PLASMA RENIN AND HYPERTENSIVE VASCULAR COMPLICATIONS: AN OBSERVATION IN THE STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT

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The hypothesis that plasma renin is a major risk factor in the development of cardiovascular complications of hypertension was evaluated in a model animal, the spontaneously hypertensive rat (SHR). Plasma renin levels and pathological findings were studied in stroke-prone and -resistant strains of SHR at various ages. The stroke-prone strain showed significantly higher plasma renin levels at and after seven months of age, while the stroke-resistant strain showed no significant differences from the control at any age. High plasma renin levels were always associated with evident vascular complications in the kidney and the brain, indicating underlying angioneclerosis and malignant transformation of hypertension. However, no evidence indicated that a higher plasma renin preceded the development of vascular lesions. Thus the high plasma renin would be a result rather than the cause of hypertensive vascular lesions.

SINCE, a possible vasculotoxic effect of plasma renin in hypertensive patients was suggested by *Brunner et al.*^{1,2} it has been a subject of controversy.³⁻⁷ Be it the case that plasma renin levels indicate prognosis of the patients, it may not be easy to determine in human subjects whether an elevated plasma renin is a cause or a result of serious vascular lesions. Fortunately *Okamoto et al.*⁸ recently succeeded in breeding the stroke-prone strain of the spontaneously hypertensive rat (SHR), of which

more than 80 per cent developed cerebral hemorrhage and/or infarction. As it is an excellent model of hypertensive vascular complications we intended to determine the relationship between plasma renin levels and vascular lesions at various stages of hypertension of these rats.

METHODS

Experimental Animals: Males of stroke-prone (SP) and stroke-resistant (SR) strains of SHR and normotensive Wistar-Kyoto (WK) rats of matched ages from 7 weeks to 12 months were subjected to this study. Each group consisted of five to eleven rats. The rats were fed with a standard chow (Oriental MF) and tap water. Some rats at ages of 4, 5 and 7 months were killed by decapitation to examine pathological changes, within 24 hours following the blood collection for the plasma renin determination. Morphological changes in the brain the heart and

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Fig.1. Influence of blood loss on plasma renin of the rat.

Observations in five normotensive WK rats weighing around 200g.

* $p < 0.05$, ** $p < 0.01$ by paired t-test

the kidney were examined macroscopically. Histological observations were made on brain and kidney tissues.

Collection of Blood and Blood Pressure Recording: A polyethylene catheter was indwelled in the abdominal aorta *via* the femoral artery

the day, before the blood collection. The rats were kept in individual cages without any other restraint. The catheter was connected to a syringe moistened with 15% ammonium EDTA for the blood collection with the least manipulation of the rat in conscious state. The amount of the blood withdrawn was restricted within 0.5 per cent of the body weight, since more blood loss than this limit was found to significantly increase the plasma renin level (Figure 1). Mean arterial pressure was recorded by connecting the catheter to a pressure transducer and an electro-manometer (Nihon-Koden).

Plasma Renin Concentration (PRC): Method of *Nasjletti and Masson*⁹ was modified as follows. To a 0.1 ml of plasma were added 10 μ l of 10% dimercaprol (in peanut oil), 5 μ l of 5% di-isopropyl fluorophosphate (in isopropyl alcohol), and 0.5 ml of rat renin substrate solution (equivalent to approximately 1,500 ng of angiotensin II) in 0.1 M phosphate buffer (pH 6.5) containing 0.01 M EDTA, 0.15 M NaCl and 5 μ M phenylmercuric acetate. The mixture was incubated at 37°C for 1 hour, and the concentration of angiotensin I was determined by

TABLE I PLASMA RENIN CONCENTRATION AND BLOOD PRESSURE^a

Age	Control (WK)	Stroke-resis.	Stroke-prone
7 weeks	36.4 $\pm$ 11.6 (91 $\pm$ 4, n=8)	51.2 $\pm$ 27.5 (126 $\pm$ 5, n=8)	27.1 $\pm$ 14.1* (114 $\pm$ 7, n=8)
12 weeks	27.8 $\pm$ 12.1 (97 $\pm$ 8, n=7)	20.1 $\pm$ 7.0 (143 $\pm$ 12, n=7)	20.9 $\pm$ 4.7 (152 $\pm$ 18, n=5)
4 months	16.7 $\pm$ 11.6 (97 $\pm$ 3, n=6)	20.1 $\pm$ 7.1 (166 $\pm$ 19, n=7)	21.5 $\pm$ 9.1 (173 $\pm$ 16, n=7)
5 months	12.6 $\pm$ 6.4 (102 $\pm$ 5, n=9)	18.9 $\pm$ 4.8 (157 $\pm$ 16, n=6)	29.4 $\pm$ 27.3 (182 $\pm$ 14, n=7)
7 months	17.2 $\pm$ 8.6 (101 $\pm$ 5, n=8)	15.2 $\pm$ 6.6 (154 $\pm$ 12, n=11)	46.5 $\pm$ 14.8*** (178 $\pm$ 18, n=9)
10 months	22.9 $\pm$ 1.7 (99 $\pm$ 7, n=5)	33.6 $\pm$ 24.7 (179 $\pm$ 27, n=7)	63.5 $\pm$ 25.2** (176 $\pm$ 32, n=5)
12 months	16.1 $\pm$ 5.5 (105 $\pm$ 4, n=5)	25.4 $\pm$ 10.4 (172 $\pm$ 24, n=5)	————— b

