Effect of sodium depletion on the steroidogenic and pressor actions of angiotensin in the rat

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Summary

1. To investigate the relative roles of angiotensin II (AII) and des-Asp$^1$-angiotensin II (angiotensin III) in the control of blood pressure and aldosterone release, the effects of seven angiotensin agonists on mean arterial blood pressure and serum aldosterone concentrations were compared in normal and sodium-depleted, conscious rats.

2. In normal rats, angiotensin I, $\alpha$-Asp$^1$-angiotensin II, $\beta$-Asp$^1$-angiotensin II, and angiotensin II-amide were equipotent in elevating mean arterial blood pressure. Angiotensin III, des-Asp$^1$-angiotensin I, and poly-O-acetylserine-angiotensin II were 25%, 25%, and 41% as potent as angiotensin I, respectively. After sodium depletion, pressor responses to these angiotensin peptides were reduced approximately 60-80% when compared with control responses. In contrast, pressor responses to noradrenaline were not significantly affected by sodium depletion.

3. Angiotensin II, $\beta$-Asp$^1$-angiotensin II, angiotensin II-amide, and angiotensin III were equipotent in increasing serum aldosterone concentrations in normal animals. Angiotensin I was 59% and des-Asp$^1$-angiotensin I only 5% as potent as angiotensin II in their abilities to release aldosterone. After sodium depletion, control serum aldosterone concentrations increased as did the slope of the dose-response curve for each angiotensin peptide. Angiotensin II was the most potent steroidogenic peptide in sodium-depleted rats with angiotensin III and $\beta$-Asp$^1$-angiotensin II being 27%, angiotensin I 7%, angiotensin II-amide 3%, and des-Asp$^1$-angiotensin I 1% as potent as angiotensin II in releasing aldosterone. Poly-O-acetylserine-angiotensin II has less steroidogenic effect than angiotensin II or III in both normal and sodium-depleted animals.

4. Infusions of the angiotensin II antagonist, Sar$^1$-Ile$^8$-angiotensin II, and the angiotensin III antagonist, Ile$^7$-angiotensin III, enhanced aldosterone release in normal rats without altering blood pressure. After sodium depletion, Sar$^1$-Ile$^8$-angiotensin II decreased blood pressure without affecting aldosterone release whereas Ile$^7$-angiotensin III diminished aldosterone release without altering blood pressure.

5. These data suggest that angiotensin II, independent of its conversion into angiotensin III, is an important regulator of steroidogenesis in the rat in normal sodium states. In sodium depletion, the octapeptide retains significant steroidogenic activity; however, the contribution of angiotensin III to its steroidogenic effects is increased.

Key words: angiotensin III, des-Asp$^1$-angiotensin I, $\beta$-Asp$^1$-angiotensin II, Sar$^1$-Ile$^8$-angiotensin II, poly-O-acetylserine-angiotensin II, Ile$^7$-angiotensin III.

Abbreviations: AI, angiotensin I; AII, angiotensin II; AIII, angiotensin III.

Introduction

In sodium depletion, the physiological responses to exogenous AII differ from those observed in the normal sodium state. Most notably, the ability of
AII to elevate the blood pressure is suppressed and its ability to stimulate aldosterone release is enhanced (Hollenberg, Chenitz, Adams & Williams, 1974; Oelkers, Brown, Fraser, Lever, Morton & Robertson, 1974; Deheneffe, Cuesta, Briggs, Brown, Fraser, Lever, Morton, Robertson & Tree, 1976; Kinson & Singer, 1968; Campbell, Schmitz & Itskovitz, 1977a). By comparison, angiotensin III (des-Asp'-AII) has less vasopressor activity than AII but equal or greater aldosterone-releasing ability in normal animals and adrenal cell suspensions (Blair-West, Coghlan, Denton, Funder, Scoggins & Wright, 1971; Campbell, Brooks & Pettinger, 1974; Campbell & Pettinger, 1976; Freeman, Davis, Lohmeier & Spielman, 1976; Chiu & Peach, 1974; Peach, Sarstedt & Vaughan, 1976). After sodium depletion, adrenal cells are much more sensitive to AIII than to AII in releasing aldosterone (Peach et al., 1976), but we have shown that exogenous AII is more potent than AIII in raising aldosterone concentrations in the sodium-depleted rat (Campbell et al., 1977a). The nonapeptide des-1-Asp-AI can also produce vasoconstriction and enhance aldosterone release when infused into conscious rats (Campbell, Schmitz & Itskovitz, 1977b; Larner, Vaughan, Tsai & Peach, 1977). However, the vasopressor and steroidogenic effects of des-1-Asp-AI appeared to depend primarily on its conversion in vivo into AIII since its vasoconstrictor and aldosterone-releasing activities were greatly reduced by inhibition of converting enzyme.

