Changes in Renal α_2 -Adrenoceptor in Experimental Hypertension in Rats

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α_2 -Adrenoceptors were studied in renal membrane fractions from spontaneously hypertensive (SHR), two-kidney, one clip hypertensive (2K, 1C HT) and DOCA-salt hypertensive (DOCA-salt HT) rats, using radioligand binding method. α_2 -Adrenoceptor concentration in the kidney measured by [3 H]yohimbine binding was significantly increased in SHR at 4 weeks old (41.5 ± 2.8 fmol/mg protein, mean $\pm$ SEM, $p < 0.01$), 12 weeks old (54.9 ± 2.5 fmol/mg protein, $p < 0.01$) and 35 weeks old (59.8 ± 3.4 fmol/mg protein, $p < 0.01$) as compared with age-matched Wistar-Kyoto rats (WKY, 31.5 ± 2.5 , 40.9 ± 1.8 , 47.8 ± 2.0 fmol/mg protein, respectively). There were no significant differences in binding affinity and 5'-nucleotidase activity (plasma membrane marker enzyme) between SHR and WKY at any age. In 2K, 1C HT rats, α_2 -adrenoceptor concentration in the clipped kidney was higher than that of control rats, but α_2 -adrenoceptor concentration in the unclipped kidney was unchanged. Binding affinity and 5'-nucleotidase activity showed no significant changes in renal hypertensive rats. In DOCA-salt HT rats, no significant change was found in concentration and affinity of renal α_2 -adrenoceptor. The observed increase in renal α_2 -adrenoceptor concentration in SHR may contribute to the pathogenesis and maintenance of hypertension through increased sodium and water reabsorption in the kidney.

It is suggested that the sympathetic nervous system may contribute to the pathogenesis and development of hypertension. Changes in catecholamine metabolism and the responsiveness to adrenergic stimuli have been reported. Some of these changes can be explained by the alteration of adrenoceptor, and we have already reported a decrease in cardiac β -adrenoceptor concentration in spontaneously hypertensive rats (SHR) and two-kidney, one clip hypertensive (2K, 1C HT) rats.¹ Renal sympathetic nerves are thought to participate to the pathogenesis of

hypertension through changes in water and electrolyte balance, renin release and renal blood flow. Adrenergic receptors exist in the kidney, and renal α_2 -adrenoceptor is recently noticed as a regulating factor of renal sodium reabsorption.^{2,3} To clarify the participation of renal adrenoceptor in the pathogenesis of hypertension, we studied renal α_2 -adrenoceptor in 3 types of experimental hypertensive rats using the direct radioligand binding method.

MATERIALS AND METHODS

Experiments were carried out for 3 types of experimental hypertension in rats.

1) Spontaneously hypertensive rats of Okamoto and Aoki strain (SHR): Four-, 12- and 35-week-old male SHR were studied, and age-

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TABLE I CONCENTRATION AND AFFINITY OF α_2 -ADRENOCEPTOR AND 5'-NUCLEOTIDASE ACTIVITY IN RENAL MEMBRANE FRACTIONS FROM SPONTANEOUSLY HYPERTENSIVE (SHR) AND WISTAR-KYOTO RATS (WKY)

	Age	Renal weight (mg)	Renal α_2 -adrenoceptor		5'-Nucleotidase activity (μ M/mg protein/hour)	No. of experiments
			B_{max} (fmol/mg protein)	K_d (nM)		
SHR	4W	288 $\pm$ 8	41.5 $\pm$ 2.8**	1.45 $\pm$ 0.19	8.03 $\pm$ 0.12	8
WKY	4W	314 $\pm$ 8	31.5 $\pm$ 2.5	1.56 $\pm$ 0.33	8.11 $\pm$ 0.26	7
SHR	12W	845 $\pm$ 20	54.9 $\pm$ 2.5**	1.97 $\pm$ 0.10	7.59 $\pm$ 0.03	9
WKY	12W	970 $\pm$ 24	40.9 $\pm$ 1.8	1.73 $\pm$ 0.08	7.61 $\pm$ 1.19	9
SHR	35W	1547 $\pm$ 38	59.8 $\pm$ 3.4**	4.90 $\pm$ 0.43	7.10 $\pm$ 0.10	4
WKY	35W	1611 $\pm$ 63	47.8 $\pm$ 2.0	5.03 $\pm$ 0.31	7.10 $\pm$ 0.26	5

