The Renin-Angiotensin System and Aldosterone Secretion during Sodium Depletion in the Rat

By William S. Spielman and James O. Davis

ABSTRACT

The role of the renin-angiotensin system in the control of aldosterone secretion was studied in the sodium-depleted rat. Administration of angiotensin II produced a significant increase in aldosterone secretion and arterial blood pressure in normal rats; simultaneous infusion of the angiotensin analogue, 1-sarcosine-8-alanine-angiotensin II, blocked both the pressor and the steroidogenic actions of angiotensin. Since the angiotensin II analogue was effective in blocking exogenous angiotensin II, an attempt was made to block endogenously formed angiotensin II in the sodium-depleted rat; infusions of large doses of the analogue produced a significant fall in arterial blood pressure, but aldosterone secretion failed to change. Bilateral nephrectomy also failed to decrease aldosterone secretion in the sodium-depleted rat even though arterial blood pressure fell. Since the secretion of corticosterone in these rats was high, it seemed likely that the failure of aldosterone secretion to fall resulted from an overriding influence of adrenocorticotropic hormone (ACTH). To test this hypothesis, the renin-angiotensin system was again blocked in sodium-depleted rats with three levels of anterior pituitary function. With high or intermediate rates of corticosterone secretion, a nonapeptide converting enzyme inhibitor (CEI) failed to influence aldosterone secretion. However, when the influence of ACTH was completely eliminated by hypophysectomy in sodium-depleted rats, the nonapeptide CEI produced a striking fall in aldosterone secretion. In contrast, arterial blood pressure was significantly reduced by CEI in rats with all three levels of anterior pituitary function. The data suggest a role for angiotensin II in the regulation of aldosterone secretion and the maintenance of arterial blood pressure in the sodium-depleted rat.

KEY WORDS  
angiotensin II  
angiotensin II analogue  
corticosterone secretion  
deoxycorticosterone secretion

Aldosterone secretion by the adrenal cortex is influenced by at least four important factors: the renin-angiotensin system, adrenocorticotropic hormone (ACTH), the plasma concentration of sodium, and the plasma concentration of potassium (1-3). However, considerable controversy exists concerning the relative importance and the possible interrelationships of these controlling factors. Extensive data in man, dog, and sheep indicate parallel activation of the renin-angiotensin system and aldosterone secretion by sodium depletion and suggest that elevated angiotensin II levels are primarily responsible for the stimulation of aldosterone secretion in the sodium-depleted animal (1, 4, 5). However, in the rat, although a response of increased aldosterone secretion to sodium depletion is accompanied by increased activity of the renin-angiotensin system (6-8), the question has been raised as to the role of angiotensin II in hyperaldosteronism. Even though angiotensin II is a potent stimulator of aldosterone in man (9, 10), dog (11-14), sheep (15), rabbit (16), and opossum (17), its role in the rat has been questioned (18-20).

Recently, several blocking agents for the renin-angiotensin system have become available (21). The most useful types of blocking agents act as specific competitive antagonists of angiotensin II or block the action of the converting enzyme and thus inhibit the formation of angiotensin II. Since the activity of the renin-angiotensin system is elevated in the sodium-depleted rat (6-8), it was reasoned that administration of angiotensin inhibitors might decrease any event stimulated by endogenous angiotensin II.
otensin II. The present study was undertaken to evaluate the role of the renin-angiotensin system in the sodium-depleted rat and to determine whether the increased plasma renin activity is a concurrent phenomenon or a causative factor in the control of aldosterone secretion.

In a preliminary study (22), the angiotensin analogue, 1-sarcosine-8-alanine-angiotensin II (1-Sar-8-Ala-angiotensin II), lowered arterial blood pressure in the sodium-depleted rat but had no effect on the secretion of adrenal steroids. The data were interpreted as a dissociation in the response of the peripheral arteriolar receptors and the receptors of the zona glomerulosa. However, the secretion of corticosterone in these rats was high, indicating an increased secretion of ACTH. It was hypothesized that the angiotensin II analogue was ineffective in lowering adrenal steroid secretion because the increased ACTH level maintained an elevated secretion rate of aldosterone even when the adrenal cortex receptors for angiotensin II were blocked. To investigate this hypothesis, rats with three levels of anterior pituitary function were studied to evaluate the influence of ACTH or other hormones from the adenohypophysis. In these studies, a nonapeptide converting enzyme inhibitor (CEI) was used to prevent the formation of angiotensin II. By this experimental design, it was possible to separate the influence of the anterior pituitary from that of the renin-angiotensin system.

