Markedly elevated specific renin levels in the adrenal in genetically hypertensive rats

(nephrectomy/renin activity)

MITSUHIDE NARUSE and TADASHI INAGAMI*

Department of Biochemistry and Hypertension Center, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232

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ABSTRACT The specific renin (EC 3.4.99.19) activity in the adrenal of spontaneously hypertensive rats was determined by a method that is capable of distinguishing renin from nonspecific renin-like activity of proteases by using specific antibody to renin. The renin level in the adrenals of adult spontaneously hypertensive rats with established hypertension was found to be 6–8 times as high as that of the normotensive control Wistar-Kyoto strain. The large difference in the adrenal renin level was observed even in 3-wk-old rats in which hypertension had not yet developed. The adrenal renin level was increased by bilateral nephrectomy in both the hypertensive and normotensive strains. A larger quantity of renin was found in the adrenal cortex than in the medulla, and the difference between the hypertensive strain and the normotensive strain was more prominent in the cortex than in the medulla. These results suggest possible involvement of adrenal renin in the development and in the early maintenance phase of hypertension in this animal model of human essential hypertension by affecting the adrenocortical or adrenomedullary activity, or both.

The etiology of hypertension in spontaneously hypertensive rats (SHR) (1) has been of great interest in view of its resemblance to essential hypertension in humans. Elevated levels of dopamine β-hydroxylase (EC 1.14.17.1) activity in plasma (2), mesenteric vessels, and adrenals (3) and decreased levels of dopamine β-hydroxylase activity (3) and norepinephrine (4) in the noradrenergic regions of the brain observed before the development of hypertension indicate a significant role of neurogenic factors in the initiation of the hypertension of this animal model (5). The aberrations in the levels of these neurogenic substances are no more than 2-fold. On the other hand, normal or subnormal levels of plasma renin (EC 3.4.99.19) have indicated the lack of a causative role for the renin–angiotensin system in spontaneous hypertension (6–10).

However, the recent finding that the angiotensin-converting enzyme inhibitor, captopril (D-3-mercaptop-2-methylpropanoyl-L-proline), normalizes the blood pressure of patients with essential hypertension and that of the SHR (11, 12) suggests a possible role of the renin–angiotensin system in this type of essential hypertension. Because the plasma level of renin activity is not increased and because captopril is known to be taken up by tissues (13), such a renin–angiotensin system may exert its effect by its local function.

Although the earlier observation of a renin-like enzyme within various extrarenal tissues (14) may have been mostly due to cathepsin D (EC 3.4.23.5) (15, 16), we have demonstrated the presence of specific renin, which is inhibitable by antibodies to renin, in various tissues of hogs (17) and rats (18). A particularly high renin activity was found in the rat adrenal (18).

Therefore, in an attempt to assess the possible role of the tissue renin–angiotensin system in the peripheral system of spontaneous hypertension, we have compared the renin levels in the adrenals of SHR of Okamoto–Aoki strain (1) and its control strain. Markedly increased renin levels were observed in the adrenal of SHR.

MATERIALS AND METHODS

Animal Models and Study Protocols. Colonies of the SHR and the normotensive Wistar–Kyoto strain (WKY) were developed from the breeding pairs obtained from the National Institutes of Health. Both strains were raised under the identical conditions with ad lib access to regular rat chow diet and tap water. Groups of male rats were matched with respect to age for each experiment. The systolic blood pressure of the conscious rat was measured by a tail-cuff sphygmomanometer couple and Statham recorder.

At age 3–48 wk, the rats were killed by exsanguination under pentobarbital anesthesia (50 mg/kg) before or after bilateral nephrectomy. Blood samples obtained by cardiac puncture were placed in a test tube in ice, and plasma was separated and frozen at −80°C until use. The adrenal glands were quickly removed after the perfusion with ice-cold saline, frozen on dry ice, and stored at −80°C.

Tissue Extraction. Adrenals were homogenized at 4°C in 5–7 vol of 0.01 M pyrophosphate buffer, pH 6.5/0.1 M NaCl in a Polytron by three cycles of 20-sec duration at the maximum speed and were centrifuged at 39,000 x g for 90 min with a Beckman JA20 rotor to obtain a clear extract.

Antiserum. Specific antibodies to renin were produced in Dutch-belted rabbits by using pure rat renin as antigen. The pure renin was prepared by a published method (19). The enzyme preparation satisfied multiple criteria of purity, which included single bands upon polyacrylamide gel electrophoresis, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, isoelectric focusing, double immunodiffusion, and a symmetric chromatographic elution pattern. This preparation (1.0 mg) was conjugated to 0.5 mg of tetanus toxoid with 25 μg of the watersoluble N-ethyl-N’-dimethylaminopropyl carbodiimide and was exhaustively dialyzed. Rabbits were immunized with aliquots containing 80 μg of the conjugated renin mixed with an equal volume of complete Freund’s adjuvant and were injected intradermally at multiple sites in the back. After six biweekly boosters, each with a 10-μg equivalent of conjugated renin, antiserum was collected. Tested at dilutions greater than 1:500, these antibodies did not crossreact with human renin or rat ca-

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto rats.

