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INCREASED PRODUCTION OF ANGIOTENSIN II IN THE ADRENAL GLAND OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS WITH MALIGNANT HYPERTENSION

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Angiotensin(Ang) contents in the adrenal gland of stroke-prone spontaneously hypertensive rats(SHRSP) and age-matched Wistar Kyoto rats(WKY) were determined using reverse phase high performance liquid chromatography combined with a specific radioimmunoassay. In normotensive 5 wk-old SHRSP, the adrenal renin activity was about 3 times higher than that of age-matched WKY while the adrenal Ang I and Ang II concentrations did not differ from those of WKY. In the severely hypertensive 25 wk-old SHRSP, the adrenal Ang II and Ang I, and plasma aldosterone concentrations were about 5-fold, 2-fold and 4-fold, respectively, increased compared with levels in the WKY. In the 25 wk-old SHRSP 24 h after bilateral nephrectomy, the adrenal Ang II and plasma aldosterone levels were not decreased and were 10 and 3 times, respectively, higher than those of nephrectomized control WKY. Thus, the enhanced local generation of Ang II in the adrenal gland may contribute to the increased release of aldosterone in SHRSP with malignant hypertension.

Various extrarenal tissues, including the adrenal gland, brain, heart, and blood vessels possess the endogenous renin-angiotensin(Ang) system, independently of the circulating system regulated by renin of renal origin(1). It has been postulated that the tissue renin-Ang system rather than the circulating system may contribute to the pathophysiology of hypertension, because inhibitors of the renin-Ang system significantly reduce the blood pressure of human hypertensive patients and genetically hypertensive rats, with no high plasma

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Abbreviations used in this paper: SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats; Ang, angiotensin; HPLC, high performance liquid chromatography; RIA, radioimmunoassay; TFA, trifluoroacetic acid; PRC, plasma renin concentration.

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renin activity(2-4), and there is a dissociation between the hypotensive effect of these inhibitors and plasma Ang II levels(5-6). In spontaneously hypertensive rats(SHR), a model of human essential hypertension, the renin activity in the adrenal gland is higher than that in control Wistar Kyoto rats(WKY)(7). This finding, taken together with the observations that all components of the renin-Ang system are present in the adrenal gland(8-13) and that Ang II potently stimulates the synthesis and release of both aldosterone and catecholamine in the adrenal gland(14-15), suggest that the adrenal renin-Ang system may participate in the pathogenesis of hypertension. However, there seems to be no documentation on the adrenal Ang II concentration in the SHR and it is unknown whether adrenal renin is a limiting factor for the local generation of Ang II, as plasma renin.

Stroke-prone spontaneously hypertensive rats(SHRSP), a substrain of SHR established by Okamoto et al.(16), develop more severe hypertension than do the SHR, and have cerebrovascular and renal vascular lesions, hence are a useful animal model of human malignant hypertension(16-19). We measured Ang content in the adrenal gland of SHRSP and obtained evidence that adrenal Ang II levels are significantly enhanced in SHRSP with malignant hypertension.

MATERIALS AND METHODS

Materials: ^{125}I-labeled Ang I and Ang II were purchased from New England Nuclear(Boston, MA). Ang peptides were obtained from Peptide Institute, Inc.(Osaka, Japan).

Animals: SHRSP and WKY were kindly donated by Dr. K. Okamoto(Kinki University School of Medicine, Osaka, Japan) and maintained by selective mating. Five wk and 25 wk-old male animals of both strains were sacrificed by decapitation. In addition, 25 wk-old male animals of both strains were bilaterally nephrectomized and 24 h after nephrectomy they were decapitated. Trunk blood was collected into prechilled tubes containing Na_2EDTA(2 mg/ml) and the plasma was separated by centrifugation at 3,000 rpm for 15 min at 4 °C. After this collection, the adrenal glands were rapidly removed, were weighed, frozen in liquid nitrogen and stored at -80°C until use.