We have now studied the roles of AII and AIII in the control of blood pressure and aldosterone release in normal and sodium-depleted states. The physiological effects of AII were compared with those of the other naturally occurring peptides AI, des-Asp1-AI and AIII as well as with three synthetic analogues of angiotensin in normal and sodium-depleted animals. Since AII can be readily converted into AIII by plasma and adrenal aminopeptidases, the synthetic analogues of AII, β-Asp1-AI and poly-O-acetylserynine-AII were selected for these studies because of their resistance to this degradation. These analogues allowed us to examine selectively the physiological effects of the octapeptide molecule. Finally, the physiological role of endogenous angiotensins was studied by using antagonists of AII and AIII.

**Methods**

We studied groups of six male Sprague–Dawley rats (250–300 g) that were placed in individual metabolism cages and kept on a normal sodium diet for 3 days followed by 5 days sodium depletion. On the normal diet, the rats ingested 3.7 ± 0.1 mmol of Na/day and 6.6 ± 0.2 mmol of K/day (Purina Rat Chow: 150 mmol of Na/kg, 270 mmol of K/kg). On the sodium-deficient diet, they ingested 0.14 ± 0.1 mmol of Na/day and 3.2 ± 0.1 mmol of K/day (Nutritional Biochemical Co.: 10 mmol of Na/kg, 220 mmol of K/kg). Frusemide (30 μmol/kg) was administered intraperitoneally during the first day of dietary alteration in the sodium-depleted group. During the first 2 days after dietary alteration, the rats were in negative sodium balance. Thereafter, sodium output matched sodium input. Sodium depletion did not alter potassium balance.

In the studies involving blood pressure measurements, chronic catheters (Weeks, 1973) were placed in the jugular vein of each rat for intravenous injections, and in the descending aorta for measurement of blood pressure 2–5 days before the experiment (Campbell & Pettinger, 1976; Campbell et al., 1977b). Angiotensin I (Beckman), des-Asp1-AI (Campbell et al., 1977b), α-Asp1-AII (Beckman), β-Asp1-AII (Beckman), angiotensin II-amide (Ciba–Geigy), poly-O-acetylserynine-AII (Cleveland Clinic), angiotensin III (Beckman), noradrenaline (Sigma Chemical) or 0.28 mol/l glucose solution was injected intravenously in a constant volume (10 μl) into groups of normal and sodium-depleted rats. The potency of each peptide was determined by the ED_{50} (the dose required to cause a 20 mmHg increase in blood pressure), and compared with that of α-Asp1-AII. In another group of rats, an injection of the angiotensin antagonist Sar1-Ile8-AII (Beckman) (1033 pmol/kg) or Ile2-AIII (Beckman) (1116 pmol/kg) was given subcutaneously followed after 5 min by a 20 min infusion of the same antagonist at a rate of 517 (Sar1-Ile8-AII) or 558 (Ile2-AIII) pmol min⁻¹ kg⁻¹. The control animals were infused with 0.28 mol/l glucose at a rate of 50 μl/min for 20 min. The effects of the peptide on mean arterial blood pressure were monitored with a Narco RP-1500 pressure transducer and a Grass model 7 Polygraph.