Values are given as mean $\pm$ SEM. * p < 0.05, ** p < 0.01

Fig.1. Association and dissociation of $[^3\text{H}]$ yohimbine to renal membrane fraction, Scatchard plot, and inhibition of $[^3\text{H}]$ yohimbine binding by adrenergic agonists and antagonists.

matched Wistar-Kyoto rats were used as controls.

2) Two-kidney, one clip hypertensive (2K, 1C HT) rats: Male Wistar rats weighing 150–200 g were anesthetized with pentobarbital (30 mg/kg, intraperitoneally). The left renal artery was dissected free and clipped with a silver clip (2 mm wide, 0.17–0.20 mm gap). The right renal artery was left intact. A loose clip (0.5 mm gap) was applied to the left renal artery for control rats. Renal α_2 -adrenoceptors were

studied 6 weeks after the operation.

3) DOCA-salt hypertensive (DOCA-salt HT) rats: Male Wistar rats weighing 150–200g were anesthetized with pentobarbital and the left kidney was removed. After nephrectomy, 10 mg of deoxycorticosterone-acetate (DOCA) was injected subcutaneously once a week for 5 weeks and a 1% saline solution was given for drinking water *ad libitum*. Unilaterally nephrectomized rats were used as controls. Renal α_2 -adrenoceptor

TABLE II CONCENTRATION AND AFFINITY OF α_2 -ADRENOCEPTOR AND 5'-NUCLEOTIDASE ACTIVITY IN RENAL MEMBRANE FRACTIONS FROM TWO-KIDNEY, ONE CLIP HYPERTENSIVE (2K, 1C HT) AND CONTROL RATS

	Weeks after operation	Renal weight (mg)	Renal α_2 -adrenoceptor		5'-Nucleotidase activity (μ M/mg protein/hour)	No. of experiments
			Bmax (fmol/mg protein)	Kd (nM)		
2K, 1C HT Controls	6W U	1010 $\pm$ 22**	55.0 $\pm$ 5.9	2.61 $\pm$ 0.33	6.67 $\pm$ 0.09	11
	6W U	815 $\pm$ 21	51.5 $\pm$ 4.3	2.46 $\pm$ 0.29	6.99 $\pm$ 0.10	11
2K, 1C HT Controls	6W C	699 $\pm$ 30**	75.2 $\pm$ 4.6*	3.83 $\pm$ 0.15	7.43 $\pm$ 0.30	11
	6W C	814 $\pm$ 17	63.3 $\pm$ 4.1	3.47 $\pm$ 0.21	7.25 $\pm$ 0.14	11

Values are given as mean $\pm$ SEM. U = unclipped side of kidney; C = clipped side of kidney; * p < 0.05, ** p < 0.01

was studied 5 weeks after nephrectomy.

Systolic blood pressure was measured by the tail-cuff plethysmographic method.

Membrane Preparation

Renal plasma membrane fractions were prepared according to the method of Williams et al.⁴ Rats were killed under pentobarbital anesthesia (50 mg/kg, intraperitoneally), and the kidneys were rapidly removed and washed with ice-cold saline. Kidneys were then minced with scissors and homogenized in ice-cold medium (0.25 M sucrose, 1 mM MgCl₂, 5 mM Tris-HCl buffer, pH 7.4) using Polytron PT-10 (Kinematica, Switzerland) at setting 7 for 20 sec. The homogenates were centrifuged at 400 $\times$ g for 10 min at 4°C, and the pellets were discarded. The supernatants were centrifuged at 30,000 $\times$ g for 10 min at 4°C. The pellets were washed twice with incubation buffer containing of 50 mM Tris-HCl at pH 7.4. The final pellets were resuspended in the same buffer to a final protein concentration of about 2 mg/ml.

