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Effects of Bevantolol HCl on Immobilization Stress-Induced Hypertension and Central β-adrenoceptors in Rats

M. TAKITA, S. KIGOSHI AND I. MURAMATSU[1]

Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan

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TAKITA, M., S. KIGOSHI AND I. MURAMATSU. *Effects of bevantolol HCl on immobilization stress-induced hypertension and central β-adrenoceptors in rats.* PHARMACOL BIOCHEM BEHAV 45(3) 623–627, 1993.—Effects of chronic treatment with bevantolol, a β-adrenoceptor blocker, and of repeated immobilization stress on blood pressure, body weight, and [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to the cerebral cortex were examined in rats. Systolic blood pressure increased to approximately 150 mmHg when stress was applied for 14 days (2 h day^{-1}). This increase was inhibited by chronic treatment with bevantolol (250 mg kg^{-1} daily). However, bevantolol did not suppress the inhibition of body weight gain by stress. The maximum number of [^{3}H]DHA binding sites (B_{max}) in the cerebral cortex was decreased by stress without changing the affinity, and the decrease in B_{max} mainly reflected the reduction of β_1-adrenoceptors. Bevantolol treatment (250 mg kg^{-1}) increased the B_{max} to 137% and completely inhibited the downregulation of β-adrenoceptors by stress. These results show that bevantolol can inhibit both the hypertension and downregulation of the central β_1-adrenoceptors induced by stress.

Bevantolol HCl Immobilization stress Central β_1-adrenoceptor Hypertension

β-ADRENOCEPTOR antagonists have long been used for the clinical treatment of hypertension (14). However, why β-adrenoceptor antagonists are effective in hypertensive therapy has not yet been clarified, although various mechanisms have been proposed such as, for example, inhibition of cardiac output, inhibition of prejunctional β-adrenoceptors, inhibition of renin release, and reduction of sympathetic activity through the CNS (2,6,14,21).

Like the mechanism of action of β-adrenoceptor antagonists, causes of essential hypertension itself are also not known. However, stress is considered by many to be one of the contributing factors to induce essential hypertension. In fact, repeated stress can produce chronic hypertension in experimental animals (7,9,10).

The CNS is a primary site of action for stress, and various kinds of neurones are activated during stress (3,5,18). Therefore, it is likely that persistent changes in activity in the CNS may be produced during chronic stress, giving rise secondarily to abnormal responses (such as hypertension) in the periphery. We previously reported a persistent decrease in the β-adrenoceptor number in the rat cerebral cortex after repeated immobilization stress (23).

The aims of the present study were to determine whether a β-adrenoceptor antagonist, bevantolol HCl (17,22), can inhibit the peripheral (hypertensive) and central (β-adrenoceptor reductive) effects induced by immobilization stress and then find some clue for substantiation of the central mechanisms of action of β-adrenoceptor antagonists in hypertensive therapy.

METHOD

Stress and Bevantolol Treatment

The experimental protocol was essentially the same as described previously (23). In brief, male Wistar rats (6 weeks of age: 160–180 g) were housed in groups of two or three animals with free access to the usual chow diet and tapwater. One week later, six different experimental groups were designed (Table 1). The four groups, bevantolol treated (groups III and V) or stressed bevantolol treated (groups IV and VI), were provided with tapwater containing bevantolol (357 or 1,783 mg l^{-1} as bevantolol base) for 5 weeks, while the other two groups, nontreated (group I) or stressed (group II), received only tapwater (Fig. 1). The daily consumption of bevantolol was estimated to be 50 or 250 mg kg^{-1}, based upon a mean water intake of 35 ml day^{-1} for a 250-g rat. The actual plasma concentration of bevantolol was measured at the end of study (see Table 1). Rats in the stressed groups received immobilization stress (2 h daily) for the last 2 weeks of the 5-week experimental period (Fig. 1). Blood pressure was measured with sphygmomanometry (Muromachikikai, MK-1000, Japan) be-

