Differential Effects of an Angiotensin II Analogue on Pressor and Adrenal Receptors in the Rabbit

By John M. Steele, Jr. and Jerome Lowenstein

ABSTRACT
The l-sarcosine-8-alanine analogue of angiotensin II (l-Sar-8-Ala-angiotensin II) was infused at 1 and 5 μg/kg min⁻¹ into conscious rabbits on normal or sodium-deficient diets. Blood pressures during the control period were comparable in both groups; plasma renin activity, angiotensin II concentration, and aldosterone concentration were higher in the rabbits on the sodium-deficient diet than they were in the rabbits on the normal diet. The analogue caused a 6-mm Hg fall in mean arterial blood pressure in sodium-depleted rabbits. Plasma renin activity increased in both groups to eight to ten times the control values with the higher rate of infusion. Angiotensin II concentration paralleled plasma renin activity. Plasma aldosterone concentration increased after infusion of the analogue at 1 μg/kg min⁻¹ to three to four times the control values but decreased from these high levels after an additional 90-minute infusion at 5 μg/kg min⁻¹ to only one to two times the control values in both groups. Infusion of angiotensin II during the administration of the analogue caused a clear-cut increase in aldosterone concentration without a change in blood pressure. The persistence of elevated aldosterone levels during inhibitor blockade in the sodium-depleted rabbits does not prove that factors other than angiotensin participate in the aldosterone response to sodium deprivation. However, the data do indicate that l-Sar-8-Ala-angiotensin II is a less effective antagonist of angiotensin II at the adrenal receptors than it is at the vascular smooth muscle receptors and suggest that the pressor and adrenal receptors differ.

KEY WORDS
angiotensin II antagonist
feedback control of renin
aldosterone
plasma renin activity
l-Sar-8-Ala-angiotensin II
des-1-Asp-angiotensin II
anephric rabbit
sodium depletion

The renin-angiotensin system is widely considered to be the physiological regulator of the aldosterone response to changes in sodium balance (1-3). However, studies in various systems including isolated adrenal cell preparations (4-7), sheep (8-10), normal man (11), and anephric man (12, 13) have raised questions about the primacy of the renin-angiotensin system in the control of the aldosterone response to sodium depletion. This question has previously been examined in rabbits actively immunized against angiotensin II (14); although the immunized rabbit is hyporesponsive to both the pressor and the steroidogenic effects of infused angiotensin II, it can nonetheless respond to dietary salt deprivation with an increase in plasma aldosterone concentration comparable to that seen in the normal rabbit.

The recent availability of specific competitive inhibitors of angiotensin II offers a new opportunity to evaluate the role of angiotensin II in the aldosterone response to dietary sodium deprivation. The l-sarcosine-8-alanine analogue of angiotensin II (1-Sar-8-Ala-angiotensin II) described by Pals et al. (15) has been shown to be an effective inhibitor of the pressor effects of angiotensin II both on excised aortic strips (15) and in vivo (16). The effects of this analogue and of closely similar analogues on adrenal steroidogenesis have been less extensively documented. Chiu and Peach (17) have demonstrated that l-Sar-8-Ile-angiotensin II is a specific antagonist of the steroidogenic effect of angiotensin II on a suspension of rabbit capsular adrenal cells. Williams et al. (18) have shown that 1-Sar-8-Ala-angiotensin II is a specific competitive inhibitor of the angiotensin II effect in vitro on strips of rabbit aorta and the zona glomerulosa cells of rat adrenal glands. Johnson and Davis (19) have reported that in dogs the elevated aldosterone secretion rate seen after 6 days of dietary sodium...
restriction undergoes abrupt and reversible suppression during infusion of 1-Sar-8-Ala-angiotensin II. In the present study, we attempted to examine further the role of angiotensin in the aldosterone response to sodium depletion by studying the effects of infusing 1-Sar-8-Ala-angiotensin II into rabbits on a normal diet and rabbits subjected to 10 days of salt deprivation.

