Role of the Renin-Angiotensin System in the Control of Vasopressin and ACTH Secretion during the Development of Renal Hypertension in Dogs

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SUMMARY Experiments were performed to determine if the activation of the renin-angiotensin system that occurs during the development of two-kidney, one clip Goldblatt hypertension in dogs is accompanied by increases in vasopressin and adrenocorticotropic hormone (ACTH) secretion. Following renal artery constriction, there were marked increases in arterial blood pressure, plasma renin activity (PRA), and plasma angiotensin II (All) concentration. These changes were accompanied by an increase in plasma vasopressin concentration during the second week following constriction, and there were significant correlations between plasma vasopressin concentration and PRA ($r = 0.57$, $p < 0.001$), and between plasma vasopressin concentration and plasma All concentration ($r = 0.59$, $p < 0.001$). In contrast, plasma corticosteroid concentration, used as an index of changes in ACTH secretion, did not change significantly. Acute blockade of the renin-angiotensin system with captopril or saralasin produced the expected changes in blood pressure, PRA, and plasma All concentration but did not decrease plasma vasopressin or corticosteroid concentrations. These results indicate that during the development of renal hypertension in dogs, there may be an interaction between the renin-angiotensin system and vasopressin, but not between the renin-angiotensin system and the pituitary-adrenal axis. They also show that the antihypertensive action of captopril in this experimental model is not mediated via suppression of vasopressin, ACTH, or corticosteroid secretion. (Hypertension 6: 35-41, 1984)

KEY WORDS • angiotensin II • captopril • saralasin • pituitary • corticosteroids

The activity of the renin-angiotensin system is increased in a variety of forms of experimental and human hypertension. Administration of agents that block the formation or actions of angiotensin II (All) lowers blood pressure in these hypertensive states, indicating that the hypertension is dependent, at least in part, on the renin-angiotensin system.

The hypertensive action of All is largely the result of its direct action to constrict vascular smooth muscle. However, All may also influence arterial pressure by a number of indirect mechanisms. For example, it is known to stimulate the secretion of vasopressin and adrenocorticotropic hormone (ACTH), two peptides that can have marked effects on blood pressure. It is therefore possible that the elevated All levels in some hypertensive states increase the secretion of vasopressin and ACTH, which in turn act to increase blood pressure. Moreover, it is possible that the antihypertensive effect of blockade of the renin-angiotensin system is partly due to inhibition of the stimulatory action of All on vasopressin and ACTH secretion.

The present experiments were designed to test these possibilities. This was accomplished by monitoring the plasma levels of renin, All, vasopressin, and corticosteroids (used as an index of ACTH secretion) during the development of two-kidney, one clip Goldblatt hypertension in dogs. In addition, the effect of blockade of the renin-angiotensin system with captopril or saralasin on the secretion of these hormones was studied at different times during the development of hypertension.

Methods

The experiments were performed on five mongrel dogs of both sexes, weighing 18–34 kg. They had free access to water and were fed a diet of Purina dry dog chow (Ralston Purina Company, St. Louis, Missouri), which provided approximately 80 mEq sodium/day. Under sodium pentobarbital anesthesia (30 mg/kg,
Tygon cannulas (0.5 mm i.d.) were chronically implanted in a femoral artery and vein. Both cannulas were led subcutaneously to a point between the shoulders where they were exteriorized and protected by a vest (Medical Arts, Los Angeles, California). On alternate days, the cannulas were flushed with sterile isotonic saline and filled with heparinized saline (1000 U/ml). During the 4 days following surgery, the dogs were treated with penicillin and streptomycin (Combipic, Pfizer, New York, 3.0 ml/day). At least 2 days were allowed for recovery.

