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Inhibition of Catecholamine Synthesis Depresses Behavior of Rats in the Holeboard and Forced Swim Tests: Influence of Previous Chronic Stress

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GIL, M., J. MARTI AND A. ARMARIO. *Inhibition of catecholamine synthesis depresses behavior of rats in the holeboard and forced swim tests: Influence of previous chronic stress.* PHARMACOL BIOCHEM BEHAV **43**(2) 597–601, 1992. – Catecholaminergic pathways in the brain are activated during stress and are presumably involved in the control of physiological and behavioral changes triggered by stress. When repeatedly stressed, adaptive changes have been observed in catecholaminergic activity in the brain. In the present experiment, it was assessed whether or not chronic exposure to immobilization (IMO) altered the influence of catecholamines on behavior in the holeboard and forced swim test by administering α-methyl-*p*-tyrosine (an inhibitor of catecholamine synthesis). Adult Sprague-Dawley rats were used. Chronic stress amortiguated the inhibitory effect of acute IMO on some but not all behaviors in the two tests. Whereas previous chronic IMO exacerbated the effects of the drug on struggling and immobility in the forced swim test, no change in response to the drug as a consequence of chronic IMO was observed in the holeboard test. The present data suggest that chronic IMO-induced changes in the catecholaminergic control of some behaviors might be related to depression-like states in rats. The actual physiological meaning of these changes and the specific receptors involved remain to be elucidated.

Chronic stress Immobilization Adaptation Catecholamines α-Methyl-*p*-tyrosine
Holeboard Forced swim test

ANIMALS exposed to severe acute stressors showed an inhibition of their active behavior in tests of activity/exploration such as the open-field and the holeboard tests (5,7,17, 18,26,31). This stress-induced inhibition of activity extends to the forced swim test (5,33). Although the latter test has been primarily used for the screening of antidepressant drugs (21,22,24), at present it should not be disregarded as an animal model of depression as nobody has reported decisive evidence against this hypothesis [see (35) for review].

When repeatedly exposed to the same stressor, animals usually showed a reduced endocrine response to the stimulus (3,4,6,9), most likely due to the reduced emotional response elicited by daily facing the same stressor. However, the existence of adaptation to chronic stress at a behavioral level remains controversial (25,28,32), perhaps because adaptation is not a unitary concept and might depend upon the particular variable under investigation (6,20).

One of the most well-characterized changes after chronic stress is an increase in the synthesis of both adrenal and brain catecholamines (12,30), which allow animals to face severe stressors without a decline in CNS noradrenaline (NA) levels. Changes in catecholamines and especially brain NA levels have been repeatedly associated with behavioral deficits caused by acute stress exposure (1,2,31–34). However, it is unclear at present whether stress-induced behavioral deficits are due to reduced or exacerbated noradrenergic activity in forebrain regions innervated by noradrenergic neurons (29). In addition, it appears possible that chronic stress could have modified the extent to which catecolamines are involved in the control of behavior. If this was the case, abrupt inhibition of catecholaminergic activity would have different consequences in rats depending upon their previous history of stress. For these reasons, in the present experiment stress-naive and chronically immobilized (IMO) rats were administered α-methyl-*p*-thyrosine (αMpT), an inhibitor of catecholamine synthesis, and the behavior of these animals in the holeboard and forced swim tests was studied in unstressed conditions and immediately after acute IMO. The holeboard

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test was chosen as an index of locomotor and exploratory activities and the forced swim test because it joins the possible interaction between chronic stress and catecholamines, both issues repeatedly related to depression, in a putative animal model of depression.

METHOD

Adult, male Sprague-Dawley rats derived of IFFA-CREDO stocks and reared in the breeding center of our university were used. They were approximately 60 days old upon arrival at the laboratory and were housed (two per cage) in a controlled environment (lights on from 0700–1900 h, temperature 22°C) for 12 days before starting the experiment. Food and water were provided ad lib.

