The Mechanism of Estrogen Hypertension*

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SINCE the first case of high blood pressure related to oral contraceptives containing estrogen was noted by *Brownrigg*¹ in 1962, a number of papers²⁻⁸ have confirmed the association. We⁹ also confirmed this association and reported that 10 of 56 normotensive women developed hypertension during administration of an estrogen-containing oral contraceptive.

As the possible mechanism for this hypertension, studies have been centered around the renin-angiotensin system. As first reported by *Helmer*,¹⁰ estrogen increases the synthesis of renin substrate. The amount of angiotensin formed per unit of renin increases progressively with the increase in renin substrate. According to *Skinner et al.*¹¹ the increased amount of angiotensin formed in the presence of high level of renin substrate feeds back to suppress the release of renin, thereby bringing the amount of angiotensin back toward normal. They further postulated that the

hypertensive effect of estrogen might be due to a failure of the feed-back mechanism to suppress renin release.

In our previous studies, we could confirm their suggestion in a number of women who became hypertensive on the pills. In this study, we tried to make hypertension in rats by estrogen and in addition we further studied the role of renin release in the development of estrogen hypertension.

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MATERIALS AND METHODS

Experiment Procedure 0.8

Forty of male Wistar rats weighing 135–140 g were used for this study. They were divided into 4 groups of 10 animals each, and the following procedures were done in each group. 1) Animals of the first group were fed on stock chow diet and given tap water to drink and no treatment was done. 2) Animals of the second group were

Renin Activity	: By the method of Skinner	ng/ml/hr
Renin Concentration:	By the method of Skinner	units/ml
	Uniform renin substrate was prepared from	
	12 hrs nephrectomized rats	
Renin Substrate	: By the method of Skinner	ng/ml
	Standard rat renin was made by the method	
	of Brown et al.	
Renal Renin Content:	By the method of Haas et al.	μg/g tissue
	Uniform renin substrate was prepared from	
	12 hrs nephrectomized rats	

Fig.1. Components of the renin-angiotensin system measured in this study.

Key Words: Estrogen Hypertension
Renin-Angiotensin System

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TABLE I RENIN ACTIVITY, RENIN CONCENTRATION, AND RENIN SUBSTRATE IN ALL GROUPS OF THE RATS

1) Control			
	RA	RC	RS
1	4.8	10.5	290
2	4.4	10.8	410
3	3.8	9.0	340
4	4.4	10.2	410
5	4.5	9.3	320
6	3.7	10.5	400
7	4.5	10.8	320
8	4.0	10.7	460
9	4.6	10.5	400
10	4.2	7.8	320
Mean $\pm$ SD	4.2 $\pm$ 0.3	9.9 $\pm$ 1.0	367 $\pm$ 56

2) Estrogen			
	RA	RC	RS
1	7.3	9.9	1100
2	8.0	7.5	800
3	6.7	7.8	840
4	6.7	8.4	800
5	6.0	9.0	900
6	6.2	6.9	1000
7	7.5	8.4	1100
Mean $\pm$ SD	6.8 $\pm$ 0.7	8.4 $\pm$ 1.2	937 $\pm$ 138

became hypertensive			
	RA	RC	RS
1	7.8	9.0	1200
2	10.0	11.0	800
3	6.6	9.0	1100
Mean $\pm$ SD	8.2 $\pm$ 1.8	9.9 $\pm$ 1.2	1033 $\pm$ 210

