Central action of angiotensin in stimulating ADH release

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MOUW, DAVID, JEAN-PHILIPPE BONJOUR, RICHARD L. MALVIN, AND ARTHUR VANDER. Central action of angiotensin in stimulating ADH release. Am J Physiol. 220(1): 239-242 1971.—Experiments were done in which angiotensin was infused either into the carotid artery or intravenously. It was shown that intracarotid infusions of angiotensin were more potent in stimulating ADH release than intravenous infusions. This was so in spite of the fact that both routes of infusion resulted in the same elevation of blood pressure and neither altered plasma osmolality, sodium concentration, nor hematocrit. Solutions of angiotensin were also perfused through the ventricular cisternal system. All concentrations of angiotensin, from 0.3-443 ng/ml, resulted in significant release of ADH. These data are interpreted to mean that some area of the brain is responsive to local concentrations of angiotensin, such that an increased concentration leads to increased release of ADH.

Angiotensin has long been known to be involved in the control of Na balance by stimulating aldosterone secretion (7). In addition, it has been shown to have a direct renal action (3, 8, 10). More recently it has been shown that infusions of either renin or angiotensin result in increased levels of circulating ADH (2), which suggests that the renin-angiotensin system may be involved in the control of ADH secretion. However, no information is available as to the location of the angiotensin receptor. The work presented here was designed to obtain information relating to the location of that receptor.

Methods

All experiments were performed on mongrel dogs weighing between 10 and 22 kg, anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg). Two types of experiments were carried out.

Intravascular infusion. Three dogs were prepared in the following way after induction of anesthesia. One femoral artery was isolated and catheterized with polyethylene tubing. The catheter was connected with a three-way stopcock to a pressure transducer and an appropriate recording apparatus. In this way arterial blood pressure was continually monitored. Blood samples for analysis were taken from the third outlet of the stopcock. A femoral vein was catheterized for the infusion of test solutions. One common carotid artery was isolated and a 23-gauge hypodermic needle inserted through the artery wall in the direction of flow. The other end of the needle was connected to polyethylene tubing. Venous and carotid catheters were connected to a constant-infusion pump set to deliver 0.2 ml/min into each catheter. During control periods both infusions consisted of 75 mm NaCl and 150 mm glucose. This infusion was continued for at least 1 hr following surgery, at which time an arterial blood sample was drawn for ADH estimation, Na concentration, osmolality, and hematocrit. Immediately after the first sample angiotensin (Hypertensin, Ciba) was mixed with the infusion solution of the common carotid catheter so that 10-17 ng per min were delivered to the dog. After 30 min of infusion a second blood sample was taken and the infusion changed to saline-glucose only. Another 30 min was allowed and a third blood sample was taken. Immediately after this, the same amount of angiotensin was infused into the femoral vein catheter for 30 min and the fourth sample was obtained. Angiotensin infusion was then terminated and a final control sample was taken after 30 additional minutes. Thus, in these experiments the dog's response to angiotensin infused directly into the carotid artery was compared to the same dose infused intravenously.

Ventriculocisternal perfusion. Seven dogs were positioned in a stereotaxic device and catheters were placed as described by Leusen (9) and Pappenheimer et al. (11) so that lateral ventriculocisternal perfusion could be initiated. Four dogs were used to study the effects of ventriculocisternal perfusion of angiotensin on ADH concentrations in plasma, and three were used for measuring the rate of disappearance of angiotensin from the perfusate. An artificial CSF, gassed with 5% CO₂ and 95% O₂, was perfused through the ventriculocisternal system. The composition of the fluid, in millimoles per liter was as follows: Na, 151; K, 3; Ca, 1.14; Mg, 0.8; PO₄, 0.5; Cl, 132; HCO₃, 25; and glucose, 4.15. The tip of the outflow cannula was fixed at the level of the ear bars so that CSF pressure in the ventricles was kept close to zero.

Synthetic angiotensin was added to the artificial dog CSF so as to achieve various angiotensin concentrations, ranging from 0.3 to 443 ng/ml. All dilutions of angiotensin were carried out in siliconized vessels (Clay-Adams, Inc., Silicad), and all parts of the perfusion system coming in contact with the angiotensin-containing CSF were either plastic or siliconized glass. These precautions minimize angiotensin loss from the artificial CSF. Care was taken to flush the perfusion apparatus following angiotensin administration so as to prevent contamination of the control solution with angiotensin during the recovery period.

The perfusion rate was the same throughout a given
experiment; rates of 0.21 or 0.3 ml/min were used for angiotensin concentrations greater than 15 ng/ml; all other perfusions were 0.59 ml/min. The experimental protocol consisted of a period of control ventriculocisternal perfusion (artificial dog CSF without angiotensin), followed by a period of perfusion with artificial CSF containing angiotensin; each perfusion period was 40–50 min long. We usually perfused 1 or 2 different concentrations of angiotensin in a given dog. Each period of angiotensin perfusion was bracketed by periods of control perfusion. Blood samples were taken 35–45 min after beginning the perfusion of each solution from a catheter in the fmcnoral region. These were analyzed for plasma ADH activity, plasma renin activity, osmotic pressure, and sodium concentration.

