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# The Effect of Age and Norepinephrine on Renin Release by Rat Kidney Slices *In Vitro* (39140)[1]

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(Introduced by E. E. Selkurt)

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Although the spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki (1) has been studied extensively as a model of human essential hypertension, the role of the renin–angiotensin system in the etiology and maintenance of the hypertension is obscure. In the spontaneously hypertensive rat, elevated plasma renin activity has been reported by one investigator (2), but reduced activity has been reported by others in rats old enough to have documented hypertension (3, 4). A variety of factors, such as age, dietary sodium intake, anesthesia, and even differences in local genetic strains, have been invoked to explain the differing observations of renin activity reported by various investigators. Furthermore, a variety of normotensive strains have been used by other investigators for comparison with the SHR. These include Sprague–Dawley, Wistar, and Kyoto–Wistar, each with marked individual characteristics.

To minimize many of the factors known to influence plasma renin activity, an isolated kidney preparation appropriate for the study of basal renin release would be useful. A consistent, sensitive, and specific method for the *in vitro* study of renin release by isolated factors by the rat kidney slice has been developed in our laboratory (5–7). Previous studies utilizing this system have demonstrated stimulation of renin release from the slices by physiologic concentrations of catecholamines in kidney slices from Sprague–Dawley rats (7), but no significant effect on renin release by alterations of media concentrations of sodium, potassium, or calcium when osmolarity is maintained constant (6).

The present studies were designed to compare the effect of age and body weight on basal renin release by the kidney slice in rats of the spontaneously hypertensive, normotensive Kyoto–Wistar and Sprague–Dawley strains. In addition, the effect of age on the responses of renin to stimulation by norepinephrine was also examined in kidney slices from spontaneously hypertensive and normotensive Kyoto–Wistar rats.

*Methods. Kidney slice Preparations.* Twenty-eight male SHR and Kyoto–Wistar rats were utilized at 1.5 to 7 months of age, and 45 male rats of the Sprague–Dawley strain were also used as age-matched controls. All rats were fed on regular rat chow.

Kidney slices were prepared and incubated by a method previously described (6). In brief, both kidneys were removed under pentobarbital anesthesia of the Sprague–Dawley rats and following decapitation of both the SHR and Kyoto–Wistar rats and placed in ice-cold saline. Two 10 to 20-mg (dry weight) slices (0.5 mm thick) were taken from the superficial cortex of each kidney using a Stadie–Riggs microtome and were incubated in 2.0 ml of Robinson's medium containing 5.65 m*M* glucose at 25° in a shaking incubator saturated with 95% $O_2$–5% $CO_2$. The pH of the medium was 7.4 and the osmolality was maintained at 350 mOsm/liter by the addition of mannitol. Previous studies have demonstrated osmolar stimulation of renin release (5), and for this reason, media osmolarity was kept constant throughout all control and experimental periods to permit the addition of the experimental agents.

Each slice was preincubated for 15 min. The preincubation medium was aspirated and the slice was incubated with 2.0 ml of

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fresh Robinson's medium for four successive 20-min periods (A, B, C, and D). The incubation medium was aspirated after each period and added to a tube containing 20 $\mu$l of dimercaprol (100 mg/ml), 20 $\mu$l of 8-hydroxyquinoline (0.34 *M*), and 0.1 ml of 4% EDTA. These agents inhibit both angiotensinase and converting enzyme.

In both the spontaneously hypertensive and Koyto–Wistar rats, the effect of norepinephrine on renin release *in vitro* was studied. Since norepinephrine is rapidly oxidized at alkaline pH (8), an antioxidant, ascorbic acid ($6 \times 10^{-5}$ *M*) was added to the Robinson's media. Ascorbic acid alone does not have any effect on renin release by kidney slices nor any influence on angiotensin I generation (7). Norepinephrine bitartrate (Calbiochem) was added to the Robinson's medium in period C in concentrations of 1.5 to $10^{-9}$ *M* (7).

*Angiotensin I generation and quantitation of renin release.* Renin-containing medium (0.05 ml) from the kidney slice incubate was added to 1.25 n*M* hog renin substrate (Pentex, Inc.) with angiotensinase and converting enzyme inhibitors and incubated for 1 hr at 37° in a shaking incubator. The amount of angiotensin I thus generated was determined by a radioimmunoassay technique previously described (6, 7). Total renin activity was corrected by the use of substrate- and renin-containing media blanks and expressed as nanograms angiotensin I generated per milligram of dry kidney weight. Norepinephrine alone was previously shown not to influence the angiotensin I generation or the radioimmunoassay of angiotensin I.