Protein concentration was determined by the Lowry method⁵ with bovine serum albumin as a standard. 5'-Nucleotidase activity were examined as a marker enzyme of membrane to check the relative purity of membrane preparation by the method of Emmelot et al.⁶

Receptor Binding Assay

Renal plasma membrane fractions were incubated with 0.5–5 nM [³H]-yohimbine (New England Nuclear Co., specific activity 89.7 Ci/mmol) at 25°C for 15 min in total volume of 200 μ l. At the end of the incubation, samples were filtered through Whatman GF/C glass fiber filters and washed with 10 ml of ice-cold incubation buffer. The filters were then dried, placed in the scintillation vials and counted in 10 ml of Triton-toluene based scintillation mixture. Specific binding was defined as [³H]-yohimbine binding displaced by 10 μ M phentolamine. Binding data were analyzed by the method of Scatchard.⁷

All values are expressed as mean $\pm$ SEM. Data were checked by the analysis of variance. When a significant (p < 0.05) F-ratio was obtained, Welch's test was used to evaluate the statistical significance. Otherwise, Student's t -test was used.

RESULTS

Blood Pressure

Fig. 2. Changes in renal α_2 -adrenoceptor concentration in spontaneously hypertensive (SHR), and two-kidney, one clip hypertensive (2K, 1C HT) rats.

Systolic blood pressure of SHR was significantly higher at 14 weeks old (193 ± 6 mmHg, $p < 0.01$) and 35 weeks old (213 ± 8 mmHg, $p < 0.01$) than that of age-matched WKY (129 ± 2 and 116 ± 3 mmHg, respectively).

In 2K, 1C HT rats, blood pressure was also significantly elevated to 234 ± 7 mmHg ($p < 0.01$) 6 weeks after the operation as compared with sham-operated rats (121 ± 3 mmHg).

In DOCA-salt HT rats, blood pressure was also raised (183 ± 7 mmHg, $p < 0.01$) at 5 weeks after nephrectomy in comparison with unilateral nephrectomized control rats (120 ± 4 mmHg).

[3 H] Yohimbine Binding to Rat Renal Membrane Fraction

Binding of [3 H]yohimbine to rat renal membrane fraction was rapid and saturable, and reversibly dissociated by the addition of $10 \mu\text{M}$ phentolamine. Scatchard plot yielded a straight line. The order of potency for inhibiting [3 H]yohimbine binding by adrenergic agonists and antagonists were (–) noradrenaline $\geq$ (–) adrenaline $>$ (–) isoproterenol, and yohimbine $\geq$ phentolamine $>$ propranolol, respectively.

Changes in Renal α_2 -Adrenoceptor in Experimental Hypertensive Rats

As shown in Table I and Fig. 2, renal α_2 -

adrenoceptor concentration in SHR was significantly increased at 4, 12 and 35 weeks old as compared with age-matched WKY ($p < 0.01$). In both SHR and WKY, the concentration (Bmax) and affinity (Kd) tended to increase with age. 5'-Nucleotidase activities were not different between SHR and WKY at any age.

In 2K, 1C HT rats, concentration of α_2 -adrenoceptor was significantly increased ($p < 0.01$) in the clipped kidney, but unchanged in the contralateral unclipped kidney (Table II, Fig. 2). There was no significant difference in 5'-nucleotidase activity between hypertensive and control rats. Concentration and affinity (Kd values) of α_2 -adrenoceptor in the unclipped kidney were significantly elevated in the clipped kidney as compared with the unclipped kidney in both 2K, 1C HT and sham-operated rats.

In DOCA-salt HT rats, renal α_2 -adrenoceptor concentration was not changed 5 weeks after the operation (hypertensive rats 47.7 ± 4.9 fmol/mg protein, $n = 5$; control rats 40.2 ± 5.3 fmol/mg protein, $n = 5$). No significant difference was also found in binding affinity (2.0 ± 0.1 nM and 2.2 ± 0.6 nM, respectively).

