Effect of Angiotensin III on Blood Pressure, Renin-Angiotensin-Aldosterone System in Normal and Hypertensive Subjects

Shin Suzuki, M.D.,* Yutaka Doi, M.D., Wataru Aoii, M.D., Morio Kuramotochi, M.D., and Kunitake Hashiba, M.D.

SUMMARY

The biological actions of angiotensin III (AIII) in animals have been reported to be stimulation of aldosterone secretion and vasoconstriction. However, the biological actions of AIII in human essential hypertension (EH) have not been evaluated. Twenty ng/Kg/min of AIII was infused intravenously for 30 min into 6 normal subjects and 24 patients with EH. The systolic blood pressure was elevated significantly, from 116±5 (mean±SD)/68±4 to 137±9/74±5 mmHg in normal subjects and from 155±29/95±17 to 176±26/106±20 mmHg in EH patients. The elevation in systolic BP of low-renin EH patients was significantly larger than that of normal-renin EH patients. Plasma renin activity (PRA) decreased significantly from 1.64±1.07 to 1.21±1.05 ng/ml/hr in normal subjects and from 0.88±0.66 to 0.76±0.63 ng/ml/hr in EH. Plasma aldosterone concentration (PAC) increased significantly from 57±34 to 116±34 pg/ml in normal subjects and from 66±56 to 91±24 pg/ml in EH. There was no significant difference between the increase of PAC in low-renin EH and in normal-renin EH. Plasma cortisol concentration (PCC) did not change in these subjects. There were no significant relationships between the changes of PRA and PAC or PRA and blood pressure. These results suggest that the pressor action of AIII appeared in relation to the basal PRA in EH. In EH, PRA is suppressed by the direct action of AIII in the kidney and neither by increased PAC nor by increased blood pressure. The small changes in blood pressure caused by AIII infusion suggest that a test using an AIII infusion for aldosterone stimulation would be preferable to an angiotensin II infusion.

* To whom reprints should be addressed.
Received for publication September 3, 1982.
Manuscript revised March 22, 1983.

From the Third Department of Internal Medicine, Nagasaki University School of Medicine, 7-1 Sakamoton-machi, Nagasaki City 852, Japan.
Additional Indexing Words:
Angiotensin III  Plasma renin activity  Plasma aldosterone concentration  Essential hypertension

AIII is the des-aspartic acid derivative of angiotensin II. Its plasma concentration has been measured in human\textsuperscript{11} and experimental animals.\textsuperscript{23} The main biological actions of AIII are thought to be vasoconstriction, stimulation of aldosterone secretion, and suppression of renin release in experimental animals.\textsuperscript{31,44} In addition the biological actions of AIII in normal human subjects have been evaluated by Kono et al\textsuperscript{5} and Carey et al.\textsuperscript{61} However, the action of AIII in essential hypertension (EH) has not been reported. The present study was carried out to evaluate the effect of AIII on renin-angiotensin-aldosterone system in EH.

Subjects and Methods

Six healthy subjects (4 males and 2 females, aged from 16 to 52 years), 24 patients with EH (15 males and 9 females, aged from 28 to 55 years), 3 patients with renovascular hypertension (2 males and 1 female, aged from 16 to 28 years), and 1 patient with primary aldosteronism (female, 51 years old) were studied. These subjects were admitted to the hospital. Drugs were withdrawn and a normal salt diet (12 Gm NaCl/day) was given for at least 7 days before the study. Patients with EH were divided into 3 groups (low-renin group: \( \text{PRA}<0.40 \text{ ng/ml/hr} \), normal-renin group: \( 0.40 \leq \text{PRA} \leq 1.98 \text{ ng/ml/hr} \), and high-renin group: \( \text{PRA}>1.98 \text{ ng/ml/hr} \)), based upon the basal PRA level which was measured at 8:00 AM after 2 hours of supine rest. A low sodium diet (3 Gm NaCl/day for 3 days) with 4 hours ambulation was imposed to detect the PRA response of the low renin group. Patients whose PRA increased more than 1.0 ng/ml/hr by this renin stimulation were excluded from this study.

Infusion of AIII:
AIII was obtained from the Protein Research Foundation (Osaka, Japan) and was sterilized. AIII infusion tests were performed at 10:00 AM in the fasting state after 30 min supine rest. AIII (20 ng/Kg/min) was administered intravenously for 30 min. Five ml of venous blood for the measurement of PRA, PAC, and PCC was collected before and during AIII infusion for 30 min.

