Effects of Angiotensin III (DES-1-Asp-Angiotensin II) and Angiotensin III Analogue (DES-1-Asp-8-Ile-Angiotensin II) upon Adrenal Steroidogenesis and Blood Pressure

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Effects of angiotensin III and angiotensin III analogue upon adrenal steroidogenesis and blood pressure were studied in rats, rabbits and a man. Pressor effect of angiotensin III was about one fifth of that of angiotensin II in all the species. Degradation rate of pressor effect of angiotensin III in plasma was more rapid than that of angiotensin II. Different from the effects of angiotensin III upon blood pressure, its effect upon aldosterone was similar to that of angiotensin II. The effect of angiotensin III upon other adrenal steroids, such as DOC and cortisol, however, seemed to be slightly less than that of angiotensin II. Angiotensin III produced an additive effect to that of ACTH, but it didn't produce an additive effect to that of angiotensin II. Angiotensin III analogue, itself, stimulated adrenal steroidogenesis, but it inhibited the effects of angiotensin III and angiotensin II upon aldosterone. Effects of ACTH upon plasma DOC and cortisol were not inhibited by angiotensin III analogue, but the effect of ACTH upon aldosterone was blunted slightly.

Recent studies\(^1\)\(^-\)^\(^8\) have suggested that a metabolite of angiotensin II, des-Asp\(^1\)angiotensin II (angiotensin III) is related to adrenal steroidogenesis as well as angiotensin II, although pressor effect of angiotensin III is significantly less than that of angiotensin II. It is proposed that angiotensin II and angiotensin III act upon aldosterone synthesis in the same way, but the mechanism of action is still unknown.

In this study, angiotensin III and angiotensin III analogue (des Asp\(^1\)-Ile\(^5\)-angiotensin II) upon plasma aldosterone, desoxycorticosterone (DOC) and cortisol were studied in rabbits. Furthermore, antagonistic action of angiotensin III analogue to the effects of angiotensin II, angiotensin III and ACTH upon adrenal steroidogenesis was studied. Pressor effects of angiotensin III were also studied in rats, rabbits and a man.

MATERIALS AND METHODS

Experiment 1:

In order to study the pressor effects of angiotensin III in a variety of species, effects of angiotensin II (Hypertensin Ciba), angiotensin III, and angiotensin III analogue upon blood pressure were studied in Wistar rats and rabbits anesthe-
tized with sodium pentobarbital, and in a man. Furthermore, degradation rate of pressor effect of angiotensin III was compared with that of angiotensin II. One hundred ng of angiotensin III or 10 ng of angiotensin II was incubated with human plasma at 37°C for 10, 20 and 30 minutes. After then, pressor effects of the incubated samples were determined by bioassay using rats. Experiments 2:

Studies on the effects of angiotensin III and angiotensin III analogue upon adrenal steroidogenesis were done in fourteen rabbits weighing 2.5—3.5 kg. All the rabbits were fed a standard laboratory diet before use. On the day of the experiment, the rabbit was anesthetized with sodium pentobarbital, and polyvinyl catheters were placed in the femoral artery, vein and ear vein to allow the recording of blood pressure, the infusion of reagents and the collection of blood samples.

All studies began with a 60-minute control period. In one infusion, the control period was followed by an infusion of 30-minute of angiotensin III at 5 ng/kg/min, 15 ng/kg/min, 30 ng/kg/min and 60 ng/kg/min, in order, with an infusion of 30—60 minutes of 5% glucose solution (0.08 ml/min) between each period of angiotensin III. In three rabbits, the control period was followed by an infusion of 30-minutes of angiotensin II at 5 ng/kg/min in the same way with the experiments on angiotensin III. In two rabbits, the control period was followed by an infusion of 30-minute of angiotensin III at 30 ng/kg/min and after then an infusion of angiotensin II at 30 ng/kg/min was added. After 30 minutes, the infusion of angiotensin III and angiotensin II was interrupted and displaced by the infusion of 5% glucose solution (0.08 ml/min). After the infusion of 60-minute of 5% glucose solution, an infusion of angiotensin III at 30 ng/kg/min was started again and 0.05 mg/kg of ACTH (a synthetic alpha 1—24 preparation) was injected as a single shot 30 minutes later. Thirty minutes after the administration, blood was collected.

In other six rabbits, the control period was followed by an infusion of 30-minute of angiotensin III analogue at 50 ng/kg/min or 200 ng/kg/min. Under the infusion of angiotensin III analogue at 200 ng/kg/min, an infusion of 30-minute of angiotensin II or angiotensin III at 30 ng/kg/min was added, or 0.05 mg/kg of ACTH (a synthetic alpha 1—24 preparation) was administered.

