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RENIN-LIKE ACTIVITY IN SUBMAXILLARY GLAND IN SEVERAL STRAINS OF RATS INCLUDING THE SPONTANEOUSLY HYPERTENSIVE RAT

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Abstract—High levels of a renin-like enzyme were found in submaxillary gland of spontaneously hypertensive (SH) male rats. The submaxillary renin-like activity increased with age. Similar high levels were measured in the normotensive strain of rats from which the SH rats were derived, while low levels were found in two other normotensive strains. Enzyme levels were not markedly lower in females and gonadectomized males, as has been observed in mice. It is concluded that the submaxillary gland renin-like activity in the SH rat appears to not be linked to the development of genetic hypertension.

THE OCCURRENCE of a renin-like enzyme in submaxillary gland has been described in mice.[1–5] Enzyme levels increase with age and show considerable variation in different mouse strains.[1,4,6,7] In male mice the enzyme level in submaxillary gland is about 10- to 20-fold higher than in the female.[6,8,9] Castration of male mice results in reduced levels of enzyme which are restored by administration of testosterone.[9] The presence of renin-like activity in submaxillary gland of rats has not previously been reported. However, recent observations in this laboratory indicated that an extrarenal source of renin exists in spontaneously hypertensive (SH) rats, since it was found that plasma renin activity was still present after nephrectomy.[10] This finding led us to investigate whether submaxillary gland in SH rats, like that in mouse, contains a renin-like enzyme. Data will be presented to show that in SH rats and in the strain of Wistars from which the SH rats were derived, a renin-like enzyme occurs in the submaxillary gland and that the amount of enzyme increases with age.

MATERIALS AND METHODS

Drugs used and sources were as follows: phenoxybenzamine hydrochloride, Smith, Kline & French Inc.; pentolinium tartrate, Wyeth Laboratories Inc.; atropine sulfate, CalBiochem; Val^5-angiotensin II (Hypertensin), CIBA Inc.; and propranolol hydrochloride, Ayerst Laboratories Inc. Doses are expressed in terms of salt.

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Animals. The spontaneously hypertensive (SH/Kyoto) rats were derived from the Wistar/Kyoto by selective inbreeding.[11] Some of the SH/Kyoto rats were brought to the National Institutes of Health (NIH) in 1966 (F_{13}) and have since been maintained as a separate strain by continuous inbreeding,[12] and are referred to as the SH/NIH.

Male and female SH/NIH rats (F_{23-26} generations) and normotensive Wistar rats (NIH) of the same age were used. Body weight and weight of submaxillary glands of the SH rats did not differ from that of the normotensive controls. Male Sprague–Dawley rats were obtained from Zivic-Miller Laboratories Inc. (Allison Park, Pa.) at an age of 8 weeks. Breeders of the normotensive Wistar Kyoto/strain and frozen submaxillary glands of male rats of this strain and SH/Kyoto rats were provided by Dr. Y. Yamori of Kyoto, Japan. Experiments were done at an age of 14–16 weeks, unless otherwise specified. Rats were caged in groups of 4–8 and maintained under standardized conditions; they had unrestricted access to food (Purina laboratory chow containing 0·5% sodium chloride) and distilled water.

Methods. Blood pressure was determined on unanesthetized rats using a tail plethysmographic technique.[13] Removal of submaxillary glands, nephrectomy and gonadectomy (testes and epididymis) were performed under ether anesthesia. To obtain basal values of plasma renin activity, 2–3 rats per cage were kept overnight in a quiet rat room. Between 8 and 9 a.m. the following day, the animals were decapitated immediately upon removal from the cage and blood was collected from the neck for plasma renin studies. Organs were removed and immediately frozen on dry ice and kept at −16° until used.

Plasma renin activity was measured by the method of Pickens *et al.*[14] with modifications described in detail previously.[15,16] Two ml plasma was incubated, under conditions known to completely inhibit angiotensinase activity,[14] for 4 hr at pH 5·5 and 37°. The angiotensin generated from endogenous substrate was then estimated by blood pressure bioassay.[17] The animals used for assay were nephrectomized 6–16 hr before use and treated with autonomic blocking agents (atropine sulfate, 4 mg/kg; phenoxybenzamine hydrochloride, 8 mg/kg; pentolinium tartrate, 8 mg/kg) for optimal sensitivity in the response to angiotensin. Val^5-angiotensin II was used as standard in all bioassays. Propranolol hydrochloride (10 mg/kg) was administered subcutaneously three times daily for 3 days.