In the steroid studies, normal and sodium-depleted groups of rats had chronic catheters implanted into their jugular veins 18 to 24 h before experimental procedures (Campbell & Pettinger, 1976; Campbell et al., 1977b; Weeks, 1973). All subsequent studies were performed without anaesthesia between 08.00 and 12.00 hours to minimize variations in steroid concentrations due to diurnal
rhythm, anaesthetic and surgical stress (Campbell et al., 1974; Campbell & Pettinger, 1976). Control animals were infused intravenously with glucose (0-28 mol/l) at a rate of 50 µl/min for 20 min. Experimental animals were infused at a similar rate with one of the angiotensin polypeptides in glucose solution (0-28 mol/l). The potency of the peptides was assessed in terms of their abilities to increase in serum aldosterone by 60 pmol/dl and compared with that obtained with α-Asp-AII. Immediately after each infusion, the animals were decapitated and aortic blood was collected into plastic tubes in ice. The blood was allowed to clot at 4°C, centrifuged in the cold, and the serum removed. The serum was stored at -20°C until thawed on ice for determination of aldosterone concentrations by radioimmunoassay using a modified method of Gomez-Sanchez, Kem & Kaplan (1973). Since the metabolic clearance rate of aldosterone is not altered by sodium depletion (Bojesen, 1966), changes in serum aldosterone reflected the changes in aldosterone secretory rate.

Statistical differences in responses between each group of rats were assessed by unpaired t-test whereas an analysis of covariance was used for statistical assessment of dose-response curves (Snedecor & Cochran, 1967). Values are given as mean ± SEM.

Results

The mean arterial blood pressure was 108 ± 6 mmHg in normal and 103 ± 12 mmHg in sodium-depleted rats in the control periods. AII and noradrenaline caused dose-related increases in mean arterial blood pressure in both groups of rats (Fig. 1). In normal animals, AII was 50 times more potent than noradrenaline in its ability to increase mean arterial pressure by 20 mmHg. Sodium depletion diminished the pressor activity of AII by 65% (P < 0-01) whereas the pressor activity of noradrenaline was reduced by only 18% (P > 0-1).

AII, AIII, and des-Asp-AI caused dose-related increases in mean arterial pressure in both groups of rats (Fig. 2). With normal salt intake, AII and AIII were equipotent in their ability to increase blood pressure. Significant increases in mean arterial pressure were measured at doses of 3 pmol/kg (P < 0-001). In contrast, AIII and des-Asp-AI were 25% as potent as AII (P < 0-01). After sodium depletion, dose–response curves for each of the angiotension peptides were shifted to the right. Doses of 30 pmol/kg for AII and AII and 300 pmol/kg for AIII and des-Asp-AI were then required to increase blood pressure significantly. In comparison with normal animals, the pressor activity of AII was reduced by 64% (P < 0-01), AII by 66% (P < 0-01). AIII by 85% (P < 0-01), and des-Asp-AI by 79% (P < 0-01) after sodium depletion.

Comparisons of the pressor activities of three synthetic analogues of AII modified at the N-terminal position are shown in Fig. 3, in normal and sodium-depleted rats. β-Asp-AII and AII-amide were equipotent with AII in pressor ability in normal rats, requiring a threshold dose of 3 pmol/kg. AIII was 28% (P < 0-01) and poly-O-acetylserine-AII was 41% (P < 0-01) as potent as AII, requiring threshold doses of 30 pmol/kg to induce significant blood pressure elevations. Whereas sodium depletion significantly reduced the pressor activities of each of these peptides (P < 0-01), their relative potencies were of the same order as in normal animals except for AIII, which was only 11% (P < 0-01) as potent as AII.

In normal rats, AII and AIII increased the serum aldosterone concentration at doses of 3 pmol min⁻¹ kg⁻¹ (P < 0-001, Fig. 4). For des-Asp¹-AI, a dose of 30 pmol min⁻¹ kg⁻¹ was required to attain a significant elevation of serum aldosterone (P < 0-001, Fig. 4). Analysis of the dose–response curves indicates that AII was the most potent for stimulation of aldosterone with AIII being 80% (P < 0-01).

