Lack of Increased Renin-Angiotensin Activity in Rats with Spontaneous Hypertension[1] (34971)

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There are only a few reports dealing with the pathogenesis of spontaneous hypertension, such as that occurring in rats of the Okamoto–Aoki strain (1) apparently on a genetic basis. Aoki (2) found that removal of the pituitary, adrenals, or thyroid in the prehypertensive stage prevented development of hypertension while removal during hypertension caused lowering of the blood pressure to normotensive levels. However Nola-Panades and Smirk (3) concluded that since the high blood pressure was maintained in adrenalectomized rats given 1% saline for drinking it was not dependent on the adrenal glands. Sokabe (4) reported that the renin activity of the kidney of spontaneously hypertensive rats was about the same as for normal rats in the early stage of high blood pressure but later decreased significantly in comparison with the controls. Renin activity was determined by the extractable renin content of the kidney and by the pressor activity of the venous effluent of the kidney as obtained by grafting the organ onto a bilaterally nephrectomized recipient animal and noting the rise in blood pressure in the recipient (4).

The renin–angiotensin system of spontaneously hypertensive rats was also investigated in the present study, using different parameters of activity from those employed by Sokabe. The role of this system is especially important because spontaneous hypertension in the rat appears to resemble human benign essential hypertension more closely than other types of experimentally induced high blood pressure. Three parameters of activity were measured, namely, the juxtaglomerular index of the kidney, the vasopressor activity of renal vein blood as determined by bioassay, and the quantitative levels of renin in renal vein blood.

Method. Spontaneously hypertensive rats of the Okamoto–Aoki strain were used.[2] In addition, a female breeder of this strain was mated in our laboratory with a normotensive white male rat of the Sprague–Dawley strain, and the hypertensive offspring were then inbred through several generations, just as the Okamoto–Aoki strain was developed, so as to yield numerous spontaneously hypertensive animals. The latter were then used for determination of renin–angiotensin activity which was carried out in rats, both male and female, 6 weeks to 12 months of age whose blood pressures ranged from 134–200 mm Hg. A number of tests were performed on rats 6–8 weeks old at which time the blood pressure was 134–144 mm Hg and rising toward hypertensive levels, *i.e.*, $\geqq$ 150 mm Hg. Most of these animals were subsequently retested weeks or months later when they were clearly hypertensive. All blood pressures were obtained under anesthesia by introducing a No. 50 polyethylene catheter into the femoral artery and connecting this to a Hg manometer.

Juxtaglomerular index. Juxtaglomerular cell granulation was measured by the juxtaglomerular index (JGI) according to the method of Hartroft and Hartroft (5). The indices were determined either on a resected kidney or on a cylindrical biopsy of renal tissue approximately 5 × 3 mm which was removed with a dermatome after the organ was exposed surgically. Step serial sections of

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[2] Obtained from the Laboratory Aids Branch of the Division of Research Services of NIH.

the biopsy specimen provided 100 different glomeruli for counting. Control values were obtained on 20 normal rats whose age distribution was approximately the same as for the spontaneously hypertensive animals.

Renal vein plasma renin activity. This was determined on 0.25 ml of renal vein plasma according to the method of Gould and coworkers (6). The plasma was dialyzed overnight against 0.003 molar EDTA (Ethylene diamine tetracetate) in 1% saline at pH 7.0 and 5°, then incubated for 16 hr at 38° and pH 6.0 with an excess of hog renin substrate. The amount of reaction product, angiotensin II, was measured by a direct rat pressor assay in comparison with the pressor effect produced by a known amount of synthetic angiotensin II. The results were expressed as nanograms of angiotensin II formed per milliliter of plasma per 16 hr incubation. Control values were obtained on renal vein plasma from normal rats and from the ischemic left kidney of rats with acute renal hypertension for a period of 1 hr to 2 days. The high blood pressure was produced by constricting the aorta just above the ostium of the left renal artery while the contralateral kidney remained *in situ* (7).

Vasopressor activity of renal vein blood. For bioassay 0.5 ml of blood was withdrawn from the renal vein of hypertensive rats and injected immediately into the femoral vein of a recipient rat previously given pentolinium tartrate (4 mg/100 g body weight subcutaneously) for ganglionic blockade (8). The response was the rise in arterial pressure of the recipient as obtained on a Hg manometer connected by polyethylene catheter to the femoral artery. Each assay was controlled by injecting the same recipient with 0.5 ml of renal vein blood from a normal animal. The increment in pressor response in millimeters of Hg of the hypertensive over the normotensive control blood represented the pressor activity of the hypertensive blood and an increment greater than 8 mm Hg was considered a positive assay. For a positive control, recipients were injected with 0.5 ml renal vein blood from the ischemic left kidney of rats with acute renal hypertension. In the latter the blood was withdrawn between 1 hr and 2 days after constricting the aorta just above the ostium of the left renal artery while the contralateral kidney remained *in situ*. Also each recipient responded to an intravenous injection of 0.004 μg synthetic angiotensin II with a blood pressure elevation of 12–24 mm Hg.

TABLE I. Juxtaglomerular Index of Rats with Spontaneous Hypertension.

Rat	No.	JGI[a] Mean	JGI[a] Range
Spontaneous hypertension	20	23.6 ± 4.2	19–35
Normotensive controls	20	33.1 ± 6.6	22–44

[a] Mean ± SD.

