN AND I. H. PAGE

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio

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SUMMARY

1. Synthetic angiotensin II was infused chronically through catheters implanted into a vertebral artery and an external jugular vein of unanaesthetized dogs. Arterial pressure was recorded daily from an indwelling catheter in the abdominal aorta before, during and after the infusions.

2. Infusion of angiotensin 1 μg kg⁻¹ day⁻¹ into a vertebral artery caused mean arterial pressure to increase by an average of 12 mmHg in five dogs, and the rise was sustained throughout the infusion period of 7 days in all dogs.

3. When the infusion rate of angiotensin was increased to 10 μg kg⁻¹ day⁻¹, arterial pressure during vertebral artery infusion rose initially to a slightly greater extent but decreased at the end of the infusion, indicating tachyphylaxis.

4. During infusion of angiotensin into the vertebral circulation, arterial pressure rose rather than fell during sleep; awakening was accompanied by sharp fall in pressure.

5. It is concluded that there is an area in the central nervous system responsive to angiotensin that can cause a sustained rise in arterial pressure.

Arterial pressure is increased in rabbits (Dickinson & Yu, 1967; Rosendorff, Lowe, Lavery & Cranston, 1970), dogs (Scroop & Lowe, 1969; Ferrario, Dickinson & McCubbin, 1970) and man (Ueda, Uchida, Ueda, Gondaira & Katayama, 1969) when angiotensin is infused into a vertebral artery in amounts that are ineffective when given intravenously. It is not known if this action of angiotensin is involved in the initiation, or maintenance, of renal hypertension. The experiments reported here were done to determine whether or not continuous infusion of small amounts of angiotensin into the vertebral arteries of unanaesthetized dogs could cause a rise in arterial pressure that would be sustained for days.

METHODS

All surgical procedures were done while the dogs (20–27 kg) were anaesthetized with sodium pentobarbital (30 mg/kg i.v.). Indwelling catheters were passed into the abdominal aorta.
through the right iliac artery for recording arterial pressure and into the left jugular vein and
the right vertebral artery for infusions. Catheters for recording arterial pressure were made of
10 cm of Teflon tubing (7F) inserted into 75 cm of Tygon tubing (i.d. 0.16 cm, o.d. 0.32 cm) and
cemented together with Silastic glue; the total volume of the catheters was less than 1.5 ml. The
catheters for infusions (Teflon: i.d. 0.06 cm, o.d. 0.09 cm, length 5 cm) were similarly inserted
into Tygon tubing (i.d. 0.06 cm, o.d. 0.1 cm, length 70 cm) with an internal volume of less than
0.4 ml. The right vertebral artery was exposed through an incision in the supra-clavicular area.
A catheter was then inserted through a small hole made by a 20 gauge needle after occluding
the vessel above and below the point of insertion. A small sheet of Dacron bonded to the

catheter was sutured to the adventitia, then wrapped around the artery and bonded to the

surrounding tissue with biological glue (isobutyl-2-cyanoacrylate monomer, Ethicon). This
procedure prevented bleeding and ensured that the catheter would remain in place for a pro-
longed period of time. The arteries remained patent as indicated by angiography.

The free ends of the catheters were led subcutaneously to emerge at the back of the dog's
neck and were filled with heparin-streptokinase solution (heparin 25 U/ml, streptokinase
200 U/ml) to prevent clotting; the exposed tips of the catheters were occluded with stainless-
steel plugs.