Extraction of adrenal glands: For the measurement of Ang peptides, one adrenal gland of each animal was boiled in 0.5 ml distilled water for 5 min to prevent the degradation of Angs by proteases and then homogenized in 0.05N HCl(Total volume, 1 ml). The supernatant, obtained by centrifugation at 30,000 X g for 1 h at 4 °C, was applied to Sep-Pak C_{18} cartridges(Waters Associates, Millford, MA), as described below. For the measurement of renin concentration, the contralateral adrenal gland of each animal was homogenized in 50 mM sodium phosphate buffer/pH 7.0/0.15 M NaCl/5 mM Na_2EDTA/2 mM phenylmethanesulfonyl fluoride/2 mM potassium tetrathionate(20% homogenate). The supernatant was obtained by centrifugation at 30,000 X g for 1 h at 4 °C to measure the renin concentration.

Determination of plasma and adrenal Angs: Measurement of Angs was carried out by reverse phase high performance liquid chromatography(HPLC) combined with specific radioimmunoassays(RIAs), a modification of the method of Kawamura et al.(20). Briefly, the samples of plasma(1-2 ml) and adrenal extracts(about 1 ml) were applied to Sep-Pak C_{18} cartridge columns which had been pretreated consecutively with 10 ml methanol, 10 ml tetrahydrofuran, 10 ml hexane, 10 ml methanol and finally equilibrated with 10 ml 0.1% trifluoroacetic acid(TFA). The column was washed with 10 ml 0.1% TFA and 10 ml of the mixture of methanol/water/TFA(10/89.9/0.1, vol/vol). The Angs, retained by the cartridge, were eluted with 4 ml of the mixture of methanol/water/TFA(80/19.9/0.1, vol/vol). The eluates were dried in a vacuum centrifuge evaporator(CC-180, TOMY SEIKO, Japan) and dissolved in 250 µl 10 mM phosphoric acid/pH 3.4, and chromatographed on an ODS-80TM C_{18} reverse phase HPLC column(4.6 mm X 25 cm, Tosoh, Japan). The separation of Angs were effected by using a linear gradient of methanol concentration from 30 to 75% in 10 mM phosphoric acid/pH 3.4 over a period of 20 min at the flow rate of 1.0 ml/min. Fractions of 0.3 ml were collected into bovine serum albumin(BSA)-coated polypropylene tubes, dried in a vacuum centrifuge evaporator. The fractionated samples were dissolved in RIA buffer(70 mM sodium phosphate/pH 7.1/50 mM NaCl/2 mM Na_2EDTA/100 µM diisopropylfluorophosphate/0.3% BSA) and subjected to RIA of either Ang I or Ang II. The retention time of each Ang peptide was determined by respective synthetic peptides. The method of specific RIA of Ang II and Ang I has been published in (21) and (22), respectively. The cross reactivities of anti-Ang II serum used were: Ang III, 100%; Ang(3-8) hexapeptide, 60%; Ang(4-8)pentapeptide, 40%; Ang(5-8)tetrapeptide, less than 0.01%; Ang I, 0.02%. The cross reactivities of anti-Ang I serum were: Ang II, 0.05%; Ang III, 0.05%; Ang(3-8), 0.04%; Ang(4-8), 0.04%; Ang(5-8), 0.002%. The sensitivity of RIA of Ang II and I was 0.3 pg and 1.5 pg/tube, respectively. In preliminary experiments, through the entire procedure, the recovery of ^{125}I-labeled Ang I and Ang II added to plasma was 65±4(n=3) and 68±5%(n=3), respectively. The recovery of ^{125}I-labeled Ang I and II added to the adrenal gland was 72±3(n=3) and 76±3%(n=3), respectively.

Measurement of renin concentration: The plasma renin concentration(PRC) and the renin concentration of the adrenal extracts were measured as the rate of generation of Ang I from rat angiotensinogen(2 µM) at 37 °C, as reported(23). The generated Ang I was measured by RIA.

Inhibition of adrenal renin activity by anti-renin serum: To determine whether the adrenal renin activity is due to true renin, the adrenal extracts were incubated with specific anti-rat renin serum(24)(dilution;1:20) or nonimmunized rabbit serum as control at 4 °C for 24 h, according to the published method(23). The residual renin activity was measured, as described above.

Determination of plasma aldosterone: The plasma aldosterone concentration was determined by RIA using a commercially available kit(SORIN BIOMEDICA S.p.A, Italy).

