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β-Adrenergic Receptor Subtypes in Stress-Induced Behavioral Depression

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PANDEY, S. C., X. REN, J. SAGEN AND G. N. PANDEY. *β-Adrenergic receptor subtypes in stress-induced behavioral depression.* PHARMACOL BIOCHEM BEHAV 51(2/3) 339-344, 1995.—The purpose of this study was to examine the role of β-adrenergic receptors in an animal model of stress-induced behavioral depression. β-Adrenergic receptors in several brain regions and leukocytes of rats were determined by receptor binding techniques using ^{125}I-cyanopindolol (cyp) as ligand and propranolol as displacer for total β-adrenergic receptors, and ICI 86,406 for β_1- and ICI 118,551 for β_2-adrenergic receptors. We observed that the maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) of ^{125}I-cyp binding to total β-adrenergic receptors were increased in hippocampus of stressed rats with escape deficits (48 h after training) as compared to control rats. This increase was due to an increase in B_{max} and K_d of ^{125}I-cyp binding to β_1-adrenergic receptors but not to β_2-adrenergic receptors. There was no significant difference in β_1-adrenergic receptors in cortex and cerebellum or β_2-adrenergic receptors in hippocampus, cortex, cerebellum, or leukocytes of stressed (48 h after training) rats with escape deficits as compared to control rats. Interestingly, it was observed that β_1- and β_2-adrenergic receptors in various brain regions (cortex, cerebellum, and hippocampus) and β_2-adrenergic receptors in leukocytes of stressed rats (10 days after training) were not significantly different from control rats, although escape deficits were still present. These results suggest that abnormalities in adrenergic neurotransmission are associated with an upregulation of β_1-adrenergic receptors, which in turn may be involved in the early stages of behavioral deficits caused by uncontrollable shock.

Escape-deficit Behavioral depression β-Adrenergic receptor subtypes Rat brain Leukocytes Depression

SEVERAL lines of evidence suggest the involvement of β-adrenergic receptors in the pathophysiology of depression. Almost all antidepressants cause downregulation of β-adrenergic receptors in rat brain after chronic treatment (10,15,22,35). This suggests that depression may be associated with the upregulation of β-adrenergic receptors and that the therapeutic effect of antidepressants may be related to their ability to downregulate β-adrenergic receptors in the brain. Animal models of depression have been used extensively to test hypotheses regarding the neurobiology of depression and the mechanism of action of antidepressant drugs (7,30,38). One of the most commonly used animal models of depression is learned helplessness. Animals often show a deficit in learning to escape a controllable stressor after exposure to an uncontrollable stressor (38). This maladaptive behavior is called learned helplessness (LH) or stress-induced behavioral depression. It has been demonstrated that there is a behavioral correlation between the LH model of depression and vegetative symptoms of clinically depressed patients (1,36). Furthermore, the behavioral deficit induced in rats by exposure to inescapable shock is specifically reversed by chronic treatment with antidepressant drugs (25,27,30,33). However, the underlying neurochemical mechanisms are not well known.

It was shown recently that β-adrenergic receptors and this receptor-mediated cAMP formation are increased in the hippocampus but not in the cortex of LH rats in comparison with normal rats (17). Furthermore, Molina et al. (21) showed that cortical β-adrenergic receptors are increased in the brain of stressed rats. However, it is not clear whether an increase in total β-adrenergic receptors in depressive illness is due to an increase in β_1- or β_2-adrenergic receptor subtypes, or both.

β_2-Adrenergic receptors and agonist-stimulated cAMP formation in leukocytes and/or lymphocytes have been shown to be decreased in depressed patients when compared with normal controls (14,16,23,24). On the other hand, β-adrenergic receptors are increased in the brains of suicide victims when compared with normal controls (3,4). Thus, the relationship between changes in β-adrenergic receptors in leukocytes and the brain is not clear. This anomaly may be due to the differential distribution of β-adrenergic receptor subtypes in various

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tissues and/or to different regulatory mechanisms. Therefore, the objectives of the present investigation were to examine: a) the changes in β_1- and β_2-adrenergic receptors in various brain regions of escape-deficit rats and control rats; and b) the changes in β_2-adrenergic receptors in leukocytes of these groups of rats.

