Role of the Renin-angiotensin System in Pathogenesis of Experimental Renal Hypertension*

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Plasma renin activity (PRA) was measured in rabbits by a modification of Picken's method. In the acute stage, PRA increased in 5 of 11 animals with unilateral constriction of renal artery but it did not increase in 13 of 15 animals with bilateral constriction of renal arteries. No clear correlation was found between PRA and blood pressure. In the chronic stage, PRA increased in 6 of 32 animals with unilateral constriction of renal artery. There were a positive correlation between PRA and blood pressure, and a negative correlation between PRA and serum potassium level. However, there were hypertensive animals without increase in PRA. Removal of the clipped kidney was followed by a fall of blood pressure in chronic hypertensive animals. The fall of blood pressure did not depend upon the previous level of PRA. These results suggest that the renin-angiotensin system does not play a primary role in the pathogenesis of experimental renal hypertension and unknown renal mechanism may be involved in it.

THE participation of the renin-angiotensin system in the pathogenesis of experimental renal hypertension remains still unclear. Until the recent years the renin-angiotensin system had been believed to play a primary role in the initial rise of blood pressure in experimental renal hypertension. This concept, however, has been criticized by studies which used more specific methods for renin measurement¹⁻⁴. In the present study we measured plasma renin activity in both acute and chronic stage of experimental renal hypertension.

METHODS

Male rabbits weighing 2.0-2.5 Kg were used. Blood pressure was measured by an indirect method from the central artery of the ear.

Key Words: Experimental Renal Hypertension
Renin-Angiotensin System
Plasma Renin Activity

Blood samples were withdrawn from the ear vein of resting unanesthetised animals. Plasma renin activity was measured by the method of *Pickens et al.*⁵ with a minor modification⁶. Values for renin activity were expressed as nanogram angiotensin II equivalents generated per ml of plasma. Average difference of duplicated measurements was 7.8 per cent in 9 samples and a coefficient of variation was 18.3 per cent for a pooled plasma. Serum electrolytes were analysed with a flame photometry. Surgical procedures were performed through a small flank incision under a pentobarbital anesthesia (30 mg/Kg). The study consists of three experiments.

(I) The Acute Stage

Animals were fed a diet containing 0.2 g of sodium chloride pro day per animal. Plasma renin activity was measured during the first

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week after the surgery. The animals were divided into four groups.

Group 1 (15 animals): Preoperative normal animals.

Group 2 (11 animals): Left renal artery was constricted with a silver clip (diameter: 0.9 mm) and right kidney was left untouched. Plasma renin activity was measured 3 to 7 days after the surgery.

Group 3 (15 animals): Both renal arteries were constricted at the same time with silver clips (diameter: 0.9 mm). Plasma renin activity was measured in 7 animals 2 days and in 8 animals 6 days after the surgery.

Group 4 (8 animals): The abdominal aorta was constricted just above the renal arteries with a silver clip (diameter: 1.6 mm). Plasma renin activity was measured 3 to 6 days after the surgery.

(II) *The Chronic Stage*

Animals were fed a salt-rich diet containing 0.6 g of sodium chloride pro day per animal, because a feeding on a salt-rich diet increases the rise of blood pressure after the unilateral constriction of renal artery in the rabbit⁶. Left renal artery was constricted with a silver clip (diameter: 0.9 mm) and right renal artery was left untouched. Plasma renin activity and serum electrolytes were measured in the chronic stage. The term chronic stage is applied to the period beyond 10 weeks after the surgery.

(III) *Removal of the Clipped Kidney*

The clipped kidney was removed in 20 animals with the chronic stage of renal hypertension. Plasma renin activity and serum electrolytes were measured just before and 2 weeks after the removal of the clipped kidney.

RESULTS

(I) *The Acute Stage*

Plasma renin activity and simultaneously measured blood pressure were shown in Fig. 1. Blood pressure in the normal animals ranged between 60 mmHg and 105 mmHg, and plasma renin activity in the normal animals ranged between 0.8 ng/ml and 18.0 ng/ml. The upper normal limit was settled as 110 mmHg for blood pressure and as 20 ng/ml for

Fig. 1. Blood pressure and plasma renin activity in the acute stage of experimental renal hypertension.

plasma renin activity. Plasma renin activity increased above the upper normal limit in 5 of 11 animals with the unilateral constriction of renal artery, whereas blood pressure still remained within the normal limits in those animals. On the other hand, plasma renin activity remained within the normal limits in all animals with 2 exceptions both 2 and 6 days after the bilateral constriction of renal arteries, while blood pressure remained within the normal limits in all 7 animals 2 days after the surgery and increased above the upper normal limit in 5 of 8 animals 6 days after the surgery. Similar results were obtained in animals with the constriction of abdominal aorta. Plasma renin activity remained within the normal limits in all 8 animals, whereas blood pressure increased in 3 of 8 animals 3 to 6 days after the surgery. There was no clear correlation between plasma renin activity and blood pressure in animals with the acute stage of experimental renal hypertension.