**Methods**

For collection of adrenal venous blood, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). The trachea was intubated with polyethylene tubing to facilitate respiration and airway clearance. The carotid artery was cannulated, and arterial blood pressure was measured with a Statham P23Db strain gauge and a Hewlett-Packard model 7702B recorder. A catheter (P.E. 90) was inserted into the left femoral vein and advanced retrograde through the inferior vena cava to the level of the left renal vein. The abdomen was opened to allow visualization and manipulation of the catheter tip into the left adrenal vein. At this time, the rat was given 0.2 ml of aqueous heparin (1,000 units/ml). The tip of the catheter was advanced as far as possible into the adrenal vein beyond the entrance of the adrenal vein tributaries. The left renal vein and kidney were not disturbed by the surgical procedure. During periods of adrenal venous blood collection, maintenance of blood pressure was achieved by infusion of whole blood from a donor rat, volume for volume, through a catheter in the external jugular vein. All rats were kept on a heating pad throughout the experiment, and rectal temperature was monitored. Rats were given dexamethasone phosphate (1.0 mg/kg) (Decadron, Merck, Sharp & Dohme) 2-4 hours prior to the experiment unless otherwise indicated; this amount of dexamethasone is four times the dose reported to result in complete suppression of ACTH release during pentobarbital anesthesia in rats subjected to an abdominal incision (23).

Following the timed collection of approximately 2.5 ml of whole blood from the adrenal vein, the exact volume was recorded, the sample was centrifuged at 4°C, and the plasma was stored at −20°C. The adrenal venous plasma concentrations of aldosterone, corticosterone, and, in some experiments, deoxycorticosterone were measured by the double isotope derivative method of Kliman and Peterson (24). Adrenal steroid secretion rates were calculated as the product of adrenal venous plasma flow and the concentration of the steroid in the effluent adrenal plasma. Blood samples for determination of plasma electrolyte concentrations and hematocrit were taken from the adrenal vein catheter. Plasma sodium and potassium concentrations were measured by flame photometry.

**EXPERIMENT 1: ADRENAL STEROID SECRETION IN NORMAL AND SODIUM-DEPLETED RATS GIVEN DEXAMETHASONE**

Male Sprague-Dawley rats (250-350 g) were divided into two groups: control (N = 21) and sodium-depleted (N = 18). All rats were housed individually in metabolism cages to measure their urinary excretion of sodium and potassium. Fecal electrolytes were not determined because, in the rat, excretion of sodium and potassium in the feces contributes only slightly to overall balance (25). The control rats were fed a normal laboratory rat chow (Purina) for at least 4 days, and the sodium-depleted rats received a low-sodium diet with a normal potassium content (Hartroft Formula, General Biochemicals) and distilled water ad libitum for 7-10 days. Daily electrolyte intake for the rats on the low-sodium diet was 0.042 ± 0.002 mEq of sodium and 4.05 ± 0.12 mEq of potassium compared with 2.89 ± 0.18 mEq of sodium and 4.90 ± 0.52 mEq of potassium for the rats maintained on the normal diet. Sodium balance was negative for the first 2-3 days in rats on the low-sodium diet; after 3 days sodium excretion approximately equaled sodium intake. Potassium balance in the rats on the low-sodium diet was essentially the same as that in the rats on the normal diet. A sample of adrenal venous blood was collected for measurement of steroids.

**EXPERIMENT 2: EFFECTS OF NEPHRECTOMY ON ADRENAL STEROID SECRETION IN SODIUM-DEPLETED RATS GIVEN DEXAMETHASONE**

Six rats were sodium-depleted as described for experiment 1. Following collection of a control sample of adrenal venous blood, the catheter was withdrawn from the adrenal vein to allow the adrenal effluent to flow directly into the circulation. The rats were then bilaterally nephrectomized. Ninety minutes after nephrectomy, the catheter was reinserted into the adrenal vein to collect the second blood sample for steroid analysis.

