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Different Effects of a Long-term Administration of β-blockers, Bopindolol and Atenolol, on the Binding Characteristics of β_1-and β_2-adrenoceptor Subtypes in the Kidney of Spontaneously Hypertensive Rats (SHR)

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Summary. **The effects of a chronic administration of a non-selective β-blocker, bopindolol, on the binding characteristics of radiolabeled CGP-12177 (^{3}H-CGP12177) to each β_1-and β_2-adrenoceptor subtype in the kidney of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) were determined using the radioligand binding assay method and compared with those of a β_1-selective β-blocker, atenolol. Bopindolol (1 mg/kg/day and 3 mg/kg/day) and atenolol (50 mg/kg/day) were given to 10-week-old SHR for 12 weeks. The changes in Kd and Bmax values of β_1-and β_2-adrenoceptors in the renal membranes of SHR treated both with and without these drugs were assessed by Scatchard analysis, and Bmax values and the ratios of β_1-and β_2-adrenoceptor subtypes were also calculated from displacement curves using ICI-118, 551. Bmax values of β_1-and β_2-adrenoceptors in the renal membranes of SHR were significantly higher than those of WKY. The elevated Bmax values of β_1-and β_2-adrenoceptors in renal membranes of SHR were reduced by an administration of bopindolol (1 mg/kg/day and 3 mg/kg/day) with no changes in the Kd values, these changes being especially marked in β_2-adrenoceptors. No effects of the β_1-selective antagonist atenolol on these parameters were observed. These findings showed that the non-selective antagonist bopindolol decreased the Bmax values of β-adrenoceptor subtypes in renal membranes of SHR, suggesting that this drug may partially contribute to hypotensive effects.**

Key words—bopindolol, atenolol, β-blocker, β-adrenoceptor, spontaneously hypetensive rats (SHR), kindly, blood pressure, radioligand binding assay.

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INTRODUCTION

The kidney has been considered one of the tissues with the greatest impact on long-term blood pressure regulation, although blood pressure is regulated by various organs like the heart, the blood vessels, and the central and peripheral nervous systems. The β-adrenoceptor system is also a major mechanism for the neural and humoral control of kidney functions. The importance of the β-adrenergic system in the renal function of spontaneously hypertensive rats (SHR) has been confirmed[1,2] and β-adrenoceptors in kidneys exist presynaptically[3], though many are found on tubular cells[4].

Our previous study[5] suggested that a long-term administration of bopindolol (4-(benzoyloxy-3-tert-butylaminopropyl)-2-methylindol hydrogenmalomate), a non-selective β-adrenoceptor antagonist with partial agonist activity, may contribute to the reduction of hypertension, mediated through a decrease in cardiac β-adrenoceptor density because hypertension occurs due to sympathetic stimulation. Several investigators have already reported enhanced Bmax values of renal β-adrenoceptors in SHR[6,7] or stroke-prone SHR (SHRSP)[8].

Therefore, this study was undertaken to examine the different effects of a chronic administration of bopindolol and atenolol on the binding characteristics of each β_1-and β_2-adrenoceptor subtype in the kidneys of SHR and Wistar Kyoto Rats (WKY) using the radioligand binding assay.

MATERIALS AND METHODS

Radioligands and drugs

^{3}H-CGP12177 (1.85 TBq/mmol) was purchased from New England Nuclear Corp. (Tokyo, Japan) Bopindolol and atenolol were kindly supplied by Novartis Pharma A.G. (Tokyo, Japan) and AstraZeneca (Osaka, Japan), respectively. The volume of the drug administration was adjusted every week.

Rats WKY and SHR were supplied by Charles River Corp. (Kanagawa, Japan).

Administration of drugs

Administration of drugs was performed as described in our previous paper[5]. Ten-week-old SHR were given bopindolol (1 mg/kg/day and 3 mg/kg/day) or atenolol (50 mg/kg/day) by oral administration for 12 weeks and WKY were also used as a control. At the end of the experiments, all animals were killed by decapitation, and their kidneys were removed, frozen in liquid nitrogen, and stored at −80 °C until use (22-week-old SHR and WKY).