METHODS

Animal Training for Stress-Induced Behavioral Depression and Testing For Escape

Male Sprague-Dawley rats (200–225 g) were used in the study and housed for 1 week before use. Stress-induced behavioral depression was induced using inescapable shock in an operant chamber with a stainless steel grid floor as previously described by Sagen et al. (29). The operant training chamber was 9.5 × 12 × 11.5 inches in size. An initial training session consisted of the delivery of a series of 2.5-mA foot-shocks (50 s) through the grid floor of a testing chamber over a period of 60 min alternating with an intershock interval (randomized duration, 50 s average). Using this schedule, the total number of trials per training session averaged 36. Either 48 h or 10 days after training, rats were tested for shock escape in a novel environment in which a foot-shock could be terminated by escaping to the nonshock side of the shuttle-box (dimensions: 8.5 × 11.5 × 10.5 inches). At the start of each escape trial, a 2.5-mA shock was initiated simultaneously with the opening of the escape panel. Rats that did not escape within 25 s were considered escape failures, and the shock was terminated. Fifteen escape trials separated by random intertrial intervals averaging 50 s were used to assess escape-deficits. Rats having 10–15 failures to escape were considered to be deficient in the escape response and were considered escape-deficit rats. Naive control rats received no uncontrollable shock but were also tested in the shock-escape test.

Rats were decapitated either 48 h or 10 days after training; their brains were removed, and cerebral cortices, hippocampi, and cerebelli were dissected out. We collected blood from the same rats and isolated leukocytes using the dextran method (see subsequent description for details). To assess both the dynamic receptor changes and the long-term stable changes, four groups of rats were used: a) escape deficit group (48 h after training); b) control group tested in parallel; c) escape-deficit group (10 days after training); and d) control group tested in parallel. Several studies have demonstrated that β-adrenergic receptors are increased only in response-deficient rats (i.e., having 10–15 escape failures) but not in nondeficient rats (7,17). Nondeficient rats (n = 5) were therefore not included in the study.

Determination of β-Adrenergic Receptor Subtypes in Rat Brain

β_1-, β_2-, and total β-adrenergic receptors in the different brain regions were determined by the procedure of Ordway et al. (22) with some modifications. Cortices, hippocampi, and cerebelli were homogenized in 10.0 ml of 0.32 M sucrose and centrifuged at 1000 × g for 10 min. The resulting supernatant was centrifuged at 49,000 × g for 15 min, and the pellet thus obtained was suspended in 10 ml of Tris-NaCl buffer (50 mM Tris HCl and 120 mM NaCl, pH 7.5). The suspension was centrifuged at 49,000 × g for 15 min. The final pellet was suspended in the incubation buffer (50 mM Tris HCl and 120 mM NaCl, pH 7.5) and used for the binding assay. The receptor binding assay was carried out in duplicate tubes containing incubation buffer, six different concentrations of ^{125}I-cyanopindolol (^{125}I-cyp, 10–100 pM), and 50 μl of membrane suspension in the presence or absence of 1 μM of propranolol in a total volume of 500 μl. To block the binding of ^{125}I-cyp to 5-HT$_{1B}$ receptors, 5-HT (10^{-6} M) was added to all tubes, which were then incubated for 60 min at 37°C. The incubation was terminated by rapid filtration over Whatman GF/B filters (Brandel, Biomedical Research & Developmental Laboratories, Inc., Gaithersburg, MD), followed by washing (three times) with 5.0 ml of washing buffer (50 mM Tris HCl, and 120 mM NaCl, pH 7.5). The filters were dried and the radioactivity was determined in a γ-counter. The nonspecific binding to ^{125}I-cyp was determined in the presence of propranolol (10^{-6}M), and specific binding ranged from 95–85% depending on the concentration of the ligand.

The binding of ^{125}I-cyp to β_1- or to β_2-adrenergic receptors was defined as the binding of ^{125}I-cyp in the presence of the β_1-antagonist ICI 86,406 (70 nM) or the β_2-antagonist ICI 118,551 (50 nM), respectively.