a. PRC is expressed by nanograms of angiotensin I produced in 1 hour per milliliter of plasma. Mean arterial pressures in mmHg and sample sizes are shown in parentheses. Values are mean $\pm$ s.d..

b. Most SP rats do not survive at this age.

*** Significantly different from both control and SR ($p < 0.001$)

** Significantly different from control ($p < 0.02$)

* Significantly different from SR ($p < 0.05$)

radioimmunoassay. The unit was expressed by nanograms of angiotensin I generated in 1 hour by 1 ml of plasma. Rat renin substrate was prepared according to the method of *Boucher et al.*¹⁰

Statistical Analysis: Student's t-test, paired t-test and, when the variances were not homologous, Fisher-Behrens' test were employed for the determination of significance of the difference between two means.

RESULTS

1. Plasma Renin Concentration of Substrains of SHR (Table I)

Plasma renin concentrations of SP strain younger than 5 months were not significantly different from the controls. In seven-week age groups, however, PRC of this strain was significantly less than that of SR strain ($p < 0.05$). Some SP rats in 5-month group had high PRCs. In 7-month and older groups this strain showed definitely higher PRCs than those in the controls.

The SR strain showed no significant difference in PRC from the control in any age groups, although some rats had high PRCs at and after 10 months.

2. Pathological Findings and Plasma Renin Levels (Table II)

No macroscopic vascular lesions or angioneurosis were found in both strains of SHR autopsied at 4 months of age, and PRCs of these

rats remained within the control range.

At 5 months, one SP rat had a prominently high PRC (No. 2 in Table II). Autopsy revealed that the brain of this rat manifested an area of softening in the cerebral cortex and a medium sized lesion of bleeding accompanied by edema in the basal ganglia (Figure 2A). The kidneys showed a marked feature of nephrosclerosis with severe microscopic vascular lesions: angioneurosis, perivascular hemorrhage and extensive fibrosis (Figure 3A). Another rat (No. 1 in Table II) which showed a moderately high PRC had a small area of cerebral softening (Figure 2B) and moderate nephrosclerosis. No macroscopic damages in either brain or kidneys were found in the other rats with normal PRCs.

Out of four SP rats autopsied at 7 months, the one which showed the highest PRC (No. 7 in Table II) had areas of cerebral softening and edema as well as marked nephrosclerosis (Figure 3B). Two rats with moderate nephrosclerosis (No. 9 and No. 10) showed moderately high PRCs. Plasma renin of the other one (No. 8) which had small fibrosis in the heart but no macroscopic lesions in the brain and the kidneys remained within the control range. Although some SR rats autopsied at 7 months had fibrosis in the heart or small scars in the kidney, their PRCs were normal.

Microscopic examination of kidney tissue revealed angioneurosis in all of the cases which showed high PRCs. Microscopic findings in SP

TABLE II FINDINGS IN STROKE-PRONE RATS

Age	Rat No.	Mean Arterial Pressure (mmHg)	Plasma Renin Conc. (ng/ml/hr)	Autopsy Findings*
5 months	1	180	32.5	CL (+), AN (++)
	2	200	88.5	CL (++), AN (+++)
	3	180	11.6	CL (-), AN (-)
	4	180	16.2	CL (-), AN (-)
	5	160	20.9	CL (-), AN (-)
	6	194	26.7	CL (-), AN (+)
7 months	7	190	62.1	CL (++), AN (+++)
	8	180	22.6	CL (-), AN (-), HF (+)
	9	205	46.9	CL (-), AN (+), HF (+)
	10	165	47.3	CL (-), AN (+), HF (+)

* CL: cerebral lesions; AN: angioneurosis; HF: fibrosis in the heart

Fig.2. A. The basal surface of the brain of a 5-month-old SHRSP; fresh hemorrhage is noted at the basal part of left caudate-putamen complex. (PRC: 88.5)
 B. The surface of the brain of a 5-month-old SHRSP; arrows indicate small lesions of softening in the left frontal cortex. (PRC : 32.5)

rats with normal PRCs were predominantly medial and intimal hyperplasia of arterioles with occasional hyalinization and fibrosis (Figure 3C). Renal arterioles of SR rats of these ages showed no pathological lesions (Figure 3D).