Animals were assigned to control and chronic IMO groups. Those assigned to the latter group were daily subjected in the morning to 2 h IMO in woodboards as described previously (16). On the morning of the 12th day, after IMO in chronically stressed rats, half the rats from each experimental group were administered saline IP and the other half 250 mg/kg α-methyl-p-thyrosine (Sigma Chemical Co., St. Louis, MO). On the following day, all rats were tested first in the holeboard for 4 min and second in the forced swim test for 5 min. These tests were carried out after two acute treatments: no stress or 2 h IMO.

The holeboard apparatus was similar to that described by File and Wardill (10) except the floor was divided into 16 areas of approximately the same size. The number of areas crossed, rearings, and head-dips were manually recorded. A dip was considered to take place when the head was introduced into the holes at least to the level of the eyes. In the forced swim test, rats were introduced in transparent cylindrical tanks similar to that described by Porsolt et al. (21,22,24), with water (25°C) up to 15 cm, and their behavior recorded by videotape. Behavior was scored from videotape by one experimenter unaware of the treatments of animals. The time spent making the following behaviors was measured with a stopwatch: a) struggling, which occurred when rats were diving, jumping, or strongly moving all four limbs, the front limbs breaking around the surface of the water or scratching the walls; b) mild swim, which occurred when rats swam around the tank while moving all four limbs; c) immobility, which occurred when rats remained motionless except to maintain the head out of the water.

The statistical significance of the results was evaluated by three-way analysis of variance (ANOVA), with previous chronic treatment (control, chronic IMO), drug (saline, αMpT), and acute treatment (no stress, IMO) as the main factors. Where appropriate, individual comparison of means was done with Student's t-test.

RESULTS

Table 1 indicates the results of the three-way ANOVA for the behavior in the holeboard, depicted in Fig. 1. In all cases, significant effects of the acute treatments were found, acute exposure to IMO always resulting in inhibition of behavior. Similarly, the drug exerted a profound inhibitory effect on all variables under investigation. The overall effects of previous chronic treatment reached statistical significance only in the case of rearing, but the interaction of chronic × acute treatment was significant for areas and rearings. Because of this interaction, the number of areas and rearings were directly compared after acute IMO in control and chronic IMO rats not treated with the drug: It was found that acute IMO induced less inhibition of both parameters in chronic IMO than in control rats ($p < 0.01$ in both cases). Any other interaction was significant.

TABLE 1

RESULTS OF THREE-WAY ANOVA OF BEHAVIOR IN THE HOLEBOARD

Factor	Areas	Rearing	Head-Dips
Chronic treatment (C)	NS	$p < 0.005$	NS
Acute Treatment (A)	$p < 0.001$	$p < 0.001$	$p = 0.05$
Drug	$p < 0.001$	$p < 0.001$	$p < 0.001$
C × A	$p < 0.02$	$p < 0.01$	NS
C × drug	NS	NS	NS
A × drug	$p < 0.05$	NS	NS
C × A × drug	NS	NS	NS

NS, not significant.

Table 2 indicates the results of the three-way ANOVA for the forced swim test measures (Fig.2). The drugs clearly potentiated immobility and decreased both struggling and mild swim activities. Acute treatment only exerted an overall significant inhibitory effect on struggling and the chronic treatment a negative effect on immobility. However, the interaction of chronic × acute treatment was highly significant for struggling. This interaction was clearly due to the fact that acute IMO induced an inhibition of struggling in control rats but not in chronic IMO rats irrespective of the drug treatment. The interaction of chronic treatment × drug was also significant for struggling and immobility in that the effect of the drug was more marked in chronic IMO than in control rats. To directly demonstrate this statistically, the performance of drug-treated rats was expressed as percentage of those of respective saline-treated rats and the effect of the drug in control and chronic IMO rats was directly compared with Student's t-test. It was found that either the inhibitory effect of the drug on struggling or the drug-induced potentiaton of immobility was less in control than in chronic IMO rats ($p < 0.001$ for the two variables).