* To whom reprint requests should be addressed.
the specific, immunosuppressible renin activity was estimated by the extent of inhibition of renin-like activity by specific antibodies to renin. Extract (25 μl) was preincubated with the anti-renin antiserum (1:4,500 dilution) for 20 hr at 4°C and then was allowed to react with renin substrate at 37°C. Angiotensin I generated by the antibody-treated extract was subtracted from the value obtained with the untreated extract. The difference was defined as specific, immunosuppressible renin activity. The protease activity in the rat adrenal gland measured at pH 3–9 with [14C]carboxymethylated bovine hemoglobin was unaffected by the antibodies to renin. On the other hand, greater than 90% of angiotensin I-generating activity in the adrenal extract was inhibited by the antibodies (18). Thus, the activity of specific, immunosuppressible renin activity was measured reliably by this method.

Plasma renin activity was measured by incubating 100 μl of plasma at 37°C and pH 6.0 in the presence of 7 mM Na₂EDTA/2 mM phenylmethanesulfonyl fluoride. The angiotensin I formed was quantitated by radioimmunoassay (21).

Protein Concentration. The protein concentration of the extract was estimated by the method of Lowry et al. (22) with bovine serum albumin as standard.

Statistical Analyses. Results of determinations are given as means ± SEM, with n as the number of experimental animals. Significance of differences was determined by the unpaired Student t test.

RESULTS

The specific, immunosuppressible renin activity in the adrenal of 3-wk-old SHR with normal blood pressure (81.4 ± 4.7 mm of Hg, n = 5) and with intact kidneys was markedly increased to levels 5 times as high as those of the control WKY of the same age (P < 0.05) (Fig. 1). A similar pronounced increase was observed in the adrenal renin levels of adult (age 17 wk) SHR with established hypertension (192.5 ± 2.3 mm of Hg, n = 4). Adrenal renin levels were affected by nephrectomy. Bilateral nephrectomy caused a marked increase in adrenal renin levels of both 17-wk-old SHR and WKY over a period of 36 hr (Fig. 2). The difference in the adrenal renin levels between the two strains was maintained during this increase. Plasma renin levels decreased precipitously in the first 6 hr after nephrectomy, followed by a slow decrease during the subsequent period. Little difference was observed between the plasma renin levels of the hypertensive and normotenstive strains before or after the nephrectomy. Although the marked difference in the adrenal renin was observed both before and after the bilateral nephrectomy, in order to eliminate the possible participation of blood-borne renin, age-dependent changes in adrenal renin levels were compared between the bilaterally nephrectomized SHR and WKY. The adrenal renin activity in SHR was 5–10 times higher than that in WKY from age 3 to 17 wk (Fig. 3). Therefore, the adrenal renin decreased with age more rapidly in SHR than in WKY. Still, a 2-fold difference was observed at age 48 wk.

FIG. 1. The specific, immunosuppressible renin activity (ng of angiotensin I per mg of protein per hr) in the adrenal of 3-wk-old and 17-wk-old SHR (○●) and WKY (●●) before (A) and after (B) bilateral nephrectomy. With 3-wk-old rats, the effect was measured 24 hr after nephrectomy. With 17-wk-old rats, 36 hr were allowed after nephrectomy. Values are mean ± SEM for the number of determinations in parentheses. *, P < 0.05; **, P < 0.01; ****, P < 0.001 vs. the corresponding WKY.

The distribution of renin between adrenal cortex and medulla was determined with 17-wk-old rats. The two regions were separated under a dissecting microscope. The cortex-to-medulla ratio of the specific activity of renin per mg of protein was 3.4
The localized increase of renin within tissues that play significant roles in blood pressure regulation other than the kidney may explain intriguing observations reported in the past, such as (i) the failure in normalizing blood pressure by bilateral nephrectomy (25) or by intravenous administration of angiotensin II antagonists (12) and (ii) the success in correcting hypertension by synthetic converting enzyme inhibitors (12, 13, 26), which seem to penetrate tissues. Angiotensin I-generating activities have been reported in the adrenal gland (14, 27, 28). However, these tissues contain large amounts of cathepsins, which show nonspecific renin-like activity (16). This nonspecific activity often accounts for a sizeable proportion of the total angiotensin I-generating activity of tissue extract. This proportion is affected by choice of assay pH and by the source and purity of renin substrate. Also, if blood is left in the tissue, blood-borne renin can contribute to the angiotensin I generation. Erroneous conclusions also may arise because of the choice of inappropriate control rats. In order to eliminate these sources of potential errors, age-matched SHR and WKY raised and treated under identical conditions were used. Prior nephrectomy and exhaustive washing of the tissues, use of high pH for angiotensin I generation, suppression of catheptic activity by the use of unfractionated rat plasma as substrate (29), and identification of the contribution of true renin by inhibition with specific antibodies were used.

