Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 2, pp. 225–232, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00301-7

Effects of Propranolol and Atenolol on Immobilization Stress-Induced Hypertension and Down-Regulation of Central β-Adrenoceptors in Rats

MANABU TAKITA,* YASUO ODA,† SHIGERU KIGOSHI* AND IKUNOBU MURAMATSU*[1]

*Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan; and
†Faculty of Pharmaceutical Sciences, Kinki University, Higashi-Osaka 577, Japan

Received 29 April 1994

TAKITA, M., Y. ODA, S. KIGOSHI AND I. MURAMATSU. *Effects of propranolol and atenolol on immobilization stress-induced hypertension and down-regulation of central β-adrenoceptors in rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 225–232, 1995. – Effects of chronic treatment with propranolol or atenolol on stress-induced changes in blood pressure, body weight, and cerebral β-adrenoceptors in rats were examined and compared with the effects of chronic treatment with prazosin. Immobilization stress (2 h daily for 2 weeks) induced a moderate elevation of blood pressure, loss of body weight gain, and downregulation of cerebral β-adrenoceptors, but produced no changes in the cerebral α_1-adrenoceptors. Chronic administration of propranolol (5 or 50 mg · kg^{-1}), atenolol (5 or 50 mg · kg^{-1}) or prazosin (2 or 20 mg · kg^{-1}) inhibited stress-induced hypertension but did not affect loss of body weight gain. Propranolol increased the density of cerebral β-adrenoceptors by 77% and reduced the downregulation induced by stress. Atenolol also increased the density of cerebral β-adrenoceptors by 34% and abolished the stress-induced downregulation in cerebral β-adrenoceptor density. In contrast, prazosin had no effect on the cerebral β-adrenoceptors in nonstressed or stressed rats. These results suggest that the antihypertensive action of propranolol and atenolol may be partly associated with the inhibition of stress-activated central β-adrenoceptor transmission.

Hypertension Stress Propranolol Atenolol Prazosin Down-regulation of β-adrenoceptor

SINCE propranolol was first found to have an antihypertensive effect, many β-blockers have been used for the clinical treatment of hypertension (11,27). Various central and peripheral mechanisms have been proposed for the effectiveness of β-blockers, such as the inhibition of cardiac output, of prejunctional β-adrenoceptors, and of renin release, and reduction of sympathetic activity through the CNS (1,12,27,33). Central mechanisms were first proposed because propranolol can easily penetrate into the brain (23). However, as hydrophilic atenolol is also effective in hypertensive therapy (7,11), peripheral mechanisms are now considered to be dominant compared with the central ones (9).

Stress is thought to activate various parts of the CNS including those affecting adrenergic nerves, and to cause hypertension (5,15,17). Furthermore, destruction of the central adrenergic system by intracisternal 6-hydroxydopamine prevents the development of hypertension in rabbits and rats (4,14). Therefore, activation of the central adrenergic system may be associated with the development and/or maintenance of hypertension. Recently, we found that chronic immobilization stress caused both hypertension and downregulation of cerebral β-adrenoceptors in rats, and that both responses were completely inhibited by bevantolol, a β-blocker. Furthermore, bevantolol itself caused upregulation of cerebral β-adrenoceptors (31). We concluded that central mechanisms of β-blockers in hypertensive therapy should not be ignored.

The aim of this study was to examine how both hydrophobic propranolol and hydrophilic atenolol with different penetrating ratios into the CNS affect the peripheral (hypertension) and central (downregulation of β-adrenoceptors) responses in-

[1] To whom requests for reprints should be addressed.

duced by immobilization stress. To find some clue for substantiation of the central mechanisms of action of β-blockers, we also compared the effects of the α_1-blocker, prazosin, on stress-induced responses. The effects of stress on central α_1-adrenoceptors were also investigated.

METHOD

Stress and Drug Treatment

The experimental protocol was essentially the same as that described previously (31,37). In brief, male Wistar rats (6 weeks of age, 160–180 g) were housed in groups of two or three with free access to the usual chow diet and tapwater. One week later, six different groups—nontreated (group I), stressed (group II), drug-treated (groups III and V), or stressed drug-treated (groups IV and VI)—six to 10 rats in each group, were formed for experiments with each drug (propranolol, atenolol, or prazosin) (Table 1). Propranolol (41 or 406 mg · liter^{-1}) or atenolol (36 or 357 mg · liter^{-1}) was dissolved with tapwater. Prazosin (16 or 156 mg · liter^{-1}) was dissolved with tapwater containing 0.01% Tween-80, and was taken freely. The daily consumption of propranolol and atenolol was estimated to be 5 or 50 mg · kg^{-1}, and that of prazosin was estimated to be 2 or 20 mg · kg^{-1}, based on a mean intake of 35 ml · day^{-1} of water for a 250-g rat. Actual plasma concentrations of the drugs were measured at the end of experiments (Table 1). Blood pressure was measured with a sphygmomanometer (MK-1000; Muromachikikai, Japan) before and at the ends of the 4th and 5th week after commencing the experiments, to avoid extra stress. Briefly, rats were preheated at 30°C for 13 min to dilate the tail artery, and blood pressure was measured three times at 35°C. To eliminate the acute effect of stress on blood pressure, the measurement of blood pressure was performed at 1400–1700 h(more than 3 h after immobilization stress). In the preliminary study, the chronic elevation of blood pressure in the stressed group had been confirmed by measurement 24 h after the final loading of stress. Rats in the stressed groups received immobilization stress for the last 2 weeks of the 5-week experimental period. The immobilization stress was induced by restraining the rats in plastic tubes (length 185 mm, internal diameter 59 mm), which were from a Heiner Borgwaldt (R14.01; Hamburg, Germany) inhalation apparatus. In this method, the rat was restrained in a prone position by pressing the hind part of the animal from one end of the tube, using a small, circular plate manipulated through a rubber tube-stopper. The head motion was partially limited by wide metal loops fixed over the neck area at the other end of the tube. Immobilization stress was forced between 0900 and 1100 h. The rats were decapitated 2 h after completing the last stress session. The cerebral cortex was rapidly dissected on ice and stored at −80°C until used for binding assays.