**Methods**

Male New Zealand rabbits weighing 2.7–3.9 kg were studied without general anesthesia. The marginal ear vein was cannulated under local lidocaine anesthesia to allow infusion of substances via a pump delivering material at the rate of 1.2 ml/hour, and the central ear artery was cannulated to allow the recording of blood pressure and the collection of blood samples. Blood for measurement of plasma renin activity and angiotensin II concentration was drawn into chilled 2,3-dimercaptopropanol-ethylenediaminetetraacetic acid solution (20); blood for measurement of plasma sodium, potassium, and aldosterone concentration was heparinized. Angiotensin I and angiotensin II were measured by radioimmunoassay of material extracted from plasma by Fuller’s earth (20); plasma renin activity was measured as the amount of angiotensin I generated during a 3- or 4-hour incubation at native pH and 37°C (21). The cross-reactivity of 1-Sar-8-Ala-angiotensin II1 in our assay was less than 0.1%. Aldosterone was measured by radioimmunoassay after methylene dichloride extraction and paper chromatography; the assay was based on the method of Mayes et al. (22). Blood pressure was measured by a Statham pressure transducer and recorded photographically; mean arterial blood pressure was estimated as the diastolic pressure plus one-third of the pulse pressure.

Most rabbits were studied once while they were on their normal pellet diet (Purina Rabbit Chow, sodium content 12.3 mEq/100 g, potassium content 35.5 mEq/100 g) and once after 10 days on a diet of dried rolled oats with a sodium content of 0.24 mEq/100 g and a potassium content of 8.5 mEq/100 g. Furosemide (10 mg, iv) was administered on the seventh day of the sodium-deficient diet. All studies began with a 3-hour control period during which dexamethasone was infused at 40 μg/hour to establish suppression of adrenocorticotropic hormone; all subsequent infusions also contained dexamethasone. In nine rabbits on the normal diet and eight rabbits on the sodium deficient diet, the control period was followed by an infusion of 1-Sar-8-Ala-angiotensin II at 1 μg/kg min⁻¹. In six rabbits on the normal diet and four rabbits on the sodium-deficient diet, this 90-minute infusion period was followed by another 90-minute infusion of the analogue at 5 μg/kg min⁻¹. Four rabbits in each group received a third infusion of 1-Sar-8-Ala-angiotensin II at 5 μg/kg min⁻¹ with 1-Asp-5-Ile-angiotensin II (Schwarz-Mann) added at 0.2 μg/kg min⁻¹. In five rabbits, the 90-minute infusion at 1 μg/kg min⁻¹ was followed by another control period during which dexamethasone alone was continued. In two rabbits, another control period followed the analogue infusion at the higher dose. A separate group of three rabbits on the sodium-deficient diet was studied 24 hours after bilateral nephrectomy; following the control period, these rabbits received 1-Sar-8-Ala-angiotensin II at 1 μg/kg min⁻¹ for 90 minutes. All blood samples were taken within the last 5 minutes of the infusion period. Blood pressures were measured graphically, and the values of mean arterial blood pressure for an entire period were averaged. The statistical significance of observed changes was calculated using the paired t-test (23).

**Results**

**EFFECTS OF SODIUM DEPLETION**

Eleven rabbits were studied both while they were on their normal pellet diet and after 10 days on a sodium-deficient diet (Table 1); an interval of at least 3 weeks elapsed between repeated studies. On the normal pellet diet, 24-hour urinary sodium excretion, which can be taken as an approximation of intake, averaged 14.7 mEq/day (range 12 to 32 mEq/day). During the final 2 days of the sodium-deficient diet, sodium excretion averaged 1.1 mEq/day (range 0.2 to 3.9 mEq/day). Sodium depletion was also manifest by a weight loss that averaged 11.1% of body weight (range 3.9 to 19.5%) and a significant decrease in both plasma sodium concentration and plasma potassium concentration. Plasma renin activity increased approximately threefold during sodium depletion. Plasma angiotensin II concentration increased in six of ten rabbits, but the magnitude of the change was uncertain because many of the values determined during the normal diet were at or below the lower limit of our assay (12–13 pg/ml). Plasma aldosterone concentration increased four- to fivefold during sodium depletion.

