Comparison of Two Angiotensin II Analogues in Normal Subjects and Hypertensive Patients

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Synopsis

Two angiotensin II analogues, i.e., 1-sarcosine, 8-isoleucine angiotensin II (Sar¹, Ile⁸-AII) and 1-sarcosine, 8-alanine angiotensin II (Sar¹, Ala⁸-AII), are now available in the clinical study. Comparative studies of the antagonistic potency and the agonistic effect of these two AII analogues were made in normal subjects on three sodium balances and in hypertensive patients with various etiologies on sodium depletion. Both AII analogues had an agonistic pressor effect in normal subjects. This effect changed with different sodium balances. In the low sodium state, this agonistic action was minimized. The agonistic pressor effect of Sar¹, Ile⁸-AII was greater than that of Sar¹, Ala⁸-AII in all sodium states. There was found an agonistic activity of both AII analogues not only on blood pressure, but also on renin and aldosterone secretion, and renal vasculature in normal subjects on a regular diet. The antagonistic depressor potency of both compounds was also varied by changing sodium balance, being greatest in the low sodium state. In hypertensive patients on sodium depletion, the blood pressure responses of individual patients to these two AII analogues were significantly correlated ($r=0.8$, $n=20$). These results indicate that pretreatment of sodium depletion is necessary to prevent the side effect caused by the agonistic pressor action of AII analogue, and also to predict renin dependency in hypertensive patients efficiently.

The specific competitive inhibitor of angiotensin II, angiotensin II analogue (AIIA), is a useful tool for understanding a role of renin-angiotensin-aldosterone system in the physiological and pathophysiological conditions (Davis et al., 1974; Haber, 1976).

At present two AIIA’s are clinically available: 1-sarcosine, 8-isoleucine angiotensin II have been used in Japan (Ogihara et al., 1974), 1-sarcosine, 8-alanine angiotensin II in western areas (Brunner et al., 1973; Streeten et al., 1976). From the aspect of clinical application of them, it is interesting to compare the pharmacological actions of these two AIIA’s. Furthermore, this structural analogue seems to be useful for the assessment of angiotensin II and angiotensin II-receptor interaction (Williams et al., 1974; Hollenberg et al., 1976). In this study we compared the effects of these two AIIA’s in normal subjects on three sodium balances and also in hypertensive patients with various causes on sodium depletion.

Materials and Methods

Study in normal subjects

Subjects studied were five male volunteers aged 25 to 35 years. The following experiments were made in three different sodium balances, i.e., i) normal sodium state; unrestricted diet, ii) high sodium state; regular diet supplemented with 6 g/day of sodium, and iii) low sodium state; regular diet supplemented with 2 g/day of sodium.
sodium chloride, and iii) low sodium state; sodium-free diet with 40 mg of furosemide given orally on the first day. Experiments were done on the 3rd and 4th day in each sodium state. Urinary sodium excretion (UNaV), plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were monitored each day.

Experiment 1: Sar\(^1\), Ile\(^8\)-AII and Sar\(^1\), Ala\(^8\)-AII were infused at a graded rate from 120 to 2,400 ng/kg/min, respectively. Each dose was infused for 10 min.

Experiment 2: After assessing the rise in mean blood pressure at 30 mmHg above the basal level by angiotensin II infusion, both analogues were then superimposed at the same rate as in Experiment 1.

Experiment 3: Either Sar\(^1\), Ile\(^8\)-AII or Sar\(^1\), Ala\(^8\)-AII at a rate of 600 ng/kg/min, and AII at a rate of 20 ng/kg/min were infused for 30 min. Before and at the end of the infusion PRA, PAC and one-hour creatinine clearance were measured. The effects of these compounds on various parameters were expressed as percent changes following them.