3) Estrogen + NaCl			
	RA	RC	RS
1	4.2	5.7	1120
2	5.6	4.5	720
3	3.9	7.4	920
4	4.6	5.1	1200
5	5.0	5.7	920
6	5.4	6.7	960
7	3.4	8.4	1200
8	3.8	5.1	1100
9	5.0	7.8	860
Mean $\pm$ SD	4.6 $\pm$ 0.8	6.3 $\pm$ 1.2	1000 $\pm$ 93

became hypertensive			
	RA	RC	RS
1	6.0	9.0	880

4) NaCl			
	RA	RC	RS
1	3.8	9.0	400
2	3.6	6.6	500
3	4.8	7.5	400
4	3.8	7.8	500
5	3.3	6.9	460
6	4.0	9.0	440
7	3.4	9.0	380
8	4.6	7.8	440
Mean $\pm$ SD	4.0 $\pm$ 0.4	7.9 $\pm$ 0.7	440 $\pm$ 4.6

became hypertensive			
	RA	RC	RS
1	4.2	9.0	400

fed in the same way and received one daily injection of 0.2 mg of estriol intramuscularly and 2 mg of stilbestrol disulfate intraperitoneally. 3) Animals of the third group were fed on stock chow diet and given tap 1% NaCl as the drinking solution, and in addition they received one daily injection of 0.2 mg of estriol and 2 mg of stilbestrol disulfate. 4) Animals of the fourth group were fed on stock chow diet and given tap 1% NaCl as the drinking solution.

Body weight and blood pressure of all animals

were checked twice weekly. All rats were killed after 14 days, and blood was drawn and both kidneys were removed for renin assay.

Blood Pressure

Measurements were made with the modified tail-water plethysmographic method (Okamoto and Aoki).¹² The three readings were averaged. The criteria for development of hypertension is to have the increase of at least 20 mmHg to a level of the blood pressure before treatment on

TABLE II CHANGES IN BODY WEIGHT, BLOOD PRESSURE, AND RENIN-ANGIOTENSIN SYSTEM IN ALL GROUPS OF RATS

		No.	Increase of weight (g)	Blood pressure Before (mmHg)	After	Serum renin activity (ng/min/hr)	Serum renin concentration (units/ml)	Renal renin (μg/g)	Serum renin substrate (ng/ml)
1	Control	10 Normotensive 0 Hypertensive	66 ± 15	125 ± 7	127 ± 9	4.2 ± 0.3	9.9 ± 1.0	17.3 ± 3.3	367 ± 56
2	Estrogen	7 Normotensive 3 Hypertensive	19 ± 11 16 ± 6	127 ± 10 121 ± 5	133 ± 8 163 ± 4	6.8 ± 0.7 8.2 ± 1.8	8.4 ± 1.2 9.9 ± 1.2	15.6 ± 4.3 18.4 ± 3.3	937 ± 138 1033 ± 210
3	Estrogen NaCl	9 Normotensive 1 Hypertensive	25 ± 5 24	123 ± 14 12.0	125 ± 6 15.0	4.6 ± 0.8 6.0	6.8 ± 1.2 9.0	14.7 ± 2.8 16.0	1000 ± 93 880
4	NaCl	8 Normotensive 1 Hypertensive	57 ± 12 30	128 ± 5 126	128 ± 5 170	4.0 ± 0.4 4.2	7.9 ± 0.7 9.0	16.2 ± 3.7 15.3	440 ± 46 400

two sequent measurements.

Renin Measurements

Various components of the renin-angiotensin system measured in this study were outlined in Fig.1. Renin activity, renin concentration and renin substrate were measured by the method of Skinner.¹³ Renal renin content was measured by the method of Haas *et al.*¹⁴ The standard rat renin for the measurement of renin substrate was prepared by the method of Brown *et al.*¹⁵ The uniform of renin substrate for the measurement of serum renin concentration and renal renin content was prepared from 12 hours nephrectomized rat serum.

Bioassays

All bioassays were done in pentolinium-treated rats anesthetized with sodium pentobarbital. Blood pressure changes were measured by a catheter in the left carotid artery coupled in a Nihon Kohden MP-4 transducer. The incubation mixture was assayed for pressor activity against aspartyl valyl, angiotensin II-β-amide (Hyperten-sin, Ciba).

RESULTS

Of the 40 rats, a rat died during the experiment. Therefore results on 39 rats were analysed.