In all experiments the sequence followed was: (1) after cannulation of the right iliac artery,
the dogs were trained to lie quietly on a soft pad while arterial pressure was recorded daily
during 1–2 weeks; (2) the left external jugular vein was cannulated and the left vertebral artery
ligated to promote blood flow to the brain stem by the right vertebral artery; (3) arterial
pressure was then measured daily during infusion of isotonic saline for 3 days; (4) angiotensin
was then infused intravenously for 7–14 days and arterial pressure recorded daily during the
infusion and for 1–3 days thereafter; (5) the right vertebral artery was cannulated and systemic
pressure recorded daily during a control infusion of isotonic saline into the artery for 3 days;
(6) angiotensin was then infused into the vertebral artery for 7–14 days and the arterial pressure
recorded daily during the infusion and for 3 days thereafter. Infusions were made with a small
pump worn in the dog's harness and driven by electrolytically-evolved gas (Sage instruments);
the pump delivered 9–10 ml/24 h. Synthetic angiotensin II (Hypertensin, CIBA) was dissolved in
isotonic saline with 1% Bacitracin added to prevent adsorption of angiotensin on glass; Bacitracin
was also added to the control infusions of saline. For both intravenous and vertebral
artery infusions, the initial dose of angiotensin was 1 μg kg−1 day−1 for 7 days; the rate was
then increased to 10 μg kg−1 day−1 for another 7 days.

For reassurance that the dogs remained in good health, they received regular examinations
by a veterinarian, and rectal temperatures were measured daily. Because of the number of
implanted cannulae and the attendant possibility of infection, antibiotics were given pro-
phylactically in all experiments. On the first post-operative day, the dogs received i.m. injections
of Benzathine penicillin G 1·2 M/U, procaine penicillin 600 000 U and streptomycin 0·5 g;
thereafter they received oral chloramphenicol (0·5 g daily until the end of the experiment).

Arterial pressure was recorded from the indwelling catheter in the iliac artery by using a
Statham P23Db transducer fastened at the dog's heart level by a shoulder harness. Although
the connecting cables were sufficiently long for the dogs to explore the laboratory at will, the
animals soon preferred to lie quietly on the pad placed for them. Measurements were always
made at the same time each day for 30–60 min with the dogs lying in approximately the same
position. Mean arterial pressures obtained by electronic integration were read at ten different
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points at 2 min intervals on each record and averaged; because of the possibility of damping of the pressure pulse due to the length of the implanted catheter, systolic and diastolic pressures are not reported.

Experiments were considered satisfactory in five of ten dogs; the other five dogs were excluded either because a catheter came out of a vessel or because of infection or embolism.

RESULTS

Average pressures of the five dogs during the training period fell, then stabilized, and neither intravenous nor vertebral artery infusions of saline caused further significant change (Table 1).

**Table 1. Response of arterial pressure to control infusions of isotonic saline**

<table>
<thead>
<tr>
<th>Infusion of isotonic saline with 1% bacitracin</th>
<th>Average mean pressure ± SD (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog 1</td>
</tr>
<tr>
<td>Before</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>During, jugular vein</td>
<td>87 ± 1</td>
</tr>
<tr>
<td>During, vertebral artery</td>
<td>85 ± 2</td>
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</tbody>
</table>

**Fig. 1. Increase of mean arterial pressure when angiotensin (1 μg kg⁻¹ day⁻¹) was infused into vertebral arteries (solid line) and jugular veins (broken line).**

**Effect of route of infusion on the pressor response to angiotensin**

Pressor responses caused by the infusion of angiotensin into a vertebral artery at the rate of 1 μg kg⁻¹ day⁻¹ were consistently larger than when it was infused intravenously at the same rate (Figs. 1 and 2; Table 2). During infusion into a vertebral artery, mean arterial pressure was elevated by an average of 12 ± 1.2 (SE) mmHg 1 day after starting the infusion and remained at approximately this level for the durations of the infusions. The same dose of angiotensin infused intravenously did not cause a significant change in pressure the first day after starting the infusion but there was transient increase on the second and third days, the maximum rise
Fig. 2. Changes in arterial pressure before and during infusion of angiotensin. Uppermost panel recorded during control infusion of saline; middle panel, during infusion of angiotensin $1 \mu g \text{ kg}^{-1} \text{ day}^{-1}$; lowermost panel, during infusion of angiotensin $10 \mu g \text{ kg}^{-1} \text{ day}^{-1}$. In each panel the upper tracing is mean pressure, the lower tracing pulsatile pressure. With infusion into the vertebral artery at a rate of $1 \mu g \text{ kg}^{-1} \text{ day}^{-1}$, there was a significant increase in arterial pressure; there was no change during intravenous infusion. On the sixth day of infusion of $10 \mu g \text{ kg}^{-1} \text{ day}^{-1}$ the pressor response was more prominent with intravenous infusion than with infusion into a vertebral artery.
being 5 ± 1.4 (SE) mmHg. Arterial pressure then declined to control levels during the remainder of the infusion period.

