Plasma Renin and the Antihypertensive Effect of Angiotensin-II Antagonist in Experimental Hypertension

JIN YAMAMOTO, MASATO MATSUNAGA, AKIRA HARA, CHUNO PAK, JUN KIRA, KOICHI OGINO, AND CHUICHI KAWAI

In order to elucidate the role of the renin-angiotensin system in the pathogenesis of experimental hypertension, plasma renin concentration (PRC) and the antihypertensive effect of 1-Sar-8-Ile-angiotensin II, a specific competitive antagonist of angiotensin II, were investigated in various types of experimental hypertension of rats in their conscious state. Some of these animals which had acute sodium-depletion following the administration of furosemide were also studied, since it has been reported that angiotensin-dependency of blood pressure is related to the state of sodium balance.

Experimental Procedure
The subjects of this experiment were 1) female Wistar rats weighing approx. 180g with one- and two-kidney Goldblatt hypertension 4–5 weeks after the clipping of the left renal artery, 2) female Wistar rats weighing approx. 200g with one- and two-kidney Loomis hypertension 2 or 4–5 weeks after the ligation of the posterior branch of the left renal artery, 3) male 7–8-month-old stroke-prone spontaneously hypertensive rats (SHRSP) (body weight, approx. 300g) and male 8-month-old stroke-resistant spontaneously hypertensive rats (SHRSR) (body weight, approx. 320g), and 4) male Wistar rats (body weight, approx. 120g) with hypertension induced by 10 days of 1% saline loading and repeated injections of kidney extracts prepared from adrenalectomized rats ("Kidney extract and salt" hypertension) and their controls which received injections of physiological saline instead of the kidney extract. The rats other than the 4th group were given a regular diet and tap water ad libitum. When the systolic pressure measured by the tail plethysmographic method was greater than 160 mmHg, the animal was considered to be hypertensive, and was used for this experiment.

Animals were cannulated in the jugular vein and the femoral artery with a polyethylene tube. A few days after the catheterization, a small amount of blood for determination of PRC was collected via the indwelling aortic catheter. The same catheter was connected to a transducer to record the mean arterial pressure (MAP). The jugular vein catheter was connected to an infusion pump for the infusion of 1-Sar-8-Ile-AII (Fig. 1). Following a 20–30 minute control period, a solution of 1-Sar-8-Ile-AII (dissolved in sterile physiological saline) was infused at a constant rate of 0.074 ml/min for 20 min. An 8.6 µg/ml solution was infused into the two-kidney Goldblatt animals (0.64 µg/min), and a 43 µg/ml solution was into all other animals (3.2 µg/min). A 215 µg/ml solution was used for re-infusion into some rats of SHRSP group (16 µg/min). When the animals showed a constant reduction in MAP of more
Fig. 1. The rats were harnessed and the catheters were protected by a pliable spring wire. The animals can walk about in the cage.

than 20 mmHg during the infusion period, the antihypertensive effect of the antagonist was considered significant (Fig. 2). Furosemide (50 mg/Kg) was injected intraperitoneally into rats with one-kidney Goldblatt hypertension or one- and two-kidney Loomis hypertension 4–5 weeks after the operation, when no significant reduction in MAP was found during the infusion of the antagonist.

PRC was determined using radioimmunoassay of angiotensin I, of which details were described elsewhere.

Statistical analyses were performed according to Student’s t-test (P < 0.05; significant). All values were expressed as mean ± SD.

RESULTS AND DISCUSSION

1) Goldblatt Hypertension. PRC of the two-kidney animals was 161.5 ± 116.0 ng/ml/hr (n = 8), and significantly higher than that (25.7 ± 27.3 ng/ml/hr, n = 9) of the one-kidney animals. The infusion of 1-Sar-8-Ile-AII (0.64 μg/min for each rat) produced a significant fall in MAP in 6 of the 8 two-kidney animals. However, the infusion of a five-fold dosage of the antagonist (3.2 μg/min for each rat) decreased MAP in only 2 of the 9 one-kidney animals. There was a significant correlation between the blood pressure response to the antagonist and the pre-infusion level of PRC in both models. These results were in agreement with previous reports, indicating that the renin-angiotensin system significantly contributes to the pathogenesis of hypertension in the two-kidney model, but only little in the one-kidney model.