**EFFECTS OF INFUSION OF 1-SAR-8-ALA-ANGIOTENSIN II**

The plasma renin activity in both groups of rabbits was increased four- to sixfold by the end of 90 minutes of infusion of 1-Sar-8-Ala-angiotensin II at 1 μg/kg min⁻¹ and about tenfold by the end of the infusion at 5 μg/kg min⁻¹ (Table 2). The responses of the individual rabbits are shown in Figure 1. The progressive increase cannot be interpreted as a dose response, because we cannot be certain that plasma renin activity had reached equilibrium at the end of the first 90 minutes of infusion.

The measured plasma angiotensin II concentrations also increased progressively in both groups.
TABLE 1
Comparison of Rabbits on Normal Diets and after 10 Days on a Sodium-Deficient Diet

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>N</th>
<th>Np*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>3.30</td>
<td>0.13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2.89</td>
<td>0.14</td>
<td>11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Plasma sodium (mEq/liter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>143.1</td>
<td>1.1</td>
<td>9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>D</td>
<td>134.8</td>
<td>1.4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma potassium (mEq/liter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>3.87</td>
<td>0.08</td>
<td>9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>D</td>
<td>2.94</td>
<td>0.08</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Urinary sodium excretion (mEq/day)</strong></td>
<td></td>
<td></td>
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<tr>
<td>R</td>
<td>14.74</td>
<td>1.45</td>
<td>4</td>
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<tr>
<td>D</td>
<td>1.14</td>
<td>0.39</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma renin activity (ng angiotensin I/ml/hour⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>1.03</td>
<td>0.27</td>
<td>8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>D</td>
<td>2.89</td>
<td>0.51</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma angiotensin II (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>30</td>
<td>6</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>D</td>
<td>51</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma aldosterone (ng/100 ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>3.8</td>
<td>0.9</td>
<td>11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>D</td>
<td>18.6</td>
<td>4.3</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

R - Sodium-repleted rabbits (normal diet), D - sodium-depleted rabbits, and NS - not significant.

*Np* - Number of rabbits for which paired measurements were available.

†P - Significance estimated by paired t-test for Np pairs.

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Effect of Infusion of 1-Sar-8-Ala-Angiotensin II

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1-Sar-8-Ala-angiotensin II (1 μg/kg min⁻¹)</th>
<th>1-Sar-8-Ala-angiotensin II (5 μg/kg min⁻¹)</th>
<th>1-Sar-8-Ala-angiotensin II plus angiotensin II (0.2 μg/kg min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean arterial blood pressure (mm Hg)</strong></td>
<td>67.4</td>
<td>66.1</td>
<td>69.5</td>
<td>69.0</td>
</tr>
<tr>
<td>Plasmap renin activity (ng angiotensin I/ml hour⁻¹)</td>
<td>1.03</td>
<td>3.92</td>
<td>8.57</td>
<td>2130</td>
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<tr>
<td><strong>Angiotensin II (pg/ml)</strong></td>
<td>30</td>
<td>52</td>
<td>357</td>
<td>688</td>
</tr>
<tr>
<td>Alldosterone (ng/100 ml)</td>
<td>3.8</td>
<td>11.8</td>
<td>7.9</td>
<td>23.7</td>
</tr>
<tr>
<td><strong>Mean arterial blood pressure (mm Hg)</strong></td>
<td>68.2</td>
<td>65.9</td>
<td>62.1</td>
<td>62.0</td>
</tr>
<tr>
<td>Plasmap renin activity (ng angiotensin I/ml hour⁻¹)</td>
<td>2.89</td>
<td>17.86</td>
<td>28.02</td>
<td>4376</td>
</tr>
<tr>
<td><strong>Angiotensin II (pg/ml)</strong></td>
<td>51</td>
<td>254</td>
<td>509</td>
<td>708</td>
</tr>
<tr>
<td>Alldosterone (ng/100 ml)</td>
<td>18.6</td>
<td>62.2</td>
<td>29.5</td>
<td>75.0</td>
</tr>
</tbody>
</table>

NS - Not significant (P > 0.05).