Following recovery from surgery, the dogs were brought to the laboratory on at least two occasions for control measurements. In the laboratory the dogs were allowed to stand while loosely restrained by a sling (Medical Arts, Los Angeles, California). Mean and pulsatile blood pressure and heart rate were continuously recorded for at least 30 minutes with a Grass polygraph (Grass Instruments, Quincy, Massachusetts) and a tachograph, which was triggered by the blood pressure pulse wave. Two 12 ml blood samples separated by 30 minutes were collected from the femoral artery and replaced with an equal volume of sterile isotonic saline. All samples were taken at approximately the same time each day (1000 to 1400 hours) in order to minimize possible hormonal changes due to circadian rhythms.

After control measurements had been made, the dogs were anesthetized with sodium pentobarbital. The right kidney was exposed through a flank incision, and a clay-filled ameroid constrictor (Three Points Products, Montreal, Canada) was placed around the renal artery. Following implantation, the ameroid gradually constricts the artery and hypertension results.8 Following surgery, the dogs were again treated with penicillin and streptomycin for 4 days. During the 30-day period following ameroid implantation, the dogs were brought to the laboratory every 2 to 3 days for blood pressure and heart rate recording and collection of blood samples as described above.

The effects of acute blockade of the renin-angiotensin system with a converting-enzyme inhibitor, captopril, or an angiotensin II receptor antagonist, saralasin, were also studied in these animals. The effects of captopril were studied 4–7, 9–14, and 15–21 days following placement of the ameroid. Two control blood samples were collected and then captopril (Squibb) dissolved in sterile isotonic saline was injected i.v. in a single dose of 5.0 mg/kg. Additional blood samples were collected 15, 30, 45, and 60 minutes following the injection. Blood pressure and heart rate were monitored continuously. The effects of saralasin were studied 7–9, 11–14, and 17–23 days following ameroid implantation. After collection of control blood samples, saralasin (Bachem) dissolved in sterile isotonic saline was infused i.v. in a dose of 2.0 μg/kg/min for 30 minutes. Blood samples were collected at 15 min intervals during the infusion and for 30 minutes following cessation of the infusion.

The blood samples were divided into two aliquots: 1) 4.5 ml was added to 0.5 ml 0.3 M EDTA in a chilled centrifuge tube for the determination of PRA and All concentration; 2) the remaining blood was added to a heparinized centrifuge tube for the determination of plasma vasopressin and corticosteroid concentrations and osmolality. The blood samples were centrifuged and the plasma separated and frozen until analysis.

Plasma vasopressin concentration was measured by radioimmunoassay of extracted plasma.9 Plasma corticosteroids were measured by radioimmunoassay,10 and changes in plasma corticosteroid concentration were used as an index of changes in ACTH secretion. Plasma renin activity was measured using a radioimmunoassay for AI and expressed as nanogram AI generated per ml plasma during a 3-hour incubation at 37°C (ng/ ml/3 hr).11,12 Plasma All concentration was measured by radioimmunoassay. Angiotensin II was extracted with the bentonite procedure used for the vasopressin assay.9 The radioimmunoassay was performed as described previously.13 In this assay, displacement of tracer by All extracted from plasma is parallel to that of synthetic All (Figure 1). The All antibody used in the assay cross reacts 100% with All and 10% with AI. The intra- and interassay coefficients of variation average 6.9% and 11.6% respectively. Recovery of angiotensin II during the extraction procedure averages 65% and all values were corrected for this. Plasma osmolality was measured by freezing point depression with a Fiske osmometer (Fiske Associates, Uxbridge, Massachusetts).

All results are expressed as means ± SE. Unless indicated otherwise, statistical evaluation of the data was performed using one-way analysis of variance for repeated measures.14 Values for PRA and plasma All concentration obtained during the 30-day period following renal artery constriction showed a significant (p < 0.01) positive skewness. This skewness was eliminated by log transformation of the data. Comparisons between specific time points and control values were made using Dunnett's t test.14

![Standard curve for the angiotensin II radioimmunoassay showing parallel displacement of tracer by extracted plasma.](image-url)
Results

Cardiovascular and Endocrine Changes During the Development of Hypertension

The cardiovascular and endocrine changes that occurred during the development of renal hypertension are summarized in Figure 2. Mean arterial pressure increased progressively from the control value of 118 ± 7 to 166 ± 9 mm Hg on Day 9 (p < 0.01). Blood pressure remained between 149 and 165 mm Hg for the remainder of the 30-day period. There was no significant change in heart rate.