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Plasma aldosterone and DOC were determined by radioimmunoassay.\textsuperscript{10} Plasma cortisol was determined by protein binding assay\textsuperscript{11} Plasma renin activity was determined by the method of Skinner\textsuperscript{12} using bioassay.

RESULTS

Pressor effects of angiotensin III and angiotensin

![Graph](image1)

![Graph](image2)

Fig.1. Pressor effects of angiotensin II, angiotensin III and angiotensin III analogue in a rat and a rabbit anesthetized with sodium pentobarbital. The tracing of blood pressure at the upper part of the figure was obtained in a rat.

A II . . . . . . . angiotensin II
A III . . . . . angiotensin III
A III A . . . . . angiotensin III analogue

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Fig. 2. Pressor effect of angiotensin II under the infusion of angiotensin III analogue in a rabbit. Angiotensin III analogue didn't prevent the pressor effect of angiotensin II.

Fig. 3. Effects of angiotensin II and angiotensin III upon blood pressure, plasma aldosterone and DOC in a 22-year-old man. The dotted lines show the results brought about by angiotensin II.

Fig. 4. Degradation rate of pressor effects of angiotensin II and angiotensin III in human plasma. (Results are Mean ± SD, n = 3).

Fig. 5. Effects of angiotensin III upon plasma aldosterone, DOC and cortisol. (Results are Mean ± SD, n = 3).

III analogue:
Pressor effects of angiotensin III, angiotensin III analogue and angiotensin II in a rat were shown in Figure 1. A large amount (250 ng) of angiotensin III analogue elevated about 5 mmHg of mean arterial pressure, but 50 ng of angiotensin III analogue was ineffective. The pressor effect of angiotensin III was significantly less than that of angiotensin II. Pressor effect of 5 ng of angiotensin II was similar to that of 50 ng of angiotensin III.

In rabbits, the infusion of 50 ng/kg/min of angiotensin III elevated about 5 mmHg of arterial
blood pressure. In the same rabbit, 50 ng of angiotensin II elevated about 25–30 mmHg of arterial blood pressure.

As shown in Figure 2, the pre-infusion of 200 ng/kg/min of angiotensin III analogue couldn't prevent the increase in blood pressure by the infusion of 30 ng/kg/min of angiotensin II.

Figure 3 shows the pressor effects of angiotensin III and angiotensin II in a man. The infusion of 10 ng/kg/min of angiotensin III did not cause a significant change in blood pressure, although the same amount of angiotensin II elevated the systolic blood pressure from 118 mmHg to 148 mmHg and the diastolic blood pressure from 74 mmHg to 98 mmHg. The infusion of 25 ng/kg/min of angiotensin III elevated the systolic blood pressure from 132 mmHg to 138 mmHg and the diastolic pressure from 80 mmHg to 88 mmHg. After the infusion of angiotensin III, plasma aldosterone increased from 11 ng/100 ml to 19 ng/100 ml, but plasma DOC did not change significantly.

Degradation rate of the pressor effects of angioten-

Fig.6. A combined effect of angiotensin III and angiotensin II, or angiotensin III and ACTH upon plasma aldosterone.

Fig.7. Effects of angiotensin III analogue upon plasma aldosterone, DOC and cortisol in rabbit. (Results are Mean ± SD. n = 3).

Fig.8. Effects of angiotensin II and angiotensin III upon plasma aldosterone, and cortisol under the infusion of angiotensin III analogue. (Results are Mean ± SD. n = 3).
Effects of angiotensin III and angiotensin II upon the adrenal steroidogenesis:

The effects of 5 ng/kg/min, 15 ng/kg/min, 30 ng/kg/min and 60 ng/kg/min of angiotensin III upon plasma aldosterone, DOC and cortisol were compared with those of angiotensin II. The effect of angiotensin III upon plasma aldosterone was almost same to that of angiotensin II, as shown in Figure 5. The effects of angiotensin III upon plasma DOC and cortisol were, however, less than those of angiotensin II.

Figure 6 shows a combined effect of angiotensin III and angiotensin II, or angiotensin III and ACTH upon plasma aldosterone. Angiotensin III produced an additive effect to that of ACTH, but it didn’t produce an additive effect to that of angiotensin II.

Effects of angiotensin III, angiotensin II and ACTH upon adrenal steroidogenesis under the infusion of angiotensin III analogue:

Angiotensin III analogue, itself, increased plasma aldosterone, DOC and cortisol significantly at 200 ng/kg/min (Figure 7).