TABLE 1

PLASMA CONCENTRATIONS OF PROPRANOLOL, ATENOLOL AND PRAZOSIN IN RATS

Group	Treatments	Concentration of Drugs: *n*	Concentration of Drugs: (ng · ml^{-1})
I	Control	6	Not detected
II	Stress	13	Not detected
III	5 mg · kg^{-1} propranolol	4	*
IV	5 mg · kg^{-1} propranolol + stress	4	*
V	50 mg · kg^{-1} propranolol	6	50.8 ± 9.5
VI	50 mg · kg^{-1} propranolol + stress	5	32.6 ± 7.7
III	5 mg · kg^{-1} atenolol	6	224 ± 38
IV	5 mg · kg^{-1} atenolol + stress	6	242 ± 33
V	50 mg · kg^{-1} atenolol	6	590 ± 128
VI	50 mg · kg^{-1} atenolol + stress	6	405 ± 82
III	2 mg · kg^{-1} prazosin	6	4.5 ± 1.1
IV	2 mg · kg^{-1} prazosin + stress	6	4.6 ± 1.6
V	20 mg · kg^{-1} prazosin	6	14.0 ± 2.2
VI	20 mg · kg^{-1} prazosin + stress	6	8.1 ± 1.2

Data shown are mean ± SEM. *n* = number of rats.
*Under detection limit.

α_1- and β-Adrenoceptor Binding

β-Adrenoceptors of rat cerebral cortex were measured in the binding assays with ^{3}H-dihydroalprenolol (^{3}H-DHA), as described previously (30,31). In brief, the cerebral cortex was homogenized with a polytron in 80 vol. of homogenizing buffer (50 mM Tris/HCl, 100 mM NaCl, and 2 mM EDTA, pH 7.4). The homogenates were filtered through gauze and centrifuged at 80,000 × *g* for 30 min at 4°C. The pellets were resuspended in the same volume of assay buffer (50 mM Tris/HCl, 1 mM EDTA, 0.1% ascorbic acid, and 5 mM $MgCl_2$, pH 7.4), incubated at 37°C for 15 min, and centrifuged at 80,000 × *g* for 30 min. The final pellets were resuspended in the same buffer, and the membrane fraction was used in binding assays. The homogenates (about 100 μg protein) were incubated with ^{3}H-DHA for 45 min at 30°C in a total volume of 1 ml. Reactions were terminated by rapid filtration through using a Brandel cell harvester (Brandel Biochemical Research-Developmental Lab, Gaithersburg, MD) and Whatman GF/C filters (Whatman International, Ltd, Maidstone, Kent, UK). The filter was then washed three times with ice-cold washing buffer (50 mM Tris/HCl, pH 7.4), and filter-bound radioactivity was determined. Nonspecific binding was defined as binding in the presence of 10 μM propranolol.

When the α_1-adrenoceptor was examined, protocols were the same as in the case of β-adrenoceptor binding except for the assay buffer (50 mM Tris/HCl and 1 mM EDTA, pH 7.4) and radioligand (^{3}H-prazosin), as described previously (30). ^{3}H-prazosin was used as a ligand, and specific binding was determined by 10 μM phentolamine. Assays were conducted in duplicate.

Statistical Analysis

All values are expressed as mean ± SEM. Data were analyzed by the weighted least-squares iterative curve-fitting program, LIGAND (22). The data were first fitted to one- and then two-site models; when the residual sums of squares were statistically less for a two-site fit of the data than a one-site fit, as determined by *F*-test comparison, the two-site model was accepted. P values < 0.05 were considered significant. Proteins were assayed according to the method of Bradford using bovine serum albumin as the standard (3).

Plasma Concentration of Drugs

Plasma concentrations of propranolol and atenolol were determined according to the method of Winkler et al., with some modifications (35). The plasma concentration of pra-

zosin was determined according to the method of Wood et al., with some modifications (36).