Isolation of Rat Leukocytes and Determination of β-Adrenergic Receptors

About 10 ml of blood was obtained from each rat. Leukocytes were isolated from the blood according to the method of Bourne and Melmon (5), with some modifications (24). The intact leukocyte pellet was homogenized in 2.0 ml of Tris buffer (5 mM Tris buffer, pH 7.5) in ice. The homogenate was spun at 49,000 × g for 10 min at 4°C. The leukocyte pellet thus obtained was suspended in 2 ml of incubation buffer (50 mM Tris HCl, 120 mM NaCl, pH 7.5) using a polytron (PT-7) at setting 5 for 10 s and diluted to 4 ml and spun at 49,000 × g for 10 min at 4°C. The pellet was finally suspended in 1–2 ml of incubation buffer such that 50 μl of the suspension contained 10–40 μg of protein. ^{125}I-cyp binding was carried out according to the procedure described subsequently. An aliquot of the membrane suspension (50 μl) was incubated with five different concentrations of ^{125}I-cyp (10–100 pM) in the incubation buffer (50 mM Tris HCl, 120 mM NaCl, pH 7.5) in a final volume of 0.4 ml in duplicate, with or without L-propranolol (10^{-6} M), for 1 h at 37°C. The incubation was terminated rapidly by adding 4 ml of ice-cold incubation buffer and filtering through Whatman GF/B filters, followed by three washings with 4 ml of incubation buffer. The filters were placed in a tube and counted in a γ-counter. The specific binding was defined as the difference between the radioactivity bound in the absence and presence of 10^{-6} M L-propranolol in the incubation mixture. The specific binding was observed to be generally in the range of 80–50%, depending on the concentration of the ligand.

In all binding assays, the maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) were computed by Scatchard analysis using the EBDA program (19). Protein content was determined using the method of Lowry et al. (13).

Statistical Analysis

Statistical analyses were performed using two independent sample t-tests. A value of $p < 0.05$ was considered to be significant.

RESULTS

Behavioral

Figure 1 shows the average latency-to-escape response during each of the 15 trials for control and escape-deficit (48 h or

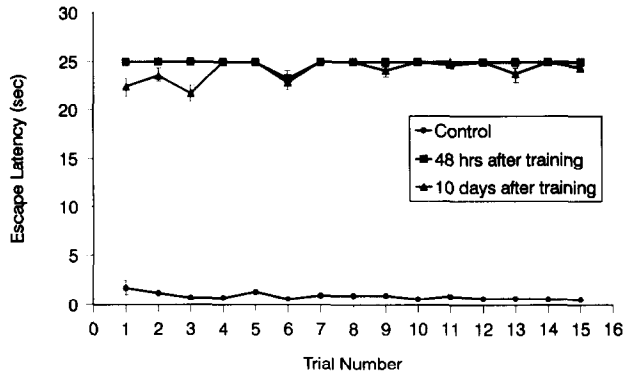

FIG. 1. The average latency (in seconds) to escape-deficit response during each of 15 trials for control and escape-deficit groups (48 h after training or 10 days after training).

10 days after training) rats. The mean escape latencies for escape-deficit groups (48 h or 10 days after training) were significantly ($p < 0.001$) different from the escape latencies for control rats. There were no significant differences between the mean escape latencies of the two escape-deficit groups.

Distribution of β_1- and β_2-Adrenergic Receptors in Different Brain Regions of Rat

Because ^{125}I-cyp has similar affinities for β_1- and β_2-adrenergic receptors, the total concentration of β-adrenergic receptors was determined in the brain using this ligand and propranolol or isoproterenol as displacer (20,22). We differentiated the β_1- and β_2-adrenergic receptors by using specific antagonists for these receptors: ICI 86,406 (70 nM) to determine β_1-adrenergic receptors and ICI 118,551 (50 nM) for β_2-adrenergic receptors. We observed that in control rats the calculated ratios of β_1:β_2 receptors were: cortex 80 : 20, cerebellum 19 : 81, and hippocampus 82 : 18. These ratios are similar to those reported in the literature by other investigators (20,22). After blocking the β_1-adrenergic receptors by ICI 86,406 (70 nM), we observed that the binding of ^{125}I-cyp in cortex and hippocampus was saturable. The Scatchard plot from a typical saturation experiment indicates a single class of high-affinity binding sites both in cortex (with a K_d of 34 pM and a B_{max} of 118 fmol/mg protein) and hippocampus (with a K_d of 42 pM and a B_{max} of 61 fmol/mg protein). In the cerebellum, after blocking the β_2-adrenergic receptors by ICI 118,551 (50 nM), we observed that ^{125}I-cyp binding was saturable. A Scatchard plot from a typical saturation experiment indicated a single class of high-affinity binding sites, with a B_{max} of 93 fmol/mg protein and a K_d of 25 pM.

