Arch. Pharm. Res. Vol.18, No.3, pp. 169-172, 1995

Changes in Adrenal Angiotensin II Receptors in Renin-dependent Hypertensive Rats

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(Received December 27, 1994)

The changes in blood pressure may relate to the alterations of the responsiveness to vasoconstrictors and vasodilators, and these alterations can arise the modifications in the properties of angiotensin II (AII) receptor. In order to examine the changes of AII receptor in the hypertensive mechanism of renin-dependent hypertensive rats (RHRs; two-kidney, one-ligated type), we compared the equilibrium binding characteristics of [^{3}H]AII in adrenal cortex and medulla from RHRs and normotensive rats. The dissociation constants of AII binding in both tissues of RHRs were very similar to those in the respective tissue of normotensive rats. However, the maximum binding was increased from 805 to 1050 fmole/mg protein in the adrenal cortex of RHRs, and decreased from 172 to 126 fmole/mg protein in the adrenal medulla of RHRs. These results imply that the up- and down-regulation of the AII receptor population on the cell surface of adrenal glands from RHRs are consorted with the elevation of blood pressure and the activation of renin-angiotensin system.

Key words : RHR, Adrenal, AII receptor, Binding analysis, Angiotensin II

INTRODUCTION

The renin-dependent hypertensive rat (RHR) is one of the animal models that are thought to mimic human hypertension of renal origin (Fernandes *et al.*, 1976). The RHRs are frequently used to elucidate the pathophysiologic mechanism of renal hypertension. In this animal model, blood pressure elevation exhibits biphasic profile, the drastic increase in blood pressure in 6-8 days after renal artery ligation (acute phase) followed by the maintenance of moderately increased blood pressure (chronic phase). Hypertension in the acute phase is characteristically accompanied by the elevated plasma renin activity (PRA), suggesting a causative role of renin-angiotensin system (RAS) in the induction of hypertension in the acute phase. Several other factors, such as the lack of vasodepressor substances and the production of a renal vasopressor factor, were proposed to be possible underlying mechanisms for the chronic phase (Cangiano *et al.*, 1979). However, the mechanism for this biphasic phenomenon is not understood completely.

The RAS is one of the hormonal systems involved in the regulation of pressure and volume homeostasis. Activation of the renin-angiotensin cascade begins with the renin secretion from the juxtaglomerular apparatus of the kidney and culminates in the formation of angiotensin II (AII), the primary active substance of this system. AII binds to specific receptors on cell surfaces to initiate numerous physiological responses including smooth muscle contraction, steroidogenesis, and glycogenolysis (Devynck and Meyer, 1978). This multiplicity of actions suggests a complex interaction of AII with single or multiple types of receptor (Timmermans *et al.*, 1993; Peach, 1977; Douglas, 1987). The existence of multiple types of receptor is suggested by a difference in the order of potency for AII and peptide analogs in adrenal gland and vascular smooth muscle as well as by the differential inhibition of adrenal cortical responses to AII by receptor antagonists (Chiu *et al.*, 1990; Peach and Ackerly, 1976). Two distinct AII binding sites (AT_1 and AT_2) in adrenal cortex and medulla have been described to exhibit reciprocal affinities for DuP 753 and WL 19, AT_1 and AT_2 specific receptor antagonist, respectively (Wiest *et al.*, 1991). The populational ratio of AT_1 and AT_2 in rat adrenal gland is 7 : 3 in adrenal cortex and 3 : 7 in adrenal medulla.

The fact that RAS is involved in the blood pressure homeostasis led us to explore the possibility of altered AII binding profile in the adrenal gland of RHR. The alterations of AII receptors in RHRs have not

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been identified yet. In the present work, the equilibrium binding properties of [^{3}H]AII to membrane preparations of the adrenal cortex and medulla from RHRs and normotensive rats have been sought to figure out the changes in the adrenal AII receptor.

MATERIALS AND METHODS

Materials

[^{3}H]AII (5-L-isoleucine, 65 Ci/mmole) and [^{125}I]Angiotensin I radioimmunoassay kit were purchased from DuPont NEN (Boston, MA). AII (human) was purchased from Sigma (St. Louis, MO). Lumagel scintillation cocktail was obtained from Lumac*LSC B.V. (Olen, Belgium). All other chemicals were of the highest purity commercially available.

Animals

Male Sprague-Dawley rats weighing 300-400 g were used in this study. They were supplied by Animal Research Lab., Korea Research Institute of Chemical Technology (KRICT) and kept in a storage room under the conditions of constant temperature, relative humidity and illumination (12-h light, 12-h dark cycle) until the day of experiment, with free access to food and tap water.

Preparation of renin-dependent hypertensive rats

Hypertension was produced by the ligation of left renal artery as described previously (Lee *et al.*, 1994; Wong *et al.*, 1990). Briefly, rats were anesthetized with ketamine·HCl (125 mg/kg, i.p.), and the left renal artery was ligated completely using 4-0 silk suture, without damaging the left kidney and left renal vein.

