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Stress Adaptation and Hypersensitivity in 5-HT Neuronal Systems After Repeated Foot Shock

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OHI, K., M. MIKUNI AND K. TAKAHASHI. *Stress adaptation and hypersensitivity in 5-HT neuronal systems after repeated foot shock.* PHARMACOL BIOCHEM BEHAV **34**(3) 603–608, 1989.—The relationship between adaptation to stress and change in sensitivity of the 5-hydroxytryptamine (5-HT) neuronal system was studied in rats exposed to repeated foot shock stress for up to 10 days. Although hypolocomotion, freezing behavior and loss of weight were observed after in the initial stress, relief from these behavioral changes developed by the 3rd and persisted for another 7 days, indicating the development of stress adaptation. Following an IP injection of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), rats exposed to the stress for 10 days, but not for 5 days, displayed enhanced forepaw treading, tremor and Straub tail compared to control rats. These results suggest that the hypersensitivity of the 5-HT system after repeated stress may be in part related to the neuronal mechanism of stress adaptation. However, since hypersensitivity was not observed after exposure for 5 days, when adaptation was maximal, it is proposed that the 5-HT system may participate in the maintenance of adaptation rather than its development. On the other hand, no change in 5-HT1, 5-HT1a and 5-HT2 receptor binding assays was found after chronic stress, suggesting that the hypersensitivity of 5-HT system may not be accompanied with changes in the numbers of 5-HT receptor binding sites. The results of beta-adrenergic receptor binding determined simultaneously were also discussed with reference to previous reports of stress-induced reduction in beta-adrenergic receptor density.

Stress adaptation 5-Hydroxytryptamine (5-HT) 5-HT-dependent behavior
5-Methoxy-N,N-dimethyltryptamine (5-MeODMT) 5-HT receptors Beta-adrenergic receptor

IT is well known that initial exposure to a stressor provoked such responses as reduced locomotor activity, marked anorexia, decreased growth rate and hypersecretion of corticosterone in animals. Behavioral studies suggest that repeated exposure to a stressor results in a diminished stress response following subsequent exposure to the same stressor compared to the stress response induced by the initial exposure (2, 14, 16). This desensitization to the stressor stimulus during repeated exposure is referred to as stress adaptation, and has been suggested to be a mechanism to protect an organism against recurrent stress.

In order to elucidate the neurochemical basis underlying this stress adaptation, several authors have investigated changes in enzymes which regulate the synthesis of neurotransmitters as well as the density of neurotransmitter receptors (1, 5, 7, 12, 29, 31). Particularly, Stone *et al.* reported that a 2.5 hr/day restraint stress for 7 days resulted in decrease in [^{3}H]dihydroalprenolol (DHA) binding sites which was positively correlated with the appearance of stress adaptation; these changes in beta-adrenergic receptors during repeated exposure to stress have been suggested to be a factor responsible for stress adaptation (41,42). Some investigators have reported that other stressors such as foot shock or tail shock could also produce a reduction in beta-adrenergic receptor density (12,24). Cohen *et al.*, however, reported that they were unable to find statistically significant reduction in beta-adrenergic receptor density in hypothalamus, brain stem or cortex after seven days of repeated 1 hr of foot shock. Thus, the changes in the number of beta-adrenergic receptor during repeated stress is still in controversy.

On the other hand, the effect of chronic or repeated stress on the brain 5-HT system has not been sufficiently investigated. Recently, Kennett *et al.* have reported that the behavioral deficits induced by a single 2-hr restraint stress are reversed during repeated restraint and that this adaptation process is associated with the development of enhanced postsynaptic behavioral sensitivity to the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (15,16).

In the present study, we observed stress adaptation following repeated exposure to foot shock stress for 1 hr/day and simultaneously examined the behavioral response to 5-MeODMT. Further, we carried out binding assays of the 5-HT1, 5-HT1a, and 5-HT2 as well as the beta-adrenergic receptor, in frontal cortex or hippocampus, or both.

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METHOD

Wistar male rats obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan) were housed in a temperature-/humidity-controlled facility maintaining a dark-light cycle (light: 0800–2000 hr), for at least 4 weeks prior to the start of the experiment. In the experiment, animals (250–300 g) were housed one to a cage with food and water available ad lib and allowed a week of habituation in their new environment with handling.