DISCUSSION

Concerning the plasma renin level of SHR, no consistent result have been obtained.¹¹⁻¹⁵ According to our opinion, the major source of the confusion among the past studies is variety and inappropriateness of the methods used for blood sampling. Plasma renin of the rat is readily susceptible to anesthesia, stresses, excess blood loss *etc.* during the blood collection (Figures 1,4,5). The blood taken by our method contained distinctly less renin than that obtained by decapitation or any other method, and is considered to closely reflect an intact

condition.¹⁶ Strains of rats used as controls in the past studies were not appropriate either. Wistar-Kyoto strain from which SHR was derived should be employed as the control. Genetic variation has been noted in catecholamine metabolism of the rat¹⁷ and could also occur in renin-angiotensin system.

In the present study, PRCs of SR strain of SHR showed no significant differences from the controls at any age from 7 weeks to 12 months. It has been noted that the development of vascular lesions in this strain is considerably variable in the advanced stage of hypertension and spontaneous incidence of stroke is less than 10 per cent. High PRCs found in some older rats appeared to correspond to the development of vascular lesions.

If the hypertensive vascular complications, however, were resulted from the vasculotoxic effect of circulating renin, the stroke-prone

Fig.3. A. Histology of the kidney of the same SHRSP as shown in figure 2A; extensive productive arteriolitis with angioneclerosis and marked perivascular fibrosis is observed.
 B. The kidney of a 7-month-old SHRSP; moderate or severe productive arteriolitis with angioneclerosis is noted. This case showed moderate cerebral edema. (PRC: 62.1)
 C. The kidney of a 5-month-old SHRSP; an arrow indicates the thickening of arterial wall without angioneclerosis. The brain had no vascular lesions. (PRC: 16.2)
 D. The kidney of a 7-month-old SHRSR with no vascular lesions. No cerebrovascular lesions were observed in this case. (PRC: 10.2)

Fig.4. Effects of anesthesia on plasma renin.

Blood samples were withdrawn from the indwelled catheter in conscious state, and the same volume of saline was injected into the catheter to replace the lost blood. One hour later the rats were anesthetized and blood samples were taken as soon as they lost righting reflexes. Ether and methoxyflurane were inhaled in glass jars; sodium amobarbital, 100mg/kg, was administered intraperitoneally. Each group comprised five rats.

* $p < 0.05$, ** $p < 0.01$ by paired t-test

strain should have a higher plasma renin than the stroke-resistant one prior to the development of significant vascular lesions. Results obtained in this study did not support this view. The PRC of SP rats was even lower than that of SR rats in an early stage of hypertension. Some vascular changes were seen without any significant increase in PRC, and no significant rise of PRC was detected before the development of evident vascular complications. Cardiac lesions did not appear to be related with plasma renin levels. On the other hand, high plasma renin levels always accompanied by severe vascular lesions in the kidney and the brain, particularly angioneclerosis in the kidney. And it could be said that high plasma renin is an index of underlying angioneclerosis and consequently of poor prognosis. It is well known that the majority of malignant or accelerated hypertension is associated with high plasma renin.¹⁸ The present result represents the counterpart of this clinical finding. Some reports pointed out that malignant hypertension could develop without high plasma renin or even in low renin states.¹⁹⁻²² Obviously, in these cases, the low renin level does not imply benign prognosis and the development of angioneclerosis cannot be attributed to vasculotoxic action of renin.²² Thus a high plasma renin is not prerequisite to angioneclerosis. And the high plasma renin levels could be a result but not the cause of serious

Fig.5. Comparison of renin levels in blood samples taken by decapitation and by indwelled catheter from conscious rats.

Decapitation was performed in a moment with sharp big scissors without head blows. Blood was collected during four seconds following the decapitation.

vascular lesions. However, there remained a possibility that angioneclerosis and the elevation of plasma renin level might share a common cause, such as exaggerated natriuresis seen in malignant hypertension.²²⁻²³ The possibility of this mechanism in the development of vascular complications in SHR is under investigation.

ADDENDUM

After the preparation of this paper, we obtained a report of *Carvalho et al.*: Methods for serial study of renin-angiotensin system in the unanesthetized rat (*Am. J. Physiol.* 228: 369-375, 1975). Their findings on the factors influencing plasma renin level of the rat are essentially the same as ours. The advantages of our methods over theirs are as follows. Laparotomy is avoided in our method for catheterization so that the influence of the operation is minimized. In addition, when restrained with a harness connected to a piece of spring wire, hypertensive rats are often weakened and the blood pressure tends to be decreased. And we protected the catheter only by plastering the free end on the back. The rat can be kept quite well for a long period in this manner.

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