Fig. 2. Blood pressure, plasma renin activity and serum electrolytes in the chronic stage of experimental renal hypertension.

(II) The Chronic Stage

Blood pressure, plasma renin activity and serum electrolytes were simultaneously measured in 32 animals beyond 10 weeks after the unilateral constriction of renal artery. The results were compared with those of normal animals (Fig. 2). Blood pressure in 40 normal animals ranged between 77 mmHg and 107 mmHg, and plasma renin activity in 31 normal animals ranged between 0.8 ng/ml and 9.2

ng/ml with 2 exceptions. Blood pressure increased above 120 mmHg in 24 of 32 animals with the unilateral constriction of renal artery. Marked increase in blood pressure above 150 mmHg was observed in 10 animals. Plasma renin activity increased above 10 ng/ml in 6 of 32 animals with the unilateral constriction of renal artery. All 6 animals which had higher values for plasma renin activity showed high blood pressure above 120 mmHg. How-

Fig. 3. Correlation between blood pressure and plasma renin activity in 32 rabbits with chronic renal hypertension.

Fig. 4. Correlation between blood pressure and serum potassium in 32 rabbits with chronic renal hypertension.

Fig. 5. Correlation between plasma renin activity and serum potassium in 32 rabbits with chronic renal hypertension.

ever, neither signs suggesting malignant hypertension nor increased blood urea nitrogen were observed in these animals. Serum sodium level ranged between 132 mEq/L and 151 mEq/L in 40 normal animals, and between 129 mEq/L and 147 mEq/L in 32 animals with the unilateral constriction of renal artery. Mean value with the standard error was 139.2 ± 0.6 mEq/L for the former and 141.5 ± 0.6 mEq/L for the latter. There was a large range of overlap between two groups. Serum potassium level ranged between 3.5 mEq/L and 4.9 mEq/L in 40 normals with one exceptional value of 3.2 mEq/L, and between 2.6 mEq/L and 4.7 mEq/L in 32 animals with the unilateral constriction of renal artery. Mean value with the standard error was 3.90 ± 0.06 mEq/L for the former and 3.71 ± 0.10 mEq/L for the latter. Hypokalemia below 3.5 mEq/L was observed in 8 of 32 animals with the unilateral constriction of renal artery.

The correlations between blood pressure and plasma renin activity, between blood pressure and serum potassium level and between plasma renin activity and serum potassium level were showed in Fig. 3, 4 and 5. Plasma renin activity remained in the lower level in several animals which had marked hypertension above 150 mmHg, although a positive correlation ($r=0.51$, $p<0.01$) was found between blood pressure and plasma renin activity. There were negative correlations between blood pressure and serum potassium level ($r=-0.57$,

$p<0.01$) and between plasma renin activity and serum potassium level ($r=-0.71$, $p<0.01$). Serum potassium level was consistently below 3.5 mEq/L in animals with higher values above 10 ng/ml for plasma renin activity. No significant correlations were found between blood pressure and serum sodium level ($r=0.193$, $p>0.05$) and between plasma renin activity and serum sodium level ($r=0.02$, $p>0.05$).

(III) Removal of the Clipped Kidney

Clipped kidney was removed in 20 animals with the chronic stage of renal hypertension. Blood pressure, plasma renin activity and serum electrolytes were measured before and after the nephrectomy (Fig. 6). Values for blood pressure were averages of 3 measurements with a weekly interval. Values for plasma renin activity and serum electrolytes were those of one measurement before and after the nephrectomy respectively. Removal of the clipped kidney was followed by a fall of blood pressure in almost all animals. However, blood pressure still remained in higher level above the upper normal limit in some animals even after the nephrectomy. Increased renin activity fell into the normal range and decreased serum potassium tended to return to the normal level. These results suggest that the clipped kidney plays a primary role in the maintenance of chronic renal hypertension and the mechanism may be unrelated with the renin-angiotensin system.