Plasma renin activity increased from the control value of 7.2 ± 2.8 to 51.0 ± 21.2 ng/ml/3 hr on Days 10–12, and remained between 41.2 and 67.8 ng/ml/3 hr for the remainder of the period of observation. Prior to constriction of the renal artery, plasma All concentration averaged 11.1 ± 4.2 pg/ml. It increased to 98.3 ± 44 pg/ml on Days 10–12 (p < 0.01) and remained between 65.7 and 146.9 pg/ml for the remainder of the 30-day period. The changes in plasma All closely paralleled those in PRA (Figure 2) and, overall, there was a very close correlation between these two variables: Plasma All = 2 (PRA) − 6 (r = 0.96, p < 0.001) (Figure 3). There were also significant correlations between mean arterial pressure (MAP) and PRA (r = 0.77, p < 0.001) and between MAP and plasma All concentration (r = 0.71, p < 0.001).

Plasma vasopressin concentration increased by at least 50% in all animals but the time at which the maximal increase occurred varied from animal to animal. However, the maximum always occurred between Days 8 and 16, and the maximal increase from 2.6 ± 0.5 to 5.4 ± 0.5 pg/ml was statistically significant (p < 0.05, paired t test). During the 30-day period, there were significant correlations between plasma vasopressin concentration and plasma All concentration (r = 0.59, p < 0.001) (Figure 4) and between plasma vasopressin concentration and PRA (r = 0.57, p < 0.001). There was a lower but statistically significant correlation between MAP pressure and plasma vasopressin concentration (r = 0.37, p < 0.002).

Plasma corticosteroid concentration, used here to reflect changes in ACTH secretion, did not change significantly. Plasma osmolality also did not change significantly, remaining between 293 and 299 mOsm/kg H₂O throughout the whole study.
Figure 5. Cardiovascular and endocrine responses to administration of captopril (•—•) or saralasin (O—O) 4—9 days after renal artery constriction. Captopril was injected i.v. in a single dose of 5.0 mg/kg at 0 minutes, while saralasin was infused i.v. at 2.0 μg/kg/min from 0—30 minutes. Values represent the means ± SE of observations made in five dogs. Asterisks refer to comparisons with the 0 minute control value. *p < 0.05; **p < 0.01.

Effects of Captopril and Saralasin

The cardiovascular and endocrine effects of captopril and saralasin are summarized in Figures 5—7. Administration of captopril between Days 4 and 7 caused a marked fall in arterial pressure from 160 ± 7 to 128 ± 6 mm Hg (p < 0.01) (Figure 5). Heart rate increased from 68 ± 11 to 83 ± 6 bpm (p < 0.05). PRA increased from 15.7 ± 3.3 to a maximum of 37.8 ng/ml/3 hr (p < 0.01). Despite this large increase, plasma All concentration did not change significantly, indicating effective blockade of converting enzyme. Neither plasma vasopressin nor corticosteroid concentrations were altered by captopril (Figure 5). Administration of saralasin between Days 7 and 9 had no significant effects on any of the measured variables (Figure 5).