2) Loomis Hypertension. Two-weeks after the operation, PRC of the two-kidney animals was 41.2 ± 15.8 ng/ml/hr (n = 5), and tended to be higher than that of the one-kidney animals (23.2 ± 13.1 ng/ml/hr, n = 5). MAP of the one- and two-kidney animals was 140 ± 8 mmHg and 137 ± 10 mmHg, respectively, and these values were not significantly different. Four to five weeks after the operation, PRC of the one- and two-kidney animals was 42.6 ± 27.5 ng/ml/hr (n = 6) and 31.6 ± 14.2 ng/ml/hr (n = 6), respectively. MAP was 143 ± 6 mmHg and 142 ± 9 mmHg, respectively. There was no significant difference in both PRC and MAP between the two models. 1-Sar-8-Ile-AII (3.2 μg/min) failed to produce a significant fall in MAP in any animals. These results suggest that the renin-angiotensin system appeared to have no role in the maintenance of Loomis hypertension later than 2 weeks after the operation, though Ebihara’s work offered controversial suggestion.

3) SHRSP and SHRSR. PRC of SHRSP was 58.7 ± 19.7 ng/ml/hr (n = 14), while that of SHRSR was 25.1 ± 14.9 ng/ml/hr (n = 7). Significant difference was found between the two.
Fig. 2. Representative experiments of 1-Sar-8-Ile-Ala infusion into conscious rats.
The darkened horizontal bars indicate a dosage of 3.2 µg/min of the antagonist infusion, 
and the open bar indicates a dosage of 0.64 µg/min. A) a one-kidney Goldblatt rat 4–5 
weeks after the clamping of the left renal artery, B) a two-kidney Goldblatt rat 4–5 
weeks after the operation, C) and D) stroke-prone spontaneously hypertensive rats aged 
7–8-months. Mean arterial pressure is recorded in all but case A. A significant reduction 
in the mean arterial pressure is produced in cases B and D. An initial transient 
pressor response can be seen at the start of the infusion.

substrains. MAP of SHRSR was almost equal
(175 ± 15 mmHg and 175 ± 13 mmHg, respectively). 1-Sar-8-Ile-Ala (3.2 µg/min) caused a 
significant reduction in MAP in 5 of the 14 SP 
rats, but in none of the SR rats. There was no 
significant correlation between PRC and the 
blood pressure response. Further infusion of the 
five-fold dosage of the antagonist failed to 
decrease MAP in the 9 rats of the SP strain which 
showed no response to the infusion of the lower 
dose. We have already reported that the SP strain 
exhibited significantly higher PRCs after 7 
months of age, whereas the SR strain exhibited 
no difference from the controls up to 12 months 
of age. Therefore, the renin-angiotensin system 
seems to have played no essential role in the 
genesis of hypertension in SHR, especially in the 
SR strain, while it seems to be partly involved in the 
maintenance of the hypertension in the 
7–8-month-old SP rats.
4) “Kidney extract and salt” Hypertension. 
MAP was 153 ± 15 mmHg (n = 5) and 
significantly higher than the controls (103 ± 5 mmHg, 
n = 5). PRC was 59.5 ± 28.3 ng/ml/hr, and sig-
sificantly higher than the controls (9.3 ± 5.6 
ng/ml/hr). 1-Sar-8-Ile-Ala failed to decrease MAP 
in both these hypertensive rats and the controls. 
Our earlier work suggested that renin may be 
implicated in the pathogenesis of this type of 
hypertension. Nonetheless, these rats with high 
PRCs did not respond to the antagonist. And 
this hypertension could not be attributable to 
a direct vasoconstricting action of angiotension II. 
Since the renin-angiotensin system has multiple 
actions on the blood pressure, other mechanisms 
—including sodium and water retention—should 
be studied.
5) Effects of Furosemide. Furosemide 
injection significantly decreased body weight and 
MAP, and significantly increased PRC in all three 
types of hypertension. Especially, PRC was 
markedly increased, and the value was 132.2 ± 
33.2 ng/ml/hr in the one-kidney Goldblatt group, 
136.8 ± 15.7 ng/ml/hr in the one-kidney Loomis 
group and 108.1 ± 24.8 ng/ml/hr in the two-
kidney Loomis group, respectively. Concerning the 
mean arterial pressure after the injection of 
furosemide, the one- and two-kidney Loomis
groups showed the nearly normal level, while the one-kidney Goldblatt group remained hypertensive. The infusion of the antagonist caused a significant reduction in MAP in 3 of the 7 one-kidney Goldblatt rats, in 1 of the 5 one-kidney Loomis rats and in 2 of the 5 two-kidney Loomis rats. There was no significant correlation between PRC and the blood pressure response. These results indicated that acutely sodium-depleted animals did not always respond to the infusion of the antagonist in spite of their prominent high PRCs.