DISCUSSION

The present data indicate that chronic IMO did not alter measures of activity/exploration in the holeboard as measured approximately 20 h after the last exposure to IMO. In the forced swim test, struggling behavior was not modified but chronic IMO animals had lower levels of immobility than control rats. Therefore, no obvious behavioral deficit was found in chronic IMO rats on the day following the last stress session, in keeping with previous results (20).

Acute IMO exerted a clear inhibitory effect on the number of areas crossed and rearings but less effects on more specific parameters of exploration such as head-dipping (10). The protective effect of previous chronic IMO on the acute IMO-induced behavioral deficit was significant for rearings and areas crossed but not for head-dips, indicating that a partial adaptation to the negative effects of acute IMO became apparent in chronically stressed rats, but not to the same extent in all parameters. The protective effect of chronic IMO was clear with regard to struggling behavior in the forced swim test. All these results fit well with previous data from our laboratory (20) and suggest that different behaviors have different dynamic patterns of adaptation to chronic repeated stress.

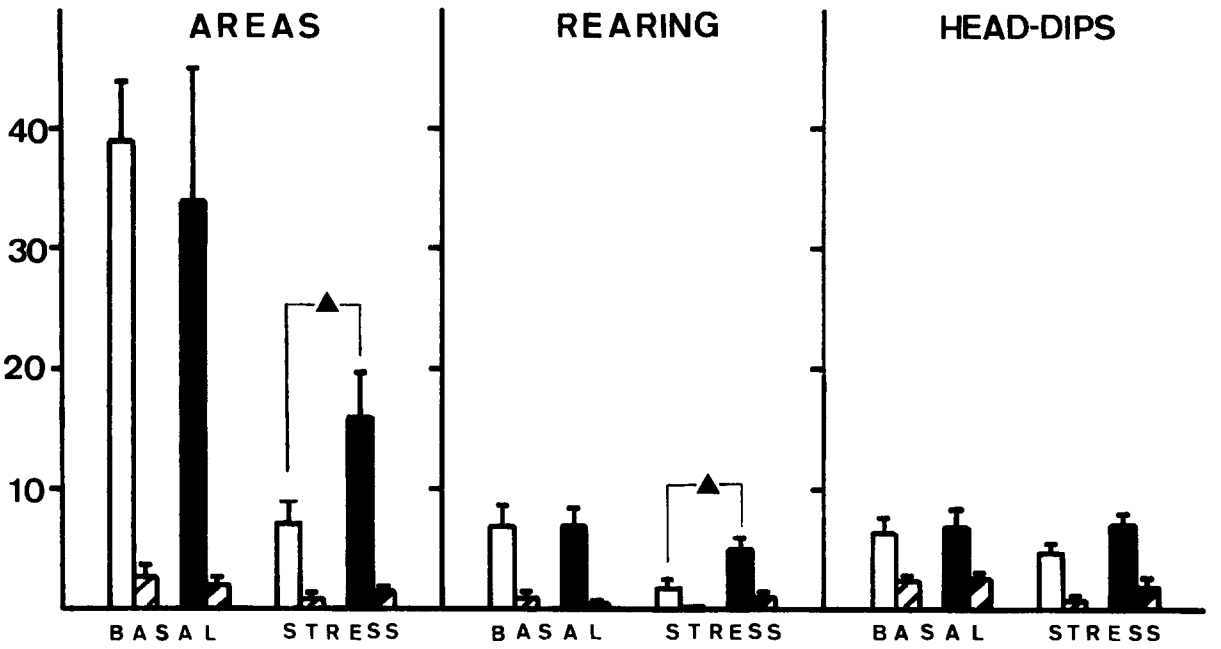

FIG. 1. Influence of previous chronic IMO on the effects of inhibition of catecholamine synthesis with αMpT on behavior of rats in the holeboard. Means and SEM (n = 6–10 per group except for control-saline rats subjected to acute IMO, in which n = 15) are represented. The groups were as follows: (□), control-saline; (▨), control-αMpT; (■), chronic IMO-saline; (▩), chronic IMO-αMpT. (▲)$p < 0.01$ between the signalled groups. For statistical analysis, see the text.