The effects of captopril administration on Days 9—14 were similar to those on Days 4—7 but more marked (Figure 6). Blood pressure decreased from 162 ± 13 to 124 ± 6 mm Hg (p < 0.01) and heart rate increased from 73 ± 11 to 119 ± 15 bpm (p < 0.01). There was a marked increase in PRA from 41.2 ± 19.0 to 208.5 ± 47.5 ng/ml/3 hr (p < 0.01) but, despite this, plasma All concentration actually decreased from 80.6 ± 38.2 to 36.8 ± 23.2 pg/ml (p < 0.05). Plasma vasopressin and corticosteroid concentrations tended to increase following injection of captopril but, overall, there were no statistically significant changes. Administration of saralasin on Days 11—14 tended to decrease blood pressure but, again, this effect was not statistically significant. Heart rate did not change. PRA in-
increased from 46.7 ± 13.7 to 111.5 ± 32.5 ng/ml/3 hr (p < 0.05) and plasma All concentration increased from 68.0 ± 17.9 to 231.4 ± 88.2 pg/ml (p < 0.05). Plasma vasopressin concentration increased from 4.4 ± 0.4 to 6.5 ± 0.3 pg/ml (p < 0.05) but plasma corticosteroid concentration did not change significantly.

The effects of captopril administration between Days 15–21 were generally similar to the responses observed on the other two occasions. Blood pressure decreased from 155 ± 10 to 122 ± 6 mm Hg (p < 0.01) and heart rate increased from 77 ± 11 to 100 ± 11 bpm (p < 0.05). PRA increased from 38.9 ± 18.6 to 237.6 ± 54.5 ng/ml/3 hr (p < 0.01) but plasma All concentration did not change significantly. Plasma vasopressin concentration increased from 3.8 ± 1.0 to 7.9 ± 3.3 pg/ml (p < 0.05) but plasma corticosteroid concentration did not change significantly. Administration of saralasin caused no significant changes in any of the measured variables except for an increase in plasma corticosteroid concentration from 1.2 ± 0.4 to 3.0 ± 0.6 μg/dl (p < 0.05).

The relationship between plasma All concentration and PRA before and after treatment with captopril is shown in Figure 8. Before captopril, the relationship was:

\[
\text{plasma All} = 2 \times \text{PRA} - 4 \quad (r = 0.97, p < 0.001)
\]

while after captopril, the relationship was:

\[
\text{plasma All} = 0.13 \times \text{PRA} + 22 \quad (r = 0.80, p < 0.001).
\]

Thus, the slope of the relationship after captopril was only 7% of that before captopril, and this corresponds well to the 10% cross reactivity of the All antibody for AI. This suggests that captopril completely blocked conversion of AI to All and explains why plasma AI levels apparently did not decrease to zero following captopril administration.

**Discussion**

The changes in blood pressure, PRA, and plasma AI all concentration that occurred during the development of two-kidney, one clip hypertension in the present study are generally similar to those observed in other models of renal hypertension.\(^{15,16}\) The changes in blood pressure correlated closely with the changes in PRA (r = 0.77) and plasma AI concentrations (r = 0.71), suggesting an important role for the renin-angiotensin system in this form of hypertension. As expected,\(^4\) administration of the converting-enzyme inhibitor captopril at different times during the development of renal hypertension produced prompt and marked falls in arterial pressure. In contrast, administration of the AI receptor antagonist saralasin produced little change in blood pressure. This discrepancy between the effects of converting-enzyme inhibition and angiotensin receptor blockade has been observed by other investigators\(^{17}\) and is thought to reflect the intrinsic agonist activity of saralasin and the bradykinin-potentiating action of captopril.

There have been several reports that plasma vasopressin concentration or urinary vasopressin excretion is elevated in animal models of hypertension in which there is increased activity of the renin-angiotensin system.\(^{18,19}\) It has also been reported that plasma vasopressin levels are increased in patients with malignant hypertension.\(^{20,21}\) Indeed, Thibonnier and associates\(^{22}\) reported a close correlation between plasma vasopressin concentration and PRA in a group of patients with severe hypertension. In general, the results of the present study confirm and extend these previous findings. Activation of the renin-angiotensin system by constriction of one renal artery led to an increase in plasma vasopressin concentration. The increase in plasma vasopressin concentration was slow to develop, reaching a maximum between Days 8 and 16, when PRA and plasma AI concentration were at or near their maximal values. Over the time course of the present study, there were significant correlations between plasma vasopressin concentration and PRA and between plasma vasopressin concentration and plasma AI concentration.