β_1- and β_2-Adrenergic Receptors in Different Brain Regions of Rats With Stress-Induced Behavioral Depression and Control Rats

We determined the β_1- and β_2-receptors in cortex, hippocampus, and cerebellum of escape-deficit rats and control rats. We studied changes in β-adrenergic receptors in escape-deficit rats at two time points: a) 48 h after training and b) 10 days after training. It was observed that 48 h after training, there was a significant increase in the B_{max} ($t = 2.25$, $df = 14$, $p = 0.04$) and the K_d ($t = 2.29$, $df = 14$, $p = 0.04$) of ^{125}I-cyp binding to total β-adrenergic receptors in hippocampus of escape-deficit rats when compared with control rats (Table 1). Assessment for the specific β-adrenergic receptor subtype revealed that this increase was primarily due to an increase in β_1-adrenergic receptors and not in β_2-adrenergic receptors in hippocampus of rats with stress-induced behavioral depression (Table 1 and Fig. 2).

However, 10 days after training, these increases in the B_{max} and the K_d of β-adrenergic receptor binding sites reverted to baseline levels in the hippocampus of escape-deficit rats (Table 2). The B_{max} and the K_d of β_1-, β_2-, and total β-adrenergic receptor binding sites were not significantly changed in the cortex and cerebellum of escape-deficit rats after 48 h or 10 days of training when compared with their respective control groups (Tables 1 and 2).

β-Adrenergic Receptors in Leukocytes of Rats With Stress-Induced Behavioral Depression and Control Rats

We observed that the binding of ^{125}I-cyp in rat leukocytes is saturable, and Scatchard analysis from a typical saturation experiment indicates a single class of high-affinity binding sites with a K_d of 21 pM and B_{max} of 97 fmol/mg protein. To examine whether β-adrenergic receptors are downregulated in leukocytes of stress-induced behavioral depressed rats, similar to depressed patients, we studied the β-adrenergic receptors in leukocytes of escape-deficit rats after 48 h or 10 days of training. We found no significant changes in B_{max} or K_d of ^{125}I-cyp binding to β-adrenergic receptors in leukocytes of escape-deficit rats after 48 h or 10 days of training when compared to their respective control groups (Table 3).

DISCUSSION

A recent study (17) revealed that β-adrenergic receptors and this receptor-mediated cyclic AMP formation are increased in hippocampus of response-deficient rats (which do not learn to terminate the controllable stressor) when compared to control rats. The significance of this finding is unclear, but it suggests that increased β-adrenergic receptors may

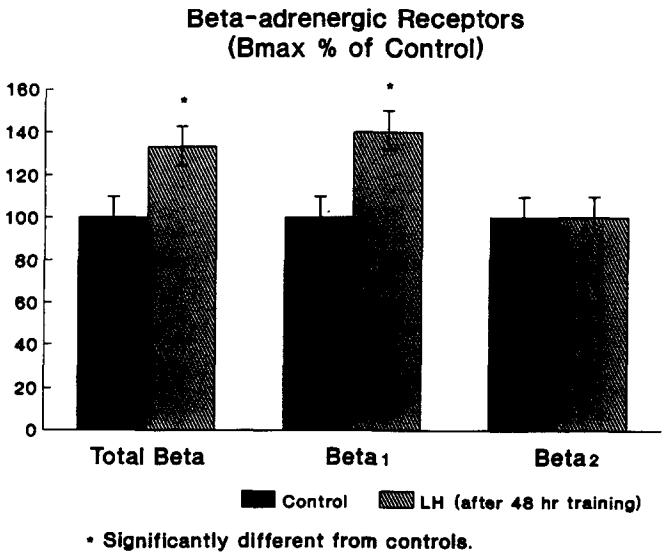

FIG. 2:. The maximum number of binding sites (B_{max}) of β_1-, β_2-, and total β-adrenergic receptors (percent normal control) in the hippocampus of escape-deficit (48 h after training) and control rats. *Significantly different from normal controls ($p = 0.04$).

TABLE 1

β-ADRENERGIC RECEPTOR BINDING PARAMETERS IN DIFFERENT BRAIN REGIONS OF ESCAPE-DEFICIT (48 h AFTER TRAINING) AND CONTROL RATS

	^{125}I-cyp Binding					
	Total β-Receptors		β_1-Adrenergic		β_2-Adrenergic	
Brain Regions	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
Cortex						
Control group	133 ± 5.9	38 ± 6.7	104 ± 4.3	38 ± 6.5	29 ± 3.2	–
Escape deficit group	120 ± 6.3	33 ± 3.0	96 ± 6.3	37 ± 4.8	24 ± 1.6	–
Cerebellum						
Control group	101 ± 2.9	24 ± 1.8	15 ± 1.2	–	87 ± 2.6	22 ± 1.6
Escape deficit group	100 ± 3.7	23 ± 1.2	14 ± 1.1	–	86 ± 3.7	21 ± 1.1
Hippocampus						
Control group	63 ± 4.6	29 ± 3.2	50 ± 3.3	29 ± 3.9	14 ± 0.89	–
Escape deficit group	83 ± 7.7*	43 ± 5.1*	69 ± 6.8†	50 ± 6.4†	14 ± 1.26	–