Materials

We used the following drugs: ^{3}H-dihydroalprenolol (^{3}H-DHA) (specific activity 107.0 Ci · mmol^{-1}) and ^{3}H-prazosin (specific activity 76.2 Ci · mmol^{-1}) (NEN, Boston, MA); propranolol hydrochloride (Nakalai, Kyoto, Japan), prazosin hydrochloride and atenolol (Sigma, St. Louis, MO), phentolamine mesylate (Ciba, Basel, Switzerland), WB-4101 hydrochloride [2-(2,6-dimethoxy-phenoxyethyl)-aminomethyl-1,4-benzodioxane hydrochloride] and 5-methyl-urapidil (Funakoshi, Tokyo, Japan), and ICI-89, 406 [1-(2-cyanophenoxy)-3-β-(3-phenylureido) ethylamino-2-propanol] (a gift from ICI Pharma, Cheshire, UK).

RESULTS

Water Intake and Plasma Concentrations of Propranolol, Atenolol, and Prazosin

To avoid extra stress, propranolol, atenolol, or prazosin was dissolved in tapwater and was taken freely, as described in Method. Therefore, actual plasma concentrations of the drugs were determined when rats were decapitated at the end of the experiments (Table 1). Plasma concentrations of all drugs were not significantly different between nonstressed and stressed rats.

Effects of Propranolol, Atenolol, and Prazosin on Stress-Induced Changes of Blood Pressure and Body Weight Gain

Immobilization stress for 14 days (2 h · day^{-1}) during the 4th and 5th weeks of the experimental period caused moderate hypertension in rats. Propranolol, atenolol, and prazosin had little effect on blood pressure in nonstressed rats, but completely inhibited the rise in systolic blood pressure induced by stress (Table 2).

Body weight gain of rats during the experimental period was not changed by propranolol, atenolol, or prazosin. Immobilization stress caused a suppression of body weight gain, which was not affected by treatment with the drugs tested (Table 3).

Effects of Propranolol, Atenolol, and Prazosin on the Stress-Induced Change of ^{3}H-DHA Binding to Rat Cerebral Cortex

Saturation experiments with ^{3}H-DHA ranging from 100–3000 pM were carried out in the rat cerebral cortex membranes. In stressed rats, the maximum number of ^{3}H-DHA binding sites (B_{max}) was significantly decreased to 69% ($p < 0.05$), without changing affinity (Table 4). The administration of propranolol (5 mg · kg^{-1}) increased the B_{max} to 177% of control, although affinity was not significantly different from that of control rats. Furthermore, no significant decrease in β-adrenoceptor number by stress was observed in propranolol-treated rats.

Both concentrations of atenolol (5 and 50 mg · kg^{-1}) also caused a small but significant increase in cerebral β-adrenoceptor density in nonstressed rats (133 and 132%, respectively). The downregulation in cerebral β-adrenoceptor density by stress was completely abolished in rats treated with both concentrations of atenolol. In contrast, prazosin treatment caused no effect in the density of β-adrenoceptors in nonstressed rats, and did not affect the decrease in the density of β-adrenoceptors induced by stress (Table 4).

Competition experiments with ICI-89, 406, a β_1-selective

TABLE 2

EFFECTS OF STRESS AND DRUGS ON BLOOD PRESSURE IN RATS

Group	Treatments	n	Systolic Blood Pressure		
			Before	28 days	35 days
I	Control	6	118 ± 1.2	132 ± 3.3	132 ± 1.9
II	Stress	10	118 ± 6.4	145 ± 3.1*	146 ± 2.3*
III	5 mg · kg^{-1} propranolol	6	116 ± 2.0	128 ± 2.2	135 ± 1.2
IV	5 mg · kg^{-1} propranolol + stress	6	118 ± 3.2	130 ± 1.4	134 ± 1.0
V	50 mg · kg^{-1} propranolol	6	118 ± 3.2	132 ± 2.3	134 ± 1.2
VI	50 mg · kg^{-1} propranolol + stress	6	118 ± 2.5	131 ± 1.7	133 ± 0.7
I	Control	6	119 ± 0.6	138 ± 1.5	133 ± 1.7
II	Stress	7	118 ± 2.5	146 ± 3.9*	148 ± 3.8*
III	5 mg · kg^{-1} atenolol	6	119 ± 3.6	133 ± 2.5	133 ± 2.6
IV	5 mg · kg^{-1} atenolol + stress	6	120 ± 2.9	135 ± 1.9	133 ± 3.0
V	50 mg · kg^{-1} atenolol	6	120 ± 1.9	133 ± 2.9	134 ± 1.8
VI	50 mg · kg^{-1} atenolol + stress	6	120 ± 0.9	133 ± 3.5	127 ± 3.9
I	Control	6	121 ± 1.6	135 ± 2.8	136 ± 2.9
II	Stress	10	122 ± 1.7	148 ± 3.6*	154 ± 3.0*
III	2 mg · kg^{-1} prazosin	6	120 ± 2.7	136 ± 3.5	136 ± 1.3
IV	2 mg · kg^{-1} prazosin + stress	6	120 ± 2.7	137 ± 1.7	134 ± 2.7
V	20 mg · kg^{-1} prazosin	6	122 ± 1.2	135 ± 2.6	139 ± 1.5
VI	20 mg · kg^{-1} prazosin	6	124 ± 0.7	140 ± 2.5	142 ± 0.5

Data shown are mean ± SEM. n = number of rats.
*Significantly different from control (group I in each experiment) ($p < 0.05$).