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

TABLE 1
PLASMA CONCENTRATION OF BEVANTOLOL IN RATS

Group	Treatments	n	Concentration of bevantolol (ng ml^{-1})
I	Control	8	not detected
II	Stress	13	not detected
III	Bevantolol (50 mg kg^{-1})	6	76 ± 23
IV	Bevantolol + stress (50 mg kg^{-1})	5	50 ± 9
V	Bevantolol (250 mg kg^{-1})	5	241 ± 60
VI	Bevantolol + stress (250 mg kg^{-1})	6	343 ± 52

Data shown are mean ± SEM. n, number of rats.

fore and at the ends of the fourth and fifth weeks after commencing the experiments. Briefly, rats were preheated at 30°C for 13 min to dilate the tail artery and the blood pressure was measured three times at 35°C. Body weight was also measured at the time of blood pressure measurement. Rats were killed by decapitation 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.

β-Adrenoceptor Binding

β-Adrenoceptors from the rat cerebral cortex were measured using binding assays with [^{3}H]dihydroalprenolol ([^{3}H]DHA) as described previously (17). In brief, the cerebral cortex was homogenized with a polytron in 80 vol 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 volume of assay buffer and the membrane fraction was used in binding assays. The homogenates 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 and Whatman GF/C filters (Whatman, Clifton, NJ). The filter was washed three times with ice-cold washing buffer (50 mM Tris-HCl, pH = 7.4), and then the filter-bound radioactivity was determined. Nonspecific binding was defined as binding in the presence of 10 μM propranolol. Assays were conducted in duplicate.

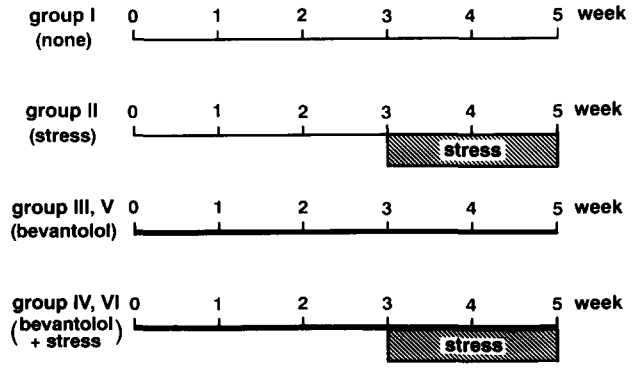

FIG. 1. Experimental schedule for treatments with bevantolol and immobilization stress.

Statistical Analyses

All values were expressed as mean ± SEM. Data were analyzed by the weighted least-squares iterative curve fitting programme LIGAND (12). The data were first fitted to one- and then two-site models, and if the residual sums of squares were statistically less for a two-site fit of the data than for a one-site, as determined by an F-test comparison, then the two-site model was accepted. p values less than 0.05 were considered significant. Proteins were assayed according to the method of Bradford using bovine serum albumin as standard (4).

Materials

The following drugs were used: [^{3}H]DHA (specific activity 111.5 Ci/mmol, New England Nuclear, Boston, MA), propranolol HCl (Nacalai, Kyoto, Japan), bevantolol HCl (Nippon-Chemiphar, Tokyo, Japan), and 1-(2-cynophenoxy)-3-β-(3-phenylureido)ethylamino-2-propanol (ICI-89,406; a gift from ICI Pharma, Cheshire, UK).

RESULTS

Water Intake and Plasma Concentration of Bevantolol

To avoid extra stress, bevantolol was dissolved in tapwater and taken freely. The daily intake of water by each animal during experiments were 35–63 ml and 32–48 ml day^{-1} in control and 50 mg kg^{-1} groups, respectively. However, the intakes decreased to 10–24 ml the first 3 days after switching tapwater to bevantolol solution and thereafter recovered to 21–33 ml in 250-mg kg^{-1} groups. Therefore, plasma concentrations of bevantolol were determined when rats were decapitated at the end of experiments. As shown in Table 1, the plasma concentrations of bevantolol in two nonstressed groups (50 and 250 mg kg^{-1} daily) were, respectively, 76 ± 23 ng ml^{-1} and 241 ± 60 ng ml^{-1}, which were not significantly different from those in stressed bevantolol treated rats.