Previous studies utilizing this technique have demonstrated the stability of renin release over four consecutive 20-min incubation periods (6), thus permitting each kidney slice to serve as its own control (Periods A, B, and D) for the experimental period (C). Renin release during period B was utilized as basal renin release. The basal renin release from each of four kidney slices from one rat was added and expressed as nanograms angiotensin I generated per milligram dry kidney weight. These data were then compared on the basis of age by linear regression analysis and the regression lines compared for the three strains studied. In the norepinephrine experiments, the statistical significance of the experimental observations was evaluated using a paired *t* test, comparing the experimental period to the preceding control period.

*Results. Growth rates within strains.* Figure 1 compares growth rates of SHR, normotensive Kyoto–Wistar, and Sprague–Dawley rats at ages 45 to 220 days (1.5 to 7 months). All three strains demonstrated a two-slope growth curve, with rapid early growth and a slower second phase. Sprague–Dawley rats completed their initial growth curve at 85 days, while SHR and Kyoto–Wistar strains grew more slowly, completing the initial phase at 130 days. After 4 months of age, body weight in the SHR did not increase significantly with age, but that of the Kyoto–Wistar rats increased

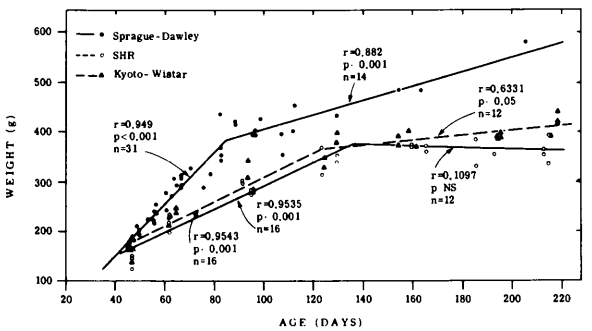


FIG. 1. Growth rates for Sprague–Dawley, SHR, and Kyoto–Wistar rats. Symbols for strains are indicated in the key; correlation coefficients, significance level, and number of observations for each slope are also indicated.

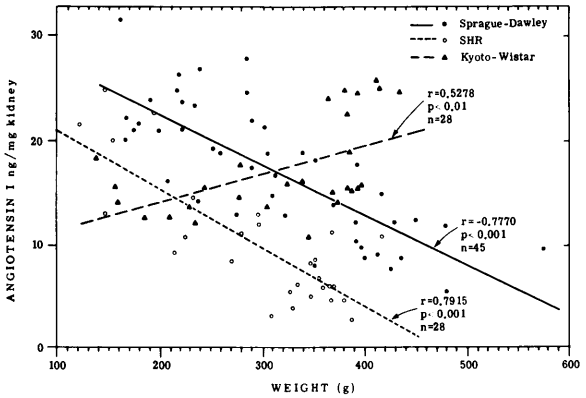


FIG. 2. Relationship between basal renin release of kidney slices and body weight in the three strains of rats. Symbols as for Fig. 1 and indicated in the key; correlation coefficients, significance level, and number of observations for each strain are also indicated.

at a very slow rate. A comparison of the body weight–age relationships between spontaneously hypertensive and Kyoto–Wistar rats showed no significant difference, but both strains were consistently smaller than Sprague–Dawley rats of comparable age.

*Relationship between basal renin release and body weight.* Figure 2 demonstrates the relationship between kidney slice basal renin release and body weight in the three strains. As shown in the figure, basal renin release decreased significantly ($P < 0.001$) with increasing body weight in the spontaneously hypertensive and the Sprague–Dawley rats. Furthermore, basal renin release of kidneys of spontaneously hypertensive rats was significantly less on a body weight basis than that of Sprague–Dawley rats. In contrast, basal renin release of Kyoto–Wistar rats increased significantly ($P < 0.01$) with augmentation of body weight.

Figure 3 demonstrates the comparison of basal renin release among the spontaneously hypertensive, Sprague–Dawley and Kyoto–Wistar rats of comparable age. Basal renin release of the Sprague–Dawley rats decreased significantly ($P < 0.001$) until 3 months of age, similar to the decrease seen in SHR. There was no significant difference in the basal renin release between the spontaneously hypertensive and Kyoto–Wistar rats at 1.5 and 2 months of age, but when 3–7 month old SHR were

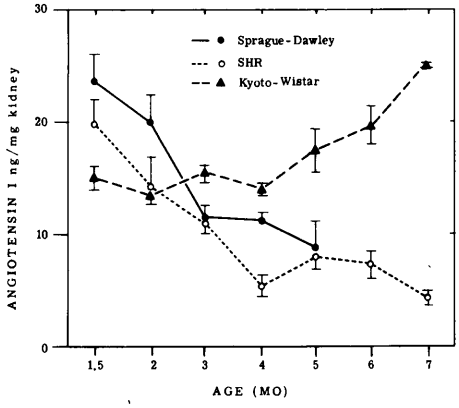


FIG. 3. Relationship between basal renin release of kidney slices and age in the three strains of rats. Symbols as for previous figures and indicated in the key.

compared to age-matched Kyoto–Wistar controls, significant differences ($P < 0.05$ to $< 0.001$) were observed.