Measurement of PRA:
One ml of plasma sample with 20 \( \mu l \) of 0.13\% 8-hydroxyquinoline sulfate and 6 \( \mu l \) of 2,3-dimercaptopropanol were divided into 2 aliquots. One
of them was incubated for 1.5 hour at 37°C and the other was incubated for 1.5 hour at 4°C. Then, the 100 µl aliquots were taken for radioimmunoassay of generated angiotensin I using CIS (CEA-IRE-SORIN, Gif-sur-yvette, France) kits. PRA was calculated by subtraction of the 4°C-incubated sample value from 37°C-incubated sample value and expressed as ng angiotensin I generation/ml/hr.

Measurement of PAC:

One ml of plasma sample was put into a cholesterol tube with 4 ml of dichloromethane and was shaken for 10 min and centrifuged. Two ml of the dichloromethane layer was collected and dried by nitrogen gas and was used for radioimmunoassay of aldosterone. The aldosterone standard, 3H-aldosterone, and aldosterone antibody were contained in CIS radioimmunoassay kits.

Measurement of PCC:

Ten µl of plasma and 200 µl of 0.6% glutamic acid, pH 3.3, were mixed well. Then cortisol antibody and 125I-cortisol were added for radioimmunoassay of cortisol. Cortisol radioimmunoassay kits were obtained from Eiken Immunochemical Co (Osaka, Japan).

Statistical analyses employed the paired and unpaired test, and results with p<0.05 were considered significant.

---

Fig. 1. Changes of blood pressure during angiotensin III infusion.
A: A patient with low renin essential hypertension (LREH).
B: A patient with renovascular hypertension (RVH).
PRA = plasma renin activity.
Fig. 2. Changes of blood pressure during angiotensin III infusion in 6 normal subjects (Normal), 20 patients with essential hypertension (EH), and 4 patients with secondary hypertension (Secondary).

B: before angiotensin III infusion.
A: during angiotensin III infusion.

SBP = systolic blood pressure; DBP = diastolic blood pressure; O = renovascular hypertension; X = primary aldosteronism.

Bars in Normal and EH represent mean value ± SD.

*p < 0.05, **p < 0.01.

RESULTS

Blood pressure changes:

Two examples of blood pressure changes are shown in Fig. 1. The upper panel shows the blood pressure changes during AIII administration in a patient with low-renin EH. The blood pressure rose from 172/98 mmHg to 212/114 mmHg after administration of AIII for 10 min. After stopping the administration of AIII, the blood pressure returned to the previous level in 4 min. This patient showed marked elevation of blood pressure by AIII. The lower panel shows the blood pressure changes during AIII infusion in a patient with renovascular hypertension. This patient had an elevated PRA (8.0 ng/ml/hr at rest) and showed no elevation of blood pressure after AIII administration. The blood pressure changes during AIII administration in
Changes in PRA:

Fig. 3 shows the AIII-induced changes in PRA. In healthy subjects, PRA decreased from 1.64 ± 1.07 ng/ml/hr to 1.21 ± 1.05 ng/ml/hr (p < 0.05) after AIII administration. In patients with EH, PRA decreased from 0.88 ± 0.95 ng/ml/hr to 0.65 ± 0.82 ng/ml/hr (p < 0.01).
Changes of plasma aldosterone concentration during angiotensin III infusion in 6 normal subjects (Normal), 18 patients with essential hypertension (EH), and 4 patients with secondary hypertension (Secondary).

B: before angiotensin III infusion.
A: during angiotensin III infusion.

Open circles in Secondary indicate renovascular hypertension and cross primary aldosteronism.

Bars in Normal and EH represent mean value ± SD.

*p<0.05, **p<0.01.

0.66 ng/ml/hr to 0.76 ± 0.63 ng/ml/hr (p<0.01). In 3 patients with renovascular hypertension and in the patient with primary aldosteronism PRA decreased after AIII administration.

Changes in PAC:
Changes in PAC after AIII administration are shown in Fig. 4. In healthy subjects the PAC increased from 57 ± 34 pg/ml to 116 ± 34 pg/ml (p<0.05) after AIII administration. There were no significant differences in the AIII induced PAC increment between healthy subjects and patients with EH. In patients with EH, PAC increased from 66 ± 56 pg/ml to 91 ± 24 pg/ml (p<0.01). In 3 patients with renovascular hypertension and 1 patient with primary aldosteronism, PAC also increased after AIII administration.

Changes in PCC:
The PCC did not change significantly in either healthy subjects or in patients with EH, renovascular hypertension, or primary aldosteronism (Fig.
Fig. 5. Changes of plasma cortisol concentration during angiotensin III infusion in 6 normal subjects (Normal), 11 patients with essential hypertension (EH), and 4 patients with secondary hypertension (Secondary).