TABLE 3

EFFECTS OF STRESS AND DRUGS ON BODY WEIGHT OF RATS

Group	Treatments	n	Body Weight (g) 21 days	28 days	35 days
I	Control	6	393 ± 5	417 ± 6	446 ± 5
II	Stress	10	384 ± 4	385 ± 4*	400 ± 5*
III	5 mg · kg^{-1} propranolol	6	397 ± 7	415 ± 6	427 ± 7
IV	5 mg · kg^{-1} propranolol + stress	6	394 ± 7	391 ± 5*	394 ± 6*
V	50 mg · kg^{-1} propranolol	6	376 ± 9	399 ± 6	425 ± 11
VI	50 mg · kg^{-1} propranolol + stress	6	382 ± 6	381 ± 5*	382 ± 5*
I	Control	6	405 ± 10	442 ± 10	465 ± 12
II	Stress	7	399 ± 6	388 ± 8*	399 ± 8*
III	5 mg · kg^{-1} atenolol	6	409 ± 12	442 ± 15	472 ± 17
IV	5 mg · kg^{-1} atenolol + stress	6	375 ± 7	383 ± 6*	384 ± 4*
V	50 mg · kg^{-1} atenolol	6	385 ± 8	422 ± 5	445 ± 6
VI	50 mg · kg^{-1} atenolol + stress	6	408 ± 7	406 ± 10*	407 ± 8*
I	Control	6	405 ± 11	434 ± 13	461 ± 15
II	Stress	10	399 ± 6	392 ± 6*	402 ± 8*
III	2 mg · kg^{-1} prazosin	6	394 ± 10	423 ± 12	447 ± 16
IV	2 mg · kg^{-1} prazosin + stress	6	387 ± 3	385 ± 8*	397 ± 7*
V	20 mg · kg^{-1} prazosin	6	391 ± 1	420 ± 9	441 ± 11
VI	20 mg · kg^{-1} prazosin + stress	6	387 ± 1	396 ± 9*	406 ± 9*

Data shown are mean ± SEM. n = number of rats.
*Significantly different from control (group I in each experiment) ($p < 0.05$).

TABLE 4

EFFECTS OF STRESS AND DRUGS ON ^{3}H-DHA BINDING TO β-ADRENOCEPTORS OF RAT CEREBRAL CORTEX

Group	Treatments	n	^{3}H-DHA binding pK_d	B_{max}
I	Control	6	9.00 ± 0.06	209 ± 10
II	Stress	7	9.13 ± 0.10	143 ± 11*
III	5 mg · kg^{-1} propranolol	6	8.98 ± 0.05	370 ± 28*
IV	5 mg · kg^{-1} propranolol + stress	5	8.91 ± 0.05	348 ± 11*
V	50 mg · kg^{-1} propranolol	4	8.85 ± 0.10	319 ± 30*
VI	50 mg · kg^{-1} propranolol + stress	6	9.00 ± 0.11	279 ± 14*
I	Control	5	8.82 ± 0.08	170 ± 8
II	Stress	3	8.96 ± 0.04	126 ± 11*
III	5 mg · kg^{-1} atenolol	5	9.04 ± 0.02	227 ± 8*
IV	5 mg · kg^{-1} atenolol + stress	5	8.97 ± 0.09	212 ± 10*
V	50 mg · kg^{-1} atenolol	5	8.89 ± 0.04	225 ± 8*
VI	50 mg · kg^{-1} atenolol + stress	5	8.88 ± 0.07	216 ± 11*
I	Control	5	9.00 ± 0.07	182 ± 4
II	Stress	4	8.88 ± 0.02	156 ± 4*
III	2 mg · kg^{-1} prazosin	3	9.25 ± 0.01	226 ± 17
IV	2 mg · kg^{-1} prazosin + stress	3	8.97 ± 0.08	159 ± 9*
V	20 mg · kg^{-1} prazosin	3	9.10 ± 0.02	180 ± 10
VI	20 mg · kg^{-1} prazosin + stress	3	9.11 ± 0.04	151 ± 11*

Scatchard curves were individually analyzed by the LIGAND program.
Data shown are mean ± SEM. n = number of experiments; pK_d-negative log of equilibrium dissociation constant; B_{max}-maximum number of ^{3}H-DHA binding sites (fmol · mg^{-1} protein).
*Significantly different from control ($p < 0.05$).