For the determination of tissue renin content, tissues were homogenized in ice-cold 0·9% NaCl (5–10 ml/g of tissue) and the homogenates were centrifuged for 20 min at 30,000 *g* at 4°. An aliquot of the supernatant (4–40 mg tissue) was added to 2 ml plasma with a high renin substrate concentration (3·0 to 4·0 μg angiotensin II equivalents per ml). This plasma was obtained from male Sprague–Dawley rats which had been nephrectomized 16–20 hr earlier. The mixture was incubated under conditions for assay of plasma renin activity as described above. Angiotensin generated was expressed as angiotensin II equivalents. Renin content of kidney was assayed directly by the pressor response of the homogenate (20 mg) in nephrectomized rats.[15] Renin substrate in plasma was determined by a substrate exhaustion technique as described by Nasjletti *et al.*[18] Partially purified rat kidney renin (1 Goldblatt units/mg) was prepared as reported previously.[19]

Data are expressed as means ± standard error of the mean (S.E.M.). Significance of observed differences was analyzed by Wilcoxon's two sample test.[20]

RESULTS

We have previously reported that plasma renin activity was substantially higher in spontaneously hypertensive (SH/NIH) rats than in Wistar/NIH or Sprague–Dawley control animals.[10] As shown in Table 1, nephrectomy reduced plasma renin activity of

TABLE 1. EFFECT OF NEPHRECTOMY ON PLASMA RENIN ACTIVITY AND RENIN SUBSTRATE OF NORMOTENSIVE WISTAR AND SPONTANEOUSLY HYPERTENSIVE (SH) RATS*

Animals	No. of rats	Plasma renin activity (ng/2 ml/4 hr)	Renin substrate (ng/ml)
Wistar/NIH			
Sham-operated	5	3·1 ± 0·2	611 ± 53
Nephrectomy	9	undetectable	2506 ± 149
SN/NIH			
Sham-operated	7	10·3 ± 2·2	719 ± 39
Nephrectomy	8	11·1 ± 2·8	2613 ± 134

* Values are means ±S.E.M. Plasma renin activity and renin substrate are expressed as angiotensin II equivalents and are measured 18 hr after nephrectomy.

normotensive rats to an undetectable level (< 0·8 ng/2 ml/4 hr), while no reduction was observed in SH rats. The renin substrate levels increased after nephrectomy to the same extent in both groups of rats. It may be that the plasma renin content decreased slightly after nephrectomy, since the elevated substrate would have increased the apparent renin activity. These findings suggest that in the SH rats there is a source of renin other than the kidneys. Therefore a survey was made of several tissues for the presence of renin-like activity. Tissue homogenates of 14- to 16-week-old Wistar/NIH and SH/NIH rats were incubated for 4 hr in the presence of excess renin substrate (nephrectomized plasma). A considerable amount of renin-like activity was found in submaxillary salivary glands of SH/NIH rats (300–700 ng/10 mg/4 hr), but not in the normotensive Wistar/NIH rats. Levels in other tissues, which included parotid gland, lung, thymus, spleen, liver, mesenteric artery, adrenals and pancreas, were low (< 50 ng/10 mg/4 hr). To determine whether submaxillary glands were a source of plasma renin activity in SH rats, plasma renin activity was measured 18 hr and 3 days after the removal of the submaxillary glands in 14- to 16-week-old animals. In an additional experiment, submaxillary glands were removed in 5-week-old SH rats and these animals were killed after 7 weeks. Plasma renin activity was reduced to an undetectable level by the combined nephrectomy and removal of submaxillary glands, while it was decreased to about 40 per cent ($P < 0·01$ compared to sham-operated group) 18 hr after removal of submaxillary glands only (Table 2). However, 3 days after the latter operation, plasma renin activity was partially restored to about 60 per cent ($P < 0·05$ compared to sham-operated group), and in the animals studied 7 weeks after the operation no differences were observed (Table 2). Macroscopic inspection indicated that there was no regrowth of submaxillary tissue. Blood pressure was not significantly affected by removal of the submaxillary gland.

TABLE 2. PLASMA RENIN ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS AFTER NEPHRECTOMY AND REMOVAL OF THE SUBMAXILLARY GLANDS*

Animals	No. of rats	Plasma renin activity (ng/2 ml/4 hr)
Wistar/NIH		
Sham-operated	9	2·6 ± 0·3
SH/NIH		
Sham-operated	7	12·1 ± 1·2
Nephrectomy + submaxillaryectomy (18 hr)	7	undetectable
Submaxillaryectomy (18 hr)	6	4·7 ± 1·0
Submaxillaryectomy (3 days)	7	6·9 ± 1·2
Submaxillaryectomy (7 weeks)	6	10·5 ± 1·2
Sham-operated	11	11·8 ± 0·9

* Values are means ±S.E.M. Plasma renin activity is expressed as angiotensin II equivalents. Blood pressure of the SH rats showed no substantial change after the operation.