The changes of blood pressure

The development of hypertension was seen in 3 of 10 estrogen treated rats (Group 2), in 1 of 10 estrogen and salt treated rats (Group 3), and 1 of 9 salt treated rats (Group 4).

The changes of body weight

The changes of body weight are summarized in Table II. In the group 2 and 3, regardless of blood pressure, the increases of body weight were very slight compared to those of the group 1 and 4. After 14 days the increases of body weight in the group 2 and 3 were about one third of those of the group 1.

Renin-angiotensin system

The overall results of serum renin activity, serum renin concentration and serum renin substrate are detailed in Table I and summarized in Table II with renal renin content.

Renin Activity: Renin activity was significantly increased in the group 2 and especially it was high in the rats in which hypertension has developed. However, in the group 3 with a com-

bination of estrogen and salt, renin activity was almost similar to that of the group 1. Renin activity in the group 4 was the lowest.

Renin Substrate: Different from the results of renin activity, renin substrate was significantly increased in the group 2 and 3. In the group 4 with salt treatment renin substrate was also slightly increased compared to that of the group 1.

Renin Concentration: Renin concentration was decreased in most of the groups except for the rats in which hypertension has developed, and especially it was significantly decreased in the group 3.

Renal Renin Content: Results of renal renin content are summarized in Table II. The results of renal renin content were similar to those of serum renin concentration, although the differences from the results of group 1 were slight. In the group 2, renal renin in the rats in which hypertension has developed, was slightly higher than that in the rats which remained normotensive, but no statistical difference was found between these two groups.

DISCUSSION

These results indicate that estrogen induces hypertension in rat as well as in man, and renin release from the kidney which controls the rate of angiotensin formation with the increased level of renin substrate plays an important role in the development of estrogen hypertension. In addition, this study suggests that it might be possible to prevent estrogen hypertension by suppressing renin release thoroughly.

Although there remains the problem in the method to suppress renin release in this study, because salt itself induces hypertension, we couldn't find another appropriate way to suppress renin release. Hypertension has developed in 1 of 9 salt-loaded rats (Group 4) and in 3 of 10 estrogen treated rats (Group 2). However, the administration of both estrogen and salt induced hypertension in only 1 of 10 rats (Group 3). Therefore, salt loading would seem more likely to prevent the development of hypertension rather than it would increase the incidence of hypertension in estrogen treated rats.

As it is known that the renin-angiotensin system plays an important role in the development of estrogen hypertension, studies have been centered around the renin-angiotensin system. The levels of renin substrate increased to the same extent in both the group 2 and 3. However,

there were significant differences in renin activity and renin concentration between these two groups. The increase of renin activity in the group 3 was slight compared to that in the group 2, and this difference of renin activity seemed to depend on the difference of renin concentration. As all of serum renin activity, serum renin concentration and renal renin content in the group 2 were significantly higher than those in the group 3, and in addition those values in the rats in which hypertension has developed were slightly higher than those in the rats which remained normotensive, it seems to be certain that renin release and angiotensin formation play an important role in the development of estrogen hypertension. As earlier studies indicated, the increase of renin substrate by the administration of estrogen is the first step of estrogen hypertension, but, this is not the determinant factor for the development of hypertension. Renin release and the rate of angiotensin formation seem more likely to control the development of hypertension.

SUMMARY

The mechanism of estrogen hypertension was studied in rats.

After the administration of 0.2 mg of estriol and 2 mg of stilbestrol disulfate for 14 days, three of 10 rats have developed hypertension. However, in the rats with the same amount of estrogen and salt loading (1% NaCl as drinking solution), only 1 of 10 has developed hypertension. Renin substrate increased significantly in both the groups, but serum renin activity, serum renin concentration and renal renin content in estrogen treated rats were significantly higher than those in the rats with the combination of estrogen and salt loading. In addition, the rats in which hypertension has developed showed higher values of renin activity, renin concentration and renal renin content compared to those in the rats which remained normotensive. Therefore it seems to be possible to think that renin release and the rate of angiotensin formation with the high level of renin substrate play an important role in the development of estrogen hypertension.