TABLE 5

INHIBITION OF 1 nM ^{3}H-DHA BINDING TO β-ADRENOCEPTORS OF RAT CEREBRAL CORTEX BY ICI-89, 406

Group	Treatments	n	$pK_{i\text{-}high}$	$pK_{i\text{-}low}$	% High
I	Control	6	8.80 ± 0.13	6.69 ± 0.17	61 ± 2
II	Stress	7	8.68 ± 0.12	6.42 ± 0.09	50 ± 2*
III	5 mg · kg^{-1} propranolol	6	8.91 ± 0.07	6.76 ± 0.08	69 ± 2*
IV	5 mg · kg^{-1} propranolol + stress	5	8.68 ± 0.14	6.73 ± 0.05	65 ± 3
V	50 mg · kg^{-1} propranolol	4	8.62 ± 0.07	6.62 ± 0.14	72 ± 3*
VI	50 mg · kg^{-1} propranolol + stress	6	8.69 ± 0.03	6.73 ± 0.15	65 ± 1
I	Control	5	8.80 ± 0.07	6.66 ± 0.11	60 ± 1
II	Stress	3	8.55 ± 0.20	6.33 ± 0.13	53 ± 2*
III	5 mg · kg^{-1} atenolol	5	8.51 ± 0.09	6.37 ± 0.10	62 ± 3
IV	5 mg · kg^{-1} atenolol + stress	5	8.69 ± 0.07	6.65 ± 0.08	60 ± 4
V	50 mg · kg^{-1} atenolol	5	8.83 ± 0.08	6.78 ± 0.10	59 ± 3
VI	50 mg · kg^{-1} atenolol + stress	5	8.62 ± 0.11	6.38 ± 0.09	55 ± 2
I	Control	5	8.84 ± 0.09	6.59 ± 0.11	60 ± 1
II	Stress	4	8.92 ± 0.10	6.56 ± 0.07	48 ± 4*
III	2 mg · kg^{-1} prazosin	3	8.70 ± 0.10	6.62 ± 0.02	69 ± 2
IV	2 mg · kg^{-1} prazosin + stress	3	8.83 ± 0.05	6.56 ± 0.28	44 ± 3*
V	20 mg · kg^{-1} prazosin	3	8.73 ± 0.09	6.64 ± 0.07	60 ± 1
VI	20 mg · kg^{-1} prazosin + stress	3	8.88 ± 0.06	6.37 ± 0.04	48 ± 4*

Displacement curves were individually analyzed by the LIGAND program. Data shown are mean ± SEM. n = number of experiments. $pK_{i\text{-}high}$ and $pK_{i\text{-}low}$-negative log of the equilibrium dissociation constants (−log M) at high or low affinity for ICI 89, 406. %High-population binding at the high-affinity site compared with the total specific binding sites.

*Significantly different from control ($p < 0.05$).

blocker, were also carried out to distinguish between β_1- and β_2-adrenoceptor subtypes. ICI-89, 406 displaced about 61% of 1 nM ^{3}H-DHA binding with a high affinity (pKi-high = 8.80 ± 0.13) in control rats (Table 5). In stressed rats, this proportion reduced to 50% without changing the affinities for both sites. Propranolol treatment increased the total number of β-adrenoceptors and suppressed the downregulation by stress. Because the population ratio was not significantly changed by stress in rats treated with propranolol or atenolol (Table 5), the amount of β_1-adrenoceptor increased more predominantly than β_2-adrenoceptors (Fig. 1). On the other hand, prazosin treatment had no effect on changes in the proportion of β_1-adrenoceptors in nonstressed and stressed rats.

Effects of Immobilization Stress on ^{3}H-Prazosin Binding to Rat Cerebral Cortex

Saturation experiments with ^{3}H-prazosin in the range of 10–5000 pM were carried out in rat cerebral cortex membranes. As shown in Table 6, analyses using the LIGAND program revealed binding of the ligand to two classes of sites, as described previously (25). In contrast to the case of β-adrenoceptors, both the affinity and B_{max} of ^{3}H-prazosin binding were not significantly affected by stress.

Competition experiments with WB-4101, 5-methyl-urapidil, and prazosin were also examined to test for possible change in subtype ratios. ^{3}H-Prazosin (200 pM) was used to label α_1-adrenoceptors. No significant difference was observed between nonstressed and stressed rats (Table 7).

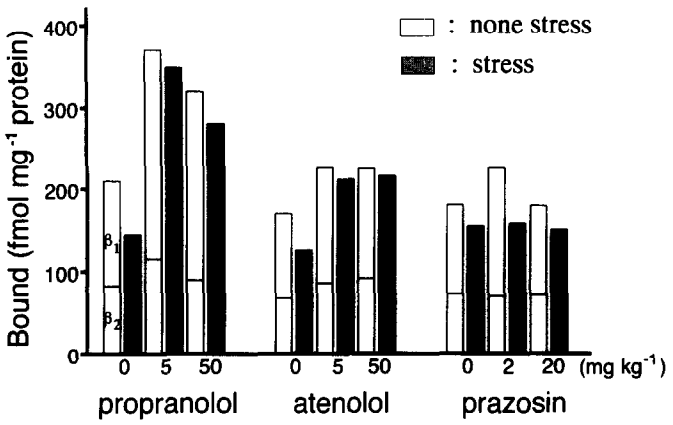

FIG. 1. B_{max} values of β_1- and β_2-adrenoceptors that were extrapolated from the proportion estimated in the displacements (Table 5) and from the total B_{max} values obtained in the saturation experiments (Table 4). Note the predominant change of β_1-adrenoceptor density.