* Significance estimated by paired t-test of difference from control period. The number of pairs equals the smaller n.

† Significance estimated by paired t-test of difference from preceding period of infusion of 1-Sar-8-Ala-angiotensin II at 5 μg/kg min⁻¹.
Effects of 1-Sar-8-Ala-angiotensin II infusions on plasma renin activity.

(Fig. 2). Mean values at the end of the infusion at 5 μg/kg min⁻¹ were about 10 times the control values for both groups of rabbits, but the wide individual variation and the fact that two rabbits on the normal diet persisted with angiotensin II concentrations below detectable limits made the magnitude of the increase uncertain. As was the case for plasma renin activity, the rabbits on the sodium-deficient diet had generally higher angiotensin II concentrations throughout the experiment. When an angiotensin II infusion was superimposed on the analogue infusion, angiotensin II concentrations rose to values tenfold or more greater than the 150-200 pg/ml measured in normal sodium-repleted rabbits receiving an angiotensin II infusion at a rate (0.012-0.025 μg/kg min⁻¹) sufficient to raise mean arterial blood pressure 8-12 mm Hg (14).

Mean arterial blood pressure was unchanged in the rabbits on the normal sodium diet during 1-Sar-8-Ala-angiotensin II infusion. The sodium-depleted rabbits showed a small drop in mean arterial blood pressure with infusion at 1 μg/kg min⁻¹ and a further small drop during infusion at 5 μg/kg min⁻¹. The decrease in mean arterial blood pressure between the control period and the second infusion period attained statistical significance at the 0.01 level (t-test). There was no blood pressure response to the superimposed infusion of angiotensin II (Table 2) in either group of rabbits, even though infusion of angiotensin II at 0.2 μg/kg min⁻¹ into rabbits not receiving the analogue causes a rise in mean arterial blood pressure of 30-35 mm Hg (14).

Plasma aldosterone concentration increased in all but one of the rabbits on the normal diet during infusion of 1-Sar-8-Ala-angiotensin II at 1 μg/kg min⁻¹ (Fig. 3); the increase averaged threefold. During the infusion at 5 μg/kg min⁻¹, plasma aldosterone fell in all rabbits from the values of the
Effects of 1-Sar-8-Ala-angiotensin II infusions on plasma aldosterone concentration in sodium-repleted rabbits. 1 = 1-Sar-8-Ala-angiotensin II infused at 1 µg/kg min⁻¹, 5 = 1-Sar-8-Ala-angiotensin II infused at 5 µg/kg min⁻¹, 5 + A-II = 1-Sar-8-Ala-angiotensin II infused at 5 µg/kg min⁻¹ plus angiotensin II infused at 0.2 µg/kg min⁻¹ preceding period to values that, although generally greater than those of the control period, were not significantly different from control values (paired t-test). When an angiotensin II infusion was superimposed on the high-dose infusion of the analogue, aldosterone concentrations again increased in all rabbits. In the sodium-depleted rabbits (Fig. 4), although the measured concentrations were in every period about three times greater than they were in the rabbits on the normal diet, the same pattern of response was observed. Aldosterone concentration increased during infusion of 1-Sar-8-Ala-angiotensin II at 1 µg/kg min⁻¹, decreased when the analogue was infused at 5 µg/kg min⁻¹, and increased despite the higher rate when angiotensin II infusion was added to analogue infusion. In seven rabbits, infusion of the analogue at 1 or 5 µg/kg min⁻¹ was followed by a control period during which dexamethasone was continued but no peptide was administered (Fig. 5). Aldosterone concentration fell during the 90 minutes following the cessation of the infusion of 1-Sar-8-Ala-angiotensin II at 1 µg/kg min⁻¹ but increased over the 90 minutes following the cessation of the infusion at 5 µg/kg min⁻¹. Plasma potassium, although significantly lower in the sodium-depleted rabbits, did not vary during the course of any experiment by more than ± 0.1 mEq/liter.