Because it is known that AI-I can stimulate the secretion of vasopressin,\(^3,5\) it seems reasonable to propose that the increase in plasma vasopressin concentration was mediated via the renin-angiotensin system. The finding of correlations between plasma vasopressin concentration and plasma renin and AI levels is consistent with this possibility. On the other hand, blockade of the renin-angiotensin system in hypertensive dogs with either saralasin or captopril failed to decrease plasma vasopressin concentration. Indeed, in some animals, plasma vasopressin levels actually increased following captopril administration, presumably because of the large fall in arterial pressure. Thibonnier et al.\(^{22}\) also reported that acute administration of captopril in hypertensive patients did not decrease plasma vasopressin concentration. However, they ob-
served significant reductions in both plasma vasopressin concentration and urinary vasopressin excretion during chronic treatment with captopril and this agrees well with observations made in rats with spontaneous hypertension by Crofton and associates. It is now clear that vasopressin is a potent vasoconstrictor, and there is considerable evidence that this peptide plays an important role in the physiological regulation of arterial pressure. There is also evidence that vasopressin may be involved in the pathogenesis of some forms of hypertension, although this point is still controversial. However, it is unlikely that vasopressin contributed in a major way to the development of hypertension in the present study because plasma vasopressin concentration did not increase significantly until the maximum increase in blood pressure had already occurred. Furthermore, there was only a weak correlation between plasma vasopressin concentration and mean arterial pressure ($r = 0.37, p < 0.002$). Pullan et al. also concluded that it is unlikely that vasopressin plays a significant direct pressor role in the pathogenesis of one-kidney Goldblatt hypertension in the dog. The present results further demonstrate that the acute antihypertensive action of captopril is not mediated via inhibition of vasopressin secretion. However, reduction in plasma vasopressin concentration may become more significant in blood pressure regulation during chronic captopril treatment.

There is little information concerning possible interactions between the renin-angiotensin system and the pituitary-adrenal axis in hypertension. Recently Atlas and associates reported that plasma cortisol concentration at 0800 hours was higher in patients with high renin essential hypertension or renovascular hypertension than in patients with normal renin or low renin forms of hypertension. In the 36 patients studied, there was a significant correlation between plasma cortisol concentration and the log of PRA. Treatment with captopril caused a reduction in plasma cortisol concentration in the patients with high renin but not in the normal or low renin groups. Angeli et al. also measured plasma cortisol concentration in six patients with essential hypertension. Analysis of their data reveals a significant correlation between PRA and plasma cortisol concentration ($r = 0.91, p < 0.01$). They reported that plasma cortisol concentration did not change significantly during treatment with captopril. Examination of their data, however, indicates that cortisol concentration did decrease in the three patients with high renin hypertension. Taken together, these observations indicate that in high renin forms of human hypertension, the renin-angiotensin system interacts with the pituitary-adrenal axis to influence the secretion of cortisol.

On the other hand, the present studies failed to reveal an interaction between the renin-angiotensin system and glucocorticoid secretion during the development of renal hypertension in dogs. Plasma corticosteroid concentration failed to increase even when PRA and plasma AII concentration had increased tenfold above their normal values. Acute blockade of the renin-angiotensin system with captopril or saralasin did not decrease corticosteroid concentration and, in some animals, corticosteroid levels actually increased, probably because of the fall in arterial pressure.

In summary, these results indicate that there may be an interaction between the renin-angiotensin system and vasopressin but not between the renin-angiotensin system and the pituitary-adrenal axis in the development of renal hypertension in dogs. The results also indicate that the antihypertensive effect of captopril in this experimental model is not mediated via suppression of vasopressin, ACTH, or corticosteroid secretion.

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