Each value is the mean ± SEM (n = 8). B_{max} values are given as fmol/mg protein and K_d values are given as pM. Membranes were prepared from different regions of brain and incubated with different concentrations of ^{125}I-cyp in the presence and absence of 10^{-6} M propranolol for determination of total β-adrenergic receptors. The $\beta_1$and β_2-adrenergic receptors were determined in the presence and absence of ICI 86,406 and ICI 118,551, respectively. Significantly different than normal controls: *p = 0.04; †p = 0.02).

be involved in a stress-induced behavioral depression in the rat. However, the studies did not specifically indicate which β-adrenergic receptor subtypes are involved in the observed upregulation of β-adrenergic receptors in the hippocampus of escape-deficit rats. The findings also did not indicate whether β-adrenergic receptors are also altered in other areas of the brain (e.g., cortex and cerebellum) or in leukocytes, which are rich in β_2-adrenergic receptor subtypes.

The evidence suggesting that β-adrenergic receptors may be involved in depressive illness is derived from studies of the effect of chronic treatment with antidepressants on β-adrenergic receptors in rat brain and studies of β-adrenergic receptors in leukocytes of depressed patients or postmortem brains of suicide victims. It has been consistently shown that chronic treatment with antidepressants causes downregulation of β_1-adrenergic receptors without any changes in β_2-adrenergic receptors in rat brain (9,22). β_1-adrenergic receptors have also been observed to be upregulated in postmortem brains of suicide victims (3,4). These studies thus suggest that depressive illness may be associated with the upregulation of β_1-adrenergic receptors. Studies of β_2-adrenergic receptors indicate that these receptor subtypes are decreased in the leukocytes of depressed patients (14,24). Martin et al. (18) reported that treatment with clenbuterol and salbutamol, which stimulate β_2-adrenergic receptors much more specifically, prevents escape deficit in learned helplessness rats, thus suggesting the involvement of β_2-adrenergic receptors in the learned helplessness behavior. These two studies therefore suggest the involvement of β_2-adrenergic receptors in depressive illness.

It appears from these studies that β_1-adrenergic receptors

TABLE 2

β-ADRENERGIC RECEPTOR BINDING PARAMETERS IN DIFFERENT BRAIN REGIONS OF ESCAPE-DEFICIT (10 DAYS AFTER TRAINING) AND CONTROL RATS

	^{125}I-cyp Binding					
	Total β-Receptors		β_1-Adrenergic		β_2-Adrenergic	
Brain Regions	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
Cortex						
Control group	155 ± 11.8	40 ± 5.2	125 ± 10.5	49 ± 7.7	30 ± 2.5	–
Escape deficit group	142 ± 11.9	36 ± 3.5	118 ± 10.9	42 ± 4.7	24 ± 1.9	–
Cerebellum						
Control group	102 ± 5.3	26 ± 1.5	16 ± 1.32	–	86 ± 4.3	24 ± 1.1
Escape Deficit group	106 ± 3.2	27 ± 1.3	16 ± 0.70	–	91 ± 2.9	24 ± 1.4
Hippocampus						
Control group	61 ± 5.6	34 ± 4.6	47 ± 4.5	33 ± 4.6	14 ± 1.2	–
Escape deficit group	64 ± 7.2	38 ± 6.2	50 ± 6.1	37 ± 6.2	14 ± 1.9	–

Each value is the mean ± SEM (n = 8). B_{max} values are given as fmol/mg protein and K_d values are given as pM. Membranes were prepared from different regions of brain and incubated with different concentrations of ^{125}I-cyp in the presence and absence of 10^{-6} M propranolol for total β-adrenergic receptor determination. The $\beta_1$and β_2-adrenergic receptors were determined in the presence and absence of ICI 86,406 and ICI 118,551, respectively.