Measurement of blood presuure: Systolic blood pressure was measured by the tail cuff method. Each value is the average of three consistent readings.

Statistics. Results were expressed as means± standard error. Statistical significance was determined by the unpaired Student's t-test.

RESULTS AND DISCUSSION

Measurement of Angs by reverse phase HPLC followed by RIA

As shown in Fig. 1, Angs I, II and III, and the related Ang fragments(Ang(3-8), Ang(4-8) and Ang(5-8)) could be completely separated by HPLC. Thus, the combination with specific RIAs allowed for the precise quantitation of Ang I and Ang II in the plasma and adrenal gland. In the plasma,

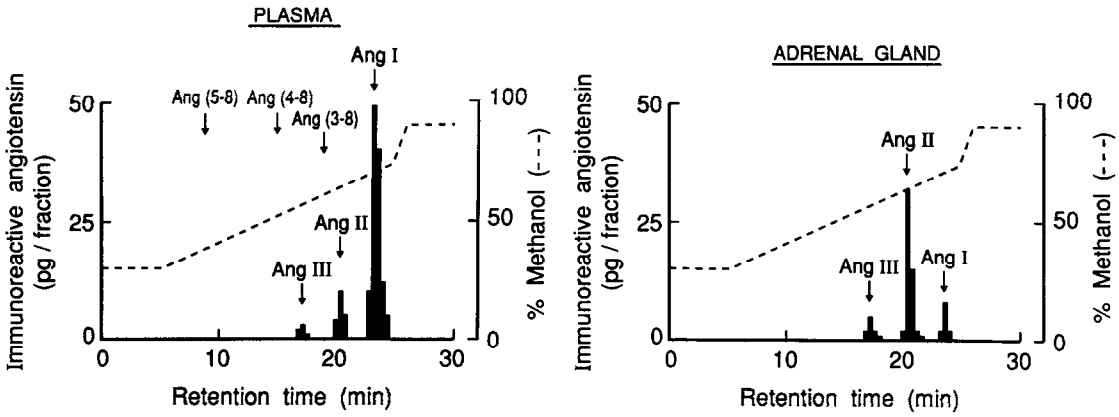

Figure 1. HPLC profiles of Angs in the plasma and adrenal gland of a 25-wk-old Wistar Kyoto rat. Arrows indicate the elution positions of synthetic Ang peptides. The elution time of Ang III, Ang II, Ang I was 16.7, 20.1, 23.7 min, respectively. The fractions between retention time of 10.0 and 22.0 min were used for RIA of Ang II. The fractions between retention time of 22.0 and 28.0 min were used for RIA of Ang I.

Ang I is the predominant Ang peptide, while in the adrenal gland Ang II is the major peptide, findings in good agreement with reported data(9).

Renin-Ang system in 5 wk-old SHRSP

The systolic blood pressure of 5-wk-old SHRSP(113±2 mmHg) was normal and not significantly different from that of WKY(106±4 mmHg). As shown in Table 1, plasma renin, Ang I, Ang II and aldosterone concentrations of normotensive 5-wk-old SHRSP were not significantly different from those of age-matched WKY. As shown in Table 2, in the adrenal gland, the renin activity of SHRSP was

Table 1. Plasma renin, Ang I, Ang II and aldosterone concentrations in 5 or 25 wk-old SHRSP and WKY

	Age	PRC (ng Ang I/h/ml)	Ang I (pg/ml)	Ang II (pg/ml)	Aldosterone (pg/ml)
WKY (n=9)	5 wk	23.3±2.0	129±30	16±2	441±53
SHRSP(n=8)	5 wk	17.5±3.4	135±19	15±2	490±41
WKY (n=7)	25 wk	14.9±1.2	110±14	14±2	317±23
SHRSP(n=9)	25 wk	93.8±11.5*	494±91**	91±15*	1351±200***
Bilaterally nephrectomized[1]					
WKY (n=5)	25 wk	0.6±0.2	17±4	4±1	469±24
SHRSP(n=5)	25 wk	7.2±1.3*	96±8*	12±2***	1614±289***

* P<0.001, ** P<0.005, *** P<0.01 compared with age-matched control WKY. PRC, plasma renin concentration. [1] Animals 24 h after bilateral nephrectomy.