**EXPERIMENT 3: EFFECTS OF SYNTHETIC ANGIOTENSIN II ON ADRENAL STEROID SECRETION IN RATS GIVEN DEXAMETHASONE**

Eleven rats maintained on a normal-sodium diet were prepared for study as previously described. A control blood sample was taken while saline was infused at 0.014 ml/min. Following the control sample, the catheter was withdrawn from the adrenal vein, and the saline was...
replaced with synthetic angiotensin II* in normal saline infused at 1.0 μg/0.014 ml min⁻¹. The synthetic angiotensin II was infused for 30 minutes prior to beginning the collection of the second steroid sample, and the infusion was maintained for the duration of the sampling period (10-20 minutes).

EXPERIMENT 4: EFFECTS OF SYNTHETIC ANGIOTENSIN II AND ITS ANALOGUE, 1-SAR-8-ALA-ANGIOTENSIN II, ON STEROID SECRETION IN RATS GIVEN DEXAMETHASONE

Eight rats were treated and prepared exactly as described in experiment 3. However, 1-Sar-8-Ala-angiotensin II (10 μg/kg min⁻¹) was simultaneously infused with the synthetic angiotensin II (1.0 μg/min). Both peptides were dissolved in normal saline and delivered at a rate of 0.014 ml/min. Collection and infusion times were the same as those described for experiment 3.

EXPERIMENT 5: EFFECTS OF THE ANGIOTENSIN ANALOGUE, 1-SAR-8-ALA-ANGIOTENSIN II, IN SODIUM-DEPLETED RATS

Series 1.—To investigate the effect of 1-Sar-8-Ala-angiotensin II on arterial blood pressure in sodium-depleted and normal rats, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and catheters were placed in the carotid artery and the jugular vein. Blood pressure was recorded for approximately 30 minutes to allow the rat to stabilize before the experiment. A 15-minute control period was followed by infusion of 1-Sar-8-Ala-angiotensin II (10 μg/kg min⁻¹) in normal saline at a rate of 0.014 ml/min for 30 minutes. At the end of 30 minutes, the infusion was stopped and a 45-minute recovery period was allowed. During the control and recovery periods, the saline diluent was infused at 0.014 ml/min.

Series 2.—The rats were sodium-depleted, divided into two groups, and prepared for adrenal vein cannulation as described earlier in this paper; all rats were treated with dexamethasone. The control group was infused with normal saline at a rate of 0.014 ml/min for 60 minutes before the adrenal venous blood samples were collected. The experimental group was infused for 60 minutes with 1-Sar-8-Ala-angiotensin II in saline (10 μg/kg min⁻¹) at a rate of 0.014 ml/min. The infusions of saline and the angiotensin analogue were maintained for the duration of the collection period.

EXPERIMENT 6: EFFECTS OF THE NONAPEPTIDE CONVERTING ENZYME INHIBITOR IN THE SODIUM-DEPLETED, DEXAMETHASONE-TREATED RAT

Ten rats were sodium-depleted and prepared for adrenal vein cannulation as described previously. Following the collection of a control adrenal venous blood sample, the nonapeptide CEI (2.0 mg/kg in 0.1 ml saline) was administered intravenously every 15 minutes until the second sample was collected; collection was begun 30 minutes after the start of CEI administration. To check the effectiveness of CEI in blocking the conversion of angiotensin I to angiotensin II, the rats were given 200 ng of angiotensin I intravenously at the end of the experiment; a pressor response failed to occur in all rats.

EXPERIMENT 7: EFFECTS OF THE NONAPEPTIDE CONVERTING ENZYME INHIBITOR IN THE SODIUM-DEPLETED, DEXAMETHASONE-MORPHINE TREATED RAT

Ten rats were sodium-depleted and treated with dexamethasone as described previously. Ten minutes after the administration of sodium pentobarbital (40 mg/kg, ip), morphine sulfate (1.25 mg/100 g body weight, im) was administered. The collection of adrenal venous blood samples, the administration of the nonapeptide CEI, and the effectiveness of CEI in preventing angiotensin II formation were the same as they were in experiment 6.

EXPERIMENT 8: EFFECTS OF THE NONAPEPTIDE CONVERTING ENZYME INHIBITOR IN THE SODIUM-DEPLETED HYPOPHYSECTOMIZED RAT

This experiment was identical to experiments 6 and 7 except that nine rats were hypophysectomized 2-4 hours before the experiment was begun and dexamethasone and morphine were not given. Cortisone acetate (2.0 mg, im) was administered at the time of hypophysectomy to maintain arterial blood pressure. Hypophysectomy was performed through the external ear canal with a stereotaxic device (Hoffman-Reiter hypophysectomy apparatus) under light ether anesthesia. Following hypophysectomy, the rats were allowed to recover from the ether anesthesia for 2-4 hours before sodium pentobarbital was administered and the study of the effect of the nonapeptide CEI on steroid secretion was begun. Completeness of hypophysectomy was determined by the rate of corticosterone secretion.