The fact that adrenal renin level increased after nephrectomy, in contrast to the marked decrease in the plasma enzyme activity, indicates that the adrenal renin is endogenous to this organ and is not derived from plasma or kidney. Penetration of plasma renin into the tissue because of a nonspecific increase in permeability under uremic conditions seems to be unlikely because of the decrease of renin activity in the liver, spleen, and lung after bilateral nephrectomy (unpublished data).

The increased level of adrenal renin in young and adult SHR and the gradual decrease with age find a parallelism in similar but less pronounced changes in adrenal dopamine β-hydroxy-

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**DISCUSSION**

The present study clearly demonstrates markedly increased levels of tissue renin in the adrenal of SHR above the levels of the normotensive control strain WKY. Of significance to the possible causative effect on the development of hypertension is the fact that the increased renin levels exist already at rat age 5 wk, prior to the manifestation of the hypertension. The persistent increase of adrenal renin after the onset of hypertension may be interpreted to indicate that, if the adrenal angiotensin plays a role in the spontaneous hypertension, it continues to exert its effect during the maintenance phase as well. It also suggests that the tissue level of renin, in contrast to plasma level, may not be sensitive to the blood pressure increase in this strain. The greatly increased renin levels in the adrenals of SHR is in contrast to normal or subnormal plasma renin levels in SHR (6–10).

To date no other organ or tissue has been found to contain an unequivocally increased level of renin in this animal model of essential hypertension compared to normotensive controls. Although an increased renin activity has been reported in the aorta of SHR with established hypertension (23, 24), the condition of the determination used in these studies seems to allow nonspecific proteases to make a significant contribution to the total renin-like activity. Moreover, studies with an opposite result have been reported.  

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**Fig. 3.** Systolic blood pressure (c, SHR; o, WKY) and adrenal specific renin activity (ng of angiotensin I per mg of protein per hr) (c, SHR; o, WKY) as a function of age. Values are mean ± SEM for the number of determinations in parentheses. *, P < 0.05; **, P < 0.005; ***, P < 0.001 vs. the corresponding WKY; †, P < 0.05 vs. 3-wk-old rats of the same strain.

± 0.8 (P < 0.05, n = 5) in SHR and 0.9 ± 0.1 (not significant; n = 5) in WKY. The SHR-to-WKY ratio of the specific activity of renin was 7.8 ± 0.8 (P < 0.01; n = 5) in the adrenal cortex and 2.1 ± 0.2 (P < 0.01; n = 5) in the adrenal medulla, respectively.

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Angiotensin II stimulates both the adrenal cortex for steroidogenesis (32–36) and the medulla for catecholamine biosynthesis (37) and release (38). However, the fact that the renin level of the adrenal cortex of SHR showed a more pronounced increase than that of the medulla may suggest a possible stimulation of adrenocortical activity. The observation that adrenalectomy prevented or markedly retarded the development of high blood pressure led Aoki to suggest that the adrenal gland may play a possible role in the pathogenesis of hypertension in SHR (39). Morphological studies also suggested adrenocortical hyperfunction in SHR (40). On the other hand, earlier studies on plasma steroids did not support these observations because no significant increase in plasma mineralocorticoid concentrations was detected in SHR (9, 41–43). In recent studies, however, higher levels of plasma aldosterone and corticosterone have been observed in SHR compared with WKY (44, 45). Although the reason for the discrepancy between the results of the earlier studies and recent studies is not immediately clear, it is likely that recent results have been obtained on the basis of more experience in the choice of control animals, the method of blood sampling, and anesthesia (44, 45). Observations suggesting a possible link of aldosterone to the development of hypertension in SHR has been reported (46). Because the plasma renin level is not increased in SHR (6–10), it is likely that the increased tissue renin–angiotensin system in the adrenal may play a role in the adrenocortical hyperfunction and, probably, in spontaneous hypertension—an intriguing hypothesis open to further investigation.

The dissecting technique used in the present studies has limitations in that the so-called corticomedullary cells (47) were not separated. Thus, whether the effect of the increased renin in the cortex is limited to the cortex or extends to the medulla remains to be clarified. The increased function of catecholamine synthesis in the adrenal of SHR (3, 30) is compatible with the increased medullary renin and with a possible corticomedullary interaction (45–50) of cortical renin.

The possibility that increased tissue renin is due to increased adrenergic activity of central origin should be considered also. However, the extent of the increase of plasma catecholamine, considered as an index of peripheral adrenergic system, seems to be much less than the increase of renin level. The extent of the increase of plasma aldosterone in SHR is 2 to 3 times the control level (45). Thus, the greater magnitude of adrenal renin increase compared with other pressor substances may be considered as an important new factor in the study of the pathogenesis and maintenance of hypertension in SHR in addition to and in relation to the several factors already implicated, such as neurogenic mechanism, renal insufficiency, and cardiovascular hypertrophy.

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