*Effect of norepinephrine on renin release of kidney slices from SHR and Kyoto–Wistar rats.* Figure 4 shows the effects of norepinephrine on renin release from kidney slices of spontaneously hypertensive and Kyoto–Wistar rats at different ages. At all points, significant stimulation of renin release was observed in both strains after addition of norepinephrine quantitatively greater in SHR. No significant differences in the magnitude of renin release was seen between the two strains at 1.5 and 2 months of age, when the SHR are not yet hyperten-

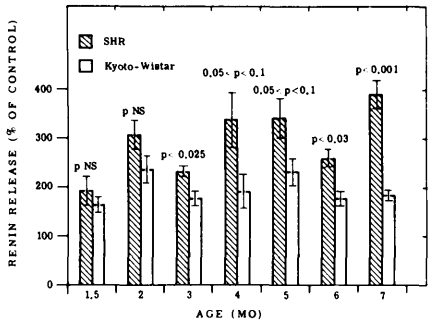


FIG. 4. Effect of norepinephrine on renin release by kidney slices of SHR (hatched bars) and Kyoto-Wistar (open bars) rats at different ages. Renin release is expressed as the percentage increase over the basal observation after addition of $1.5 \times 10^{-9}$ *M* norepinephrine to the incubation media. Significance levels for the difference in response between the two strains is indicated.

sive. At 3–7 months however, the effect of norepinephrine on renin release was noted to be greater in SHR than Kyoto–Wistar rats.

*Discussion.* The role of renin as a humoral factor involved in the development and maintenance of the hypertension in SHR is controversial. deJong *et al.* (2) reported that plasma renin activity in SHR was increased after the onset of hypertension and remained elevated with age and sustained hypertension when compared to Wistar rats. Forman and his colleagues (9) found normal levels of plasma renin activity in SHR 6–8 months old when compared to Wistar and Sprague–Dawley of a similar age during normal sodium intake, but observed a blunted response of PRA to sodium restriction in 6–8 month old SHR and Wistar rats when compared to Sprague–Dawley. Sen *et al.* (4) have demonstrated that plasma and renal renin activity were both significantly elevated before and during the initial phase of hypertension and normal or subnormal during the established phase when compared to Wistar controls. Thus, these investigators suggested that renin may play a primary role in the initiation of hypertension in SHR. Sokabe (3) has reported a low renal renin content during the hypertensive stage in SHR when compared to rats of the Kyoto–Wistar strain and Baer and his co-workers (10) have reported suppressed plasma renin activity in SHR when compared to Wistar controls. However, the age and anesthetic agent used in the latter study were not specified, precluding valid interpretation of their results.

The differences between the results obtained by other investigators may possibly be due to differences in the method of collection of blood samples as well as a variety of other factors known to influence plasma renin activity in humans and in animals, such as catecholamines and sympathetic nervous system activity (11), dietary sodium (12), anesthesia (12), surgery (13), and age (14). To minimize the effects of such influences, a system devoid of hemodynamic, neurogenic, and hormonal influences would provide a convenient method for the study of basal renin release. Such isolation can be achieved by an *in vitro* kidney slice system whose characteristics have been previously described (6, 7).

The present studies indicate that basal renin release by kidney slices in SHR and Sprague–Dawley rats significantly decreased with increasing body weight and advancing age and was significantly lower on a body weight basis in SHR than in Sprague–Dawley rats, as shown in Fig. 2. In contrast, basal renin release in Kyoto–Wistar rats increased significantly with age. Sokabe (3) and Sen *et al.* (4) have found decreased renal renin content in SHR after hypertension develops, which is consistent with our observations of basal renin release by kidney slices from SHR.

It is possible that basal renin release could have been influenced by the method of preparing the animals. In the present study, the Sprague–Dawley rats were anesthetized with intraperitoneal barbiturate, but the SHR and Kyoto–Wistar rats were decapitated. Bozovic and Efendic (15) found a fivefold increase in renin release of kidney slices from rats killed by decapitation in comparison to that from rats anesthetized with sodium thiopental. Thus, it can be inferred that decapitation would produce higher basal renin release, and barbiturate anesthesia lower renin release, observations opposite those actually seen in the present study. In addition, such methodol-

ogic differences cannot account for the differences in basal renin release observed between SHR and Kyoto–Wistar rats, since both were decapitated.

Basal renin release in relation to age of SHR was similar to that of Sprague–Dawley and different than that of Kyoto–Wistar rats. When Kyoto–Wistar rats were used for comparison, basal renin release of SHR declined with age at a time when the SHR became hypertensive (4).