6) Correlation of PRC, the blood pressure response to the antagonist, and MAP. Significant correlation between PRC and the blood pressure response was found in the Goldblatt rats, but not in the rats with the other types of hypertension. There was no significant correlation between PRC and MAP in any group of our hypertensive animals.

Acknowledgements

The authors wish to thank Emer. Prof. K. Okamoto and assist. Prof. Y. Yamori of the department of pathology, faculty of medicine, Kyoto Univ., for a generous supply of SHRP.

REFERENCES


Discussion:

Chairman: Dr. TERUO OMAE, Kyushu Univ.

Dr. T. OMAE: Now the paper is open for discussion.

Dr. S. FUKUCHI: (1) What do you think about the mechanism of hypertensive effect of l-Sar-8-Ile-angiotensin II observed in some of your rats? (2) Did you examine the change of PRC after the administration of the antagonist.

Dr. J. YAMAMOTO: In order to completely block the action of angiotensin II, we administered a dosage of the antagonist somewhat greater than that given by other investigators. As was shown in Fig. 2, most of the experimental animals showed a transient pressor response at the start of the infusion of the antagonist, and some showed a sustained rise in blood pressure. Although a significant correlation was not detected between the pre-infusion level of PRC and the pressor response to the antagonist, the animals with the lower PRCs tended to have a larger pressor response. We speculate that an agonistic effect would occur more frequently in the presence of a lower level of endogenous angiotensin II, since many vascular receptor sites for angiotensin II were left free and easily occupied by the competitive antagonist. Other targets of angiotensin II might be involved in inducing a pressor response. Recently some analogues were shown to possibly release catecholamine from adrenal medulla (Sen. S. et al. P.S.E. B.M. 147, 847, 1974).

The plasma renin level after the infusion of the antagonist was not measured in this study. Dr. A. EBIIHARA: I did not measure plasma renin activity, but measured renal renin content in the rat with Loomis type hypertension. Renal renin content increased in the region adjacent to the necrotic area within 2 weeks after the operation, but it was not so thereafter. Renal renin content of whole kidney decreased. In the rat, the hypertension of 4 to 5 weeks’ duration after the operation should be considered as a chronic stage of hypertensive process. In this stage, the renin-angiotensin system may not contribute to the mechanism of hypertension.

Dr. J. YAMAMOTO: The kidney renin content was not measured. We prepared the control animals for Loomis hypertension, receiving the sham-operation. Compared to the controls, the one-kidney Loomis animals had no significant differences in PRC, but the two-kidney Loomis animals showed a higher PRC 4–5 weeks after
the operation. The antagonist failed to alter the blood pressure significantly in any of our Loomis hypertensive animals, even if their PRCs were at times increased. Therefore, we are inclined to believe that the vascular action of angiotensin-II made no contribution to the maintenance of hypertension in these animals.

Dr. I. SAITO: Salt depletion activated the renin-angiotensin system and enhanced the depressor effect of angiotensin-II antagonist in experimental hypertension, didn't it?

Dr. J. YAMAMOTO: Though all the rats injected with furosemide exhibited high PRCs, most of them did not showed a blood pressure fall of 20 mmHg or above during the infusion of the antagonist. It should not be said that angiotensin II contributed to the maintenance of hypertension in the physiological state simply because an angiotensin-II antagonist lowered the blood pressure in the volume-depleted state in either experimental animals or in humans. The physiological states were very different from the volume-depleted states in which the renin-angiotensin system is activated in order to restore the body-fluid volume.