This study was divided into three experiments: 1) observation of stress adaptation, 2) evaluation of drug-induced behavioral response and 3) biochemical determination of neurotransmitter receptors. Such manipulations as the administration of stress, behavioral observation and the decapitation of rats were conducted between 0900 and 1200 hr.

Each day during the stress experiment, the animal was transported to the room specially designed for the observation of behavior. For the administration of stress, a shock box was used which consists of four compartments ($50 \times 50 \times 50$ cm) with electric grids (distance of 1 cm) as a floor. The rats were placed into individual compartments and suffered inescapable electric foot shocks for 1 hr daily (2 mA, fixed interval schedule with an intershock interval of 4 sec and shock duration of 1 sec), delivered by a shocker distributor (Muromachi-kikai Co., Japan).

Experiment 1: Observation of Stress Adaptation

Locomotor activity. The locomotor activity of each rat was measured with Animex-auto (Muromachi-kikai Co., Japan) for 14 days (4 days before and 10 days during the period of repeated stress).

Freezing behavior. Freezing behavior was defined as the lack of all visible body movement except for that necessitated by respiration. On days 1, 2, 3, 4, 6, 8 and 10 during the period of repeated stress, the rat was observed in the stress box for 5 min from the end of each exposure to the stress and the duration of the freezing behavior was recorded.

Change in weight. The animal was weighed every day prior to the administration of the stress. The daily change in weight was represented as the weight difference on two consecutive days.

Experiment 2: Evaluation of 5-HT-Dependent Behavior Induced by 5-MeODMT

The behavioral responses to 5-MeODMT were observed in control rats without stress and rats treated with subchronic or chronic stress. The subchronic and chronic stress were defined as the exposure to foot shock for 5 and 10 days, respectively. Twenty-four hours after the last stress, each rat was placed in a quiet room with temperature controlled at 20–24°C and was transferred from his home cage to an observation cage of the same size ($35 \times 20 \times 18$ cm) at least 45 min before the evaluation. Following intraperitoneal injection of 5-MeODMT (5 or 10 mg/kg), the 5-HT-dependent behavior was video-monitored for 30 min. Each of four parameters in the 5-HT-dependent behaviors—forepaw treading, hindlimb abduction, tremor and Straub tail—was evaluated for 1 min every 5 min after injection and scored on a 0–4 scale of relative intensity: 0—absent, 1—perceptible, 2—weak, 3—medium, 4—maximum by a blind observer, as reported by Kennett *et al.* (16).

Experiment 3: Biochemical Determination of Neurotransmitter Receptors

Twenty-four hours after either exposure to stress for 5 days or 10 days, rats were decapitated, the brain removed and then dissected into cortex and hippocampus. These areas were chosen for 5-HT receptor binding assay because fimbria-fornix lesion interrupting the ascending 5-HT pathway from raphe to hippocampus or cortex could prevent the expression of 5-HT-dependent behaviors (20). The sample was stored at −80°C until assayed. Five to 7 animals were prepared for each receptor binding study.

5-HT1 and 5-HT2 receptor binding assays were performed by the methods of Schnellman *et al.* (33) and Leysen *et al.* (19) with minor modifications to each. In brief, the brain region was weighed and homogenized in 100 volumes of Tris-HCl (50 mM, pH 7.7) including 5.0 mM EDTA at 4°C using a Brinkman Polytron (setting 6, 20 sec). The homogenate was centrifuged twice at $39,000 \times g$ for 10 min at 4°C. The pellet was suspended in 100 volumes of Tris-HCl buffer without EDTA and recentrifuged. The resulting pellet was suspended in 70 volumes of Tris-HCl buffer and used in the assay procedures. In addition, the suspension for 5-HT1 receptor binding assay was incubated at 37°C for 15 min and then added to 10 μM pargyline.

The assay mixture for 5-HT1 receptor binding consisted of 0.50 ml of tissue suspension, 0.38 ml of a solution of [^{3}H]-labelled ligand (0.4–8.0 nM [^{3}H]5-HT), 0.1 ml Tris-HCl buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid and 0.02 ml of buffer or metergoline (10 μM) as a displacing drug. Following incubation at 37°C for 10 min, the assay mixture was rapidly filtered under vacuum through Whatman glass filters (GF/B) and the filter was thrice washed with 3 ml of 50 mM Tris-HCl buffer (pH 7.4). The assay mixture for 5-HT2 receptor binding consisted of 0.50 ml of tissue suspension, 0.48 ml of a solution of [^{3}H]-labelled ligand (0.15–3.0 nM [^{3}H]ketanserin) and 0.02 ml of buffer or methysergide (10 μM) as the displacing drug. Following incubation at 25°C for 45 min, the same procedure for filtration was conducted.