To confirm the development of renin-dependent hypertension, blood pressure and PRA were measured in rats grouped by pre- or post-operation days (0, 6, 7, 8 and 28 days after renal artery ligation). Systolic blood pressure was measured by the tail cuff technique with Multichannel 8000 (TSE, Germany) from the conscious rat. One and half ml of blood was withdrawn by the heart puncture under mild ether anesthesia, and its PRA was determined by radioimmunoassay (Haber *et al.*, 1969) using [^{125}I]angiotensin I assay kit (Du Pont New England Nuclear). The PRA was expressed as ng of angiotensin I generated/ml/hr.

Preparation of the particulate membrane fraction from rat adrenal glands

Microsomal fractions of rat adrenal glands were prepared by the method of Lee et al. (1995) and used as a source of AII receptors. Briefly, rat adrenal glands from RHRs and normotensive rats were obtained after cervical dislocaton and kept in ice-cold sucrose buffer containing 0.2 M sucrose, 1 mM EDTA and 10 mM Trizma base (pH 7.2). After separation of cortex and medulla, the tissues were rinsed and disrupted with Brinkmann Homogenizer (Brinkmann Instruments, Inc.). The homogenate was spun at 3,000 x g for 10 min and supernatant was decanted through Kim-Wipes. Combined supernatant was spun at 12,000 x g for 13 min. The final supernatant was then centrifuged at 102,000 x g for 60 min. The pellet was washed with washing buffer containing 5 mM $MgCl_2$, 50 mM Trizma base (pH 7.2), and resuspended in assay buffer containing 0.25% bovine serum albumin (BSA), 5 mM $MgCl_2$, and 50 mM Trizma base (pH 7.2). All of the above steps were carried out at 4°C.

AII binding to rat adrenal particulate fractions

Binding assays were performed in triplicate. Freshly prepared particulate fraction (0.02-0.03 mg protein for adrenal cortex, 0.15-0.20 mg protein for adrenal medulla) were mixed with various concentrations of [^{3}H]AII in 13x100 mm borosilicate glass tubes in a final volume of 0.5 ml of assay buffer. Nonspecific binding was determined in the presence of 1 μM unlabeled AII. After incubation in a shaking water bath at 25°C for 60 min, the reaction was terminated by the addition of 3 ml of cold washing buffer and the bound radioactivity was separated rapidly through glass fiber filters (GF/C Whatman, prewetted with assay buffer) with a Brandel cell harvester system (Brandel M-12R). The filters were washed with an additional 3 ml of cold washing buffer and the trapped radioactivity was measured by a liquid scintillation counter (Packard Tricarb 1500C).

RESULTS AND DISCUSSION

In order to study the equilibrium binding properties of AII in RHR, we first generated RHRs by renal artery ligation. As shown in Fig. 1, systolic blood pressure was maximally increased on six to eight days (190 to 215 mmHg from basal value 155±2 mmHg, $p<0.01$) after ligation of renal artery. Likewise, plasma renin activity was significantly increased ($p<0.01$) from control level of 7.31±0.63 to 19-22 ng/ml/hr during this period. However, on day 28, it returned to the control level, despite the sustained high blood pressure. Consistent with the findings by others (Cangiano *et al.*, 1979), these results indicate the development of renin-dependent hypertension in renal artery-ligated rats.

Using these RHRs, AII binding profiles were studied in adrenal glands. The adrenals on day 8 after ligation were used for the preparation of membrane particulate fraction. The microsomal fractions of adrenal cortex and medulla from RHRs and normotensive rats

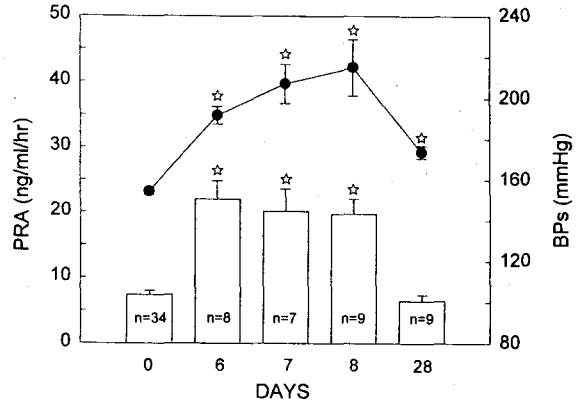

Fig. 1. Systolic blood pressure (BPs, circle) and plasma renin activity (PRA, bar) levels on the 0 (non-operative), 6, 7, 8 and 28 days after ligation of renal artery. Values are expressed as mean±S.E.M.

☆ $p<0.01$, significantly different from the corresponding control group, evaluated by ANOVA followed by the Student-Newman-Keuls test.