Under the infusion of 200 ng/kg/min of angiotensin III analogue, both 30 ng/kg/min of angiotensin III and angiotensin II didn’t stimulate plasma aldosterone and cortisol (Figure 8). However, ACTH could stimulate plasma aldosterone, DOC and cortisol under the infusion of angiotensin III analogue, although the stimulatory effect of ACTH upon plasma aldosterone was slightly blunted by the infusion of angiotensin III analogue (Figure 9).

Effects of angiotensin III and angiotensin II upon plasma renin activity:

At the infusion of low doses of angiotensin II or angiotensin III, plasma renin activity didn’t show significant changes. However, at the high dose (60 ng/kg/min), plasma renin activity was significantly suppressed (P < 0.05). There was no significant difference between the effect of angiotensin III and that of angiotensin II (Figure 10).

DISCUSSION

These studies have suggested that the pressor effect of angiotensin III is about one fifth of the pressor effect of angiotensin II in rats, rabbits and a man, but the effect of angiotensin III upon aldosterone is similar to that of angiotensin II. In addition to these findings which supported the results reported by other investigators,1–8 this study has also revealed that the degradation rate

tensin III and angiotensin II:

As shown in Figure 4, degradation rate of the pressor effect of angiotensin III in human plasma was more rapid than that of angiotensin II (P < 0.01).

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of the pressor effect of angiotensin III in plasma is more rapid than that of angiotensin II. Therefore, there is a possibility that the increased degradation rate of angiotensin III is also related to the poor effect of angiotensin III upon blood pressure, in addition to the fact that the receptor in vascular smooth muscle has a lower binding affinity for angiotensin III than for II.\textsuperscript{13}

Different from the effects of angiotensin III upon vascular smooth muscle, its effect upon aldosterone was similar to that of angiotensin II. The effect of angiotensin III upon other adrenal steroids, such as DOC and cortisol, however, seemed to be slightly less than that of angiotensin II. Therefore, it was supposed that angiotensin III acts upon aldosterone more specifically.

As a mechanism of action of angiotensin II or angiotensin III upon adrenal steroidogenesis, some investigators\textsuperscript{16} proposed that angiotensin II may act upon adrenal steroidogenesis in converting to angiotensin III. We couldn't reveal this fact, but our combined studies showed that angiotensin II didn't produce an additive effect to that of angiotensin III upon adrenal steroidogenesis, although ACTH produced an additive effect to that of angiotensin III. Furthermore, the studies using angiotensin III analogue revealed that angiotensin III and angiotensin II didn't stimulate aldosterone and cortisol under the infusion of angiotensin III analogue. Therefore, it is certain that the mechanism of action of angiotensin III upon adrenal steroidogenesis is similar to that of angiotensin II.

In the study concerning the effect of ACTH upon adrenal steroidogenesis under the infusion of angiotensin III analogue, the effects of ACTH upon DOC and cortisol seemed to be slightly different from the effect of ACTH upon aldosterone in which the effect of ACTH was slightly blunted. It is well known that the mechanism of action of ACTH upon aldosterone is different from that of angiotensin II\textsuperscript{15−17} but there might be a possibility that angiotensin and ACTH have a common receptor in the glomerulosa cells which is antagonized by angiotensin III analogue or there might be a possibility that angiotensin III analogue has a non-specific inhibition to the pathway of the effect of ACTH upon aldosterone. Further studies will be necessary to reveal this problem.

As suggested by other investigators\textsuperscript{18} a large amount of angiotensin II and angiotensin III suppressed plasma renin activity significantly.

As the degree of the suppression of plasma renin activity was not dependent on the increased levels of aldosterone, it is considered that the direct effect of angiotensin II or angiotensin III upon renin release may play an important role.

REFERENCES


Discussion:
Chairman: Dr. S. Fukuchi (Fukushima Med. College)

CHAIRMAN: Thank you for your nice presentation about the effects of angiotensin III and its analogue. The paper is now open for discussion. It is known that the rabbit adrenal gland is morphologically and functionally different from human adrenal gland. Is there any difference in the effects of angiotensin III and its analogue upon steroidogenesis between rabbit and man?

Dr. T. SARUTA: It seemed that there was no significant difference between them. In rabbits plasma DOC and cortisol seemed to be easily stimulated by angiotensins.

CHAIRMAN: In rabbits, corticosterone is the main glucocorticoid in stead of hydrocortisone. Was there any significant difference in the effects of ACTH upon those steroids between rabbit and man?