Since renin-like activity in submaxillary gland increases with age in mice,[9] male normotensive Wistar/NIH and SH/NIH rats of different ages were studied for changes in renin activity in submaxillary gland. As shown in Fig. 1, low levels of renin-like activity were found at 5 weeks of age in both strains. Significantly ($P < 0·01$) elevated levels appeared at 12 weeks of age. By 16 weeks, the enzyme level was maximal and appeared to remain at about the same level up to 40 weeks of age. Little or no difference in renal renin has been observed in SH rats through the first 20 weeks of age.[10]

Unlike the mouse, female rats had only slightly lower ($P > 0·1$) submaxillary renin-like activity than the males (Table 3). The SH rats were derived by means of selective inbreeding from the normotensive Wistar/Kyoto strain.[11,12] To determine if high submaxillary levels of renin-like activity also develop in this strain, we compared submaxillary renin-like activity in male Wistar/Kyoto, SH/Kyoto rats and in male Sprague–Dawley rats. Both Kyoto strains had high submaxillary renin activity. The normotensive Sprague–Dawley rats had low levels similar to that of the Wistar/NIH (Table 3).

To determine whether submaxillary renin-like activity develops independently of the presence of testosterone, gonadectomy was performed in 5-week-old male SH rats. The rats were killed at 16 weeks of age. No significant reduction of plasma renin activity or submaxillary renin-like activity was observed (Table 4). Blood pressure of gonadectomized rats did not increase after 10–12 weeks of age, and at the end of the experiment was slightly lower than that of the sham-operated controls. Increase in body weight was significantly less in the gonadectomized rats.

Since stimulated renin release from the kidney can be blocked by β-adrenergic blocking agents, the effect of propranolol on the renin system of SH rats was studied. A significant ($P < 0·01$) reduction of plasma renin activity occurred, while renin substrate and kidney renin content were not changed (Table 5). Propranolol treatment caused a slight reduction ($P > 0·1$) in submaxillary renin-like activity. No significant decrease in blood pressure was observed.

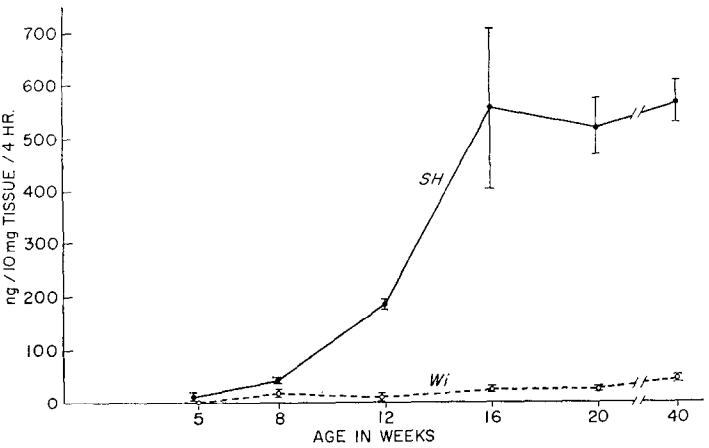

FIG. 1. Renin-like activity in submaxillary glands of normotensive Wistar (○- - - ○,Wi) and spontaneously hypertensive (●——●, SH) rats. Points represent mean values (± S.E.M.) of angiotensin generated *in vitro* in 2 ml plasma obtained from nephrectomized rats (n = 6–8).

TABLE 3. RENIN-LIKE ACTIVITY IN SUBMAXILLARY GLANDS OF SEVERAL STRAINS OF RATS*

Animals	No. of rats	Sex	Submaxillary renin activity (ng/10 mg/4 hr)
Wistar/NIH	6	♀	5 ± 2
SH/NIH	6	♀	389 ± 33
Wistar/NIH	7	♂	33 ± 5
SH/NIH	7	♂	559 ± 75
Wistar/Kyoto	9	♂	443 ± 35
SH/Kyoto	8	♂	598 ± 41
Sprague–Dawley	5	♂	59 ± 8

* Values are means ±S.E.M. The submaxillary gland renin activity is based on the amount of angiotensin generated *in vitro* in 2 ml plasma obtained from nephrectomized rats and expressed as angiotensin II equivalents. The animals used in this study were 16 weeks of age, except the Wistar/Kyoto and SH/Kyoto rats which were 28–32 weeks of age.

