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Effect of Chronic Variable Stress on Monoamine Receptors: Influence of Imipramine Administration

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MOLINA, V. A., M. VOLOSIN, L. CANCELA, E. KELLER, V. S. MURÚA AND A. M. BASSO. *Effect of chronic variable stress on monoamine receptors: Influence of imipramine administration.* PHARMACOL BIOCHEM BEHAV **35**(2) 335–340, 1990.—Adult male rats were exposed to a series of unpredictable stressors, a paradigm considered to be a model of experimental depression, with or without concurrent administration of imipramine. One day after the last stress event of the chronic regime, binding of cortical beta-adrenoreceptors and the behavioral serotonin (5-HT) syndrome induced by 5-methoxy-N,N,dimethyltryptamine (5-MeODMT) were determined in all the experimental groups. Stressed rats showed an "up-regulation" of cortical beta-adrenergic sites, while similar values to control rats were observed when stressed animals were administered imipramine. Regarding the behavioral 5-HT syndrome, comparable behavioral scores were observed between controls and chronically stressed rats. The combination of chronic exposure to different stressors with imipramine treatment resulted in a significant increase of forepaw treading and Straub tail scores. The probable facilitation of behavioral deficits induced by this scheme of chronic stress and the recovery following concurrent administration of imipramine are discussed.

Beta-adrenoceptors 5-HT_1 sites Imipramine Chronic variable stress

NUMEROUS pharmacological findings support the hypothesis that adaptive changes induced by drugs on monoamine receptors are related to their therapeutic efficacy. Within these adaptive changes, chronic antidepressant treatments including pharmacotherapy and electroconvulsive shocks, cause subsensitivity of adenylate cyclase to NA and isoprenaline (49), usually linked to a down-regulation of the number of brain beta-adrenoceptors (2,4).

Regarding the serotoninergic system, Peroutka and Snyder (36) had indicated that repeated administration of several antidepressant drugs to rats reduced the number of cortical 5-HT_2 sites. This evidence was further supported by Green *et al.* (18), who observed a decrease in the head-twitch response, a behavioral correlate of 5-HT_2 sites, following chronic treatment with antidepressant drugs.

All these adaptive changes may be crucial for clinical efficacy since a temporal correlation exists between the appearance of these changes and the clinical onset of antidepressive effects. However, a great limitation in extrapolating these observations to clinical effects is that they were obtained mainly in normal rats, whereas depressive patients may be suffering from alterations of monoaminergic transmission.

Evidence is accumulating that life events stress may precipitate or predispose the appearance of depressive disorders (5,11). In fact, those animal models of depression with sufficient validity to be used as experimental tools for research on depression are mainly based on behavioral alterations following diverse stress situations (25, 38, 51, 52). In this work we use a paradigm of chronic unpredictable stress, originally proposed by Roth and Katz (40). This regime resulted in altered locomotion and corticosterone release to acute stress that can be prevented by concurrent administration of diverse types of antidepressant drugs, but not by drugs of other classes (22, 23, 25) and it has been described to have a reasonable degree of validity (52).

In order to study potential alterations on monoamine sites and their reversion by chronic imipramine administration, animals were submitted to the chronic stress regime with or without imipramine injection; radioligand binding and behavioral studies were conducted in order to analyze cortical beta-adrenoceptor and 5-HT_1 sites, respectively.

METHOD

Animals and Experimental Treatments

Fifty-six male Wistar rats (280–350 g) were caged in the

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TABLE 1

CHRONIC STRESS REGIME

Day	Treatment
1	ear mark
2	shaker stress (high speed 1 hr)
3	cold swim (4°C-5 min)
4	switch cage (24 hr)
5	shock (½ hr)
6	remove water (24 hr)
7	tail pinch (1 min)
8	remove food (24 hr)
9	shock (½ hr)
10	remove water (24 hr)
11	individual housing (24 hr)
12	cold swim (4°C-5 min)
13	shaker stress (high speed 1 hr)
14	switch cage (24 hr)
15	behavioral study or binding assay

experimental room for at least seven days before the start of experiments. They were maintained at 22 ± 2°C, under a 12-hr light/dark cycle beginning at 07.00 a.m. The rats were housed in wire cages measuring $45 \times 30 \times 20$ cm (4–6 rats per cage) and had continuous access to food and water except for three occasions when food or water were removed from the experimental groups for 24 hr as part of the chronic stress procedure.