Preparation of renal membrane-enriched fraction

The membrane-enriched fractions of kidneys from SHR and WKY were prepared as described previously[5]. The thawed kidneys were weighed, minced, and homogenized in 10 volumes of 10 mM Tris-HCl (pH 7.4), and 250 mM sucrose with a Polytron homogenizer. The homogenates were filtered through 4 layers of gauze, and the filtrate was centrifuged at 40,000 g for 30 min. The resultant pellets were rinsed with 120 mM Tris-HCl (pH7.4), 40 mM $MgCl_2$, and homogenized in 20 ml of the same buffer. The assay for ^{3}H-CGP12177 binding was carried out using membrane-enriched fractions stored at −80°C. Protein concentrations were determined by the method by Lowry et al.[9] using bovine serum albumin as a standard.

Binding assay

The β-adrenoceptor binding assays were performed in duplicate using ^{3}H-CGP12177. The membrane suspension (0.1 -0.2 mg of protein) was incubated for 45 min at 23 °C in a total volume of 0.5 ml containing 60 mM Tris-HCl (pH7.4), 20 mM $MgCl_2$. The dissociation constant (Kd value) and maximum binding capacity (Bmax value) for ^{3}H-CGP12177 were calculated using Scatchard plots, and radioligand concentrations were 0.1 -10 nM for ^{3}H-CGP12177. At the end of the incubation period, the reaction mixture was immediately filtered through a Whatman GF/C glass fiber filter using a LM-101 cell harvester (Labo Science, Tokyo). The filter was added to a Scintizol EX-H (Dojin) for scintillation counting. The differences in mean value between total and non-specific ^{3}H-CGP12177 bindings determined in the presence of a 10 μM non-selective β-adrenoceptor blocker, l-propranolol, were taken as the specific binding.

As described previously[10], Bmax values and the ratio of each β_1-and β_2-adrenoceptor subtype were calculated by a method of displacement experiments. The displacement experiment was performed in the presence of ICI-118, 551 with a high concentration (L/Kd>10) of ^{3}H-CGP12177 in duplicate to obtain Bmax values and ratios of β_1-and β_2-adrenoceptors. Using high concentrations of the radioligand, parameters describing the competition of ICI-118, 551 (β_2-selective antagonist) with specific ^{3}H-CGP12177 binding at two sites (IC_{50} values at β_1-and β_2-adrenoceptors and % β_1-and %β_2-) were estimated by non-linear regression analysis of data that were fitted to a two-site model compared with a one-site model.

Statistical analyses of SHR and the administration of each drug were performed by a One-way ANOVA test followed by Newman-Keuls test, and a P value of less than 0.05 was considered significant.

RESULTS

As shown in Table 1, Bmax values in the renal membranes of SHR were higher than those of WKY, but there were no differences in Kd values between WKY and SHR. On the other hand, Bmax values of β-adrenoceptors were lowered by the administration of bopindolol (1 mg/kg/day and 3 mg/kg/day), with no associated changes in the Kd values. However, no effects of atenolol were observed on Bmax or Kd values.

Table 2 summarizes the effects of bopindolol and atenolol on the values of Bmax of each β_1-and β_2-adrenoceptor and the ratios of these adrenoceptor subtypes in SHR. Higher Bmax values of β_1-and β_2-adrenoceptors in renal membranes of SHR as compared with those of WKY were also observed, but no changes were seen in the $\beta_1 : \beta_2$ ratio. No changes were observed in the Bmax value or ratio of β_1-and β_2-adrenoceptors following atenolol administration, but a higher dose (3mg/kg/day) of bopindolol induced a decrease in the Bmax value of β_2-adrenoceptors. Thus, the proportion of β_1-adrenoceptors compared with β_2-adrenoceptors was increased following the administration of a high dose (3mg/kg/day) of bopindolol.

Table 1. Effects of drugs on ^{3}H-CGP12177 binding to β-adrenoceptor in the kidneys of SHR

Strain	Kd (nM)	Bmax (fmol/mg protein)
WKY(7)	0.89 ± 0.24	13.38 ± 1.56
SHR(7)	1.30 ± 0.36	22.49 ± 2.90
Bopindolol : 1mg/kg/day(6)	1.38 ± 0.42	12.50 ± 1.75**
Bopindolol : 3mg/kg/day(6)	1.67 ± 0.18	11.32 ± 2.08**
Atenolol(7)	0.99 ± 0.16	18.15 ± 1.69

Data are means±S.E. Values in parentheses represent numbers of rats.
**: $p<0.01$ vs. SHR.