![Fig. 1. Effect of angiotensin II (AII) and noradrenaline (NA) on the change in mean arterial pressure (ΔMAP) in normal and sodium-depleted, conscious rats. Each point represents the mean ± SEM for ten rats. ●, Normal animals; ○, sodium-depleted rats. ***P < 0-001, n = 8.](image-url)
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**FIG. 2.** Effect of angiotensin I (AII, ---), angiotensin II (AII, ---), angiotensin III (AIII, ••••••), and des-Asp-angiotensin I (des-Asp AII, ••••••) on the change in mean arterial pressure (ΔMAP) in normal (a) and sodium-depleted (b) conscious rats. Each point represents the mean ± SEM for eight rats. **P < 0.01; ***P < 0.001.

![Graph 1](image1)

**FIG. 3.** Effect of angiotensin II (---), β-Asp-angiotensin II (---), angiotensin II-amide (••••••), angiotensin III (••••••), and poly-O-acetylserine-angiotensin II (••••••) on change in mean arterial pressure (ΔMAP) in normal (a) and sodium-depleted (b) rats. Each point represents the mean ± SEM for eight rats. **P < 0.01; ***P < 0.001.

![Graph 2](image2)

> 0.1), AII 59% (P < 0.01), and des-Asp-AI 5% (P < 0.001) as potent as AII. After sodium depletion, the control serum aldosterone concentration increased from 7.2 ± 1.1 to 180 ± 14 pmol/dl. Aldosterone responses to angiotensin were enhanced as indicated by significant increases in the slope of the dose-response curves (P < 0.01). As with normal sodium intake, AII and AIII increased serum aldosterone concentrations significantly at a dose of 3 pmol min⁻¹ kg⁻¹ (P < 0.01).
Angiotensins in sodium depletion

700

500

300

100

0

Angiotensin (pmol min^{-1} kg^{-1})

0

3

6

9

12

15

Angiotsins (pmol/dl)

FIG. 4. Effect of angiotensin I (AI), angiotensin II (AII), angiotensin III (AIII), and des-Asp^1-angiotensin I (des-Asp-AI) on aldosterone release in normal (○) and sodium-depleted (○) conscious rats. Each point represents the mean ± SEM for eight rats. **P < 0.01; ***P < 0.001.

whereas a dose of 30 pmol min^{-1} kg^{-1} was required for AI (P < 0.01) and 300 pmol min^{-1} kg^{-1} for des-Asp-AI (P < 0.001). Des-Asp-AI significantly suppressed the serum aldosterone concentrations at the 3 (P < 0.01) and 30 (P < 0.02) pmol min^{-1} kg^{-1} doses in those studies. Sodium depletion increased the steroidogenic potency of AII to a greater extent than the other angiotensin peptides. In sodium depletion, AIII was only 27%, AI 7%, and des-Asp-AI 1% as potent as AII (P < 0.001) in their respective abilities to raise the serum aldosterone concentrations.

As with the naturally occurring angiotensin peptides, the synthetic analogues induced dose-related increases in serum aldosterone concentrations in normal and sodium-depleted rats (Fig. 5). In normal rats, β-Asp-AII, AIII, and AII-amide were equipotent with AII in their abilities to release aldosterone. All four peptides significantly increased serum aldosterone concentrations at the 3 pmol min^{-1} kg^{-1} dose (P < 0.001). After sodium depletion, there were marked differences in the response. The potency of AII increased relative to the other peptides. β-Asp-AII and AIII were next in potency (27% as potent as AII) whereas AII-amide exhibited only 3% the potency of AII for increasing serum aldosterone concentrations in sodium-depleted rats. Angiotensin III significantly increased the aldosterone concentrations at a dose of 3 pmol min^{-1} kg^{-1} (P < 0.05) whereas β-Asp-AII required a dose of 30 pmol min^{-1} kg^{-1} (P < 0.01); however, the dose-response curves for the two peptides did not differ significantly (P > 0.1).

Comparison of the steroidogenic effects of equi-pressor doses of AII (2.9 pmol min^{-1} kg^{-1}), AIII (32 pmol min^{-1} kg^{-1}), and poly-O-acetyls-L-serine-AII (41 pmol min^{-1} kg^{-1}) (Table 1), shows that in both normal and sodium-depleted rats, the poly-O-acetyls-L-serine-AII was significantly less active in stimulating aldosterone release than either AII or AIII (P < 0.05).