Table 2. Adrenal renin, Ang I and Ang II levels in 5 or 25 wk-old SHRSP and WKY

	Age	Renin activity (ng Ang I/h/g tissue)	Ang I (pg/g tissue)	Ang II (pg/g tissue)
WKY (n=9)	5 wk	131± 36 (90±4%)[1]	678± 78	1911±282
SHRSP (n=8)	5 wk	414± 60* (89±5%)	580± 92	1414± 80
WKY (n=7)	25 wk	247± 14 (93±2%)	296± 21	1599±318
SHRSP (n=9)	25 wk	1040±141* (90±5%)	701±106**	7301±828*
Bilaterally nephrectomized[2]				
WKY (n=5)	25 wk	527± 65 (93±6%)	283± 34	777± 88
SHRSP (n=5)	25 wk	1383±172* (92±5%)	556± 71**	7454±1646**

* P<0.001, ** P<0.005 compared with age-matched control WKY. [1] The values in the parentheses indicate % inhibition of the adrenal renin activity in each group of rats, by specific anti-renin serum(24). In all groups of rats, about 90% of the adrenal renin activity was inhibited by specific anti-renin antibody, thereby indicating that almost all of the enzymatic activity was due to true renin. [2] Animals 24 h after bilateral nephrectomy.

about 3-fold higher than that of control WKY, findings consistent with observations in the SHR(7). Of note is the observation that despite the elevated renin activity, there was no difference in the adrenal Ang I and Ang II contents between the two strains. This dissociation between adrenal renin and Ang II levels indicate that the regulatory mechanism for the formation of Ang II in the adrenal gland is more complex than that in the blood circulation in which the Ang II levels are controlled by renin of renal origin(25).

Renin-Ang system in 25 wk-old SHRSP

The systolic blood pressure of 25-wk-old SHRSP(250±3 mmHg) was significantly higher than that of age-matched WKY(130±2 mmHg)(P<0.001). PRC in 25 wk-old SHRSP was higher than that of WKY, hence there was the increase in plasma Ang I and II(Table 1). It is well established that SHRSP in the phase of high PRC is a useful model of human malignant hypertension(17-19, 26). However, high PRC in SHRSP results from renal angionecrosis(17, 26) and bilateral nephrectomy fails to lower the blood pressure of SHRSP(27), thereby indicating that the circulating renin-Ang system plays a minor role in malignant hypertension.

As shown in Table 2, in 25 wk-old SHRSP with malignant hypertension, the adrenal Ang II concentrations were about 5-fold higher than those of WKY, accompanied by 2 and 4-fold increases in the adrenal Ang I and renin, respec-

tively. To determine whether the elevated adrenal Ang II levels in SHRSP are due to an increase in the local generation or in uptake of circulating Ang II, the effects of bilateral nephrectomy on the adrenal Ang II were examined. As shown in Table 1, 24 h after nephrectomy, plasma renin, Ang I and Ang II levels were decreased considerably in both SHRSP and WKY, findings expected from the fact that the kidney is the predominant source of circulating renin(25). Of great interest are the observations that in spite of the marked decrease in plasma Ang II the adrenal Ang II content in SHRSP did not decrease 24 h after nephrectomy and was 10-fold higher than that in nephrectomized control WKY(Table 2). These results suggest that the adrenal Ang II derives mainly from a local generation rather than from uptake of circulating Ang II and that the production of Ang II is increased in the adrenal gland of SHRSP with malignant hypertension. As there were about 3-fold higher concentrations of plasma aldosterone in nephrectomized SHRSP than nephrectomized WKY(Table 1), the increased adrenal Ang II may contribute to increases in the release of aldosterone. In addition, adrenal Ang II may stimulate the release of adrenal catecholamine, as plasma norepinephrine is increased in the SHRSP(28). We postulate that elevation of Ang II in the adrenal gland of SHRSP likely contributes to acceleration of malignant hypertension in these animals.

This is apparently the first report on Ang peptide content in the adrenals of SHRSP. Adrenal Ang II levels are increased in SHRSP with malignant hypertension. Mechanisms related to the increased formation of adrenal Ang II are under investigation.

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