DISCUSSION

As reported previously, immobilization stress induced moderate hypertension (18–20) and the downregulation of cerebral β-adrenoceptors (28,31,32,37). The present study further demonstrates that such effects of stress were completely inhibited by the chronic administration of propranolol or atenolol.

Propranolol itself increased the cerebral β-adrenoceptor density to 177% (Table 4). Because propranolol can enter into brain easily (23), it is likely that propranolol blocks the central β-adrenoceptors resulting in upregulation (10,13). This remarkable increase also means that cerebral β-adrenoceptors are downregulated under normal (stress-unloaded) conditions

TABLE 6

EFFECTS OF STRESS ON ^{3}H-PRAZOSIN BINDING TO α_1-ADRENOCEPTORS OF RAT CEREBRAL CORTEX

Treatments	^{3}H-prazosin binding			
	$pK_{d\text{-}high}$	$pK_{d\text{-}low}$	$B_{max\text{-}high}$	$B_{max\text{-}low}$
Control	10.19 ± 0.01	8.61 ± 0.01	246 ± 23	135 ± 31
Stress	10.11 ± 0.01	8.50 ± 0.09	241 ± 25	150 ± 2

Scatchard curves were individually analyzed by the LIGAND program. Data shown are mean ± SEM of three experiments. $pK_{d\text{-}high}$ and $pK_{d\text{-}low}$-negative log of the equilibrium dissociation constants at high and low affinity for ^{3}H-prazosin. $B_{max\text{-}high}$ and $B_{max\text{-}low}$-maximum number of high- and low-affinity sites of ^{3}H-prazosin binding sites (fmol · mg^{-1} protein).

(24). Thus, stress-induced downregulation of β-adrenoceptors seems to result from further activation of the central adrenergic system by stress. Such a change by stress occurred mainly in β-adrenoceptors, especially of the β_1-adrenoceptor subtype, and no change was observed in α_1-adrenoceptors.

As in the case of propranolol, atenolol itself caused the upregulation of cerebral β-adrenoceptors. Atenolol is a hydrophilic drug, and its poor penetration into the brain has been reported (6). However, even at a low dose (5 mg · kg^{-1}), atenolol slightly but significantly increased β-adrenoceptor density and inhibited the stress-induced downregulation of cerebral β-adrenoceptors. Two possible explanations may be given for the upregulation by atenolol. One is that atenolol may also enter into the brain and then block β-adrenergic transmission, although the penetration rate is much lower than that of propranolol. In fact, central side effects were clinically reported not only in propranolol but also in atenolol (2,11,34). The smaller extent of upregulation by atenolol than propranolol may reflect this lower penetration ratio. Another explanation is that the upregulation of cerebral β-adrenoceptors may be result from the inhibition of peripheral β-adrenoceptors. If so, the stress-induced downregulation of β-adrenoceptors in the cerebral cortex must be associated with the activation of peripheral β-adrenoceptors.

Unlike propranolol and atenolol, prazosin (2 or 20 mg · kg^{-1}) had no effect on cerebral β-adrenoceptors; neither did stress. These results suggest that chronic changes by stress or drugs occur more easily in β- as compared with α_1-adrenoceptors. Such a predominant change by stress has been reported in β-adrenoceptors (28,31,32,37), but not in α_1-adrenoceptors and muscarinic receptors (20,37).

Stress-induced hypertension was observed in parallel with the downregulation of cerebral β-adrenoceptors. However, no such parallel relation was seen in prazosin-treated rats. As prazosin is a potent α_1-adrenoceptor blocker, hypertension may be suppressed by blocking the sympathetic tone at peripheral sites (8). Results in prazosin-treated rats further suggest that the downregulation of β-adrenoceptors in the cerebral cortex is not the result of an elevation in systemic blood pressure.

Various central and peripheral mechanisms of β-blockers in hypertensive therapy have been proposed, such as the inhibition of cardiac output, of prejunctional β-adrenoceptors, and of renin release, and the reduction of sympathetic activity through the CNS (1,12,27,33). Because hydrophilic atenolol is also effective in hypertensive therapy, peripheral mechanisms are now considered to be dominant. However, as mentioned earlier, the results in our experiments suggest that central mechanisms cannot be neglected, whether or not β-blockers penetrate into the brain. In fact, the central administration of the β-adrenoceptor agonist, isoproterenol, has been demonstrated to elevate the systolic blood pressure of cats (26). The injection of propranolol directly into the lateral cerebral ventricle of the conscious rabbit also produces a transient presser response followed by a prolonged fall in arterial pressure (23). Intracerebroventricular administration of propranolol prevents renal sympathetic nerve activity and antinatriuretic responses to air stress (17). Furthermore, the destruction of nor-

TABLE 7

INHIBITION OF 200 pM ^{3}H-PRAZOSIN BINDING TO α_1-ADRENOCEPTORS OF RAT CEREBRAL CORTEX BY WB-4101, 5-METHYL-URAPIDIL, OR PRAZOSIN