Dr. T. SARUTA: We have not measured plasma corticosterone. There was no significant difference in response of plasma cortisol.

Dr. T. ITO (Self-Defence Force Hosp.): According to your study, angiotensin II didn’t make any additive effect with angiotensin III on aldosterone secretion. Isn’t there any possibility that aldosterone secretion was stimulated maximally by angiotensin III to such an extent that further secretion was not induced by angiotensin II?

Dr. T. SARUTA: We are able to rule out the possibility, because we employed submaximal doses of angiotensin II and angiotensin III.

Dr. T. ITO: It is said that about 15% of total angiotensins is angiotensin III. Does the angiotensin III stem from angiotensin II or angiotensin I?

Dr. T. SARUTA: It is supposed that there are two pathways for the formation of angiotensin III. In one pathway, angiotensin III is formed from angiotensin II by the cleavage of N-terminal aspartic acid. In the other pathway, the nonapeptide is formed by removal of N-terminal aspartic acid from angiotensin I and then angiotensin III is formed by the cleavage of the C-terminal dipeptide.

Dr. K. YAMAMOTO (Osaka City Univ.): In the study on renin release, inhibitory effects upon renin release should be studied as well as stimulatory effects. As a method to study the inhibitory effects, angiotensin II and angiotensin III are available in addition to β-blocking agents. As you suggested, angiotensin II and angiotensin III act upon vascular receptors in the different ways, while they act upon the steroidogenesis in the same way. Have you examined how angiotensin III acts upon renin release? Have you studied the effects of angiotensin III analogue and II analogue upon renin release?

Dr. T. SARUTA: We have not compared the effects of angiotensin II analogue upon renin release with those of angiotensin III analogue. In future, we’ll study those effects upon renin release in an in-vitro system using kidney slices in addition to an in-vivo study.

Dr. T. TAKEDA (Tokyo Univ.): You suggested that angiotensin II and angiotensin III have almost same inhibitory effects upon renin release. Does angiotensin II act upon renin release after converting to angiotensin III? Do angiotensin II and angiotensin III have additive inhibitory effects upon renin release? Have you studied combined effects of angiotensin III and angiotensin III analogue or angiotensin II and angiotensin II analogue upon renin release?

Dr. T. SARUTA: We have not studied the combined effects of angiotensin III and angiotensin III analogue or angiotensin II and angiotensin II analogue upon renin release.

Dr. M. ISHII (Tokyo Univ.): Have you examined the effect of angiotensin III analogue upon plasma aldosterone in animals with increased levels of renin or aldosterone?

Dr. T. SARUTA: We are doing the experiment now.

Dr. M. MATSUNAGA (Kyoto Univ.): Can you answer from your data whether angiotensin II acts upon aldosterone secretion in the form of angiotensin II itself or in the form of angiotensin III?

Dr. T. SARUTA: We can’t say anything about
your question from our data.
Dr. M. MATSUNAGA: In your study, effects of angiotensin III upon DOC and cortisol were different from those of angiotensin II. Does it mean that angiotensin II acts upon DOC and cortisol without conversion to angiotensin III? Furthermore, do you think that angiotensin II acts upon the steroidogenesis through the same pathway with angiotensin III, even though angiotensin II doesn’t convert to angiotensin III?
Dr. T. SARUTA: It is difficult to say from this study whether angiotensin II didn’t convert to angiotensin III in order to stimulate DOC and cortisol. But I think that the concentration of angiotensin III or angiotensin II at the adrenal gland plays an important role, because angiotensin III is easily destroyed compared to angiotensin II.

From our combined studies of angiotensin II and angiotensin III, or angiotensin II, III and angiotensin III analogue, angiotensin III is supposed to act upon the adrenal gland in the similar way with angiotensin II. Furthermore, it seems that the pathway of steroidogenesis blocked by angiotensin III analogue is slightly wider than that affected by angiotensin II or angiotensin III, because the effects of ACTH upon the adrenal steroidogenesis is slightly blurred by angiotensin III analogue.
Dr. M. ISHII: Have you examined the metabolism of aldosterone in the liver during angiotensin II or angiotensin III infusion?
Dr. T. SARUTA: We have not done it.
CHAIRMAN: How much amount of blood did you take for measurement of various hormones?
Dr. T. SARUTA: We took 4 ml of blood in each sampling. In each rabbit, 5 to 6 samples were collected. The volume contraction was avoided by infusing about 4 ml of 5% glucose solution between one sampling and the other.