Blood sampling. All blood samples (60–80 ml) were taken into precooled plastic syringes in which 0.3 ml of heparin solution (10 mg/ml) was added to prevent clotting. Immediately after taking the first sample, a volume of isoncotic dextran (Gentran, Travenol Laboratories, Morton Grove, Ill.) in saline, equal to the volume of blood withdrawn, was injected intra-arterially. Following subsequent samples, red cells from the previous blood were added to the dextran-saline solution, and this mixture was used for replacement of the blood volume.

Disappearance of angiotensin from CSF perfusate. During four perfusions in three dogs, cisternal outflow of CSF was collected during perfusion of artificial CSF containing high concentrations of angiotensin (15, 73, and 365 ng/ml). The perfusion rate was 0.3 ml/min, and outflow samples were collected in test tubes surrounded by ice. Within 15 min after collection of 0.5–1 ml of the cisternal outflow, the samples were injected directly into anesthetized, pentolinium-treated rats, and the blood pressure response was compared to standard injections of angiotensin. Samples were assayed again after remaining 40–50 min at room temperature. No decrease in pressor activity was observed during this time. Samples of cisternal outflow during control (i.e., no angiotensin) CSF perfusion were also assayed to bioassay for pressor activity, and in addition, all CSF solutions containing angiotensin were assayed for pressor activity prior to their perfusion through the ventricles to check the accuracy of weighing and dilutions.

In these same three animals, samples of CSF cisternal outflow were collected over short timed intervals (4–6 min) immediately after perfusion of the artificial CSF containing angiotensin, i.e., after changing from the angiotensin solution to the nonangiotensin solution. These samples were also assayed for pressor activity.

Analytical methods. Concentrations of ADH were measured by the bioassay method of Share and Levy (12) as modified by Bonjour and Malvin (1). Renin assay was carried out using the method described by Brubacher and Vander (4). Hematocrit was measured in microcapillary tubes. Sodium was measured by flame photometry and osmolality was measured with a Fiske osmometer.

RESULTS

Table 1 shows the results of the three experiments in which angiotensin was infused into the carotid artery and peripheral vein. In all experiments the blood pressure response to angiotensin was the same, regardless of the route of infusion. However, the increment in the concentration of ADH in plasma was greater when angiotensin was infused into the carotid artery than in the vein. With the exception of the second control period in the third dog, all ADH concentrations returned to preinfusion values on terminating the infusion of angiotensin. We cannot explain this unusually high "control" value. In any event, these data suggest that intracarotid infusion of angiotensin is a more potent stimulus to ADH release than is intravenous infusion. In these experiments no change in the concentration of Na in plasma, osmolality, or hematocrit was observed.

Perfusion of an artificial CSF containing angiotensin in concentrations ranging from 0.3 to 443 ng/ml also resulted in a significant increase in the concentration of ADH in plasma. Figure 1 shows the results of these experiments. The changes in plasma activity of ADH were not accompanied by changes in blood pressure, pulse pressure, heart rate, plasma osmolality, or concentration of Na. No significant change in plasma renin activity was found. In five dogs, all of which responded to angiotensin with a signifi-

TABLE 1. Response to intracarotid and intravenous infusion of angiotensin in dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Control</th>
<th>Carotid Infusion</th>
<th>Control</th>
<th>Intravenous Infusion</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ADH, μU/ml</td>
<td>1.4</td>
<td>3.1</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>MBP, mmHg</td>
<td>120</td>
<td>133</td>
<td>120</td>
<td>135</td>
</tr>
<tr>
<td>2</td>
<td>ADH, μU/ml</td>
<td>0.7</td>
<td>1.3</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>MBP, mmHg</td>
<td>147</td>
<td>153</td>
<td>137</td>
<td>157</td>
</tr>
<tr>
<td>3</td>
<td>ADH, μU/ml</td>
<td>0.8</td>
<td>1.7</td>
<td>2.4</td>
<td>0.7</td>
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<tr>
<td></td>
<td>MBP, mmHg</td>
<td>88</td>
<td>124</td>
<td>96</td>
<td>128</td>
</tr>
</tbody>
</table>

Dogs 1 and 2 were infused with 10 ng/kg per min of angiotensin. Dog 3 was infused with 17 ng/kg per min.

FIG. 1. Effect of ventriculocisternal perfusion of angiotensin on concentration of ADH in peripheral plasma. Each series of 3 points represents results obtained from an experiment in which concentration of angiotensin in CSF was as shown on figure.
cantly increase in circulating ADH, the average renin activity was 20.0 ng/ml of angiotensin equivalents. During the experimental period, the average was 24.0 ng/ml, a non-significant increase.