Results

EXPERIMENT 1

The results of experiment 1 are presented in Table 1. After 7-10 days on a low-sodium diet, the sodium-depleted rats had a cumulative negative sodium balance of 1.91 ± 0.15 mEq. The secretion of aldosterone by the sodium-depleted rats was markedly elevated (P < 0.001) compared with that of the rats on the normal-sodium diet. No significant differences were observed between the two groups in secretion of corticosterone, adrenal plasma flow, mean arterial blood pressure, or plasma concentrations of sodium and potassium.

EXPERIMENT 2

The response to bilateral nephrectomy in the sodium-depleted rat is presented in Table 1. The secretion rates of aldosterone and corticosterone were very high 90 minutes after nephrectomy, but they were not significantly different from the rates in sodium-depleted rats with their kidneys intact. The high secretion rate of corticosterone indicates that dexamethasone was ineffective in blocking ACTH release. Following nephrectomy, a significant decrease (P < 0.01) was observed in

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1 The term synthetic angiotensin II refers to 5-valine-angiotensin II (Hypertensin, CIBA) unless otherwise specified.


3 Generously supplied by E. R. Squibb and Company.

4 The hypophysectomy apparatus was furnished by Dr. W. P. Palmore, Department of Physiology, School of Veterinary Medicine, University of Missouri, Columbia, Missouri.
TABLE 1
Effects of Sodium Depletion, Nephrectomy, Angiotensin II, and 1-Sar-8-Ala-Angiotensin II on Steroid Secretion and Blood Pressure in the Rat

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition</th>
<th>Aldosterone secretion (ng/min)</th>
<th>Corticosterone secretion (ng/min)</th>
<th>Adrenal plasma flow (ml/min)</th>
<th>Mean arterial blood pressure (mm Hg)</th>
<th>Plasma sodium concentration (mEq/liter)</th>
<th>Plasma potassium concentration (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal diet</td>
<td>0.66 ± 0.06</td>
<td>974 ± 62</td>
<td>0.12 ± 0.01</td>
<td>132 ± 5</td>
<td>141 ± 0.7</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sodium-depleted diet</td>
<td>7.70 ± 0.97*</td>
<td>983 ± 115</td>
<td>0.11 ± 0.01</td>
<td>128 ± 4</td>
<td>138 ± 0.7</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>Sodium-depleted diet + nephrectomized</td>
<td>8.40 ± 2.60</td>
<td>1478 ± 217</td>
<td>0.19 ± 0.02</td>
<td>138 ± 8</td>
<td>0.63 ± 0.10</td>
<td>9.00 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>Normal diet</td>
<td>1.00 ± 0.20</td>
<td>1203 ± 115</td>
<td>0.13 ± 0.02</td>
<td>131 ± 6</td>
<td>141 ± 0.7</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Normal diet + All</td>
<td>1.70 ± 0.20*</td>
<td>1165 ± 127</td>
<td>0.11 ± 0.01</td>
<td>194 ± 6*</td>
<td>138 ± 1.0</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>Normal diet</td>
<td>0.88 ± 0.30</td>
<td>1115 ± 141</td>
<td>0.12 ± 0.01</td>
<td>128 ± 6</td>
<td>143 ± 0.5</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Normal diet + All</td>
<td>0.63 ± 0.10</td>
<td>1040 ± 101</td>
<td>0.14 ± 0.01</td>
<td>117 ± 8</td>
<td>141 ± 0.8</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>Sodium-depleted diet + saline</td>
<td>9.00 ± 1.40</td>
<td>763 ± 119</td>
<td>0.09 ± 0.01</td>
<td>130 ± 7</td>
<td>138 ± 1.4</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sodium-depleted diet + 1-Sar-8-Ala-AII</td>
<td>7.60 ± 2.00</td>
<td>755 ± 148</td>
<td>0.10 ± 0.02</td>
<td>112 ± 10†</td>
<td>138 ± 1.0</td>
<td>3.7 ± 0.2</td>
</tr>
</tbody>
</table>

* P < 0.01.
† P < 0.05.

mean arterial blood pressure, although adrenal plasma flow was unchanged. In this experiment, plasma electrolyte concentrations were determined only at the end of the postnephrectomy procedures. Plasma sodium was significantly decreased (P < 0.05) and plasma potassium was significantly elevated (P < 0.05) compared with the values for the sodium-depleted rats in experiment 1. The elevated plasma potassium concentration probably reflected the fact that the rats had been anephric for approximately 2 hours prior to collection of the sample used for determination of plasma electrolytes.