TABLE 3

β_2-ADRENERGIC RECEPTORS IN LEUKOCYTES OF ESCAPE-DEFICIT AND CONTROL RATS

	^{125}I-cyp Binding	
	B_{max}	K_d
Control group (48 h)	152.03 ± 22.2	28.7 ± 1.9
Escape deficit group (48 h)	143.05 ± 19.5	27.1 ± 0.98
Control group (10 days)	91.60 ± 6.6	20.5 ± 1.5
Escape deficit group (10 days)	109.87 ± 10.1	19.9 ± 2.5

Values are mean ± SEM (n = 7). Each saturation curve was performed on leukocytes from a separate rat. Leukocyte membranes were incubated with different concentrations of ^{125}I-cyp in the presence and absence of 10^{-6} M propranolol. K_d values are given as pM; B_{max} values are given as fmol/mg protein.

may be upregulated whereas β_2-adrenergic receptors may be downregulated in depressive illness. To test this hypothesis, we determined both β_1- and β_2-adrenergic receptors in different regions of the brain as well as β_2-adrenergic receptors in the leukocytes of rats with stress-induced behavioral depression. Whereas we observed upregulation of β_1-adrenergic receptors in the hippocampus, β_1-adrenergic receptors in other areas of the brain remained unchanged. Also, β_2-adrenergic receptors in the hippocampus as well as in other areas of the brain remained unchanged. In the leukocytes of escape-deficit rats, β_2-adrenergic receptors were not significantly different from control rats. Taken together, our results suggest that β_1-adrenergic receptors are increased in the hippocampus but neither β_1- nor β_2-adrenergic receptors are changed in other areas of the brain.

Despite the sustained behavioral escape-deficits, the transient nature of β_1-adrenergic receptor upregulation in the present study is an interesting finding. These results suggest that β-adrenergic receptor changes and behavioral changes are not necessarily correlated. However, although we did not observe any changes in β_2-adrenergic receptors in any area of the rat brain or in leukocytes, or in β_1-adrenergic receptors in certain areas of the rat brain, that does not necessarily mean that β_2-adrenergic receptors are not associated with depressive illness. Because we observed a change in β_1-adrenergic receptors in escape-deficit rats only after 48 h of training, it is possible that this finding may have reflected the effects of stress. However, Martin et al. (17) observed upregulation of β-adrenergic receptors in the hippocampus of response-deficient (24 h of training) rats but not in nondeficient rats when compared to untreated control rats. Therefore, it is not clear at present whether the observed upregulation of β_1-adrenergic receptors in escape-deficit rats (48 h of training) may be related to stress or learning-deficit-to-escape response. Further study is needed to determine to what extent the changes are due to escape-deficit or stress.

Changes in adrenergic neurotransmission during stress situations have been demonstrated by several investigators. For example, unpredictable stress caused the upregulation of β-adrenergic receptors, but repeated exposure to the same stressor has been reported to decrease cortical β-adrenergic receptors (21,34). Earlier studies involving different schedules of uncontrollable stress have shown an increase in turnover and a decrease in norepinephrine (NE) levels in rat brain (2,26,37). It is likely that the postsynaptic supersensitivity of β_1-adrenergic receptors may be an adaptive mechanism to acute stress due to decreased levels of NE. In this context, it is interesting to note that changes in β_1-adrenergic receptors occurred after 48 h of training, while after 10 days of training, β_1-adrenergic receptors returned to baseline levels even though both groups of rats were deficient in learning the shock-escape response (Fig. 1). We observed long-lasting escape-deficit effects in the present study that were similar to the findings of some, but not all investigators (6,12,30,32). The fact that antidepressant drug treatment reverses the escape-deficit process, at least after 1 week of treatment (32), suggests that the escape-deficit effect in these studies may last for more than 1 week. The reason for the discrepancies in the behavioral parameters between these studies is not clear, but may be related to the use of different behavioral training paradigms. It is also possible that more than one neurotransmitter system, such as the serotonergic system, may be involved in learning-deficit-to-escape response. Alterations in the serotonergic system in this model of depression have been demonstrated recently. Animals exposed to inescapable shock had lower levels of 5-HT and higher levels of 5-HIAA and decreased release of 5-HT in brain than control animals (11,28). On the other hand, 5-HT_2 receptors are not changed but 5-HT_{1B} receptors are increased in brains of escape response–deficient rats when compared with normal rats (8,17).

In summary, our results indicate abnormalities in adrenergic neurotransmission, which are associated with an increase of both B_{max} and K_d of β_1-adrenergic receptor-binding sites in hippocampus but no change in β_2-adrenergic receptors in leukocytes and brains of rats with stress-induced behavioral depression. These results suggest that complex neuropharmacologic interactions are involved in the generation and maintenance of escape-deficit and that a more complete understanding of these processes depends on correlative neurochemical and behavioral analysis of dynamic changes in the CNS.

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