Table 2 shows the effects of the AII antagonist (Campbell et al., 1974; Campbell & Pettinger, 1976; Freeman et al., 1976; Chiu & Peach, 1974), Sar^1-Ile^8-AII, and the AIII antagonist (Sarstedt, Vaughan & Peach, 1975; Bravo, Kosia & Bumpus, 1975), Ile^7-AIII, on blood pressure and aldosterone release in normal and sodium-depleted rats. In normal animals, neither antagonist altered the blood pressure significantly at these doses. However, both peptides increased the serum aldosterone concentrations significantly in normal rats (P < 0.05). Ile^7-AIII was more active than Sar^1-Ile^8-AII in this respect. After sodium depletion,
### Table 1. Effect of equipressor doses of angiotensin II, angiotensin III, and poly-O-acetylserine-angiotensin II on serum aldosterone

Values represent the mean ± SEM for seven rats. MAP, Change in mean arterial pressure; * response significantly greater than control (P < 0.01); ** response significantly less than angiotensin II or angiotensin III (P < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Sodium-depleted rats</th>
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<tbody>
<tr>
<td></td>
<td>ΔMAP (mmHg)</td>
<td>ΔMAP (mmHg)</td>
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<tr>
<td>Serum aldosterone</td>
<td>(pmol/dl)</td>
<td>Serum aldosterone</td>
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<tr>
<td>Control</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>Angiotensin II (2:9 pmol min⁻¹ kg⁻¹)</td>
<td>5 ± 1*</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Angiotensin III (32 pmol min⁻¹ kg⁻¹)</td>
<td>8 ± 2**</td>
<td>2 ± 1</td>
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<tr>
<td>Poly-O-acetylserine-angiotensin II (41:0 pmol min⁻¹ kg⁻¹)</td>
<td>7 ± 1*</td>
<td>5 ± 1*</td>
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### Table 2. Effect of angiotensin antagonist on blood pressure and serum aldosterone

Values represent the mean ± SEM for seven rats. MAP, Mean arterial pressure; *P < 0.05; **P < 0.01; ***P < 0.001, compared with control.

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Sodium-depleted rats</th>
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<tbody>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>MAP (mmHg)</td>
</tr>
<tr>
<td>Serum aldosterone</td>
<td>(pmol/dl)</td>
<td>Serum aldosterone</td>
</tr>
<tr>
<td>Control</td>
<td>109 ± 4</td>
<td>108 ± 7</td>
</tr>
<tr>
<td>Sar¹-Ile⁸-AII (517 pmol min⁻¹ kg⁻¹)</td>
<td>109 ± 4</td>
<td>88 ± 8**</td>
</tr>
<tr>
<td>Ile⁷-AIII (558 pmol min⁻¹ kg⁻¹)</td>
<td>108 ± 5</td>
<td>105 ± 3</td>
</tr>
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</table>

Sar¹-Ile⁸-AII decreased the mean arterial blood pressure significantly from 109 ± 4 mmHg to 88 ± 8 mmHg (P < 0.01) whereas Ile⁷-AIII had no significant effect. However, Ile⁷-AIII suppressed the serum aldosterone concentration by 61% (P < 0.01) whereas this dose of Sar¹-Ile⁸-AII did not significantly alter the serum aldosterone concentrations.

### Discussion

We administered seven angiotensin agonists to normal and sodium-depleted rats, and measured changes in blood pressure and serum aldosterone. Qualitatively, the pressor responses of angiotensin were diminished by sodium depletion, whereas the aldosterone release was enhanced. Unlike the angiotensins, the pressor responses to noradrenaline were not significantly altered in normal rats as compared with sodium-depleted rats. This suggests that there is a specific relationship between sodium metabolism and angiotensin vascular responsiveness, apart from general alterations in vascular smooth muscle reactivity or hypovolaemia. This reduced responsiveness to angiotensin after sodium depletion may result from either a reduced affinity of vascular angiotensin receptors (Williams, Hollenberg & Braley, 1976), prior occupancy of the available receptors (Brunner, Chang, Wallach, Sealey & Laragh, 1972; Thurston & Laragh, 1975) or reduction in the number of vascular angiotensin receptors (Chevillotte, Devynck, Fyhrquist, Meyer, Rouzaire-Dubois & Worcel, 1975). The relative roles of these three mechanisms in determining the activity of angiotensin remains to be determined. In the adrenal gland, the enhanced response of aldosterone release to angiotensin after sodium depletion may be related to an increased conversion of corticosterone into aldosterone (Campbell et al., 1977a; Blair-West, Brodie, Coghlan, Denton, Flood, Goding, Scoggins, Tait, Tait, Wintour & Wright, 1970; Muller, 1971), an increase in the number of angiotensin adrenal cortical receptors (Douglas & Catt, 1976) or an enhanced production of AIII (Peach et al., 1976). We have now studied this last-named hypothesis.