EXPERIMENT 3
Infusion of synthetic angiotensin II resulted in a significant increase in the secretion rate of aldosterone (P < 0.005) and a marked rise in mean arterial blood pressure (P < 0.001) (Table 1). No significant change was observed in the secretion of corticosterone, the rate of adrenal plasma flow, or the plasma concentrations of sodium and potassium (Table 1).

EXPERIMENT 4
When the angiotensin analogue was infused simultaneously with synthetic angiotensin II, no difference was observed in the secretion rate of aldosterone or the mean arterial blood pressure (Table 1). These results indicate that the angiotensin II analogue was effective in blocking the steroidogenic and the pressor activity of synthetic angiotensin II observed in the previous experiment. Also, no significant differences were observed in any of the other parameters (Table 1).

EXPERIMENT 5
Series 1.—The effect of angiotensin analogue (10 µg/kg min⁻¹) on mean arterial blood pressure in five sodium-depleted and five normal rats is shown in Figure 1. In the normal rats, following a 15-minute control period, the angiotensin analogue...
produced small but consistent decreases in mean arterial blood pressure (4.5 ± 2.0 mm Hg, \( P < 0.05 \)). The infusion of 1-Sar-8-Ala-angiotensin II into sodium-depleted rats resulted in a decrease of 22.0 ± 4.2 mm Hg (\( P < 0.05 \)). The decrease observed in the sodium-depleted rats was significantly greater (\( P < 0.01 \)) than that observed in the normal control rats. During a recovery period, mean arterial blood pressure returned to the control level in both groups.

Series 2.—The steroid response of sodium-depleted rats given dexamethasone to infusions of saline (\( N = 5 \)) or of 1-Sar-8-Ala-angiotensin II (\( N = 6 \)) is presented in Table 1. The rates of aldosterone and corticosterone secretion were both very high, but they were not significantly different in sodium-depleted rats infused with the angiotensin analogue compared with those in sodium-depleted rats infused with saline. Mean arterial blood pressure was significantly decreased in the group infused with the angiotensin analogue (\( P < 0.05 \)). No differences were observed in the rate of adrenal plasma flow or in the plasma concentrations of sodium and potassium between the two groups.

EXPERIMENT 6

The results of experiment 6 are presented in Figure 2 and Table 2. The nonapeptide CEI had no effect on the secretion of aldosterone or corticosterone in this group of sodium-depleted rats. The mean arterial blood pressure was significantly reduced (\( P < 0.01 \)) by the nonapeptide CEI, but adrenal plasma flow was significantly increased (\( P < 0.01 \)). No changes were observed in the concentrations of plasma sodium or potassium.

EXPERIMENT 7

The results of experiment 7 are presented in Figure 2 and Table 2. The secretion of corticosterone during the control period in this group of sodium-depleted rats was significantly lower (\( P < 0.05 \)) than that observed in the sodium-depleted rats which did not receive morphine in experiment 6. This suppression of corticosterone production indicates that the dexamethasone-morphine regimen was, at least, partially effective in blocking ACTH release. Administration of the nonapeptide CEI failed to produce a significant change in the secretion of aldosterone, corticosterone, or deoxycorticosterone. Mean arterial blood pressure fell significantly (\( P < 0.01 \)) after CEI was given. Adrenal plasma flow was significantly elevated (\( P < 0.01 \)), but plasma electrolyte concentrations were unaltered. Although no significant change was observed in the secretion of aldosterone for the group, inspection of the data from individual rats revealed that in four of the ten rats a drop in aldosterone secretion was suggested.

EXPERIMENT 8

The results of experiment 8 are presented in Figure 2 and Table 2. Following acute hypophysectomy, the secretion of aldosterone in sodium-depleted rats (4.8 ± 0.8 ng/min) was significantly decreased (\( P < 0.05 \)) compared with that in sodium-depleted rats with their pituitaries intact (7.7 ± 0.97 ng/min, Table 1, experiment 1). However, the secretion of aldosterone was still significantly higher (\( P < 0.05 \)) in the hypophysectomized, sodium-depleted rat (4.8 ± 0.8 ng/min).
than it was in normally fed rats (0.66 ± 0.06 ng/min). Therefore, even without the anterior pituitary, aldosterone secretion was maintained at an elevated level, indicating the involvement of an extrahypophysial factor or factors in the stimulation of aldosterone.