The 5-HT1a binding site, a subtype of 5-HT1 receptor binding site, was characterized with [^{3}H]8-OH-DPAT, based on the method of Peroutka (25). In brief, the tissue was homogenized in 80 volumes of Tris-HCl (50 mM, pH 7.7) at 4°C and then centrifuged at $39,000 \times g$ for 10 min. The pellet was suspended in the same volumes of Tris-HCl buffer and incubated at 37°C for 10 min before a second centrifugation at $39,000 \times g$ for 10 min. The final pellet was resuspended in 50 volumes of Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. The assay mixture consisted of 0.45 ml of a solution of [^{3}H]-labelled ligand (0.3–6.4 nM [^{3}H]8-OH-DPAT), 0.05 ml of buffer or 5-HT (10 μM) as displacing drug and 0.5 ml of tissue suspension. Following incubation at 25°C for 30 min, the mixtures were rapidly filtered.

Beta-adrenergic receptor binding assay was done according to the method of Bylund and Snyder (3) with a partial modification. The tissue was prepared by the same procedure as in 5-HT2 receptor assay. The assay mixture consisted of 0.48 ml of a solution of [^{3}H]-labelled ligand (0.2–4.0 nM [^{3}H]DHA), 0.02 ml of buffer or (−)propranolol (200 nM) as displacing drug and 0.5 ml of tissue suspension.

Radioactivity was measured by liquid scintillation spectroscopy in 10 ml of Aquasol-II (New England Nuclear, Boston, MA) at 53% efficiency. Specific bindings were defined as the difference between the amount of radioactive ligand bound in the presence and absence of displacing drug. They were represented 50–60% of total [^{3}H]5-HT binding, 90–95% of total [^{3}H]ketanserin binding and 70–80% of total [^{3}H]8-OH-DPAT and [^{3}H]DHA binding, respectively.

The dissociation constant (K_d) and the maximum binding capacity (B_{max}) were calculated from the linear regression of Scatchard plot. A B_{max} and K_d were obtained from each animal. The protein concentrations of the tissue homogenate were determined by the method of Lowry (21).

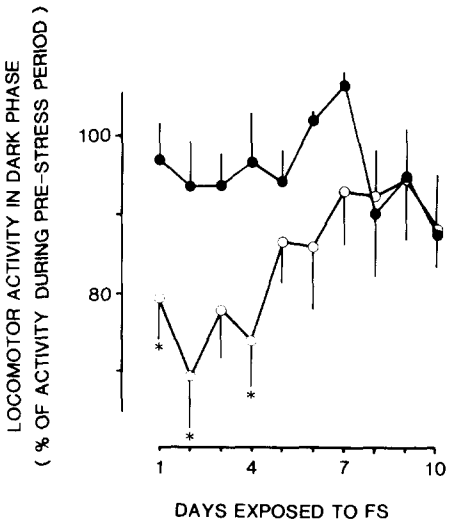

FIG. 1. Effect of foot shock stress on locomotor activity during dark phase (2000–0800 hr) over the 10-day period. Level of the ordinate indicates percent activity of corresponding mean dark phase activity for 4 days before start of stress. Open and closed circles represent the mean values for 8 rats exposed to stress and 6 control rats, respectively. Vertical lines represent the S.E.M. Asterisks indicate a significant difference between two groups ($p<0.05$).

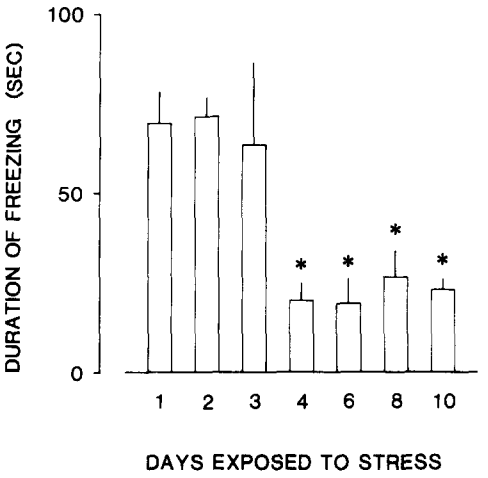

FIG. 2. Effect of foot shock stress on duration of freezing over 10-day period. Each bar indicates a mean duration time in 8 rats. Vertical lines represent the S.E.M. Asterisks indicate that the mean duration time on the day is significantly shortened, compared to those on days 1 to 3 ($p<0.05$).