Study in hypertensive patients

Twenty hypertensive patients with various etiologies, essential hypertensive: 11, renovascular hypertension: 3, renoparenchymal hypertension: 3, primary aldosteronism: 3, were studied. All medications were discontinued for at least 2 weeks before the examination except in 3 patients with primary aldosteronism who were on 200 mg/day of spironolactone. On the 4th day after a low sodium diet coupled with 80 mg/day of furosemide for 3 days, Sar\(^1\), Ile\(^8\)-AII and Sar\(^1\), Ala\(^8\)-AII were infused into each patient for 30 min at a rate of 600 ng/kg/min and 1,800 ng/kg/min, respectively. These infusion rates were based on the finding in experiment 2 that the antagonistic effect of Sar\(^1\), Ile\(^8\)-AII was three times more than that of Sar\(^1\), Ala\(^8\)-AII in normal subjects.

Both Sar\(^1\), Ile\(^8\)-AII and Sar\(^1\), Ala\(^8\)-AII were synthesized by Dr. Sakakibara, Peptide Institute, Protein Research Foundation, Osaka, Japan and were processed in injectable forms by Dai-ichi Pharmaceutical Co. Ltd. (Tokyo). Synthetic angiotensin II (Hypertensin, Ciba) and AIIA were diluted with normal saline and infused intravenously with a Harvard infusion pump or a VolumetR (Sigma Motor Co.). Blood pressure was measured every 5 min automatically by Non-Stetho 7R (Parama Co. Ltd., Tokyo). The mean blood pressure was calculated as the diastolic pressure plus one third of pulse pressure. The control blood pressure was taken as the average of at least 5 stable readings before starting the infusion. PRA and PAC were measured by radioimmunoassay using a commercial kit. The evaluation of statistical probability was done by the analysis of variance (Campbell, 1974) and Student’s t test.

Results

Study in normal subjects

The values for UNaV, basal PRA and basal PAC on each sodium balance are summarized in Table 1.

Experiment 1: Sar\(^1\), Ile\(^8\)-AII from 120 to 2,400 ng/kg/min increased in mean blood pressure from 5 to 9 mmHg, 12 to 18 mmHg and 18 to 26 mmHg in low, normal and high sodium states, respectively. The change in mean blood pressure (ΔMBP) by Sar\(^1\), Ala\(^8\)-AII from 0.1 to 10.0 μg/kg/min were from 1 to −7 mmHg, 8 to 15 mmHg and 12 to 18 mmHg in low, normal and high sodium states, respectively (Fig. 1). Sar\(^1\), Ile\(^8\)-AII had a more agonistic pressor action than Sar\(^1\), Ala\(^8\)-AII in the high sodium (P < 0.01), normal sodium (P < 0.01) and low sodium state (P < 0.05).

Experiment 2: Both analogues reduced blood pressure dose-dependently in all

<table>
<thead>
<tr>
<th>Sodium Balance</th>
<th>UNaV (mEq/day)</th>
<th>PRA (ng/ml/hr)</th>
<th>PAC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrestricted state</td>
<td>157 ± 11</td>
<td>1.5 ± 0.4</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>High sodium state</td>
<td>256 ± 24</td>
<td>0.5 ± 0.1</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Low sodium state</td>
<td>18 ± 3</td>
<td>10.4 ± 1.5</td>
<td>223 ± 22</td>
</tr>
</tbody>
</table>

Fig. 1. Agonistic effects of Sar\(^1\), Ile\(^8\)-AII and Sar\(^1\), Ala\(^8\)-AII in three sodium balances.
sodium states (Fig. 2). This antagonistic potency was greater in Sar¹, Ala₈-AII in the high sodium state (P<0.05). In the normal sodium state this effect of Sar¹, Ile₈-AII was three times greater than that of Sar¹, Ala₈-AII (P<0.05). In the low sodium state the depressor action was most striking on both compounds and this potency was not significantly different between two AIIA’s.