To block the effects of the renin-angiotensin system, the nonapeptide CEI was administered. Thirty minutes later, the secretion of aldosterone was significantly reduced (P < 0.01). The secretion of corticosterone was also decreased (P < 0.05), but the secretion of deoxycorticosterone was unchanged. Mean arterial blood pressure fell (P < 0.01) with the administration of CEI, but the adrenal plasma flow and the plasma concentrations of sodium and potassium were unaffected by CEI.

### Discussion

An alteration in sodium balance, either by dietary sodium restriction or diuretic-induced loss of body sodium, is a potent and well-known stimulus for aldosterone secretion. In most mammals studied including man, the control of aldosterone secretion secondary to alterations in sodium balance is considered to be mediated by the renin-angiotensin system (1, 25). Although the evidence for a renin-angiotensin-aldosterone pathway in the maintenance of normal sodium balance is convincing in most species studied to date, the results obtained in the rat have been conflicting. Numerous studies have indicated that the sodium-depleted rat has increased activity of the renin-angiotensin system. In 1953, it was reported (26) that rats maintained on a low-sodium diet demonstrated hyperplasia of the juxtaglomerular cells. In addition, there is convincing evidence (27) that the renal juxtaglomerular cells secrete renin, which is an important factor in the regulation of aldosterone secretion.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Experiment 6 (Dexamethasone)</th>
<th></th>
<th>Experiment 7 (Dexamethasone + morphine)</th>
<th></th>
<th>Experiment 8 (Hypophysectomized)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CEI</td>
<td>Control</td>
<td>CEI</td>
<td>Control</td>
<td>CEI</td>
</tr>
<tr>
<td>Aldosterone secretion (ng/min)</td>
<td>6.7 ± 0.8</td>
<td>7.6 ± 0.8</td>
<td>3.7 ± 0.8</td>
<td>3.7 ± 1.1</td>
<td>4.8 ± 0.8</td>
<td>2.7 ± 0.6*</td>
</tr>
<tr>
<td>Corticosterone secretion (ng/min)</td>
<td>843 ± 109</td>
<td>915 ± 85</td>
<td>162 ± 56</td>
<td>285 ± 86</td>
<td>33 ± 8</td>
<td>22 ± 5†</td>
</tr>
<tr>
<td>Deoxycorticosterone secretion (ng/min)</td>
<td>11.6 ± 6.1</td>
<td>18.2 ± 7.6</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>127 ± 4</td>
<td>116 ± 6*</td>
<td>129 ± 6</td>
<td>105 ± 6*</td>
<td>122 ± 6</td>
<td>108 ± 7*</td>
</tr>
<tr>
<td>Adrenal plasma flow (ml/min)</td>
<td>0.095 ± 0.009</td>
<td>0.142 ± 0.013*</td>
<td>0.081 ± 0.008</td>
<td>0.124 ± 0.014*</td>
<td>0.096 ± 0.009</td>
<td>0.106 ± 0.014</td>
</tr>
<tr>
<td>Plasma sodium concentration (mEq/l)</td>
<td>140 ± 0.6</td>
<td>137 ± 0.9</td>
<td>138 ± 1.0</td>
<td>138 ± 1.6</td>
<td>139 ± 0.6</td>
<td>140 ± 1.2</td>
</tr>
<tr>
<td>Plasma potassium concentration (mEq/l)</td>
<td>4.2 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.5 ± 0.3</td>
<td>4.5 ± 0.2</td>
<td>4.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± se. Student’s t-tests were done on paired observations. * P < 0.01. † P < 0.05.
aldosterone and corticosterone secretion following nephrectomy of sodium-depleted, hypophysectomized dogs (28). Using a similar approach, Eilers and Peterson (18) found that no difference in the secretion of aldosterone occurred between sodium-depleted, hypophysectomized rats and nephrectomized, sodium-depleted, hypophysectomized rats. This experiment suggests that the kidney is not involved in the control of aldosterone secretion in the sodium-depleted rat. In experiment 2 of the present study, the response to nephrectomy was evaluated in the same rat with each rat serving as its own control; neither aldosterone nor corticosterone secretion fell after removal of the kidneys. The finding of a high rate of corticosterone secretion indicated an increased secretion of ACTH secondary to surgical stress. The ACTH might have prevented the fall in aldosterone secretion even though the source of renin had been removed by nephrectomy. Indeed, it seems likely from subsequent experiments that this explanation is the correct one, but the effects of nephrectomy have not been studied in unstressed, sodium-depleted rats with a low plasma level of ACTH. Experiment 2 was further complicated because plasma potassium was elevated, and a high concentration of plasma potassium stimulates aldosterone secretion (15, 29, 30).