Effects of Bevantolol and Immobilization Stress on Blood Pressure and Body Weight

When immobilization stress was loaded for 14 days (2 h day^{-1}) during the fourth and fifth weeks in the experimental period (Fig. 1), blood pressure of rats gradually rose and at the end of experiments the systolic blood pressure reached approximately 150 mmHg ($p < 0.05$ compared with control, Table 2). Bevantolol (50 and 250 mg kg^{-1} daily) itself had little effect on the blood pressure of nonstressed rats, but at the higher dose it completely inhibited the rise in systolic blood pressure induced by stress.

The body weight of nontreated (control) rats increased by approximately 60 g during the last 2 weeks of the experimental period, an effect that was completely suppressed by stress (Table 3). Such suppression of body weight gain by stress was

TABLE 2

EFFECTS OF STRESS AND BEVANTOLOL ON BLOOD PRESSURE IN RATS

Group	Treatments	n	Systolic Blood Pressure		
			Before	28 days	35 days
I	Control	8	117 ± 1.9	131 ± 1.1	136 ± 2.8
II	Stress	13	115 ± 1.9	142 ± 2.9*	147 ± 2.4*
III	Bevantolol (50 mg kg^{-1})	6	114 ± 1.6	135 ± 6.5	136 ± 1.2
IV	Bevantolol + stress (50 mg kg^{-1})	5	116 ± 2.2	142 ± 4.6	147 ± 5.0*
V	Bevantolol (250 mg kg^{-1})	5	118 ± 1.6	124 ± 2.5*	127 ± 5.0
VI	Bevantolol + stress (250 mg kg^{-1})	6	121 ± 1.7	123 ± 2.5*	129 ± 4.4

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

also observed in bevantolol-treated rats, although bevantolol itself slightly reduced the body weight.

Effects of Bevantolol on the [^{3}H]DHA Binding to the Rat Cerebral Cortex

Saturation experiments with [^{3}H]DHA in the ranges of 30–2,500 pM were carried out in the rat cortex membranes. Figure 2 shows the Scatchard plots of the binding data. The affinity of the specifically bound [^{3}H]DHA to the cerebral cortex was not affected by stress treatment, but the maximum number of [^{3}H]DHA binding sites (B_{max}) was significantly decreased to 68% (Table 4). In bevantolol-treated rats (250 mg kg^{-1} daily), the affinity of the specific binding of [^{3}H]DHA was not different from that of control rats but the B_{max} was increased to 137% ($p < 0.05$). Increase in B_{max} by bevantolol was also produced in stressed rats and the extent was not significantly different from that in rats treated with bevantolol alone.

As [^{3}H]DHA binds to both β_1- and β_2-adrenoceptor subtypes with an equal affinity, displacement experiments with ICI-89,409, a β_1-selective antagonist, were examined. ICI-89,409 displaced about 65% of 1 nM [^{3}H]DHA binding with a high affinity ($pK_{i\text{-high}} = 8.25 \pm 0.16$) and the remaining 35% with a low affinity ($pK_{i\text{-low}} = 6.01 \pm 0.21$) in control rats (Table 5). In stress-treated rats, affinities for ICI-89,406 of both binding sites were not significantly different from those of control, but the proportion of high-affinity sites was decreased to 48 ± 4% ($p < 0.05$ as compared with control). In bevantolol-treated rats (250 mg kg^{-1} daily), both the affinity constant and proportion of high-affinity sites were almost the same as those of control rats, irrespective of the presence or absence of stress. Figure 3 shows the B_{max} values of β_1- and