Our results in vivo indicate that the pressor response to AII is reduced less by sodium depletion than the response to AIII, while its steroidogenic activity is enhanced more than that of AIII. Peach et al. (1976), using adrenal cell suspensions from normal and sodium depleted rats, observed a different steroidogenic response, finding that AII...
was only one-tenth as potent as AIII in adrenal cell suspensions from sodium-depleted animals, whereas in vivo we found AII to be four times more potent than AIII in stimulating steroidogenesis in similarly sodium-depleted animals. Thus, our studies in vivo indicate that AII functions as the predominant peptide controlling steroidogenesis in sodium depletion, whereas Peach et al. (1976) indicate that AIII exerts this function in vitro. If, on the other hand, adrenal cells in vitro are actually sensitized by sodium depletion to such an extent to the steroidogenic action of AII, our results would indicate that 97% of infused AIII must be inactivated before reaching the adrenal cortical receptors in sodium depletion. This explanation is consistent with a greater reduction in the pressor activity of AIII, as compared with AII (85% for AIII vs 66% for AII), as well as the enhanced inactivation of angiotensin in sodium depletion (Leary & Ledingham, 1970; Johnston, Mendelsohn & Doyle, 1972). These findings would then suggest that the aspartyl group in position 1 of the AII molecule functions to prolong the activity of the peptide by protecting it from the action of circulating aminopeptidases, allowing greater quantities of the active peptide to reach the receptors.

The studies with N-terminally modified analogues of AII further substantiate this contention and provide several perspectives on the relative importance of AII and AIII in states of normal and low sodium. Whereas AII, \( \beta \)-Asp-AII, and AII-amide were equipotent as steroidogenic agents in normal rats, AII was four times more potent than \( \beta \)-Asp-AII and 30 times more potent than AII-amide after sodium depletion. Although these steroidogenic differences may reflect changes in receptor affinity for the various angiotensin agonists after sodium depletion (Williams et al., 1976; Douglas & Catt, 1976), they may also be due to differences in N-terminal metabolism by plasma or tissue aminopeptidases. Since AII can be readily converted into AIII by aminopeptidase A in the plasma and adrenal cortex (Campbell & Pettinger, 1976; Bumpus, Smeyb, Page & Khairallah, 1964; Khairallah, Bumpus, Page & Smeyb, 1963; Regoli, Riniker & Brunner, 1963; Nagatsu, Gillespie, Folk & Glenner, 1965), its pressor and steroidogenic effects must be a result of the combined actions of the octapeptide and heptapeptide molecules. On the other hand, \( \beta \)-Asp-AII is resistant to aminopeptidases, but is metabolized in the adrenal by an endopeptidase (Bumpus et al., 1964; Khairallah et al., 1963; Regoli et al., 1963), which catalyses the formation of peptide fragments which have little steroidogenic activity. Thus, the actions of \( \beta \)-Asp-AII must be attributed primarily to a direct effect of the octapeptide molecule. With this line of reasoning it would appear that AII can stimulate aldosterone release independently of any conversion into AIII, since \( \alpha \)-Asp-AII and \( \beta \)-Asp-AII produced identical steroidogenic effects. After sodium depletion, both peptides retained significant steroidogenic activity; however, \( \alpha \)-Asp-AII was four times more potent than \( \beta \)-Asp-AII. Thus, unlike the normal sodium state, the steroidogenic response to AII in sodium-depleted rats seems to be a combination of the effects of the octapeptide \( \text{per se} \) as well as an additional component provided by the formation of the heptapeptide, AIII. Similarly, coupling poly-O-acetylserine to the N-terminus of AII can also protect the peptide from plasma and adrenal aminopeptidase degradation (Bumpus et al., 1964; Khairallah et al., 1963). In our studies, poly-O-acetylserine-AII was also less potent than AII or AIII in stimulating aldosterone release in both normal and sodium-depleted animals. Unlike \( \beta \)-Asp-AII, however, the interpretation of the effects of poly-O-acetylserine-AII is complicated by steric factors which may be contributed by poly-O-acetylserine. However, our results clearly show that in both normal and low sodium states, AII can stimulate aldosterone release independently of any N-terminal degradation by aminopeptidases. Finally, AII-amide undergoes rapid N-terminal degradation to the hexapeptide, and possibly to the heptapeptide, by plasma aminopeptidases, which differ from those acting on AII (Regoli et al., 1963; Nagatsu et al., 1965; McDonald, Zeitman, Callaghan & Ellis, 1974; Khairallah & Page, 1967). Although the reason for the relatively weak steroidogenic effect of AII-amide is unknown, it may be that increased proportions of heptapeptide and hexapeptide formed from AII-amide are rapidly inactivated on reaching the adrenal, to form smaller fragments similar to circulating AIII.