Although angiotensin II has been clearly shown to stimulate aldosterone secretion in man (9, 10), dog (11-13), and sheep (15), the data supporting this relationship in the rat are conflicting (31). Evidence against an aldosterone-stimulating effect of angiotensin in the rat has been summarized in a monograph by Müller (31) on the in vitro work, and there are several negative reports (18-20) from early in vivo studies. In contrast, stimulation of aldosterone by angiotensin or renin has been demonstrated in vitro (31) and in vivo (32-38). Kinson and Singer (38) reported that angiotensin II stimulated aldosterone output in sodium-depleted rats but not in sodium-repleted rats, suggesting that sodium depletion sensitized the aldosterone-secreting mechanism to angiotensin II, a situation which has also been reported for the dog (39) and human (40).

Because of the conflicting results, the effects of angiotensin II on steroid secretion were restudied. Large doses of angiotensin II were infused into normal rats; a significant increase in aldosterone secretion was observed. Although the dose of angiotensin II was very large, the response was relatively small compared with the aldosterone response to sodium depletion. It has been suggested that 7 days of sodium depletion leads to marked hypertrophy of the zona glomerulosa and that the aldosterone biosynthetic mechanisms are therefore more effective than they are in an adrenal gland stimulated by angiotensin II administration for only 30 minutes. In this regard, Ganong and Boryczka (39) demonstrated that a low-sodium diet for 14 days in dogs increased adrenal sensitivity to exogenous angiotensin II so that an augmented response in aldosterone secretion occurred. In the present study, the secretion rate of corticosterone was unaffected by the infusion of angiotensin II; this finding suggests that ACTH had already maximally stimulated the output of this hormone.

The present data agree with previous reports that large doses of angiotensin II stimulate aldosterone secretion in the rat. Moreover, recent reports (36, 37) have indicated that conscious rats respond to angiotensin II with an increase in aldosterone secretion substantially greater than that seen in anesthetized rats.

To investigate the role of the renin-angiotensin system in the increased aldosterone secretion accompanying sodium depletion, two blocking agents for the renin-angiotensin system were used. The two agents were a synthetic analogue of angiotensin II, 1-Sar-8-Ala-angiotensin II, and a synthetic nonapeptide CEI which inhibits the conversion of angiotensin I to angiotensin II. Recently, the work of Pals et al. (41) has demonstrated that 1-Sar-8-Ala-angiotensin II is an effective competitive antagonist of the pressor effects of angiotensin II in the rabbit and the rat. Similarly, it has been demonstrated that CEI blocks angiotensin I-initiated hemodynamic changes in the dog (42).

We first studied the ability of 1-Sar-8-Ala-angiotensin II to block the steroidogenic action of exogenous angiotensin II on the adrenal cortex of the normal rat; the angiotensin II analogue was infused simultaneously with angiotensin II. The results demonstrated that the angiotensin analogue was effective in blocking the action of angiotensin II at both vascular and adrenal cortex receptors. These results agree with previous reports (43, 44). Since all available data suggest that 1-Sar-8-Ala-angiotensin II is a potent specific competitive inhibitor of angiotensin II in the normal rat, this analogue was used to evaluate the importance of the renin-angiotensin system in the control of aldosterone secretion in the sodium-depleted rat. A secondary part of the study was to investigate the response of arterial blood pressure to the angiotensin analogue.