TABLE 3

EFFECTS OF STRESS AND BEVANTOLOL ON BODY WEIGHT OF RATS

Group	Treatments	n	Body weight (g)		
			22 days*	28 days*	35 days*
I	Control	8	427 ± 11	460 ± 12 (33)	489 ± 13 (62)
II	Stress	13	411 ± 6	400 ± 4† (−11)	411 ± 5† (0)
III	Bevantolol (50 mg kg^{-1})	6	429 ± 10	457 ± 10 (28)	486 ± 10 (57)
IV	Bevantolol + stress (50 mg kg^{-1})	5	418 ± 10	405 ± 10† (−13)	409 ± 11† (−9)
V	Bevantolol (250 mg kg^{-1})	5	374 ± 6	402 ± 9† (28)	431 ± 12† (57)
VI	Bevantolol + stress (250 mg kg^{-1})	6	380 ± 10	374 ± 8† (−6)	393 ± 9† (13)

Data shown are mean ± SEM. n, number of rats. Body weight gain for 1 and 2 weeks was represented in parentheses.
*Number of days of treatment.
†Significantly different from control (group I)($p < 0.05$).

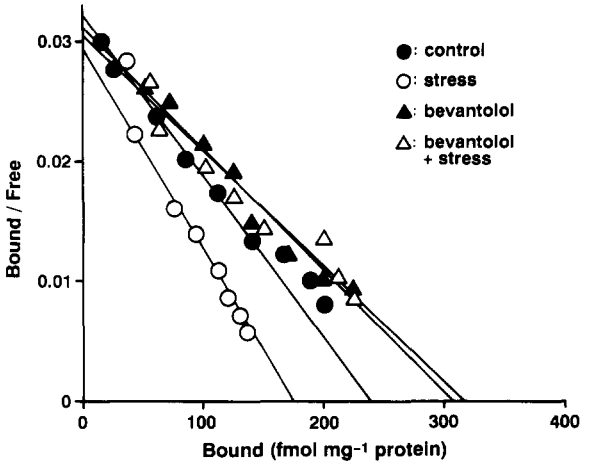

FIG. 2. Scatchard analysis of [^{3}H]dihydroalprenolol ([^{3}]DHA) binding to the cortexes of control (●), stress-treated (○), bevantolol-treated (▲), and stressed-bevantolol-treated rats (△). Saturation experiments were done with the use of 30 ~ 2,500 pM [^{3}H]DHA and were conducted in duplicate.

β_2-adrenoceptors (high- and low-affinity sites for ICI-89,406, respectively) that have been extrapolated from the proportions estimated in the displacement experiments and from the total B_{max} values obtained in the saturation experiments. The changes of number of [^{3}H]DHA binding sites predominantly reflected the changes of the β_1-adrenoceptors.

DISCUSSION

Repeated immobilization stress causes a persistent increase in blood pressure (7,9,10). In the present study, an increase in blood pressure was also seen when immobilization stress was repeatedly applied for 14 days. However, such an effect of stress was completely inhibited in rats treated with bevantolol (250 mg kg^{-1} daily).

Reduction of water intake may be related to the antihypertensive effect seen in the bevantolol group because the drinking water containing the higher drug dose left a bitter taste and the drinking volume was consequently reduced for the first few days. However, the water intake increased during the subsequent experimental period. Moreover, because bevantolol has no tranquilizing action (8), which could affect the interpretation of tail cuff blood pressure data, the observed antihypertensive effect of bevantolol appears to be caused purely through an antistress action.

Repeated immobilization stress produced a decrease in β-adrenoceptor numbers, especially β_1-adrenoceptor numbers, in the rat cerebral cortex. Such a decrease in β-adrenoceptors by stress has been reported by U'Prichard and Kvetnansky (19) and Yamanaka et al (23). Cortical β-adrenoceptors are regulated by the activity of noradrenergic neurones, which originate primarily from the locus coeruleus, and repeated

TABLE 4

EFFECTS OF STRESS AND BEVANTOLOL ON [^{3}H]DHA BINDING TO β-ADRENOCEPTORS OF THE RAT CEREBRAL CORTEX

Treatments	[^{3}H]DHA Binding	
	pK_D	B_{max}
Control	9.16 ± 0.07	219 ± 13
Stress	9.33 ± 0.05	150 ± 19*
Bevantolol (250 mg kg^{-1})	8.95 ± 0.05	301 ± 22*
Bevantolol + stress (250 mg kg^{-1})	9.04 ± 0.04	339 ± 23*