Drugs

Drugs were obtained from the following sources: 5-MeODMT, pargyline and 5-HT from Sigma Chemical Co.; methysergide hydrogenmalcinate from Sandoz Ltd.; metergoline from Farmitalia; [^{3}H]5-HT (28.2 Ci/mmol), [^{3}H]ketanserin (61.8 Ci/mmol), [^{3}H]8-OH-DPAT (156.6 (Ci/mmol) and [^{3}H]DHA (46.5 Ci/mmol) from New England Nuclear.

Statistics

Differences between the stressed and control groups were tested by the two-tailed nonparametric Williams-Wilcoxon test.

RESULTS

Behavioral Adaptation During Repeated Stress

Figure 1 shows the changes in locomotor activity measured over the experimental period. The activity was only counted during the dark phase (2000–0800 hr), an active phase of rats, because during the light phase (0800–2000 hr), the activity by such artifacts as handling or transfer of rats and administration of stress interfered with the true activity count. The control rats were individually housed similar to the stressed rats with the exception of the administration of stress. They did not display any significant changes in locomotion. On the other hand, the group exposed to the stress displayed a significant reduction in locomotor activity on days 1, 2 and 4 compared to the control group ($p<0.05$). The reduction in locomotor activity was greatest on day 2 (-31%), thereafter it gradually, but significantly, recovered to -13% on day 10 ($p<0.05$ by paired t-test). There was no significant difference between the stress and control groups in day 10 values.

Figure 2 shows the mean duration of freezing behavior observed after each exposure to the stress. On days 1 to 3, the rats exposed to the stress displayed prolonged freezing behavior which lasted for an average of 64–83 sec. On day 4, the duration time was markedly and significantly shortened to an average of 17 sec, compared to those on days 1 to 3 ($p<0.05$). This shortened duration persisted until the last day of the experiments.

Figure 3 shows the change in body weight during the repeated foot shock. The control group displayed a constant gain of about 3 g per day in weight. On the other hand, the stress group displayed a loss in weight on days 1 and 2. Thereafter, this group began to gain weight, although weight remained below the control level throughout the series of experiments.

5-HT-Dependent Behaviors Induced by 5-MeODMT

As shown in Table 1, the behavioral responses to 5-MeODMT

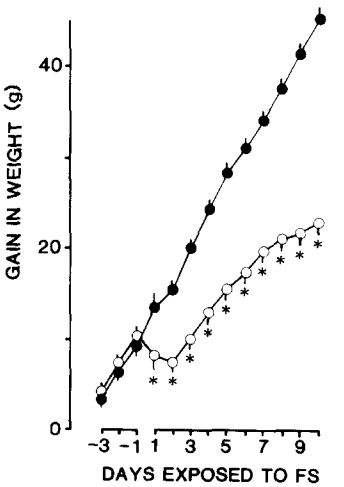

FIG. 3. Effect of foot shock stress on body weight over 14 days, 4 days before and 10 days during stress session. Level on ordinate indicates a gain in weight at end of 4 days before stress. Open and closed circles represent the mean value for 14 rats exposed to stress and 16 control rats, respectively. Vertical line represents S.E.M. Asterisks indicate a significant difference between two groups.

TABLE 1

THE EFFECT OF SUBCHRONIC AND CHRONIC FOOT SHOCK STRESS ON BEHAVIORAL RESPONSES TO 5-MeODMT 24 HR AFTER THE LAST ADMINISTRATION

	Hindlimb Abduction	Forepaw Treading	Tremor	Straub Tail
5 mg/kg, IP				
Control (9)	6.06 ± 0.86	9.11 ± 0.65	7.25 ± 0.41	1.56 ± 0.65
Subchronic (7)	9.29 ± 1.78	10.8 ± 1.22	6.83 ± 0.60	2.86 ± 0.77
Chronic (8)	8.81 ± 1.39	10.1 ± 0.61	8.21 ± 0.72	1.88 ± 0.83
10 mg/kg, IP				
Control (9)	15.2 ± 1.66	15.2 ± 0.60	10.6 ± 0.96	4.56 ± 0.97
Subchronic (7)	15.7 ± 1.11	14.8 ± 0.79	10.7 ± 0.52	2.20 ± 1.00†
Chronic (8)	15.9 ± 1.32	16.8 ± 0.46†	13.2 ± 0.58†	5.75 ± 0.39*