Furthermore, this study suggested that it might be possible to prevent estrogen hypertension by suppressing renin release completely.

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DISCUSSION:

Chairman: SOITSU FUKUCHI, Tohoku Univ.

CHAIRMAN: I would like to congratulate your accomplishment about the role of renin-angiotensin in the mechanism of hypertension induced by contraceptives. The side effect of contraceptives is now one of social problems caused by many chemicals. I think that your work is of great value in a point of suggesting to be able to prevent contraceptives-induced hypertension by suppressing renin release.

Dr. OMAE (Kyushu University): Does the excessive intake of salt suppress the development of hypertension in estrogen treated rats?

Dr. SARUTA (Keio University): The incidence of the development of hypertension in estrogen-salt treated rats was less than that in only estrogen treated rats. Therefore, we considered that the development of estrogen hypertension might be prevented by the suppression of renin release by salt loading. However, there remain problems concerning with the employment of salt loading to suppress renin release.

Dr. MATSUNAGA (Kyoto University): We have also observed the similar results of rise in both renin substrate and renin activity, but not hypertension. Therefore, I suppose that the hypertension does not related with renin-angiotensin system.

Dr. SARUTA: I don't know the reason why you have not experienced hypertension in your experiments. The difference of sensitivity of the rat may play a role in the development of hypertension.

We must consider several factors in the development of estrogen hypertension. Among of

them, the changes of renin-angiotensin system were most significant. Therefore, we tried to find the cause of estrogen hypertension in renin-angiotensin system.

Dr. KIRA (Kyoto University): Was the incidence of hypertension statistically significant? I would like to emphasize that some researchers deny a role of renin-angiotensin in the hypertension developed by contraceptives.

Dr. SARUTA: The incidence of hypertension in estrogen treated rats was significantly high. In the experiment concerning with NaCl treatment or NaCl + estrogen treatment total samples were too small to conclude this experiment. In future we'll try to do similar experiments in a large number of rats.

CHAIRMAN: 1) How do you think about the sodium-losing effect of estrogen as the cause of the rise in plasma renin activity?

2) Do you think that the increase of renin substrate was induced by the effect of estrogen on liver?

Dr. SARUTA: 1) In most of subjects on estrogen treatment, renin concentration gradually decreases, different from renin activity and renin substrate. Therefore, I don't think estrogen itself stimulates renin production or release.

2) Yes, I think the effects of estrogen upon the liver probably play an important role in the elevation of renin substrate.

Dr. YAMAMOTO (Osaka Municipal University): 1) Does angiotensin II suppress renin release or renin production?

2) Also I would like to ask you about the effect of angiotensin II on renin release from renal-cortical slice of rats.

Dr. SARUTA: According to our preliminary studies concerning with the effects of angiotensin II upon renin release and production in rat kidney slices, much amounts of angiotensin II suppressed both of renin release and production.

Dr. MOTOMURA (Kyoto University): Have you experienced irreversible or malignant hypertension in estrogen-treated patients or rats?

Dr. SARUTA: In our studies all patients who became hypertensive on estrogen showed the normalization of blood pressure of estrogen. However, there are a few reports about malignant type of hypertension or cerebral accidents on estrogen treatment in the references.

Dr. IIMURA (Sapporo Medical College): Did you find any relationship between plasma renin activity and blood pressure in hypertensive and normotensive groups? Are the normotensive sub-

jects less sensitive to angiotensin II?

Dr. SARUTA: There was a significant difference in renin concentration between the group who became hypertensive and the group who remained normotensive, but there was no significant difference in renin activity.

We expected to have the difference of the

incidence of estrogen hypertension between in the subjects with the family history of hypertension and in those without it, because most of people with the family history of hypertension are more sensitive to angiotensin and norepinephrine. However, we couldn't see any significant difference.