Table 2. Effects of drugs on ^{3}H-CGP12177 binding to β_1-and β_2-adrenoceptors in the kidneys of SHRs

Strain	Bmax (fmo/mg protein)		
	β_1	β_2	$\beta_1 : \beta_2$
WKY(7)	8.70 ± 1.02*	4.68 ± 0.55*	65 : 35
SHR(7)	14.17 ± 1.83	8.32 ± 1.07	63 : 37
Bopindolol: 1 mg/kg/day(6)	7.37 ± 1.03**	5.12 ± 0.72*	59 : 41
Bopindolol: 3 mg/kg/day(6)	8.38 ± 1.54*	2.94 ± 0.54**	74 : 26
Atenolol(7)	12.53 ± 1.16	5.63 ± 1.52	69 : 31

Data are means±S.E. Values in parentheses represent numbers of rats. *: $p<0.05$, **: $p<0.01$ vs. SHR.

DISCUSSION

Increased sympathoadrenal activity plays an important role in the development and maintenance of enhanced blood pressure in hypertensive patients and in various animal models of hypertension[1,2]. The kidneys also play an important role in the initiation and maintenance of hypertension. The present study clearly demonstrated increased densities of each β_1-and β_2-adrenoceptor subtype in the kidneys of SHR. These results agreed with those of the renal β-adrenoceptor in animal hypertension models studied by many investigators[4-6], although no report has been available describing changes in β-adrenoceptors in SHR[11]. In addition, there have been no reports concerning the effects of chronic treatment with bopindolol or atenolol on β_1-and β_2-adrenoceptor subtypes in renal tubules of SHR. Therefore, in the present study we reevaluated changes in each β_1-and β_2-adrenoceptor subtype in renal tubules of SHR, and also examined the inhibitory effects of bopindolol and atenolol on the binding characteristics of both subtypes.

The present study indicated that the non-selective β-blocker bopindolol induced decreases in β_1-adrenoceptor densities in the renal tubules of SHR. In contrast, the selective β_1-blocker atenolol did not show this effect in the present study. Bopindolol has such advantages over atenolol as improved absorption, favorable patient compliance, the relief of side effects related to the intrinsic activity (ISA), and better sustained effects[12]. In addition, β-adrenoceptors in the glomeruli of the kidney are considered to be β_1-adrenoceptors[13], and this subtype may be related to renin release[14] or abnormalities in the tubular reabsorption of sodium and water[15]. Thus, bopindolol may possess these effects mediated by a decrease in the density of β_1-adrenoceptors in SHR.

The present study also confirmed the existence of β_2-adrenoceptors in the membrane fraction of kidney tubules, as reported previously by other investigators[16,17]. Furthermore, bopindolol also decreased the density of β_2-adrenoceptors in the kidneys of SHR, while atenolol did not. Michel et al (1987)[18] suggested that the kidney contained both β_1-and β_2-adrenoceptors and that the increased β_2-adrenoceptors in the kidneys of SHR are also involved in the develop-

ment or maintenance of high blood pressure, possibly facilitating noradrenaline release. In addition, Singh et al.[19] reported that activation of this subtype caused increases in $Na^+ + K^+$-ATPase and Na^+ transport, resulting in an increased apical sodium entry. Thus, the β_2-adrenoceptor subtype may play an important role in these pharmacological functions in the kidney. Atenolol did not affect β_1-or β_2-adrenoceptors in renal tubules, suggesting that the non-selective β-blocker bopindolol may manifest pharmacological functions through a decrease of B max values of β_2-adrenoceptors as well as β_1-adrenoceptors.

Bopindolol lowered the systolic blood pressure by binding to the β-adrenoceptors in cardiac muscles of SHR[5]. Therefore, if β_2-adrenoceptors in renal tubules as well as β_1-adrenoceptors have important roles in the maintenance or development of hypertension, the non-selective β-blocker bopindolol may be useful as an anti-hypertensive drug in SHR.

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