EFFECTS OF 1-SAR-8-ALA-ANGIOTENSIN II IN NEPHRECTOMIZED RABBITS

Control period measurements in three sodium-depleted rabbits 24 hours after bilateral nephrectomy showed low circulating angiotensin II concentrations; the values did not increase during infusion of 1-Sar-8-Ala-angiotensin II at 1 µg/kg min⁻¹ for 90 minutes (Table 3). This finding provides direct evidence that 1-Sar-8-Ala-angiotensin II does not in vivo yield a metabolite capable of reacting with our angiotensin II assay and indicates that the measured increases in angiotensin II in nonnephrectomized rabbits that received the analogue represented neither the analogue nor any metabolite of it. Two rabbits had very low values for plasma renin activity that were likewise unaffected by the analogue infusion; the third rabbit

![Image](http://circres.ahajournals.org/)}
had a plasma renin activity of 1.3 ng angiotensin I/ml hour⁻¹ which increased to 2.4 ng angiotensin I/ml hour⁻¹. This response to the analogue infusion was much less than that seen in the intact rabbits. Plasma aldosterone concentration, however, increased markedly during 1-Sar-8-Ala-angiotensin II infusion in all three rabbits. These nephrectomized rabbits had plasma potassium concentrations (4.2, 4.3, and 4.2 mEq/liter) distinctly higher than those in intact sodium-depleted rabbits (2.9 ± 0.3 mEq/liter) or even those in rabbits on the normal sodium diet (3.9 ± 0.2 mEq/liter), rendering suspect any comparison of the magnitude of the aldosterone response to 1-Sar-8-Ala-angiotensin II in nephrectomized and nonnephrectomized rabbits.

**Discussion**

The administration of 1-Sar-8-Ala-angiotensin II stimulates the release of renin in rabbits on a normal diet and rabbits on a sodium-deficient diet (Figs. 1 and 6) independent of the change in mean arterial blood pressure. Since angiotensin II has been shown to be a direct inhibitor of renin release (24), this response to the analogue demonstrates that it competes successfully with angiotensin II for the intrarenal receptor and thereby diminishes the response of the short feedback control loop to circulating angiotensin II. The demonstration that 1-Sar-8-Ala-angiotensin II stimulates renin release from the isolated kidney perfused with nonrecirculating Krebs-Ringer's solution (25) suggests that the angiotensin involved in the control of renin secretion and with which the analogue presumably competes may be intrarenal rather than circulating.

The absence of any increase in blood pressure shows that, at the doses used, 1-Sar-8-Ala-angiotensin II blocks the pressor effect not only of the induced increase in endogenous angiotensin but also of the much higher concentration subsequently imposed by the angiotensin II infusion, confirming the finding of Pals et al. (15) and Johnson and Davis (19) that this analogue is an effective antagonist of the effects of angiotensin II on vascular smooth muscle. The decrease in blood pressure observed only in the sodium-depleted rabbits confirms the similar finding by Johnson and Davis (19) in sodium-depleted dogs.

The unexpected increase in plasma aldosterone

### TABLE 3

**Effect of Infusion of 1-Sar-8-Ala-Angiotensin II into Sodium-Depleted Nephrectomized Rabbits**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Control period</th>
<th>After infusion for 90 minutes at 1 μg/kg min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma renin activity (ng angiotensin I/ml hour⁻¹)</td>
<td>Angiotensin II (pg/ml)</td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>1.29</td>
<td>20</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>0.10</td>
<td>&lt;13</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>0.25</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean values for intact sodium-depleted rabbits</td>
<td>2.89</td>
<td>51</td>
</tr>
</tbody>
</table>

