Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion

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Ramsay, David J., Lanny C. Keil, Michael C. Sharpe, and Jeanette Shinsako. Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. Am. J. Physiol. 234(1): R66-R71, 1978 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 3(1). R66-R71, 1978. — The effects of intravenous infusion of Asp1-Ile2-angiotensin II on blood pressure, plasma vasopressin, ACTH and 11-hydroxycorticosteroid levels and on plasma renin activity were studied in five trained, conscious dogs. The dogs were prepared with bilateral carotid loops. Infusion of angiotensin II at rates of 5, 10, and 20 ng/kg·min raised its plasma concentration from 23 ± 7 to 48 ± 8, 125 ± 8, and 187 ± 21 pg/ml, respectively. The lowest rate of infusion was mildly pressor, the two higher rates more so. All rates of infusion promptly increased vasopressin levels and depressed renin levels. The two higher rates also stimulated ACTH, although with a latency of 30-45 min. Since the rates of infusion of angiotensin II employed produced plasma levels within the physiological range, it is suggested that peripherally generated angiotensin II may play an important role in the regulation of vasopressin, and ACTH secretion.

plasma renin activity

There is evidence in the literature that administration of angiotensin II into the brain tissue or ventricular cerebrospinal fluid results in the stimulation of vasopressin (13, 14) and ACTH secretion (15) as well as drinking (8, 18, 25) and a rise in blood pressure (24). These central effects of angiotensin depend upon giving the angiotensin (7, 14) or a substance such as renin, which can result in the generation of angiotensin (21), on the brain side of the blood-brain barrier. Peripherally generated angiotensin II does not cross the blood-brain barrier readily (18). Particularly in view of recent evidence that casts doubt on the functional significance of the brain renin-angiotensin system (6), it is important to know if variations in the plasma level of angiotensin give rise to central effects. In particular, this paper is concerned with the secretion of vasopressin and ACTH.

Although intracerebroventricular injection of angiotensin stimulates vasopressin secretion in the rat (13) and the dog (14), the information on systemic administration is conflicting. The early reports that demonstrated a rise in vasopressin concentration following infusion of renin or angiotensin (1, 16) have not always been confirmed, and there is disagreement in the literature. A recent report suggests that blood-borne angiotensin causes release of vasopressin only when combined with dehydration, and that the two stimuli may be synergistic (4).

The information on the effects of peripherally generated angiotensin on ACTH levels is more sparse (23). Angiotensin infusion leads to increased secretion of cortisol in dogs with an isolated median eminence and pituitary, but not in dogs with isolated pituitaries alone (10). In the latter the effect is no greater than in hypophysectomized dogs. Infusion of pressor doses of angiotensin increases ACTH secretion in man, but here the conclusion was drawn that the mechanism was via an angiotensin-induced inhibition of adrenal cortisol output (19). These experiments have been taken as evidence that ACTH is involved.

The present experiments were designed to test the effects of angiotensin infusions in different doses in a population of trained, conscious dogs and to combine assays of vasopressin and ACTH with direct measurement of angiotensin II. With this technique it should be possible to determine if the fluctuations of angiotensin concentration within the normal plasma range can affect the secretion of these pituitary hormones.

METHODS

Subjects. The experiments were all carried out on five trained dogs, weighing 17-25 kg. The dogs were housed individually with free access to water. Cages were cleaned daily at 0900 h and the animals were maintained on a normal lighting regime and fed at 1400 h a constant amount of synthetic dog food providing 50 meq sodium per day. All the dogs were operated on at least 4 wk before starting the experiments and, under pentobarbital anesthesia (31), were prepared with bilateral carotid skin loops. This operation allowed easy access to the carotid arteries for measurement of blood pressure and withdrawal of arterial blood samples for analysis. During the period of recovery from surgery, the animals were accustomed to being in the laboratory in a modified Pavlov stand and to minor procedures such as venipuncture and cannulation of the carotids in the previously prepared skin loops, without apparent discomfort.
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Protocol. All the experiments were carried out in the morning to minimize variations due to circadian rhythms. The dogs were brought from their cages at 0800 h, and placed in the Pavlov stand. One of the carotids was cannulated with a 20-gauge catheter (Angiocath, Deseret Pharmaceutical Co.) which allowed measurement of blood pressure and withdrawal of arterial blood for hormone analysis. A limb vein was cannu-lated with a 17-gauge catheter placement set (Intracath, Deseret) for the intravenous infusions, after which the animal was allowed 30 min to settle. Two control samples were taken with an interval of 15 min, and the intravenous infusion was begun at a rate of 0.02 ml/min with a Harvard infusion pump. Each dog received infusions of angiotensin II (Asp'Ile'-angiotensin II, Schwarz/Mann, New York City) at rates of 5, 10 or 20 ng/kg/min or the vehicle (0.15 M NaCl). These four treatments were presented to the dogs in a counterbalanced sequence and a minimum of 4 days was allowed between experiments. The infusions lasted for 60 min, during which time four samples were taken at 15-min intervals. After the infusion was stopped, the dog was left for 30 min longer, and the final sample was taken. The dog was then returned to its cage.