Rats were injected with imipramine (IMI, 10 mg/kg/day, IP) or 0.9% saline (SAL) one hour before each stressful event throughout all the chronic stress regimes, during 14 consecutive days. Control unstressed rats received SAL or IMI injections for the same period. Therefore, the experimental groups consisted of: 1) unstressed rats injected with IMI for 14 days, 2) unstressed rats injected with SAL for 14 days, 3) stressed rats injected with SAL 1 hr before each stress session and 4) stressed rats injected with IMI 1 hr before each stress session.

Chronic Stress Procedure

The regime of chronic stress was similar to that described by Roth and Katz (40). The following stressors were used: 24-hr cage switch (the rats were transported to another room and each original group was placed in a cage similar to the one described above), isolation (the rats were placed individually in $30 \times 15 \times 10$ cm acrylic cages in the housing room and after 24 hr they were returned to their home cage), 24-hr water deprivation, 24-hr food deprivation (in both cases the rats were maintained in their home cages), 30 min of unpredictable shock [average 1 mA, 1 sec duration, average one shock/min (twice)], 5-min swim in 4°C ice water (twice), 60 min of horizontal shaker (high speed) (twice) and 1-min tail pinch. All animals were stressed between 10.00 and 13.00 hr, except for cage switching, food and water deprivation and isolation, which lasted for 24 hr. The experimental protocol and the schedules of stress delivery are summarized in Table 1.

Binding Assay

Determination of cortical beta-adrenergic receptor density was performed in 20 rats submitted to a schedule of treatment similar to that described above. Each binding assay run contained frontal cortex tissue for a single rat belonging to saline, IMI, chronic stress and chronic stress + IMI groups. In all cases, animals were sacrificed 24 hr after the last injection or stressful experience, the brain was removed according to Heffner *et al.* (20) and the frontal cortex quickly dissected and preserved at −70°C for 1–2 weeks until binding assays were performed by a conventional procedure (7). Tissues were homogenized with a Polytron PT 10 (setting 6, 20 sec) in 20 vol. of ice-cold Tris buffer (0.05 M; pH 8) and centrifuged at $39000 \times g$ for 20 min at 4°C. The resulting pellets were washed in the same volume of Tris buffer and resuspended in 19 vol. of buffer. ^{3}H-Dihydroalprenolol (^{3}H-DHA, specific activity 54.8 Ci/mmol, from NEN Corp., USA) was used as radioligand for receptor binding assays. Experiments were performed in duplicate with 150 µl of membrane suspension (400–500 µg protein) and ^{3}H-DHA (0.5–5 nM) incubated at 23°C for 18 min in a final volume of 500 µl. The incubation was ended by adding 3.5 ml of cold buffer to each tube and rapidly filtering the contents under vacuum through Whatman GF/B filters. The incubation tubes were rapidly washed twice with 3.5 ml of cold buffer and the filters were dried and transferred to vials to count the radioactivity in a solution containing PPO, Triton X-100 and toluene. Specific binding was defined as the difference in radioactivity in the absence and presence of 2.0×10^{-5}M propranolol. The dissociation constant (K_d) and maximum ^{3}H-DHA binding were determined from least squares fits to Scatchard plots of the data. Proteins were assayed according to Lowry *et al.* (32).

Behavioral Study

Behavioral experiments were conducted in a quiet room at 20–24°C between 12.00 and 16.00 hr, 24 hr after the last injection or stressful experience. Thirty-six rats were used, eleven were included in the control group, eight in the imipramine group, eight in the chronic stress group and nine in the imipramine plus chronic stress group. The behavior of the rats was observed while they were in a stainless steel cage (40×60 cm) to which they had been habituated by placing them individually there for at least 30 min before the observation period. Analysis of animal behavior was made by an observer who was "blind" to the treatments. In each behavioral test, control and chronically stressed animals were scored in parallel. After injection of 5-MeODMT (5 mg/kg, IP), 5-HT-dependent behavior was recorded as follows (26). Rats were observed for continuous scoring periods of 2 min separated by 1 min nonscoring intervals over a total period of 19 min beginning 2 min after 5-MeODMT injection. Values for each behavior from the six 2-min observation periods were summed over the total period (maximum score per behavior: 25). In each scoring period, intermittent behavior (forepaw treading) was scored on a 0–4 scale: 0—absent, 1—present once, 2—present several times, 3—present frequently and 4—present continuously. Continuous behavioral responses (hindlimb abduction, tremor and Straub tail) were scored on a 0–4 scale of relative intensity: 0—absent, 1—perceptible, 2—weak, 3—medium and 4—maximal.