When the infusion rate of angiotensin was increased to 10 μg kg⁻¹ day⁻¹, experiments were considered satisfactory in two of the five dogs in which angiotensin was infused into a vertebral artery and in four in which it was infused intravenously. There was again a difference in response depending upon the route of infusion (Fig. 2; Table 3). With infusion into a vertebral artery, arterial pressure was elevated in both dogs 1 day after starting the infusion and remained elevated until the fifth day when it decreased despite continued infusion (Fig. 2); the maximum increases in pressure were 12 ± 1.7 and 24 ± 1.8 mmHg and occurred on the fourth and fifth days respectively. When the same larger dose of angiotensin was infused intravenously...
in four dogs, arterial pressure rose in two dogs on the first day and was elevated in all dogs after 5 days. There was a further increase thereafter, the average elevation being $27 \pm 2$ mmHg at the end of the infusions. The decline in pressure that occurred on the fifth to seventh day of infusion into a vertebral artery did not occur with intravenous infusion; pressure continued to rise until the end of the infusion.

When infusion of angiotensin into a vertebral artery was stopped and saline infused instead, arterial pressure of all dogs returned to control values within 24 h. With intravenous infusion it returned to control values within 24 h in three of five dogs, but in two dogs 48 h or more were required.

**Arterial pressure during sleep**

While the dogs were resting quietly on a soft pad during the recording sessions, they would often fall asleep. Normally, arterial pressure fell or was unchanged during sleep. In contrast, when angiotensin was infused into a vertebral artery, arterial pressure rose during sleep, and awakening was accompanied by sharp fall in pressure (Fig. 3). When the higher dose of angiotensin was infused intravenously, the same effect was observed but it occurred less often and, when present, the rise in pressure during sleep was less prominent (Table 4).

**Tachyphylaxis during vertebral artery infusion of angiotensin**

When angiotensin was infused into the vertebral artery at the higher rate of $10 \mu g \text{ kg}^{-1} \text{ day}^{-1}$ pressure decreased after 7 days of infusion though the infusion was continued for an additional 5 days (Table 3). These dogs also failed to respond after the fifth day to superimposed injections of angiotensin through the vertebral catheters, indicating tachyphylaxis. Two days after
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TABLE 4. Increase of mean pressure during sleep

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Dose (μg kg⁻¹ day⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Average increase of mean pressure ± SD (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>0</td>
<td>-7 ± 3 (9)</td>
<td>-6 (1)</td>
<td>-6 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Angiotensin into jugular vein</td>
<td>1</td>
<td>18 ± 2 (2)</td>
<td>6 (1)</td>
<td>10 (1)</td>
<td>-7 ± 5 (2)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>13 ± 1 (2)</td>
<td>-10 (1)</td>
<td>5 ± 3 (3)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>Angiotensin into vertebral artery</td>
<td>1</td>
<td>23 ± 14 (4)</td>
<td>6 ± 2 (4)</td>
<td>13 ± 8 (4)</td>
<td>6 ± 2 (4)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>19 ± 13 (5)</td>
<td>15 ± 3 (7)</td>
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</table>

( ) = no. of observations.