DISCUSSION

[3 H] Yohimbine binding to rat renal membrane fraction was rapid, saturable and reversible, and

Scatchard plot revealed a single binding site for this radioligand. [³H]Yohimbine binding was inhibited by the low concentration of nonselective α - and selective α_2 -adrenergic agonists and antagonists and hardly inhibited by the low concentration of β -adrenergic agonist and antagonist. These results show the validity of our radioligand binding technique for the assessment of the renal α_2 -adrenoceptor function.

The distribution and the role of renal α_2 -adrenoceptor are as yet unknown. Recently several reports have revealed the existence of renal α_2 -adrenoceptor. Müller and Barajas⁸ demonstrated the direct innervation of adrenergic nerves to the renal tubular basement membranes. Young and Kuhar⁹ showed that the α_2 -adrenoceptor was located on the proximal convoluted tubules in rat kidney using autoradiographic technique. Jarrott et al.¹⁰, Yamada et al.¹¹ and Schmitz et al.¹² also reported the existence of α_2 -adrenoceptor binding sites in the kidney by the radioligand binding method.

The denervation of renal sympathetic nerves leads to the increase of sodium and water excretion (denervation natriuresis), and the stimulation of renal sympathetic nerves increases renal sodium and water reabsorption.² These phenomena are explained by α_2 -adrenergic receptor mechanism in the kidney, and renal α_2 -adrenoceptor has been taken notice as a regulating factor of renal sodium reabsorption.^{2,3}

In SHR, renal α_2 -adrenoceptor concentration was increased as compared with age-matched WKY. Similar results were reported by Pettinger et al.³ in 10-week-old SHR and WKY. This increase in receptor concentration in SHR was already evident at the pre- or early-stage of hypertension (4 weeks old) and sustained to the chronic stage of hypertension (35 weeks old).

α_2 -Adrenoceptor concentration was increased in the clipped kidney of 2K, 1C HT rats as compared with that of sham-operated rats. The concentration and K_d values were also higher in the clipped side than the unclipped side of the kidney in both hypertensive and control rats. These changes in concentration and affinity are considered to be probably the results of denervation of renal ischemia due to the clipping operation of the renal artery. Whereas in the unclipped kidney, α_2 -adrenoceptor was not changed in 2K, 1C HT rats, regardless of high blood pressure. Renal α_2 -adrenoceptor was also unchanged in DOCA-salt HT rats.

These results suggest that the increase in renal

α_2 -adrenoceptor concentration in SHR is not induced by the sustained high blood pressure in SHR.

We have previously reported that the sympathetic nervous activity was increased in SHR, especially in young age, 2K, 1C HT and DOCA-salt HT rats,¹⁴ and cardiac β -adrenoceptor concentrations were reduced in SHR and 2K, 1C HT rats, probably due to the down regulation by the increased sympathetic nervous activity.¹ We have also reported that the renal β -adrenoceptor concentration was not changed in SHR, but increased in 2K, 1C HT and DOCA-salt HT rats.¹³ The renal β -adrenoceptor are thought to be regulated by localized factors such as salt loading to the kidney and renal blood flow, and to be unregulated by the generalized sympathetic nervous activity.

Renal α_2 -adrenoceptor, as well as renal β -adrenoceptor, appeared to be free from the regulation by the increased sympathetic nervous activity in the 3 types of experimental hypertension we studied.

Dahl's salt-sensitive rats, another genetic hypertensive rats, are also reported to have higher concentration of renal α_2 -adrenoceptor than that of control rats.³ These hypertensive rats are thought to have the genetic abnormality in renal sodium handling mechanism due to an increase in renal α_2 -adrenoceptor concentration. These results lead to the possibility of similar genetic abnormality in renal sodium reabsorption mechanism in SHR.

Since the activation of renal α_2 -adrenoceptor induces the increase in renal sodium and water reabsorption, the increase in renal α_2 -adrenoceptor concentration in SHR may contribute to the pathogenesis and maintenance of hypertension.

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