Drugs

Drugs used were 5-methoxy-N-N,dimethyltryptamine (Sigma Chemical Co., St. Louis, MO), imipramine HCl (Lab. Prest, Bs. As., Argentina), and propranolol (Sigma).

Statistical Analysis

Binding parameters were evaluated by two-way ANOVA and subsequent Student's *t*-test. Behaviors of the 5-HT syndrome were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA)

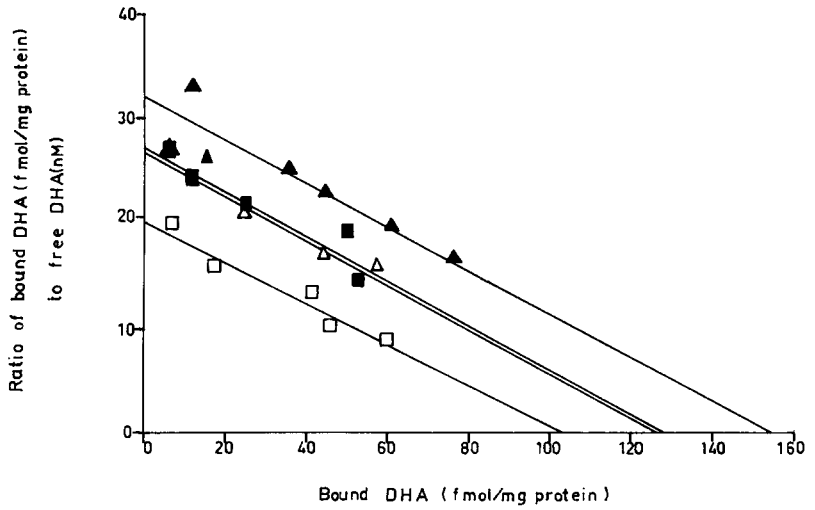

FIG. 1. Scatchard plots of data from representative assay showing beta-adrenoceptor binding performed in frontal cortex. Each assay contained tissue from a single rat frontal cortex from △: saline, □: IMI, ▲: chronic stress and ■: chronic stress + IMI.

followed by the two-tailed Mann-Whitney U-test.

RESULTS

Effect of Chronic Stress Regime and Imipramine Administration on Cortical Beta-Adrenergic Receptor Binding

As shown in Fig. 1 and Table 2, SAL-treated rats submitted to a series of unpredictable stressors showed a significant increase in the number of ^{3}H-DHA binding sites as compared to unstressed SAL-treated animals. Continuous treatment of unstressed rats with IMI during 14 days reduced the density of beta-adrenergic receptors in the frontal cortex (Fig. 1 and Table 2). In contrast, the concurrent administration of unpredictable stressors and antidepressant drug in the same period resulted in a similar concentration of sites as that observed in control animals (Fig. 1 and Table 2). In addition, the number of cortical beta-adrenoceptors of rats with the combined treatment was significantly different from B_{max} values observed after IMI alone or repeated stress events. No alterations in the apparent affinity (K_d) were observed (Table 2).

TABLE 2

EFFECT OF CHRONIC STRESS REGIME AND IMIPRAMINE ADMINISTRATION ON CORTICAL BETA-ADRENOCEPTOR BINDING

Treatment	No. of Assay	B_{max} (fmol/mg prot)	K_d (nM)
Saline	5	134.7 ± 4.5	4.3 ± 0.4
Imipramine	5	98.6 ± 8.1*	3.8 ± 0.4
Chronic Stress	5	151 ± 6.5†	4.8 ± 0.3
Chronic Stress + Imipramine	5	125 ± 6.9‡	4.3 ± 0.4

Data are mean ± S.E.M. of the number of assays conducted on homogenates prepared from a single rat frontal cortex for each assay. Each assay run contained tissue from saline, imipramine, chronic stress and chronic stress + imipramine-treated rats. Statistical differences were evaluated by two-way ANOVA and subsequent Student's *t*-test. n = 5. Different from saline group, *$p<0.005$, †$p<0.05$. Different from chronic stress and also from imipramine-treated group, ‡$p<0.025$.