B: before angiotensin III infusion.
A: during angiotensin III infusion.
Open circles in Secondary indicate renovascular hypertension and cross primary aldosteronism.
Bars in Normal and EH represent mean value ± SD.

5).

**Basal PRA and AIII induced blood pressure elevation:**

Relationship between basal PRA and AIII-induced blood pressure elevation is shown in Fig. 6. The average elevation of systolic blood pressure of the low renin group after AIII administration was 33±12 mmHg and was greater than that in the normal renin group (18±11 mmHg). On the other hand, the diastolic blood pressure elevation of the low renin group was 15±10 mmHg, which was almost identical to the normal renin group (9±8 mmHg).

**Basal PRA and AIII-induced changes in PAC:**

The AIII induced PAC increase was not statistically different between the low renin group and the normal renin group. However, the PAC increase was less than 15 pg/ml in 4 out of 5 patients of low renin group, in contrast to 4 patients out of 11 patients of normal renin group (Fig. 7).
relationship between AIII induced changes in PRA, PAC, and blood pressure is shown in Fig. 8. There were no significant relationships between changes of PRA and PAC, PRA and blood pressure.

DISCUSSION

Recent reports suggest that AIII is found in human plasma and that it may have an important role in the control of aldosterone secretion. Semple and Morton\(^1\) reported that AIII in normal human plasma was about 12% of the total peptides which react with an antibody to AII. It has been proposed that two different pathways may produce AIII. The first pathway is a conversion from angiotensin I via AII and the second pathway is a conversion from angiotensin I via des-aspartic acid angiotensin I.\(^7\) Campbell et al\(^8\) and Vaughan et al\(^9\) have shown that this 2nd pathway exists in rats. It
Fig. 7. Relationship between increment of plasma aldosterone concentration during angiotensin III infusion and plasma renin activity in patients with essential hypertension.

PAC = plasma aldosterone concentration; PRA = plasma renin activity.

Fig. 8. Relationship between plasma renin activity and plasma aldosterone concentration, systolic blood pressure, and diastolic blood pressure during angiotensin III infusion.

$\Delta$PRA: decrement of plasma renin activity.
$\Delta$PAC: increment of plasma aldosterone concentration.
$\Delta$SBP: increment of systolic blood pressure.
$\Delta$DBP: increment of diastolic blood pressure.
has been reported that vasoconstriction and aldosterone stimulation are two main actions of AIII. Furthermore, it has been reported that vasoconstricting action of AIII is less potent than that of AII in various animals\(^4,10^-12\) including rat, rabbit, and dog. In human subjects Kono et al\(^5\) have reported that the vasoconstricting action of AIII is one fifth as potent as AIII. Our results showed that 20 ng/Kg/min of AIII elevated the systolic blood pressure from 121±5 to 132±8 mmHg and the diastolic blood pressure from 68±4 to 74±5 mmHg in normal subjects indicating that AIII has a weak vasoconstricting action. Kono et al\(^5\) have reported that 20 ng/Kg/min of AIII produced a 10 mmHg increase in systolic and diastolic blood pressure in normal subjects. The elevation of diastolic blood pressure by AIII in our study was smaller than that reported by Kono et al\(^5\) and the precise reason of this difference is not clear. On the other hand our results on the systolic pressure change were nearly identical to those reported by Kono et al.\(^5\)

By contrast, in patients with EH, AIII produced a 22 mmHg elevation of systolic blood pressure. Furthermore, this systolic blood pressure elevation in the low renin group was greater than in the normal renin group. Since the basal PRA in patients with EH was lower than in normal subjects, the AIII induced systolic blood pressure elevation is related to the basal PRA levels. However, the pressor response to AIII in a patient with primary aldosteronism and a low PRA level was small. Further study is necessary to clarify this effect.

At least three possible mechanisms may explain our findings that PRA was suppressed by AIII in normal and hypertensive subjects. These include an intra-renal negative short feedback mechanism,\(^5\) an aldosterone induced volume expansion, and an elevation of blood pressure. Since there are no correlations between changes of blood pressure and PRA and/or changes of PAC and PRA, it is likely that AIII suppressed PRA by an intra-renal negative short feedback mechanism in EH.

It has been reported that AIII can increase corticosterone concentrations as well as aldosterone in rats.\(^11\) Since the main glucocorticoids in rat and man are corticosterone and cortisol respectively, it is interesting that the dose of AIII that was employed in this study had no effect on PCC in normal and hypertensive subjects. Finally, our results suggest that AIII may be a good stimulator of aldosterone secretion as clinical test, since its pressor action is less potent than that of angiotensin II.

**ACKNOWLEDGMENT**

We are greatly indebted to Miss Nobuko Kubota for typing the manuscript.
REFERENCES