Chemical assays. The following assays were carried out on all samples after separating the plasma. a) Plasma renin activity was measured with a radioimmunoassay for angiotensin I (22, 28) and expressed as nanograms of angiotensin formed per milliliter of plasma during a 3-h incubation. b) Plasma ACTH was determined by radioimmunoassay (5, 11, 20). c) Plasma 11-hydroxycorti-costeroids were measured by the competitive protein-binding radioassay of Murphy (17). In the dog, these consist mainly of cortisol plus some corticosterone. d) Plasma vasopressin was determined by radioimmunoassay on extracted plasma (12). For the assay, synthetic arginine vasopressin (357 U/mg) was used to prepare the standards.

Two samples were taken for analysis of angiotensin II with an antibody developed by J. Stockigt and an extraction procedure already published (2). The samples were taken during the preinfusion period and 45 min after beginning the infusion.

After centrifugation and removal of the plasma for analysis, the red blood cells were resuspended in an equal volume of saline and returned to the animal via the venous line. This was done to prevent the effects of hypovolemia which might otherwise have been significant. In an experiment, seven blood samples were withdrawn, each with a volume of 12 ml.

Statistical analysis. An analysis of variance for repeated measurements on the same subjects was employed (32). The significance of the differences between doses was examined using Duncan’s multiple-range test for equal n’s.

RESULTS

Blood pressure. Infusion of angiotensin II caused a dose-related increase in mean blood pressure in all animals (Fig. 1). There was no change in mean blood pressure when the saline vehicle was infused and a mild increase in mean blood pressure at an angiotensin dose of 5 ng/kg min. The two higher doses of angiotensin, however, gave much more marked and reliable increases in mean blood pressure. From this information, an infusion of 5 ng/kg min appears to be at the threshold of pressor activity.

Plasma renin activity and angiotensin II concentration. The results of infusing angiotensin on plasma renin activity are shown in Fig. 2 and in Table 1. The plasma concentration of angiotensin II in our population of normally hydrated dogs was 23 ± 7 pg/ml. Infusion of angiotensin II gave dose-related increases in its plasma concentration reaching 187 ± 21 pg/ml at the highest dose (Table 1). The plasma renin activity of the plasma or blood taken before the beginning of the infusion was 3.4 ± 0.4 ng/ml·(3 h), a reasonable value for a conscious dog in normal sodium balance. Infusion of the saline vehicle had no effect on plasma renin activity. However, infusion of angiotensin II reduced plasma renin to a minimum of 44% of the control values. These data are compatible with the inhibition of renal renin secretion in the presence of increased plasma levels of angiotensin II (26, 30).

Vasopressin. The preinfusion concentration of 3.1 pg/ml is typical of a normally hydrated conscious dog (4). Infusion of a saline vehicle had no effect on plasma vasopressin levels (Fig. 3). However, infusion of angiotensin at all doses was correlated with an increase in plasma vasopressin. The increase was statistically significant and at the highest dose of angiotensin infusion (20 ng/kg·min) became significant after 15 min of

Fig. 1. Changes in blood pressure from control values during intravenous infusion of angiotensin II at various doses. Means ± SE are shown. Significances: * P < 0.05; ** P < 0.01; *** P < 0.001 compared with saline control.
infusion. The level then remained elevated for the period of infusion. At the lowest dose of angiotensin, although there was a tendency for the vasopressin titer to rise, this did not become statistically significant until 60 min after beginning the infusion. Only one postinfusion blood sample was taken 30 min after disconnecting the infusion. As the data in Fig. 3 demonstrate, in all cases the plasma vasopressin level returned toward normal.