Effect of Chronic Stress Regime and Imipramine Administration on 5-HT-Dependent Behavior

One day after the last stressful session, 5-HT behaviors induced by 5-MeOMDT were unaltered in stressed rats treated with SAL during 14 days as compared with unstressed saline-treated animals (Fig. 2). Similarly, IMI given during 14 days in unstressed rats induced 5-HT-dependent behavior scores comparable to those observed in stressed and unstressed rats injected with SAL (Fig. 2). However, pretreatment with IMI 1 hr before each stress session caused a significant enhancement in the behavioral response to the 5-HT agonist, as assessed by Kruskal-Wallis one-way ANOVA [forepaw treading, $H(3) = 9.56$, $p<0.03$; Straub tail, $H(3) = 8.09$, $p<0.03$], followed by Mann-Whitney U-test (Fig. 2). Hind limb abduction and tremor values were not significantly different (Fig. 2).

DISCUSSION

The results of the present study showed that rats subjected to a series of unpredictable stressors had a higher number of cortical beta-adrenoceptors than unstressed animals. This effect was clearly antagonized by the concurrent administration of imipramine. In addition, a down-regulation of beta-adrenergic sites in brain frontal cortex was observed after chronic imipramine, confirming previous evidence (2).

In contrast to the up-regulation of beta-adrenoceptors after chronic unpredictable stress, repeated exposure to the same stressor has been reported to decrease cortical beta-adrenoceptor density (45), similarly to the effect exerted by a wide variety of antidepressant agents (2). In a similar way, behavioral studies have pointed out comparable changes in the reactivity of catecholaminergic receptors following persistent exposure to the same stressor or long-term administration of antidepressant drugs (8–10, 19, 41, 46). Thus, opposite actions in the modulation of adrenergic sites density may be obtained when different schemes of

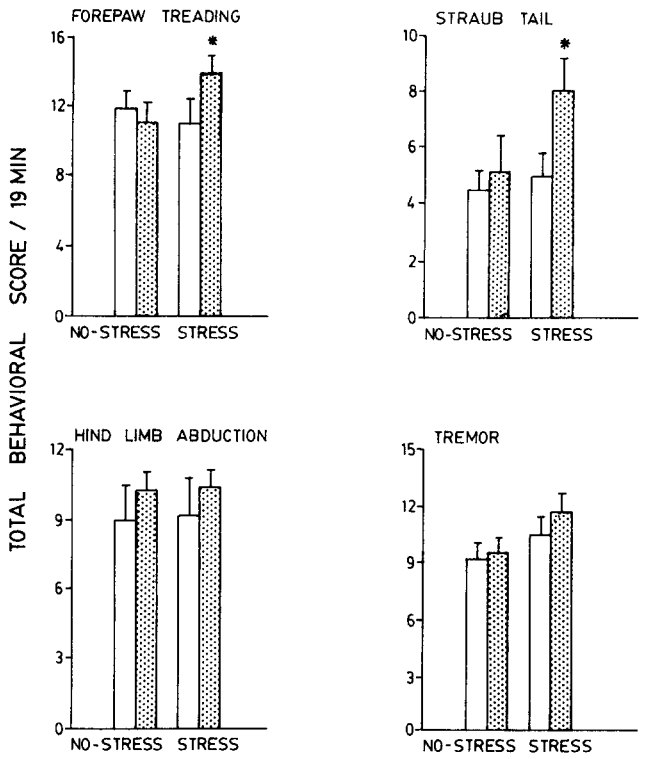

FIG. 2. Behavioral responses to 5-methoxy-N,N-dimethyltryptamine (5 mg/kg, IP) in nonstressed and stressed rats treated with saline (open bars) or imipramine (10 mg/kg, IP) (dotted bars). All scores shown as mean ± S.E.M., n = 8–11 per group. Significantly different from nonstressed and stressed saline-treated rats *$p<0.03$ (Mann-Whitney U-test).

chronic stress are applied. It has been reported that stimulation of beta-adrenergic site provokes the inhibition of several active behaviors (14,31); therefore, as noted by Stone (46), the decrease in the number of beta-adrenergic receptors (as that observed after chronic immobilization events or prolonged antidepressants), could facilitate behavioral disinhibition. According to this hypothesis, it is reasonable to suggest that when an up-regulation of beta-adrenergic sites is induced, as observed in the present study, behavioral inhibition is facilitated. In support of this suggestion, we have observed a clear increase in escape deficits in shuttle-box sessions following a comparable stress regime (Murúa *et al.*, manuscript in preparation). This deficit was blocked when the antidepressant was administered concurrently with each stress event (Murúa *et al.*, manuscript in preparation). Similarly, repeated administration of beta-adrenergic agonists reversed deficits in escape responding after inescapable shocks (34).