DISCUSSION

The occurrence of significant amounts of renin-like activity in submaxillary gland of rats has not been previously reported. The present data show that in some strains of rat the submaxillary gland contains substantial renin-like enzymatic activity and that the activity increases with age, as reported in several mice strains.[6,9] The finding of high levels in both the SH rats and the normotensive Wistar/Kyoto strain suggests that this renin-like activity is not primarily linked to genetic hypertension. The high

TABLE 4. EFFECT OF GONADECTOMY ON PLASMA RENIN ACTIVITY AND SUBMAXILLARY RENIN-LIKE ACTIVITY IN MALE SPONTANEOUSLY HYPERTENSIVE RATS*

Animals	No. of rats	Plasma renin activity (ng/2 ml/4 hr)	Submaxillary renin activity (ng/10 mg/4 hr)
Sham-operated	6	10·3 ± 1·3	455 ± 102
Gonadectomy	7	8·1 ± 1·5	381 ± 69

* Values are means ±S.E.M. Plasma renin activity is expressed as angiotensin II equivalents. The submaxillary gland renin activity is based on the amount of angiotensin generated *in vitro* in 2 ml plasma obtained from nephrectomized rats and expressed as angiotensin II equivalents. The blood pressure and body weight in sham-operated and gonadectomized rats were, respectively, 199 ± 6 mm Hg and 304 ± 6 g, and 181 ± 3 mm Hg and 263 ± 3 g.

TABLE 5. EFFECT OF PROPRANOLOL ON THE RENIN SYSTEM AND BLOOD PRESSURE IN THE SPONTANEOUSLY HYPERTENSIVE RAT*

Treatment	No. of rats	Plasma renin activity (ng/2 ml/4 hr)	Renin substrate (ng/ml)	Kidney pressor response (mm Hg)	Submaxillary renin activity (ng/10 mg/4 hr)	Blood pressure (mm Hg)
0·9% Saline	8	7·6 ± 1·8	837 ± 43	47 ± 2	700 ± 40	205 ± 6
Propranolol	8	2·7 ± 0·2	750 ± 52	46 ± 3	505 ± 84	193 ± 6

* Values are means ±S.E.M. Plasma renin activity and renin substrate are expressed as angiotensin II equivalents. The submaxillary gland renin activity is based on the amount of angiotensin generated *in vitro* in 2 ml plasma obtained from nephrectomized rats and expressed as angiotensin II equivalents.

level of renin-like activity in submaxillary gland of male mice is known to be dependent on the presence of the gonads.[9,21] In the SH/NIH rats a different control mechanism appears to exist, since levels in the female were only slightly lower than in the male and gonadectomy of male SH/NIH rats did not prevent the rise of submaxillary renin-like activity with age. Another difference in the submaxillary glands of mice and rats is that high levels of nerve-growth factor are present in mouse glands[22] but cannot be detected in SH/NIH rat submaxillary glands.* High levels of extrarenal renin-like activity have also been reported in placenta and uterus.[21] As yet, the function of renin-like enzyme activity of these different tissues is not clear.

Plasma renin activity of SH/NIH rats is higher than that of the normotensive Wistar/NIH strain[10] of 12 weeks of age and older. However, this increased plasma renin activity did not seem to be related to development of hypertension. We recently obtained animals from the normotensive Wistar/Kyoto strain from which the SH rats were derived and, in the F_1 generation of rats bred at the NIH, plasma renin activity at an age of 12 weeks was not different ($P > 0·1$) from that of the SH/NIH rats. Values (nanograms/2 ml/4 hr) for six male Wistar/Kyoto and six male SH/NIH were respectively 6·8 ± 2·4 and 10·1 ± 2·6 (means ± S.E.M.). It is concluded that the higher plasma renin activity initially observed in SH/NIH rats is related to the

* L. H. Green, W. De Jong, W. Lovenberg, unpublished observations.

Kyoto strain and not to blood pressure. This is substantiated by the fact that the decrease in plasma renin activity after nephrectomy and removal of submaxillary glands was not associated with a decrease of blood pressure. Also, propranolol treatment decreased plasma renin activity of SH/NIH rats to the level of the normotensive Wistar/NIH strain, without substantially reducing blood pressure.

A recent study in mice showed that renin activity as measured in plasma obtained by decapitation was not related to submaxillary renin-like activity in several strains in which considerable variation in submaxillary enzyme levels occurred.[6] Different findings were reported in another strain of mice with high levels of submaxillary renin-like activity in which plasma renin activity and blood pressure, as measured under pentobarbital anesthesia, decreased after removal of submaxillary glands.[2,3] In acute experiments, only nephrectomy combined with removal of submaxillary glands reduced plasma renin activity of SH/NIH rats to undetectable levels, as observed after nephrectomy in the Wistar/NIH strain with low submaxillary renin-like activity. Although acutely after removal of submaxillary glands of SH/NIH rats plasma renin activity decreased, 7 weeks after submaxillaryectomy no difference in plasma renin activity was found. No adequate explanation is available for the rise in plasma renin activity after the first day the submaxillary glands were removed. It is quite possible, however, that the source of plasma renin activity after removal of the submaxillary gland is the kidney. These findings do suggest, however, that plasma renin activity in SH rats may be derived in part from the submaxillary glands.

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