Infusion of the angiotensin analogue into so-
dium-depleted rats did not change the secretion of aldosterone or corticosterone, but a striking drop in arterial blood pressure occurred. It seems likely that the stress of laparotomy and adrenal vein cannulation stimulated the release of enough ACTH (45, 46) to mask the effect of blocking angiotensin II receptors and thus to prevent a fall in aldosterone secretion. To investigate the hypothesis that increased activity of the anterior pituitary influenced adrenal steroid secretion and prevented manifestation of the loss of activity of angiotensin II, three groups of sodium-depleted rats, each with a different degree of pituitary function, were studied. The agent used to block the renin-angiotensin system in these experiments was the synthetic nonapeptide CEI, which has previously been shown to inhibit the conversion of angiotensin I to angiotensin II (42). The nonapeptide CEI was used instead of the angiotensin analogue because the primary structure of rat angiotensin II is unknown. Therefore, it was possible that, although 1-Sar-8-Ala-angiotensin II effectively blocked the angiotensin II response in experiment 4, endogenous angiotensin II might have resisted blockade because of a different structure. The use of CEI would prevent the formation of endogenous angiotensin II and, therefore, would avoid this difficulty.

In all three sodium-depleted groups (experiments 6–8), administration of the nonapeptide CEI significantly decreased mean arterial blood pressure; this finding confirms reports (5, 47–49) indicating a homeostatic role for angiotensin II in the maintenance of arterial blood pressure. In two of the three groups of rats, administration of CEI also resulted in an increase in adrenal plasma flow. This response in the sodium-depleted rat, in the presence of a decreased arterial blood pressure reflects a decrease in adrenal arteriolar resistance due to blockade of the action of angiotensin II in the adrenal vasculature. The observation suggests that angiotensin II participates in the regulation of adrenal blood flow in the sodium-depleted rat as it does for both adrenal (5) and renal blood flow (50) in the sodium-depleted dog.

No effect of CEI on adrenal steroid secretion was observed in the group of rats given dexamethasone alone or in the group given dexamethasone plus morphine, but both aldosterone and corticosterone secretion fell significantly in the hypophysectomized rats. As pointed out previously, the high ACTH level present in experiments 6 and 7 probably prevented the response to decreased angiotensin II. These observations demonstrate, therefore, that the renin-angiotensin system is a control mechanism in the secretion of aldosterone in the sodium-depleted rat. In addition, the secretion of deoxycorticosterone in the hypophysectomized, sodium-depleted rats was not decreased by the administration of the nonapeptide CEI. This observation agrees with previous reports (51) that deoxycorticosterone is only poorly responsive to stimulation by angiotensin II. Likewise, the parallel fall in aldosterone and corticosterone secretion agrees with many previous reports (1, 2, 11–14, 28, 52) that angiotensin II influences the secretion of both of these steroids.

Although aldosterone secretion fell significantly when CEI was administered to hypophysectomized, sodium-depleted rats, the drop was quantitatively less than that observed in sodium-depleted dogs given the angiotensin analogue (5). One explanation for the failure of aldosterone secretion to fall to a low level in the rat is that some factor other than angiotensin II or ACTH also stimulates aldosterone biosynthesis. Although no changes in plasma concentrations of sodium or potassium were observed, changes in intracellular electrolyte concentrations cannot be excluded. The importance of adrenal intracellular potassium concentration in the control of aldosterone secretion has been suggested by Baumber et al. (53) for the dog, and Boyd et al. (54) have provided convincing evidence that intracellular potassium plays an important role in the response of aldosterone secretion to a number of stimuli in the rat.

Although the present study demonstrates that the renin-angiotensin system is involved in the control of aldosterone secretion in the sodium-depleted rat, the data point to a strong, overriding influence of ACTH in the control of aldosterone secretion in rats laparotomized for cannulation of the adrenal vein. The secretion rate of aldosterone in hypophysectomized, sodium-depleted rats and dexamethasone-morphine-treated, sodium-depleted rats, although still markedly elevated over that in normally fed rats, is decreased compared with that in sodium-depleted rats with their pituitaries intact and unsuppressed. This finding emphasizes the role of the anterior pituitary in the control of aldosterone secretion during sodium depletion in the rat. These data show a striking parallel with results obtained in the sodium-depleted dog (55): the rate of aldosterone secretion in the dog is three times greater when the pituitary is present than it is after hypophysectomy. The importance of the anterior pituitary is further emphasized by the finding that not until the pituitary influence was removed entirely (experiment 8).
did the effect of the renin-angiotensin system become discernible. Therefore, the results show that both the renin-angiotensin system and the anterior pituitary are involved in the control of aldosterone secretion in the sodium-depleted rat.

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The Renin-Angiotensin System and Aldosterone Secretion during Sodium Depletion in the Rat

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