*Circulation Research, Vol. 35, October 1974*
concentration with the low-dose infusion of 1-Sar-8-Ala-angiotensin II could be due to a direct agonistic effect of the compound itself on the adrenal glands. Direct stimulation of aldosterone synthesis by 1-Sar-8-Ile-angiotensin II in an isolated rat adrenal cell preparation has been reported (17) but only at concentrations 10-1000-fold greater than those required for stimulation by angiotensin II itself. Williams et al. (18), using a very similar preparation and approximately the same molar concentrations, could not demonstrate any stimulation of aldosterone release by 1-Sar-8-Ala-angiotensin II and, in fact, observed some inhibition. The observation in the present study that plasma aldosterone increased following infusion of 1-Sar-8-Ala-angiotensin II into nephrectomized rabbits seems to be clear evidence that this analogue can stimulate aldosterone secretion in the absence of kidneys or measurable angiotensin. However, agonistic action alone does not explain the results in the intact rabbits. An agonistic effect on the adrenal glands should increase with an increase in dose; instead, we found a decrease (Figs. 3, 4, and 6). The fact that this decrease was not due to having in some way exceeded the capacity of the rabbit to respond was shown by the increase that followed superimposition of angiotensin II infusion on the analogue infusion. A possible alternative explanation is that 1-Sar-8-Ala-angiotensin II infused at 1 μg/kg min⁻¹ does not inhibit the action of angiotensin on the adrenal glands and the rise in aldosterone concentration from control values following low-dose infusions is attributable largely to the unopposed or ineffectively opposed stimulatory effect of the increased concentration of endogenous angiotensin that the analogue infusion induces. The decrease in aldosterone that follows the high-dose infusion strongly suggests that 1-Sar-8-Ala-angiotensin II infused at 5 μg/kg min⁻¹ is an effective inhibitor, since at this rate of infusion the analogue can block the adrenal response despite a further increase in endogenous angiotensin. The results of the control infusions following analogue infusion support this explanation (Fig. 5). The decrease in plasma aldosterone concentration following discontinuation of the infusion of the analogue at 1 μg/kg min⁻¹ is consistent with the waning of an unblocked stimulus, whether it is angiotensin II, the analogue itself, or both. It seems reasonable to assume that the concentration of 1-Sar-8-Ala-angiotensin II begins to decrease as soon as the infusion is discontinued; however, since renin continues to generate angiotensin II, in the presence of high plasma renin activity the plasma concentration of angiotensin decreases more slowly than that of the analogue. The increase in aldosterone following the discontinuation of the infusion at 5 μg/kg min⁻¹ suggests that, as the concentration of the inhibiting analogue decreases and the elevated plasma renin activity continues to generate angiotensin, the blockade of the adrenal receptors diminishes and the stimulatory action of angiotensin II is uncovered.

Aldosterone concentration remained well above values associated with sodium repletion when 1-Sar-8-Ala-angiotensin II was administered to sodium-depleted rabbits at a rate (5 μg/kg min⁻¹) that appeared to provide significant inhibition of the adrenal response to angiotensin II. Although this finding might be taken as evidence that factors other than the renin-angiotensin system sustain the aldosterone response to sodium depletion, we feel that this assertion cannot be made without reservation. The failure of aldosterone to decrease to levels associated with sodium repletion during inhibitor blockade may be due to offsetting stimulation of the adrenal glands either by the induced increase in endogenous angiotensin if the blockade is less than complete or by direct stimulation of the adrenal glands by the inhibitor itself. The original question of the role of angiotensin in the aldosterone response to sodium depletion remains unanswered.