**TABLE 1. Effects of infusion of angiotensin II at various doses**

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Saline Vehicle</th>
<th>Angiotensin II 5 ng/kg-min</th>
<th>Angiotensin II 10 ng/kg-min</th>
<th>Angiotensin II 20 ng/kg-min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure</td>
<td>110±1</td>
<td>110±5</td>
<td>120±6</td>
<td>124±5*</td>
<td>132±4*</td>
</tr>
<tr>
<td>Torr</td>
<td>3.4±0.4</td>
<td>3.2±0.8</td>
<td>3.8±0.8*</td>
<td>1.7±0.6*</td>
<td>2.2±0.8*</td>
</tr>
<tr>
<td>Plasma renin activity, ng/ml (3 hr)</td>
<td>23±7</td>
<td>48±81</td>
<td>125±8*</td>
<td>187±21*</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II, pg/ml</td>
<td>3.1±1.0</td>
<td>2.7±0.5</td>
<td>4.4±1.1*</td>
<td>6.3±1.3*</td>
<td>13.0±3.1*</td>
</tr>
<tr>
<td>Vasopressin, pg/ml</td>
<td>50±5</td>
<td>44±11</td>
<td>66±16</td>
<td>81±15*</td>
<td>146±40*</td>
</tr>
<tr>
<td>ACTH, pg/ml</td>
<td>71±1.7</td>
<td>21±0.3</td>
<td>3.6±0.3</td>
<td>3.5±0.4*</td>
<td>5.9±0.81</td>
</tr>
<tr>
<td>11-Hydroxycorticosteroids, µg/100 ml plasma</td>
<td>20±2.3</td>
<td>11±0.4</td>
<td>3.5±0.3</td>
<td>3.8±0.4</td>
<td>6.3±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for each of the variables in the five dogs. Postinfusion values measured 45 min for beginning of infusion are shown. Significances: *P < 0.05; **P < 0.01; ***P < 0.001 when compared with saline control.

**FIG. 2.** Changes in plasma renin activity from control values during intravenous infusion of angiotensin II at various doses. Means ± SE are shown. Significances: *P < 0.05; **P < 0.01; ***P < 0.001 compared with saline control.

**FIG. 3.** Changes in plasma vasopressin concentration from control values during intravenous infusion of angiotensin II at various doses. Means ± SE are shown. Significances: *P < 0.05; **P < 0.01; ***P < 0.001 compared with saline control.

**ACTH.** These data are summarized in Fig. 4 and Table 1. The preinfusion plasma ACTH concentration was 50 ± 5 pg/ml, a reasonable value for the normal dog. Infusion of the saline vehicle had no effect on plasma ACTH levels which held remarkably constant throughout the course of infusion and recovery period. Infusion of angiotensin brought about a dose-related increase in plasma ACTH levels. Analysis of variance for repeated determination showed that the increase in ACTH concentration was statistically significant at rates of infusion of 10 and 20 ng/kg·min. At the lowest dose of angiotensin infusion, by analysis of variance, the slight increase in ACTH levels just failed to be significant at the 5% level. However, by a nonparametric test, the Wilcoxon sign test, the increase was significant at the 2.5% level even at this low rate (5 ng/kg·min) of angiotensin infusion. The increase in plasma ACTH seemed to develop more slowly than that of vasopressin. Regardless of the dose of angiotensin infused, no significant increase in ACTH was observed until 30 min, and the peak was not reached until 60 min.

As expected, plasma 11-hydroxycorticosteroid levels followed those of ACTH (Table 1). The correlation coefficient between plasma ACTH and plasma 11-hydroxycorticosteroids was 0.78 (P < 0.001). This close correlation is illustrated in Fig. 5.

In summary, infusions of angiotensin caused statistically significant increases in plasma levels of vasopressin, ACTH, and 11-hydroxycorticosteroids. Stimulation of these hormones by angiotensin takes place within the normal physiological range over which plasma angiotensin varies.
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FIG. 4. Changes in plasma ACTH concentration from control values during intravenous infusion of angiotensin II at various doses. Means ± SE are shown. Significances: \( * P < 0.05; \quad ** P < 0.01; \quad *** P < 0.001 \) compared with saline control.