The main aim of the present report was to study the role of catecholamines in the behaviors under investigation and more specifically whether or not chronic IMO could have changed the influence of catecholamines on these behaviors. With regard to the first point, our data clearly indicate that catecholamines exerted a tonic and salient positive role on the activity of animals in the two tests. Nevertheless, quantitative differences between the two tests appear to exist. Thus, in the holeboard all measures were profoundly depressed by the inhibition of catecholamine synthesis with αMpT, whereas in the forced swim test the effects of the drug were consistent but less dramatic. That αMpT effectively inhibited catecholamine synthesis was directly assessed by measuring dopamine and noradrenaline levels in various brain regions (unpublished data). The positive effect of catecholamines in both situations is in accordance with previous reports attributing a role to both noradrenaline and dopamine, although the relative contribution of either dopamine or noradrenaline is a matter of dispute (11,13–15,19,23,27). However, our data also indicate that activity in the holeboard is more dependent upon catecholamines than forced swim activity. In the latter case, other neurotransmitters would play important roles. This could explain the wide range of drugs able to alter forced swim activity in rodents (8,36).

Regarding the second point, whereas the effects of the drug on holeboard activity were not altered by chronic IMO, the effects of the drug on both immobility and struggling were potentiated by chronic IMO, suggesting that the involvement of catecholamines in an escape-oriented response such as struggling (which in our hands usually displays a behavior negatively correlated to immobility) was enhanced by chronic IMO. To our knowledge, this is the first report of a change in the catecholaminergic control of these types of behaviors as a consequence of chronic stress. It is also noteworthy that after acute exposure to IMO the significant difference in struggling behavior between control and chronic IMO rats disappeared in drug-treated animals, suggesting that catecholamines were responsible for the different responses of control and chronic IMO rats to acute IMO with regard to this particular behavior in the forced swim test.

Although the present data suggest that chronic stress alters the catecholaminergic control of some behaviors in the forced swim test, we do not know at present whether these changes are related to depressive-like behavior or are merely a reflection of chronic stress-induced changes in the control of locomotor activity. The finding that chronic stress did not induce any change in the catecholaminergic control of holeboard activities argues against the latter possibility, but more studies are obviously needed. Both the susceptibility of chronically stressed rats to catecholamine depletion in other behavioral

TABLE 2

RESULTS OF THREE-WAY ANOVA OF BEHAVIOR IN THE FORCED SWIM TEST

Factor	Struggling	Mild Swim	Immobility
Chronic treatment (C)	NS	NS	$p < 0.01$
Acute Treatment (A)	$p < 0.02$	NS	NS
Drug	$p < 0.001$	$p < 0.001$	$p < 0.001$
C × A	$p < 0.001$	NS	NS
C × drug	$p < 0.001$	NS	$p < 0.05$
A × drug	NS	NS	NS
C × A × drug	NS	NS	$p < 0.05$

NS, not significant.