Treatments	Drugs	$pK_{i\text{-}high}$	$pK_{i\text{-}low}$	% High
Control	WB-4101	9.55 ± 0.07	8.25 ± 0.06	49 ± 2
	5-Methyl-urapidil	8.96 ± 0.06	7.05 ± 0.03	46 ± 1
	Prazosin	9.66 ± 0.05		100
Stress	WB-4101	9.35 ± 0.07	8.02 ± 0.05	50 ± 5
	5-Methyl-urapidil	9.12 ± 0.06	7.12 ± 0.02	49 ± 1
	Prazosin	9.66 ± 0.05		100

Displacement curves were individually analyzed by the LIGAND program. Data shown are mean ± SEM of three experiments. $pK_{i\text{-}high}$ and $pK_{i\text{-}low}$-negative log of the equilibrium dissociation constants (−log M) at high or low affinity for drugs. % High-population binding at the high-affinity site compared with the total specific binding sites.

adrenergic neurons by intracisternal 6-hydroxydopamine prevents the development of hypertension in rabbits and rats (4,14). These results suggest that activation of the central adrenergic system, especially of β-adrenoceptors, may be closely associated with the development and/or maintenance of hypertension, and that β-blockers may caused an antihypertensive action, at least in part, by inhibiting central β-adrenoceptor-mediated transmission.

In this study, we showed that the change in β-adrenoceptor density in rat cerebral cortex induced by stress or drugs was mainly due to the changes in β_1-subtypes. Because noradrenaline-containing fibers innervate the cerebral cortex (24), a higher affinity of the β_1- than the β_2-subtype for noradrenaline (16,21) may be related to the predominant changes of the β_1-subtype by stress. It is also interesting to speculate that all β-blockers used in hypertensive therapy have a β_1-blocking activity (27).

The gain in body weight was significantly suppressed under stressed conditions, and its effect was not affected by chronic treatment with any drugs used. Under stressed conditions, the corticotropin-releasing hormone (CRH) system and the locus ceruleus-norepinephrine (LC-NE)/autonomic (sympathetic) nervous system have been proposed to be activated in CNS (5). Centrally administered CRH in moderate doses inhibits vegetative functions such as feeding and reproduction (29). A decrease in body weight gain may not be due to the LC/sympathetic nervous system.

In summary, immobilization stress induced a moderate elevation of blood pressure and the downregulation of central β-adrenoceptors. These changes were inhibited by chronic treatment with propranolol or atenolol. The inhibition of central β-adrenoceptor-mediated transmission may be associated with the antihypertensive action of β-blockers.

ACKNOWLEDGEMENTS

The authors thank N. Aoki for secretarial assistance and H. Tanaka for technical assistance. They also thank Dr. Yamanaka, Pfizer Co. Ltd., for measuring the plasma concentration of prazosin. This work was supported in part by grant from the Smoking Research Foundation of Japan.