COMMENT

The present study could not reveal that the renin-angiotensin system plays a primary role in the pathogenesis of experimental renal hypertension. There was a marked discrepancy between blood pressure and plasma renin activity in both acute and chronic stage of renal hypertension. The results agree with the previous observations in dogs^{1,2} and in sheep³. *Lever et al.*⁴ measured plasma renin activity in rabbits with renal hypertension. The animals with renal hypertension showed higher renin values than the normotensive control animals, although there was a large overlap between the groups. However there was no clear correlation between plasma renin activity

Fig. 6. Blood pressure, plasma renin activity and serum potassium before and after the removal of clipped kidney in 20 rabbits with chronic renal hypertension. Open circles indicate animals in which average blood pressure was equal or above 120 mmHg before the nephrectomy. Solid circles indicate animals in which average blood pressure was below 120 mmHg before the nephrectomy.

and the rise of blood pressure.

In the acute stage of experimental renal hypertension plasma renin activity increased in about a half of animals after the unilateral constriction of renal artery with the untouched contralateral kidney and it did not increase in almost all animals after the bilateral constriction of renal arteries. The different response of plasma renin activity between the groups may be understood by study the renin content of kidney. *Regoli et al.*⁷ measured renin content of kidney in rats. Unilateral constriction of renal artery caused an increase or no change in renin content of the clipped kidney, depending on the presence or absence of an untouched contralateral kidney. If the contralateral kidney was left untouched, renin content of the clipped kidney increased in a few days and that of the untouched kidney decreased within 3 weeks. If the contralateral kidney was previously removed, renin content of the clipped kidney did not increase.

In the chronic stage of experimental renal hypertension, the activity of the renin-angio-

tensin system has been believed to return to the normal level. Recent studies indicated that plasma renin activity increased only in malignant phase and during the first few day of experimental renal hypertension^{1,8,9}. The present study, however, showed a different result from the previous ones. Plasma renin activity increased in 6 of 32 animals with the chronic stage of renal hypertension. The animals with higher renin values did not show any signs of malignant hypertension. There were also hypertensive animals in which plasma renin activity did not increase. These results suggest that an increase in plasma renin activity does not play a primary role, although it is undeniable that plasma renin is an accelerating factor in the maintenance of the chronic hypertension. Removal of the clipped kidney was followed by a fall of blood pressure in almost all hypertensive animals. The fall of blood pressure did not depend upon the previous level of plasma renin activity. An unknown renal mechanism may be involved in the maintenance of the chronic renal hypertension.

*Brown et al.*¹ revealed a striking correlation between plasma renin and sodium balance in dogs with renal hypertension. The present study showed no correlation between plasma renin activity and serum sodium level but a significant negative correlation between plasma renin activity and serum potassium level. The special study on the electrolyte balance and aldosterone measurement was not attempted in the present study.

REFERENCES

1. BROWN, T.C., DAVIES, J.O., O'LEIGH, M.J., AND JOHNSTON, C.I.: Relation of plasma renin to sodium balance and arterial pressure in experimental renal hypertension. *Circulation Res.*, **18**: 475, 1966.
2. FASCILO, J.C., DE VITO, E., ROMEO, J.C., AND CUCCHI, J.N.: The renin content of the blood of humans and dogs under several conditions. *Canad M.A.J.* **90**: 206, 1964.
3. BLAIR-WEST, J.R., COGHLAN, J.P., DENTON, D.A., ORCHARD, E., SCOGGINS, B.A. AND WRIGHT, R.D.: Renin-angiotensin system and sodium balance in experimental renal hypertension. *Endocrinology* **83**: 1199, 1968.
4. LEVER, A.F. AND ROBERTSON, J.I.S.: Renin in the plasma of normal and hypertensive rabbits. *J. Physiol.* **170**: 212, 1964.
5. PICKENS, P.T., BUMPUS, F.M., LLOYD, A.M., SNEY, R.R. AND PAGE, I.H.: Measurement of renin activity in human plasma. *Circulation Res.* **17**: 438, 1965.
6. FUJII, J., KURIHARA, H., YAMAGUCHI, H. AND IKEDA, M.: Salt-rich diet and experimental renovascular hypertension in the rabbits. *Tohoku J. Med.* **97**: 191, 1969.
7. REGOLI, D., HESS, R., BRUNNER, H., PETER, G., AND GROSS, F.: Interrelationship of renin content in kidney and blood pressure in renal hypertensive rats. *Arch. int. Pharmacodyn.* **140**: 416, 1962.
8. BLAQUIER, P., BOHR, D.F., AND HOOBLER, S.W.: Evidence against an increase in circulating pressor material in renal hypertensive rats. *Amer. J. Physiol.* **198**: 1148, 1960.
9. KOLETSKY, S., JACKSON, E.B.JR., HESS, B.M., RIVERA-VELEZ, J.M. AND PRICHARD, W.H.: Role of a pressor substance in unilateral renal hypertension. *Proc. Soc. Exp. Biol. Med.* **122**: 941, 1966.