FIG. 5. Correlation between plasma ACTH and 11-hydroxycorticosteroid concentrations in samples withdrawn during angiotensin II infusion.

DISCUSSION

These experiments were carried out on a group of dogs that had been in the laboratory several months. They were accustomed to laboratory life and seemed un perturbed by the simple procedures involved in these experiments.

Early reports gave plasma concentrations of angiotensin II ranging from 10 to 100 pg/ml for human beings (2). With refinements of technique, normal values for human plasma were 5–35 pg/ml (3). The values in our normal hydrated dogs on a constant sodium diet were \( 23 \pm 7 \) pg/ml and appear to be compatible with the findings in other species. Furthermore, after dietary sodium depletion, plasma angiotensin II rose to \( 142 \pm 5.4 \) pg/ml (Reid and Ramsay, unpublished observations). In some pathological conditions, for example renal hypertension, plasma concentrations of 200 and as high as 500 pg/ml have been reported. In our experiments, raising the plasma concentration of angiotensin to 48, and to an even more pronounced extent to 125 pg/ml, gave statistically significant increases in the plasma titers of vasopressin and ACTH. From this evidence it would seem reasonable to suggest that changes in plasma angiotensin II which may occur in the intact animal are capable of bringing about changes in pituitary function.

The present experiments confirm the earlier reports that systemic infusions of angiotensin II may result in an increase in vasopressin secretion in the normally hydrated dog (1), as it does in normally hydrated human beings (29). In addition, it has been demonstrated that this is a dose-related phenomenon and furthermore, that the stimulation of vasopressin secretion may occur with changes in angiotensin II over the physiological range. However, the results are not in accord with some observations of Claybaugh (4). In his experiments, also on conscious dogs, infusions of renin gave rise to stimulation of vasopressin secretion only if the animals had been subjected to 48 h of water deprivation. The suggestion was therefore made, similar to earlier suggestions to explain experimental results in the goat, that angiotensin will stimulate vasopressin secretion only in conjunction with some other stimulus such as hyperosmolality (4, 27). It is difficult to explain this discrepancy. With a conversion figure of 2.5 pg of vasopressin as equivalent to 1 \( \mu \)U, the mean vasopressin level in the normally hydrated dogs reported by Claybaugh was 3.0 pg/ml. In our series, the preinfusion vasopressin concentration was 3.1 ± 1.0 pg/ml. It is likely therefore that the dogs in the two series of experiments were in a similar state of hydration. It would be interesting to know the plasma levels of angiotensin II in the experiments reported by Claybaugh, because angiotensin rather than renin itself is the agent which stimulates the vasopressin secretion. When the exogenous level of angiotensin has been increased by infusion of renin, the endogenous secretion of renin is suppressed, as in our experiments. It is therefore particularly important to estimate plasma angiotensin II concentrations.

It is interesting to speculate why both vasopressin and ACTH levels took so long to increase after starting the angiotensin infusion. The infusion was started at a constant rate, without an initial priming injection, so the plasma angiotensin level presumably rose slowly to achieve its new, higher steady-state level. It is probable...
that this lag accounts at least in part for the slow increases in vasopressin and ACTH. It would be interesting to repeat the experiments with an intravenous infusion of angiotensin as in this series, but preceded by a priming injection designed to bring about a rapid increase in plasma angiotensin concentration and then to maintain it there.

Maran and Yates (15) have recently reported that intravenous infusion of angiotensin at a dose of 1 µg/min in dogs stimulates cortisol secretion via stimulation of ACTH. In our experiments, there was stimulation of ACTH at even lower infusion rates of intravenously administered angiotensin. The relationship between the activity of the peripheral renin/angiotensin system and the secretion of ACTH is particularly interesting in view of the recent evidence which suggests that a decrease in plasma angiotensin concentration and then to maintain it there.

Our results do not allow us to comment on the site of action of the angiotensin. It is likely, however, that the effect is on the central nervous system (13–15). Angiotensin II does not cross the blood-brain barrier to the extent that it cannot be detected in cerebrospinal fluid following intravenous infusion (18). The site of action of angiotensin must therefore be at, or close to, areas where the blood-brain barrier is deficient. Whether the site of action of angiotensin is the hypothalamus, one of the circumventricular organs, or the pituitary itself, awaits experimental elucidation.

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