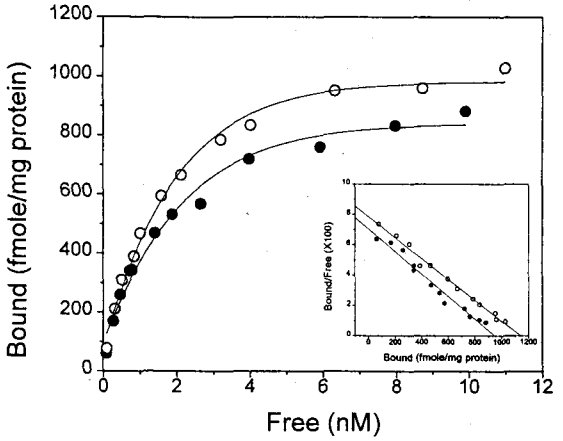

Fig. 2. Equilibrium binding of [^{3}H]AII in adrenal cortex from RHRs and normotensive rats. The concentration range of [^{3}H]AII was 0.1 to 10 nM. Inset shows the Scatchard plot of the same data: K_d, 1.10±0.11 nM (mean±S.E.M.) for control and 1.32±0.09 nM for RHRs; B_{max}, 805±63 fmole/mg protein for control and 1050±59 fmole/mg protein for RHRs. Equilibrium binding parameters were obtained using the iterative nonlinear curve fitting program EBDA-LIGAND (Munson and Rodbard, 1980). ● = adrenal cortex from normotensive rats, ○ = adrenal cortex from RHRs

were prepared by the differential centrifugation as described in Materials and Methods. The protein concentrations of receptor preparations were adjusted to 0.2-0.3 and 1.5-2.0 mg/ml for binding experiments with adrenal cortex and medulla, respectively. In our preliminary study, these ranges of protein concentration were best fitted to the study of [^{3}H]AII binding (data not shown).

The equilibrium binding analyses of [^{3}H]AII in adrenal cortex and medulla from RHRs and normotensive rats showed the saturability of specific binding

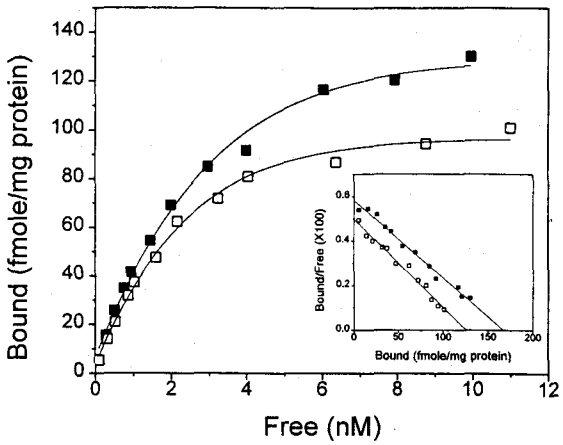

Fig. 3. Equilibrium binding of [^{3}H]AII in adrenal medulla from RHRs and normotensive rats. The concentration range of [^{3}H]AII was 0.1 to 10 nM. Inset shows the Scatchard plot of the same data: K_d, 2.97±0.15 nM (mean±S.E.M.) for control and 2.48±0.11 nM for RHRs; B_{max}, 172±6 fmole/mg protein for control and 126±4 fmole/mg protein for RHRs. Equilibrium binding parameters were obtained using the iterative nonlinear curve fitting program EBDA-LIGAND (Munson and Rodbard, 1980). ■ = adrenal medulla from normotensive rats, □ = adrenal medulla from RHRs

with [^{3}H]AII (0.1-10 nM), and Scatchard analyses on these data displayed linear distribution. The Hill slopes (Hill coefficient, nH) in all tissues were very near to the unity: rat adrenal cortex 1.045, rat adrenal medulla 0.996, RHR adrenal cortex 1.013, RHR adrenal medulla 0.981. In adrenal cortex from RHRs and normotensive rats, the dissociation constants (K_d) were determined to be 1.32±0.09 (mean±S.E.M.) and 1.10±0.11 nM, respectively, suggesting that AII binding affinity was not changed in RHRs. However, the apparent maximum binding (B_{max}) of AII was increased from 805±63 to 1050±59 fmole/mg protein in the adrenal cortex of RHRs (Fig. 2). When AII binding in adrenal medulla was examined, the K_d value of RHR (2.48±0.11 nM) also turned out to be similar to that of normotensive rats (2.97±0.15 nM). However, the B_{max} of AII binding in adrenal medulla from RHRs was rather decreased from 172±6 to 126±4 fmole/mg protein in contrast to what was seen in adrenal cortex (Fig. 3).

The up- and down-regulation of B_{max} in the adrenal glands of RHRs might reflect the changes in the populations of cell surface receptors that are available for AII binding without the changes in receptor affinity. As stated earlier, the majority of AII receptors in adrenal cortex is AT_1 receptor that mediates the major physiological functions of AII such as vasoconstriction, stimulation of aldosterone secretion and positive cardiac inotropy. Thus, the increase in receptor population in adrenal cortex of RHR correlates well

with the increase in blood pressure in this animal model with high renin and accordingly high AII. However, the physiological significance of the decrease in the receptor population in adrenal medulla of RHR with higher population of AT_2 receptor, is not understood, as the physiological functions of AT_2 receptor have not yet been known.

In conclusion, the equilibrium binding studies on AII receptor using adrenal cortex and medulla from RHRs and normotensive rats revealed 30% increase and 27% decrease in maximum binding (B_{max}) in adrenal cortex and medulla, respectively, with unchanged dissociation constant (K_d).

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