As demonstrated in Table I, plasma renin activity (PRA) in SHR reported by different investigators was variable depending on the age of the animal, the control strain and the anesthesia used. These discrepancies could be related to the factors previously mentioned. Despite the differences in strains used and methods of anesthesia and collection of blood, renal renin content was reported by other investigators to be decreased consistently at a time when SHR became hypertensive. These observations also are consistent with our findings of decreased basal renin release by kidney slices from SHR with increasing age and development of hypertension.

The causes for decreased basal renin release with age and body weight in SHR and Sprague–Dawley rats can only be speculated upon. In SHR, low renal renin content has been thought to be due to degenerative changes in the juxtaglomerular apparatus secondary to long-standing hypertension (3, 4). In normal humans the age-related decrease in plasma renin activity may be related to the reported decrease in glomerular filtration rate (16) and in functional size of the kidney with age (17).

The results of the present studies further suggest that as the SHR developed hypertension, and at a time when basal renin release demonstrated a significant decline, the effect of norepinephrine on the stimulation of renin release by the kidney slice was noted to be greater in SHR than in Kyoto–Wistar rats (3 to 7 months of age). Okamoto *et al.* (18) first pointed out the importance of the increased sympathetic vasoconstrictor discharge in the maintenance of hypertension in SHR. Haeusler and Harfely (19) found that mesenteric arteries isolated from SHR were more sensitive to norepinephrine when compared to vessels obtained from Wistar strains. It has also been shown that there is a decline in adrenergic response of vascular smooth muscle with age in normal rats (20). These findings are consistent with an enhanced renin response to stimulation by sympathetic activity in SHR which could account for the increased PRA in SHR reported by other investigators. Additional evidence suggesting an abnormality in the responsiveness of the SHR to sympathetic stimulation can be obtained from environmental experiments. Spontaneously hypertensive rats have been shown to have an increased cardiovascular reactivity to irritating stimuli (21), while being chronically maintained in a quiet dark environment retards the development of the hypertension (22). These observations all suggest that in

TABLE I. Reported Observations of Renin Activity in SHR.[a]

| Investigator | Control strain | Age | Anesthesia | Observations of renin activity in SHR |
|---|---|---|---|---|
| deJong (2) | Wistar | 12 weeks | Decapitated | ↑ PRA ↓ RRC |
| | | 35 weeks | Decapitated | ↑ PRA ↓ RRC |
| Forman *et al.* (9) | Wistar | 6–8 months | Decapitated | Normal PRA |
| | Sprague–Dawley | 6–8 months | Decapitated | Normal PRA |
| Sen *et al.* (4) | Wistar | Early (60–150 g) | Ether | ↑ PRA ↑ RRC |
| | | Late (200–300 g) | Ether | ↓ PRA ↓ RRC |
| Sokabe (3) | Kyoto–Wistar | 5 weeks | Barbiturate | Normal RRC |
| | | 11 weeks | Barbiturate | ↓ RRC |
| Baer *et al.* (10) | Wistar | Not specified | Not specified | ↓ PRA |
| Aoi *et al.* (6) | Kyoto–Wistar | 1.5–7 months | Decapitated | ↓ RR (with age) |
| | Sprague–Dawley | 1.5–7 months | Barbiturate | Similar ↓ RR (with age) |

[a] PRA, plasma renin activity; RRC, renal renin content; RR, renin release; the arrows indicate the direction of significant change for the parameter measured in SHR as compared to the indicated control strain.

SHR enhanced sensitivity to the cardiovascular and renal effects of adrenergic stimulation may play a role in the hypertensive process.

Hypertension-prone salt-sensitive rats and hypertension-resistant rats were both developed from the Sprague–Dawley strain by Dahl *et al.* (23). Recently, Iwai *et al.* (24) reported that these hypertension-prone rats had significantly lower renin activities in both plasma and kidneys than did the hypertension-resistant rats, suggesting that neither sodium intake nor hypertension itself was responsible for the renin suppression, but rather other factors such as adrenal abnormalities producing sodium retention or genetically mediated mechanisms may be involved (25).

The decreased basal renin release observed with age in SHR may be a normal response, since similar changes in basal renin release with age were also observed in Sprague–Dawley rats without hypertension, and since it is known that plasma renin activity decreases with age in normotensive humans (14). It is also possible that basal renin release in SHR may be suppressed by unidentified factors such as excessive mineralocorticoid activity or genetic factors previously cited (25).

*Summary.* Basal renin release by rat kidney slices decreases with age in rats of SHR and Sprague–Dawley strains. In contrast, Kyoto–Wistar rats (from which SHR are derived) demonstrate no decrease in renin release with age. The decline in basal renin release observed in SHR occurs at a time when the animal develops hypertension. However, the ability of renin release to respond to stimuli, such as norepinephrine, is enhanced at the time of declining basal renin release and developing hypertension.

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