Scatchard curves were individually analysed by the LIGAND programme. Data shown are mean ± SEM of three to four experiments. pK_D, negative log of the 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 THE RAT CEREBRAL CORTEX BY ICI-89,406

Treatments	$pK_{i,high}$	$pK_{i,low}$	% high
Control	8.25 ± 0.16	6.01 ± 0.21	65 ± 1
Stress	8.51 ± 0.19	6.47 ± 0.12	48 ± 4*
Bevantolol (250 mg kg^{-1})	7.86 ± 0.17	6.07 ± 0.35	70 ± 2
Bevantolol + stress (250 mg kg^{-1})	7.87 ± 0.18	5.77 ± 0.33	77 ± 2

Displacement curves were individually analysed by the LIGAND programme.

Data shown are mean ± SEM of three to four experiments. $pK_{i,high}$ and $pK_{i,low}$, negative log of the equilibrium dissociation constants (−log M) at high or low affinity for ICI-89,409. % High, population binding at the high affinity site compared to the total specific binding sites.

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

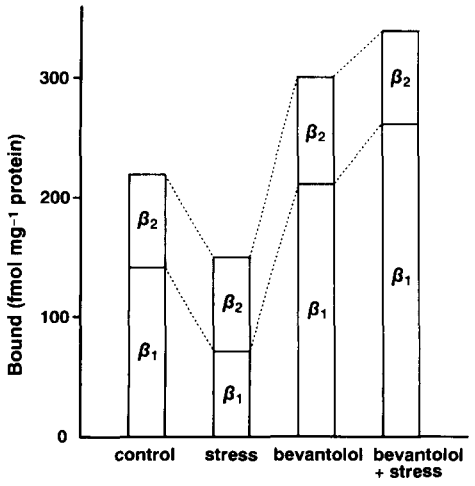

FIG. 3. B_{max} values of β_1- and β_2-adrenoceptors that have been extrapolated from the proportions estimated in the displacement experiments (Table 5) and from the total B_{max} values obtained in the saturation experiments (Table 4). Note the predominant change of β_1-adrenoceptor density.

stress is well known to activate the noradrenergic nervous systems, causing excess release of noradrenaline (1,15,16,18). Decrease in β-adrenoceptor density after stress would be associated with hyperfunction of the β-adrenoceptor system, which in turn reduces the number of receptors (downregulation) (11). Because the β_1-adrenoceptor subtype was mainly reduced by stress, it is further suggested that noradrenergic pathways that stimulate β_1-adrenoceptors may be predominantly activated during stress.

On the other hand, bevantolol treatment itself increased the number of β-adrenoceptors to 137% in nonstressed rats. This may suggest that bevantolol can enter the brain (22) and act as a β-adrenoceptor blocker, causing the upregulation (20). Because bevantolol is relatively selective to the β_1-adrenoceptor (17), the upregulation seems to develop more predominantly in the β_1-adrenoceptor.

Bevantolol completely suppressed both the decrease of β-adrenoceptors in the cerebral cortex and the hypertension induced by immobilization stress. Therefore, it appears that noradrenergic hyperfunction through β-adrenoceptors in the CNS may be of etiologic significance in stress-induced hypertension. Central administration of the β-adrenoceptor agonist isopropanol has been demonstrated to elevate the systolic blood pressure of the cat (13).

Under stress conditions, a gain in body weight was significantly suppressed and its effect was not altered by chronic treatment with bevantolol. This may suggest a limitation in antistress actions of bevantolol and/or other β-adrenoceptor blockers. The inhibition of body weight gain by stress may be associated with stimulation of the pituitary-adrenocortical system, which causes an increase in catabolism in muscle and liver (1).

In conclusion, the present results show that repeated immobilization stress causes a decrease in the number of β_1-adrenoceptors in the rat cerebral cortex and an increase in systolic blood pressure. Chronic treatment with the β-adrenoceptor blocker bevantolol eliminated both effects at the higher dose studied.

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