Results. Juxtaglomerular index. Table I shows the mean JG index (JGI) and also the range of indices for 20 rats with spontaneous hypertension and for 20 normotensive control rats. The mean JGI and standard deviation of the mean was 23.6 ± 4.2 in the former and 33.1 ± 6.6 in the latter. The difference between the two groups is highly significant ($p < .001$).

Ten of the 20 indices were obtained in hypertensive rats 2–6 months old and 10 at 7–12 months of age. The mean JGI and standard deviation of the younger animals was 24.2 ± 4.9 and of the older 22.9 ± 3.4 and the difference was not statistically significant.

Renal vein plasma renin activity. The mean and the range of plasma renin activity in the spontaneously hypertensive rats, in the normotensive control rats, and in the rats with acute renal hypertension are shown in Table II. The mean and standard deviation

TABLE II. Renal Vein Plasma Renin Activity of Rats with Spontaneous Hypertension.

Rat[a]	Renal vein PRA[b] Mean	Renal vein PRA[b] Range
Spontaneous hypertension	216 ± 93.6	60–360
Normotensive controls	196 ± 58.6	120–240
Acute renal hypertension	942 ±390	480–1320

[a] 33 determinations in each group.

[b] Mean ± SD; nanograms angiotensin II per milliliter plasma/16 hr incubation.

for the spontaneously hypertensive animals (216 ± 93.6) was not significantly different from that of the normal rats (196 ± 58.6). On the other hand, the renal vein plasma renin activity of rats with acute renal hypertension (mean 942 ± 390) was substantially higher than in rats with spontaneous hypertension and in the normal rats, and the difference in comparison with each of the latter groups was highly significant ($p < .001$).

The mean and standard deviation of renal vein plasma renin activity was obtained in 13 hypertensive rats 2–6 months of age and again when these animals were 7–12 months old. The values in the younger and older rats were 258.5 ± 102 and 147.6 ± 101, respectively and the difference was statistically significant ($p < .01$).

Vasopressor activity of renal vein blood. Forty-one bioassays of renal vein blood from rats with spontaneous hypertension were negative for the presence of a vasoconstrictor agent. As a rule there was a difference of only a few millimeters Hg, or none at all, between the rise in pressure produced in the recipient by blood from spontaneously hypertensive rats and the rise produced by blood from the normotensive rats. As shown in Table III, 20 assays yielded a small negative increment (response of the recipient to hypertensive blood was less than to the normotensive control blood), 12 were in the 0 column indicating that the hypertensive and normotensive samples gave identical responses in the recipient rat while 9 assays yielded a small positive increment of 1–8 mm Hg. In contrast, renal vein blood from the ischemic kidney of rats with acute renal hypertension yielded a rise in blood pressure in the recipient, *i.e.*, 6–24 mm Hg, which in the great majority of cases exceeded by more than 8 mm Hg the rise produced by the normal blood samples.

Discussion. The juxtaglomerular indices in the spontaneously hypertensive rats were substantially lower than in the normotensive controls, and the difference between the two groups was highly significant. However, for reasons not clear, a similar significance did not apply to renal vein plasma activity which was approximately the same in the two groups. As for pressor activity of renal vein blood, the response of recipient rats to blood from hypertensive animals was generally smaller than to blood from the normotensive controls. This indicates a reduction in pressor activity of the renal vein blood of hypertensive animals as compared to normal rats.

It was reported that the renin activity of rats with spontaneous hypertension decreased after the blood pressure was elevated for several weeks (4). In this study the juxtaglomerular indices of spontaneously hypertensive rats 2–6 months of age were not significantly different from those of animals 7–12 months old. However, when the mean renal vein plasma renin activity of hypertensive rats 2–6 months old was compared with the activity of animals which had long-standing hypertension (7–12 months of age), the latter showed a significant decrease in activity ($p < .01$). This could be due to high blood pressure of long duration and/or to the development of intrarenal vascular disease.

The results of this study indicate that the renin–angiotensin activity of rats with spontaneous hypertension whether in the prehypertensive, early hypertensive, or well-developed stage of high blood pressure, is either reduced or within normal limits. Hence, neither the development nor maintenance of the high blood pressure is due to increased

TABLE III. Pressor Activity of Renal Vein Blood in Rats with Spontaneous Hypertension.

		Increments (mm Hg) in pressor response of hypertensive over normotensive blood			
Rat	No. assays	−8 through −1	0	1–8	9–22
Spontaneous hypertension	41	20	12	9	0
Acute renal hypertension	24	0	1	5	18

activity of the renin–angiotensin system. Moreover, the consistently negative pressor assays of blood from the renal vein of hypertensive rats excludes release by the kidney of a vasoconstrictor agent other than renin–angiotensin as the cause of the high blood pressure. The status of the renin–angiotensin system in rats with spontaneous hypertension appears to be similar to that in human benign essential hypertension. Etiologically, the latter has a strong genetic factor and no evidence of participation of the renin–angiotensin system (6, 9–12).

Summary. Three parameters of renin angiotensin activity were studied in rats with spontaneous hypertension. The juxtaglomerular index of these animals in the prehypertensive, early hypertensive, and chronic stage of the high blood pressure was significantly lower than in normal rats. Bioassays of renal vein blood for a pressor agent were consistently negative in hypertensive rats 6 weeks to 12 months of age, including tests performed in animals 6–8 weeks old when the blood pressure was rising to hypertensive levels. The renal vein plasma renin activity was within normal limits except for rats with long-standing high blood pressure which showed a significant reduction in activity. The findings indicate that neither the development nor maintenance of the high blood pressure in animals with spontaneous hypertension is due to increased activity of the renin–angiotensin system.

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