In four perfusion periods in three dogs the concentration of angiotensin was measured in the perfusate and in the cisternal effluent. Table 2 presents those data. Pressor activity in cisternal fluid during perfusion of CSF with added angiotensin was considerably less than that entering the lateral ventricle. No pressor activity was detected in the control CSF or in cisternal outflow during the control perfusion, nor was there any detectable loss of pressor activity in cisternal fluid during angiotensin perfusion after remaining at room temperature for 10 to 50 min.

In two dogs the pressor activity of the cisternal outflow was determined during the recovery periods following perfusion of angiotensin at three concentrations—15, 73, and 443 ng/ml. Fifteen minutes after changing to angiotensin-free CSF no activity was detected at the lowest dose. After infusing 73 ng/ml the effluent had a concentration of 4 ng/ml 15 min after changing the reinfusion and 1 ng/ml at 30 min. Following the highest dose, the outflow had concentrations of 20 and 3 ng/ml at 15 and 30 min, respectively.

From the data in Table 2, and the known rate of perfusion (the rate of CSF outflow was within 1 % of the perfusion rate), it is possible to calculate the rate of disappearance of angiotensin from the perfusate (rate of disappearance = perfusion rate × [angiotensin]inflow − perfusion rate × [angiotensin]outflow). Figure 2 shows that the rate of disappearance is a linear function of the concentration of angiotensin in the perfusate over the range studied.

**DISCUSSION**

The experiments in which the response to intracarotid infusions of angiotensin were compared to the response to intravenous infusions indicate that the receptor, whatever its nature may be, is somewhere in the head, presumably the brain. Since the blood pressure response was the same, regardless of the route of infusion, and no changes were observed in osmolality or concentration of sodium in the plasma, we believe angiotensin has a direct central effect rather than acting indirectly by changing some cardiovascular parameter which in turn effects ADH release. However, one cannot rule out the possibility that angiotensin may exert its effect by altering the local blood flow to some area of the brain, i.e., a vascular effect. The baroreceptors do not seem to be implicated as the blood pressure response was not significantly different with intravenous and intracarotid infusions, nor does it seem likely that atrial receptors are involved.

It should be pointed out that Hodge et al. (6) showed that about half of angiotensin II infused into the carotid artery is removed in a single pass through the head. In view of this it is surprising that in our experiments there appeared to be no difference in blood pressure response between intracarotid and intravenous infusions of angiotensin. We are unable to explain this discrepancy.

Ventriculocisternal perfusions also indicate that the receptor for angiotensin is in the brain. The amount of angiotensin perfused through the ventricles was so low, that even if all of it had entered the circulation it could not have had a significant cardiovascular effect. The total amount of angiotensin perfused varied from 0.01–5 ng/kg min. Although these experiments do not localize the precise area of the brain responsive to angiotensin, we speculate that it may be in the paraventricular nucleus. We assume that angiotensin diffused out of the ventricular system and came into contact with its receptor. Of the nuclei known to be associated with ADH secretion, only the paraventricular nucleus is near the ventricular space. As a tentative hypothesis we suggest that the paraventricular nucleus is the site which responds to angiotensin. It should be noted that this does not appear to be a good correlation between the absolute increment in ADH and the concentration of angiotensin in the perfusion. However, the control levels of ADH varied considerably, and the increment seems better related to the control level of ADH. Why this is so is not clear, but the fact remains that at all concentrations tested, angiotensin resulted in an increased circulating level of ADH.

The difference in pressor activity between artificial CSF-containing angiotensin and the corresponding cisternal outflow collections almost certainly represents loss of angiotensin in passage through the brain ventricles. Pressor activity did not decrease when cisternal samples remained at room temperature, suggesting that the fall in pressor activity was not due to enzymatic degradation (angiotensinases) within the CSF.
Loss of angiotensin from CSF during perfusion could be due to: a) diffusion out of the ventricles into brain tissue and the cerebral vasculature, b) binding of angiotensin to brain tissue, or c) enzymatic degradation of angiotensin by the brain tissue. The linear relation of angiotensin loss from the ventricles to angiotensin concentration suggests that over this concentration range, the primary route of loss from the ventricles is by a process with almost limitless capacity, i.e., diffusion into the cerebral vasculature. Furthermore, the rapid fall in cisternal angiotensin concentration after returning to control perfusion suggests that only a very small fraction of the angiotensin which is not recovered could be sequestered in brain without entering the capillaries; the total angiotensin washed out of the brain during subsequent control perfusion was less than 15% of the angiotensin lost during perfusion. Most of this probably comes from mixing in the ventricles and especially in the subarachnoid space. These experiments demonstrate that a central receptor exists which is responsive to angiotensin. When angiotensin concentrations are increased the response is to stimulate ADH release. Although it is not possible to localize the exact site in the brain which responds to angiotensin, we believe the most likely nucleus is the paraventricular nucleus. However, confirmation of this must await neurophysiological evidence.

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