The difference between same stressor and variable stressor chronic stress paradigm is not restricted to effects on beta-adrenoceptor modulation. In the forced swimming test, a sensitive model for screening antidepressants, repeated immobilization sessions, induced a clear antiimmobility effect (37), while the application of a chronic stress regime similar to that used in this study induced a higher immobility time (15).

A deficiency in 5-HT neurotransmission has been implicated in depression (21,48). Numerous central functions that are modulated by the central serotoninergic system are impaired in depressive subjects, supporting the idea of an involvement of 5-HT neurotransmission in depressive disorders. Concerning experimental models, decreased 5-HT metabolism in some brain areas has been associated with learned helplessness after footshock (42,43). Consistent with this data, drugs which increase 5-HT efficacy reverse behavioral deficits in several models of depression (16, 29, 30, 39). Application of a single stress provoked a marked deficit in open-field activity and enhanced defecation (26). These behavioral effects disappear after repeated restraint sessions, suggesting that an adaptation process is occurring (26). There is evidence pointing out that an enhanced responsiveness of 5-HT postsynaptic sites is implicated in this adaptation process (26,27).

A lack of adaptation to repeated immobilization might represent a model of depression as suggested by Kennet *et al.* (27). Thus, female rats (unlike males) did not show an increased response of 5-HT sites following chronic immobilization events; this correlated with clinical observations indicating a higher risk of depression in women (28). Our findings showed comparable behavioral values in unstressed and chronic, unpredictably stressed animals following the administration of 5-MeODMT, demonstrating an absence of the increased reactivity of postsynaptic 5-HT sites. If this enhanced response of 5-HT receptors is a marker of the adaptation process to the stressor stimuli, our findings could suggest a lack of adaptation that, as suggested by Kennet *et al.* (27), may account for the behavioral deficits observed in uncontrollable stress situations. Supporting this suggestion, the administration of imipramine was capable of establishing a higher response to 5-MeODMT in stressed, but not in unstressed rats and in this way it could favour the recovery of the behavioral deficit.

Different studies have reported that some components of the 5-HT behavioral syndrome, especially forepaw treading, are mediated by the 5-HT_{1a} site (33,47). Therefore, it is probable that an adaptive change in this 5-HT subtype receptor is occurring after the association with chronic stress and imipramine. This finding is consonant with reports indicating an antidepressant effect of 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), a drug with a great affinity for 5-HT_{1a} sites, but not other 5-HT subtypes (35), in several models of experimental depression (16, 29, 30). The present result showed no alteration of 5-HT site reactivity in unstressed rats treated with imipramine. However, early evidence has demonstrated that tricyclic antidepressants attenuated the behavioral syndromes induced by 5-HT agonists; this difference may be attributed to different experimental situations, since higher doses of drugs were used in those experiments (17,44).

Finally, the application of chronic variable stressors, considered a model of depression (24,40), seems to alter the functionality of the central monoaminergic system as determined by the number of beta-adrenoceptors and the behavioral response of 5-HT_1 sites. Since the down-regulation of beta-adrenoceptors is modulated by the functionality of the 5-HT neuronal system, and on the other hand, several reports have pointed out that functional serotoninergic neurons are under stimulatory noradrenergic control (1, 3, 6, 12, 13), the present evidence may not be simple independent phenomena provoked by chronic stress. Further experiments are necessary in order to clarify a possible link between these effects.

The up-regulation of cortical beta-adrenoceptor and the absence of adaptive change on 5-HT_1 sites may lead to the expression of behavioral deficits. Since concurrent administration of antidepressant drugs normalizes both parameters and antagonizes behavioral deficits provoked by stress (38, 40, 51), these effects could be implicated in the physiopathogenesis of depressive disorders, at least of those linked to stress events.

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