Experiment 3: The effect of the 30 min infusion of either Sar¹, Ile₈-AII or Sar¹, Ala₈-AII at a rate of 600 ng/kg/min of blood pressure, PRA, PAC and creatinine clearance was summarized in Fig. 3. The response to either AIIA on various parameters was in the same direction as the response to AII on them. The ΔMBP following to Sar¹, Ile₈-AII and Sar¹, Ala₈-AII were +18±4.5 % (Mean±SE) and +7.0 ±1.1 % respectively, and the difference in these changes was significant between two AIIA’s. The change in PAC was +65±42 % in Sar¹, Ile₈-AII and +26±7.1 % in Sar¹, Ala₈-AII, and that in PRA was -24 ±8.7 % in Sar¹, Ile₈-AII and -20±9.1 % in Sar¹, Ala₈-AII. The reduction of Ccreat. was -12±6.4 % in Sar¹, Ile₈-AII and -8.0±10 % in Sar¹, Ala₈-AII. These values in PAC, PRA and Ccreat. were not statistically different between two AIIA’s.

Study in hypertensive patients

The ΔMBP on the 30-min infusion of Sar¹, Ile₈-AII at a rate of 600 ng/kg/min and Sar¹, Ala₈-AII at a rate of 1,800 ng/kg/min in hypertensive patients on sodium depletion was plotted in Fig. 4. The ΔMBP values of individual patients on infusion of Sar¹, Ile₈-AII and Sar¹, Ala₈-AII were significantly correlated.

Discussion

Both AIIA’s, i.e., Sar¹, Ile₈-AII and Sar¹, Ala₈-AII, had an agonistic pressor action in normal subjects. The pressor action of
Fig. 4. Comparison of mean blood pressure changes by two angiotensin II analogues in hypertensive patients on sodium depletion.

Sar¹, Ile⁸-AII was greater than that of Sar¹, Ala⁸-AII in all sodium balances. The pressor activity of both AIIA's varied with different sodium balances, being minimized by sodium depletion. The antagonistic effect of both AIIA's was also affected by the variations in sodium balances, which was greatest in the low sodium state.

These AIIA's had an agonistic effect on AII receptors other than vascular beds, i.e., renin release, aldosterone secretion and renal vasculature, in normal subjects on regular sodium intake. The extent of the agonistic activity by either AIIA on each AII receptor was different, suggesting that the sensitivity to AIIA of each AII receptor might differ. Furthermore the agonistic effect on peripheral vascular beds was greater in Sar¹, Ile⁸-AII than Sar¹, Ala⁸-AII, but the effect on aldosterone secretion, renin release and renal vasculature was not different between these two AIIA's. These results suggest that AII and AII-receptor interaction on various AII targets might be heterogeneous.

AIIA has been widely used for evaluating a renin dependency in hypertensive patients. This AIIA possesses an agonistic effect due to its intrinsic activity as well as a specifically, competitively, antagonistic effect due to AII, and this agonistic pressor action is undesirable because this compound is given to the patients with high blood pressure. So an agent not only potent in antagonistic action but also devoid of agonistic effect is required from the clinical aspect. Case et al. (1976) reported that the infusion of Sar¹, Ala⁸-AII showed a pressor response in about 50% of hypertensive patients they studied. In our study in which we used Sar¹, Ile⁸-AII we observed a pressor response, particularly, in patients with low renin values such as primary aldosteronism or low-renin essential hypertension (Ogihara et al., 1974).

At present we have few reports in which the effect of these two clinically available AIIA's is compared. Bravo et al. (1976) observed that Sar¹, Ile⁸-AII had a more agonistic pressor action than Sar¹, Ala⁸-AII in the comparative study of Sar¹, Ile⁸-AII and Sar¹, Thr⁸-AII in dogs and they concluded that Sar¹, Thr⁸-AII was the most ideal agent for the clinical purpose, because it had the least pressor activity. In the present comparative study in normal subjects we obtained the same findings as observed by Bravo et al. (1976).