Values are mean ± S.E.M. Significant differences from control: *$p<0.01$ and †$p<0.05$ by Williams-Wilcoxon test. Each number in parentheses indicates the number of rats in each group.

were observed 24 hr after the last stress. When receiving the intraperitoneal injection of 10 mg/kg 5-MeODMT, the rats treated with chronic stress (exposed to the stress for 10 days) displayed a significantly increased response in such 5-HT-dependent behaviors as forepaw treading, tremor and Straub tail ($p<0.05$). The injection of 5 mg/kg produced a tendency to an increase in the responses, but not significantly. The rats with subchronic stress (exposed for 5 days) displayed no significant difference compared to the control group.

Receptor Binding Assays

Table 2 shows the specific binding of [^{3}H]5-HT, [^{3}H]ketanserin, [^{3}H]8-OH-DPAT and [^{3}H]DHA in the following three groups: chronic stress, subchronic stress and control group. There was no significant difference among the three groups in both the B_{max} and K_d of each binding assay.

DISCUSSION

To our knowledge, the present study demonstrates for the first time that foot shock stress results in behavioral adaptation during repeated exposure to the stress. The findings are in good agreement with results of previous studies (16,41) on stress adaptation observed during repeated restraint stress. Accordingly, the stress adaptation may be a universal phenomenon occurring in a chronically stressful environment and this phenomenon may not be affected by differences in strain or other small differences in the experimental conditions. We have shown that the reduction of locomotor activity, freezing behavior and weight loss following the initial stress are clearly relieved between the 3rd and 5th days during the stress period, thereby indicating that the stress adaptation occurs uniformly in different behavior groups. There may be a common mechanism underlying the adaptation observed in each type of behavior.

After intraperitoneal injection of 5-MeODMT, the rats exposed to the foot shock stress for 10 days displayed significantly enhanced response in three kinds of behavior, i.e., forepaw treading, tremor and Straub tail. These findings generally correspond to the results of Kennett *et al.*, who reported that the restraint stress for 7 days (2 hr/day) could produce the enhancement of forepaw treading and tremor to 5 mg/kg of 5-MeODMT (15–17). Our results together with Kennett *et al.* indicate that frequently repeated stress can elicit hypersensitivity of the 5-HT neuronal system.

Although the physiological relevance of 5-HT neuronal system remains unclear, many reports have referred to the changes in 5-HT neuronal system after stress. Soblosky demonstrated that the increase in 5-hydroxyindole acetic acid concentration, a major metabolite of 5-HT, was due to chronic stress alone (37). Petty and Sherman reported that microinjections of 5-HT into the frontal cortex or septum increased escape behavior in rats following chronic stress (26,27). Hamilton *et al.* found that the escape deficit, which was engendered by the exposure to shock, was prolonged by the administration of the 5-HT receptor blocker

TABLE 2

EFFECT OF CHRONIC FS STRESS ON 5-HT (1) AND β-ADRENERGIC (2) RECEPTOR

	B_{max} (fmol/mg protein)	K_d (nM)
	(1)	
[^{3}H]5-HT—Cortex		
Control (7)	118 ± 17.3	1.88 ± 0.73
Subchronic (5)	117 ± 11.1	2.38 ± 0.78
Chronic (7)	124 ± 14.6	2.14 ± 0.79
[^{3}H]5-HT—Hippocampus		
Control (5)	331 ± 14.3	3.60 ± 0.54
Subchronic (5)	278 ± 81.1	3.52 ± 0.35
Chronic (5)	332 ± 30.9	4.11 ± 0.79
[^{3}H]Ketanserin—Cortex		
Control (7)	238 ± 20.6	0.544 ± 0.073
Subchronic (5)	218 ± 19.6	0.555 ± 0.084
Chronic (7)	238 ± 28.1	0.510 ± 0.046
[^{3}H]8-OH-DPAT—Hippocampus		
Control (5)	168 ± 7.49	1.05 ± 0.25
Subchronic (6)	170 ± 10.1	0.95 ± 0.20
Chronic (5)	163 ± 22.4	0.94 ± 0.14
	(2)	
[^{3}H]DHA—Cortex		
Control (5)	111 ± 12.6	0.435 ± 0.053
Subchronic (5)	101 ± 7.92	0.412 ± 0.035
Chronic (5)	102 ± 4.96	0.415 ± 0.033