Others have reported that Sar\(^1\)-AII was more potent than AII or AIII in stimulating aldosterone release in normal animals (Bravo et al., 1976; Fredlund, Saltman & Catt, 1975; Saltman, Fredlund & Catt, 1976; Bravo, Khosla & Bumpus, 1976a, b). Since Sar\(^1\)-AII resists metabolism by aminopeptidase A (Hall, Khosla, Khairallah & Bumpus, 1974), it was concluded that AII had steroidogenic activity independent of its conversion into AIII. Our observations with \( \beta \)-Asp-AII support this conclusion with one additional advantage. Although Sar\(^1\)-AII is not metabolized by aminopeptidase A, it can be converted into AIII by leucine
aminopeptidase (Hall et al., 1974), which is abundant in the plasma and adrenal cortex (Campbell & Pettinger, 1976). Therefore, the possibility that the formation of AIII contributes to its steroidogenic activity cannot be eliminated. In contrast, β-Asp-AII is not metabolized by either of these aminopeptidases (Bumpus et al., 1964; Khairallah et al., 1963; Regoli et al., 1963) so its steroidogenic activity must represent a direct action of the intact octapeptide molecule.

In normal rats, neither Sar1-Ile8-AII nor Ile7-AII affected blood pressure whereas both significantly elevated aldosterone concentrations, demonstrating their partial agonistic properties. After sodium depletion, Sar1-Ile8-AII decreased blood pressure without affecting the elevated aldosterone concentrations; however, Ile7-AIII had an opposite effect, to decrease aldosterone without altering blood pressure. This occurred despite our observation that in the same doses, Sar1-Ile8-AII was more potent than Ile7-AIII, in blocking both the pressor and steroidogenic effects of exogenous AII and AIII in normal rats (Campbell & Schmitz, 1979). Thus in sodium depletion, the AII antagonist was more potent in blocking steroidogenesis and the AII antagonist was more potent in blocking vasoconstriction (Sarstedt et al., 1974; Table 2). These findings give further evidence that AII is a major regulator of aldosterone secretion in sodium depletion, and that AIII participates in this steroidogenic activity.

In summary, our studies indicate that AII functions as a major regulator of aldosterone secretion in normal and sodium-depleted rats, and that the conversion of AII into AIII is not essential for this action. After sodium depletion, the steroidogenic responses to AII are enhanced while its pressor effects are reduced; however, it is unlikely that these effects are due only to an increased conversion of AII into AIII, since the steroidogenic and pressor responses to β-Asp-AII are similarly altered. As AII was four times more potent than β-Asp-AII in stimulating aldosterone release in sodium-depleted rats, and as Ile7-AIII was more effective than Sar1-Ile8-AII in reducing aldosterone release in sodium depletion, some of the steroidogenic effect of AII may be due to its conversion into AIII.

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References


Angiotensins in sodium depletion