The influence of the variations in sodium balance on the agonistic or antagonistic effect of both AIIA's is similar among them, i.e., reduction of agonistic action or potentiation of antagonistic effect by sodium depletion. The circulating level of AII modifies the sensitivity of the vascular AII receptors to AII (Kaplan and Silah, 1964). Recent studies suggested that the variations in sodium balance changed the affinity of vascular AII receptors for AII (Brunner et al., 1972) and the number of total vascular AII receptors (Devynck and Meyer, 1976), and that these changes were modified by the circulating AII level (Deheneffe et al., 1976) and, possibly, the local level of AII in the vascular wall (Swales et al., 1975) affected by various sodium balances.

These two AIIA's also had an ago-
nergistic effect on adrenal glomerulosa, renal vasculature and juxtaglomerular apparatus, but the intensity of this action of both AIIA's on each parameter was not different. Beckerhoff et al. (1975) infused the subpressor dose of Sar¹, Ile⁶-AII and Sar¹, Ala⁸-AII into dogs and observed that Sar¹, Ile⁶-AII caused an increase of plasma aldosterone, but Sar¹, Ala⁸-AII had no steroidogenic activity, and suggested that AII receptors on vascular beds and adrenal glomerulosa were different. In our present study there was no difference in steroidogenesis between both AIIA's, however there existed a dissociation in the agonistic action of both AIIA's on peripheral vascular beds and adrenal glomerulosa. This finding may be explained by the functional difference of AII receptors in these AII targets. In vitro Williams et al. (1974) observed by using two different AIIA's i.e., Sar¹, Ala⁸-AII and Phe⁴, Tyr⁸-AII, that Sar¹, Ala⁸-AII had an agonistic effect on neither aortic strip nor adrenal cortex, whereas Phe⁴, Thr⁸-AII showed the stimulating action of aortic strip although aldosterone secretion was unaffected by this AIIA, and they suggested by this dissociated finding that AII receptors were different in these two systems. Hollenberg et al. (1976) demonstrated that the attitude of blood pressure, renin and aldosterone secretion and renal vasculature to the graded-dose infusion of Sar¹, Ala⁸-AII was different in these systems, which implicated the difference of AII and AII receptor interaction at various AII targets. In contrast, Johnson and Davis (1973) suggested that AII exerted its effect at a similar receptor site in vascular beds and adrenal cortex on the basis of the finding that the relatively large dose of Sar¹, Ala⁸-AII inhibited both vasoconstriction and steroidogenesis of exogeneous AII in dogs. However, Bravo et al. (1975) argued against this view because in a small dose Sar¹, Ile⁸-AII blocked the vasopressor action of AII, but it showed a very weak inhibition against aldosterone secretion by AII. These inconsistent observations may be explained by the differences of the species and experimental conditions used. Further investigations are necessary to confirm whether AII and AII receptor interaction in various AII targets is heterogeneous or homogeneous.

Sodium depletion minimizes the agonistic action and potentiates the antagonistic effect of both AIIA's. The response of blood pressure to either AIIA in hypertensive patients with various causes on sodium depletion showed a good correlation between these two AIIA's. So far evaluating the involvement of renin-angiotensin system in hypertension, the pretreatment of sodium depletion is necessary and both AIIA's are equally useful when salt depleted. Recently, Anderson et al. (1977) reported that blood pressure reduction to Sar¹, Ala⁸-AII (Saralasin) was seen in 14 out of 34 hypertensive patients, most of low-renin essential hypertension during 4 or 5 weeks' diuretic therapy with hydrochlorothiazide, 50 to 100 mg/day, or spironolactone, 100 to 400 mg/day. This means excessive sodium depletion is not adequate for determination of renin dependency in hypertension. From our experience in a series of studies of hypertensive patients, a sodium-restricted diet (NaCl 5g/day) coupled with 80 mg of furosemide p.o. for 3 days is found to give a most desirable condition for this AIIA test to differentiate renin-dependent hypertension from low-reninemic hypertension (Ogihara et al., 1978).

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References