Our results are at some variance with those
reported by Johnson and Davis (19). In normal dogs they found that the pressor and the steroidogenic effects of angiotensin II infused at 0.06–0.12 μg/kg min⁻¹ were reversed by 1-Sar-8-Ala-angiotensin II infused at 6 μg/kg min⁻¹. In contrast, in our normal rabbits, 1-Sar-8-Ala-angiotensin II infused at 5 μg/kg min⁻¹ blocked the pressor effect but not the steroidogenic effect of angiotensin II infused at 0.2 μg/kg min⁻¹. Furthermore, in sodium-depleted dogs infused with 1-Sar-8-Ala-angiotensin II at 6 μg/kg min⁻¹ both blood pressure and aldosterone secretion rate decreased significantly (19); in our sodium-depleted rabbits given 1-Sar-8-Ala-angiotensin I at 5 μg/kg min⁻¹, although there was a small fall in mean arterial blood pressure, the plasma aldosterone concentration did not differ significantly from that seen in the control period. Apart from the species difference, which is not known to be important but must inevitably remain suspect, two other differences in these experiments may be significant. The experiments of Johnson and Davis (19) were carried out in anesthetized dogs, and there is evidence (26) that in some species general anesthesia affects the responsiveness of the adrenal glands to angiotensin. The second possibly important difference is in the ratios of antagonist to agonist. At a ratio of 1-Sar-8-Ala-angiotensin II to angiotensin II of 60 to 1, Johnson and Davis (19) found blocking of both the pressor and the steroidogenic effects of infused angiotensin II; at a ratio of 25 to 1, we observed blocking of the pressor effect only. Our finding that infusion of 1-Sar-8-Ala-angiotensin II at 5 μg/kg min⁻¹ effectively blocked the adrenal response to endogenous angiotensin II although infusion at 1 μg/kg min⁻¹ did not suggest that variations in the dose ratio in this range may be critical.

Our findings agree with earlier reports (18) suggesting that vascular and adrenal receptors for angiotensin differ. Although 1-Sar-8-Ala-angiotensin II infused at 1 μg/kg min⁻¹ obviously interferes with the angiotensin II feedback control of renin release and blocks the pressor response to increased endogenous angiotensin, it does not appear to block the steroidogenic effect of the increased angiotensin. At 5 μg/kg min⁻¹ the same disparity between the inhibition of the vascular smooth muscle and the adrenal receptors is evident—a superimposed infusion of angiotensin II that causes no change in blood pressure causes a large increase in plasma aldosterone concentration. The finding by Williams et al. (18) that in vitro 4-Phe-8-Tyr-angiotensin II has no effect on the adrenal response to angiotensin II even in doses much higher than those required to block aortic strip contraction but that 1-Sar-8-Ala-angiotensin II has an angiotensin II-blocking effect in both systems at roughly equal doses offers further support for the theory that the adrenal and the pressor receptors differ in their responses to an angiotensin II stimulus.

The observed differences may be attributed to differences in the steric configuration of the receptors at the two sites or, alternatively, may be explained as a reflection of the functioning of two different agonists. The observation that des-1-Asp-angiotensin II—the 2-8-heptapeptide fragment of angiotensin II that is a normally occurring metabolite (27)—is a potent stimulus to aldosterone production but almost without pressor activity (28) has led to the suggestion (17, 29) that it is the angiotensin molecule normally responsible for adrenal stimulation. The 1-Sar-substituted analogues of angiotensin II are partially protected from N-terminal cleavage by aminopeptidases (30, 31); it is tempting to speculate that 1-Sar-8-Ala-angiotensin II is a less effective blocker of the steroidogenic effect than it is of the pressor effect of angiotensin II because it cannot readily reach adrenal receptors at which des-1-Asp-angiotensin II is a physiologically important agonist. Chiu and Peach (17) have recently reported that aldosterone biosynthesis by an adrenal capsular cell preparation can be stimulated approximately equally well in vitro by either angiotensin II or des-1-Asp-angiotensin II. 1-Sar-8-Ile-angiotensin II inhibits this induced biosynthesis but is effective against des-1-Asp-angiotensin II only at much greater molar ratios, suggesting that the affinity of adrenal receptors for des-1-Asp-angiotensin II is greater than it is for angiotensin II. This observation offers further support for the idea that des-1-Asp-angiotensin II may play a physiological role in the stimulation of aldosterone biosynthesis. Our demonstration that 1-Sar-8-Ala-angiotensin II is a less effective inhibitor of the aldosterone response than it is of the blood pressure response to angiotensin II and our inference that the adrenal and the vascular angiotensin II receptors differ are consistent with this proposed role for des-1-Asp-angiotensin II.

Acknowledgment

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JOHN M. STEELE, Jr. and JEROME LOWENSTEIN

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