REFERENCES

1. American Medical Association. Drug evaluation, 6th ed. Philadelphia: W. B. Saunders; 1986:522–527.
2. Betts, T. A.; Alford, C. β-Blocking drugs and sleep. A controlled trial, Drugs 25(Suppl 2):268–272; 1983.
3. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
4. Chalmers, J. P.; Dollery, C. T.; Lewis, P. J.; Reid, J. L. The importance of central adrenergic neurons in renal hypertension in rabbits. J. Physiol. 238:403–411; 1974.
5. Chrousos, G. P.; Gold, P. W. The concepts of stress and stress system disorders. JAMA 267:1244–1252; 1992.
6. Cruickshank, J. M. The clinical importance of cardioselectivity and lipophilicity in beta blockers, Am. Heart. J. 100:160–178; 1980.
7. Cruickshank, J. M.; Neil-Dwyer, G. β-Blocker brain concentrations in man. Eur. J. Clin. Pharmacol. 28(Suppl):21–23; 1985.
8. Davey, M. J. Relevant features of the pharmacology of prazosin. J. Cardiovasc. Pharmacol. 2(Suppl 3):S287–S301; 1980.
9. Dibona, G. F. Sympathetic neuronal control of the kidney in hypertension. Hypertension 19(Supple I):I28–I35; 1992.
10. Dolphin, A.; Adrien, J.; Hamon, M.; Bockaert, J. Identity of [^{3}H]-dihydroalprenolol binding sites and β-adrenoceptors coupled with adenylate cyclase in the CNS: Pharmacological properties, distribution and adaptive responsiveness. Mol. Pharmacol. 15:1–15; 1979.
11. Foerster, E. C.; Greminger, P.; Siegenthaler, W.; Vetter, H.; Vetter, W. Atenolol vs. pindolol: Side-effects in hypertension. Eur. J. Clin. Pharmacol. 28(Suppl):89–91; 1985.
12. Frohlich, E. D. New concepts in antihypertensive drugs. Prog. Cardiovasc. Dis. 20:385–402; 1978.
13. Graubiger, G.; Lefkowitz, R. J. Elevated beta-adrenoceptor number after chronic propranolol treatment. Biochem. Biophys. Res. Commun. 78:720–725; 1977.
14. Haeusler, G.; Finch, L.; Thoenen, H. Central adrenergic neurons and the initiation and development of experimental hypertension. Experientia (Basel) 28:1200–1203; 1972.
15. Henry, J. P.; Stephens, P. M.; Santisteban, G. A. A model of psychosocial hypertension showing reversibility and progression of cardiovascular complications. Circ. Res. 36:156–164; 1975.
16. Hoffman B. B.; Lefkowitz, R. J. Catecholamines and sympathomimetic drugs. In: Goodman Gilman, A.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. Goodman and Gilman's the pharmacological bases of therapeutics, 8th ed. New York: Pergamon Press; 1990: 187–220.
17. Koepke, J. P.; DiBona G. F. Central β-adrenergic receptors mediate renal nerve activity during stress in conscious spontaneously hypertensive rats. Hypertension 7:350–356; 1985.
18. Lamprecht, F.; Williams, R. B.; Kopin, I. J. Serum dopamine-beta-hydroxylase during development of immobilization-induced hypertension. Endocrinology 92:953–956; 1973.
19. Lawler J. E.; Barker, G. F.; Hubbard, J. W.; Schaub, R. G. Effects of stress on blood pressure and cardiac pathology in rats with borderline hypertension. Hypertension 3:496–505; 1981.
20. Lynch M.; Littleton, J.; McKerman, R. M.; Durcan, M. J.; McMillan, T.; Campbell, I. C. α-Adrenoceptor number and function in rat cerebral cortex after ethanol and immobilization stress. Brain Res. 288:145–149; 1983.
21. Minneman, K. P.; Dibner, M. D.; Wolfe, B. B.; Molinoff, P. B. The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. Mol. Pharmacol. 16:21–33; 1979.
22. Munson, P. J.; Rodbard, D. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220–239; 1980.
23. Myers, M. G.; Lewis, P. J.; Reid, J. L.; Dollery, C. T. Brain concentration of propranolol in relation to hypertensive effect in the rabbit with observations on brain propranolol levels in man. J. Pharmacol. Exp. Ther. 192:327–335; 1975.
24. Ordway, G. A.; Gambarana, C.; Frazer, A. Quantitative autoradiography of central beta adrenoceptor subtypes: Comparison of the effects of chronic treatment with desipramine or centrally administered *l*-isoproterenol. J. Pharmacol. Exp. Ther. 247:379–389; 1988.
25. Oshita, M.; Kigoshi, S.; Muramatsu, I. Three distinct binding sites for [^{3}H]-prazosin in the rat cerebral cortex. Br. J. Pharmacol. 104:961–965; 1991.
26. Philippu, A.; Kittel, E. Presence of beta-adrenoceptors in the hypothalamus: Their importance for the presser response to hypothalamic stimulation. Naunyn Schmiederbergs Arch. Pharmacol. 297:219–225; 1977.
27. Prichard, B. N. C. β-Adrenergic blockade in hypertension, past, present and future, Br. J. Pharmacol. 5:379–399; 1977.
28. Stone, E. A.; Platt, J. E.; Brain adrenoceptors and resistance to stress. Brain Res. 237:405–414; 1982.
29. Sutton, R. E.; Koob, G. F.; Le Moal, M.; Rivier, J.; Vale, W. Corticotropin releasing factor produces behavioral activation in rats. Nature 297:331–333; 1982.
30. Takita, M.; Kigoshi, S.; Muramatsu, I. Selectivity of bevantolol hydrochloride toward α- and β- adrenoceptor subtypes in rat cerebral cortex. Jpn. J. Pharmacol. 58:193–196; 1992.

31. Takita, M.; Kigoshi, S.; Muramatsu, I. Effect of bevantolol hydrochloride on immobilization stress-induced hypertension and central β-adrenoceptors in rats. Pharmacol. Biochem. Behav. 45: 623–627; 1993.
32. U'Prichard, D. C.; Kvetnansky, R. Central and peripheral adrenoceptors in acute and repeated immobilization stress. In: Usdin, E.; Kvetnansky, R.; Kopin, I. J., eds. Catecholamines and stress: Recent advances. Amsterdam: Elsevier North-Holland; 1980: 299–308.
33. Van Zwieten, P. A.; Antihypertensive drugs interacting with α- and β-adrenoceptors: A review of basic pharmacology. Drugs 35: 6–19; 1988.
34. Westerlund, A. A comparison of the CNS side effects caused by lipophilic and hydrophilic β-blockers. Drugs 25(Suppl 2):280–281; 1983.
35. Winkler, H.; Ried, W.; Lemmer, B. High-performance liquid chromatographic method for the quantitative analysis of the aryloxypropanolamines propranolol, methoprolol and atenolol in plasma and tissue. J. Chromatogr. 228:223–234; 1982.
36. Wood, A. S.; Bolli, S. P.; Simpson, F. O. Prazosin in normal subjects: Plasma levels, blood pressure and heart rats. Br. J. Clin. Pharmacol. 3:199; 1976.
37. Yamanaka, K.; Muramatsu, I.; Kigoshi, S. Effect of chronic nicotine treatment against repeated immobilization stress. Pharmacol. Biochem. Behav. 26:259–263; 1987.