During recent years it has become apparent that angiotensin has a variety of actions, other than its direct vasoconstrictor one, that influence arterial pressure. Bickerton & Buckley (1961) were the first to suggest, on the basis of cross-transfusion experiments, that it acts on the central nervous system to cause a rise in arterial pressure. It was necessary to employ doses of angiotensin much larger than those that were likely to occur endogenously. This central action of angiotensin was reinvestigated by Dickinson & Yu (1967) who found that infusion of a much smaller amount into the vertebral circulation caused a rise in arterial pressure; the same amounts given intravenously were without effect. Similar results were reported later by Scroop & Lowe (1969) and Ferrario et al. (1970). These experiments were all short-term ones. The present experiments supplement the previous acute ones by demonstrating that angiotensin given chronically into the vertebral circulation results in a rise in pressure that is sustained for the 7 day period of the infusion. The rise in mean pressure is apparently mediated centrally since the same small dose infused intravenously caused essentially no sustained rise in pressure. It is likely that the pressor response is mediated by the sympathetic nervous system since Ferrario et al. (1970) found the rise usually to be accompanied by an increase in total peripheral resistance. On the other hand, Scroop & Lowe (1969) found that sympathetic blockade was ineffective in reducing the response, but section of vagus nerves or administration of atropine was effective. They suggest that the central action of angiotensin depends in large part upon withdrawal of vagus tone. It is possible that they came to this conclusion because of the use of greyhound dogs; this breed may be more dependent upon alteration of cardiac function for haemodynamic regulation than others.

Increasing the dose of angiotensin infused into the vertebral circulation by 10 times did not cause a proportionally greater increase in arterial pressure. This is consistent with the observa-
tion of Ferrario et al. (1970) that the dose–response curve flattens very quickly in acute experiments, suggesting an action in the nature of a stimulant effect on an area in the central nervous system. Gildenberg (1969) and Joy & Lowe (1970), using almost identical techniques involving diversions of intracerebral blood flow, demonstrated in acute experiments that the central site sensitive to angiotensin lies in the lower portion of the pons or medulla. Ueda (1968) had previously shown that microinjection of angiotensin, catecholamines or digitalis into the floor of the fourth ventricle, and particularly into the area postrema, caused systemic pressor responses. This led Gildenberg, Ferrario & McCubbin (unpublished observations) to determine whether the same area was involved when angiotensin was infused into the vertebral circulation. They found that destruction of the area by electrical coagulation, as confirmed by examination of serial slices at autopsy, entirely abolished the rise in pressure produced by infusion of low dosage of angiotensin into the vertebral arteries; destruction of adjacent areas in the floor of the fourth ventricle were without effect on the response.

Infusion of larger doses of angiotensin into the vertebral circulation was followed after several days by return of arterial pressure to control values, indicating that tachyphylaxis occurs in this as in other areas responsive to angiotensin. It was expected that some rise in pressure would persist due to recirculation of angiotensin into the systemic circulation; this did not occur, apparently because of its degradation in the cephalic capillary circulation. The larger dose of angiotensin infused intravenously caused sustained hypertension after several days. This was to be expected since it was similar to the dose employed by McCubbin, De Moura, Page & Olmsted (1965) who demonstrated that angiotensin has a delayed indirect effect mediated through the peripheral sympathetic nervous system which results in hypertension. A portion of the rise in pressure could, of course, be mediated by the central action of angiotensin.

An unusual finding in the present study was that arterial pressure rose, rather than fell, when sleep occurred during infusion of angiotensin. Awakening was accompanied by sharp fall. We have no explanation for this finding but the same phenomenon occurs in dogs with experimental neurogenic hypertension produced by section of the carotid sinus and aortic depressor nerves. Ferrario, McCubbin & Page (1969) reported that pressure in these dogs rose to extremely high levels during 'spindle and slow wave' sleep as determined by electroencephalographic measurements. It is possible that angiotensin in the present experiments acted upon vasomotor centres under partial control of the carotid sinus and aortic buffer afferent neurones and affected them in a manner that simulated denervation, in so far as they are influenced by changes in cerebral alertness.

ACKNOWLEDGMENT

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REFERENCES


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