methysergide and that the 5-HT releasing agent parachloramphetamine effectively ameliorated the escape deficit provoked by shock (11). In several studies (10, 30, 34, 35), it has been demonstrated that the 5-HT synthesis inhibitor parachlorophenylalanine potentiates depression of motor activity in an open field after stress. These reports, but not all (7,38), suggest that the 5-HT neuronal system may play an antistress role (28). If the stress adaptation is a behavioral phenomenon of antistress response to chronic stress, 5-HT hypersensitivity may be one of the neuronal mechanisms accounting for stress adaptation.

In our data, however, the 5-HT hypersensitivity was not observed after exposure for 5 days, when the stress adaptation was maximal. Therefore, the 5-HT hypersensitivity may not participate in the initial stage but the maintenance of the stress adaptation.

On the other hand, the enhanced response in forepaw treading, tremor and Straub tail induced by 5-MeODMT suggests an up-regulation in 5-HT receptor density. In particular, the enhanced response in forepaw treading may indicate increase of a 5-HT1a subtype receptor density, because 8-OH-DPAT, a putative 5-HT1a receptor agonist, produces forepaw treading in reserpinized rats (19,36). However, no change in either the number or the affinity of the [^{3}H]8-OH-DPAT binding sites was found after the exposure to foot shock stress for 10 days. There was also no significant difference between stressed and control rats in either [^{3}H]5-HT or [^{3}H]ketanserin binding assay. The 5-HT hypersensitivity represented by the enhanced behavioral response to 5-MeODMT is not reflected in an increased density of any 5-HT receptors.

It has been often reported in another kind of experiment that behavioral responses to 5-HT agonists are not consistent with the results from the 5-HT receptor assay. Although chronic treatment with several tricyclic-antidepressants (8, 9, 23, 32, 39, 40), but not quadri-cyclics or monoamine oxidase inhibitors (4,22), produces 5-HT hypersensitivity as represented by enhanced behavioral responses to 5-HT agonists, the increase in 5-HT receptor density is not observed. The discrepancy between behavioral responses and biochemical determination of receptors remains unclear, but there are several explanations. The first explanation is that the behavioral hypersensitivity may result from the hypersensitivity of postsynaptic effectors. The second messenger mechanism mediated by formation of cyclic adenosine-monophosphate (c-AMP) or breakdown of phosphoinositide may be sensitive to the stimulation of receptor agonist. The second explanation is that the present method for receptor binding assay is too insensitive to detect the slight, but significant alterations in receptor binding that are associated with the observed behavioral changes. The third explanation is that other brain regions except the cortex and hippocampus may be more essential for the expression of 5-HT-dependent behaviors. Because of serially transecting the rat brain rostrally to caudally, a cut at the caudal mesencephalic level failed to abolish the 5-HT-dependent behaviors (13), the descending 5-HT neurons may contribute to them.

The beta-adrenergic receptor assay also revealed no difference between the control and stress group. Many investigators have reported that a reduction of beta-adrenergic receptor density develops in various regions of the brain after chronic stress (18, 24, 41, 43). However, Cohen *et al.* recently reported that the B_{max} of the beta-adrenergic receptor assay in the cortex and hypothalamus was not significantly altered after 7 days (1 hr/day) of foot shock stress (random 0.7 mA shock for 10 sec) and 24-hr recovery. They suggested that the change in the number of beta-adrenergic receptors following stress may be affected by the type of stressor, duration and intensity of stress, strain of rat or the time of evaluation (6). Although we used more severe and prolonged stress conditions in our study than those employed by Cohen *et al.*, we still found no change in the number of beta-adrenergic receptors. Thus, the type of stressor may be a more important factor than intensity or duration. The foot shock stress may not readily produce the reduction in beta-adrenergic receptor density. Stone *et al.* proposed that a subsensitivity to catecholamine stimulation resulting from the reduction in beta-adrenergic receptor density might be related to stress adaptation (43). In the present study, however, the behavioral adaptation to stress developed without reduction in the receptor density. It seems that change in beta-adrenergic receptor is not essential for the neurochemical basis of stress adaptation.

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