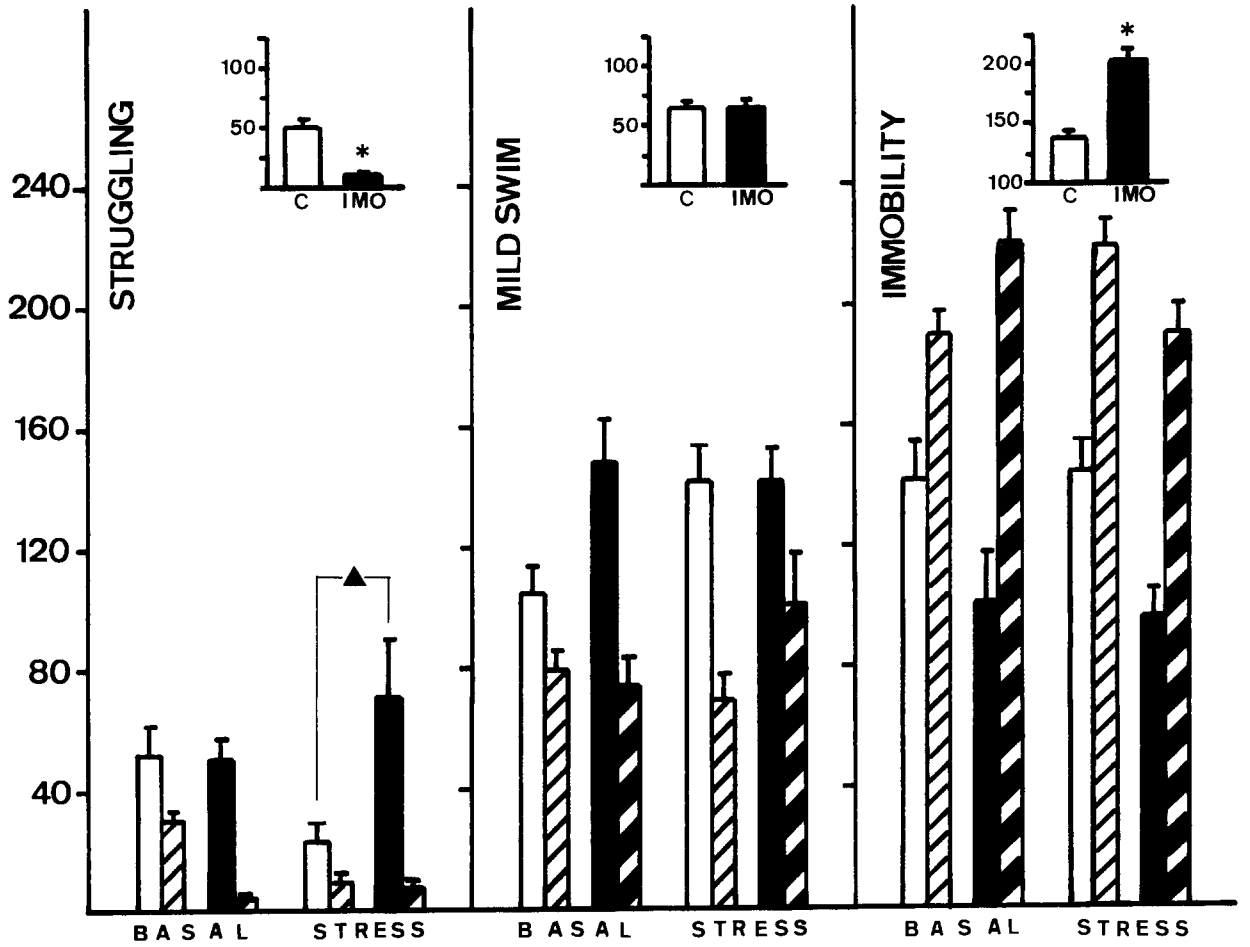

FIG. 2. Influence of previous chronic IMO on the effects of inhibition of catecholamine synthesis with αMpT on behavior of rats in a forced swim situation. Means and SEM of the time spent making each type of behavior (n = 7–10 per group except for control-saline rats exposed to acute IMO, in which n = 15) are represented in the large graphs. The groups were as follows: (□), control-saline; (▨), control-αMpT; (■), chronic IMO-saline; (◪), chronic IMO-αMpT. In the small graphs, the effect of αMpT in control (C) and chronic IMO (IMO) rats is represented as percentage of corresponding saline-treated groups (means and SEM), pooling both basal and stress values for each chronic treatment. *$p < 0.001$, (▲)$p < 0.01$ between the signaled groups. For other statistical analyses, see the text.

tasks possibly related to depression (e.g., learned helplessness) and the response of animals to dopaminergic and α- and β-adrenergic drugs could and should be directly tested in further studies.

These shifts in the control of struggling behavior do not appear to be due to enhanced tonic catecholaminergic activity in the brain of chronic IMO rats because neither MHPG-SO_4 levels in the pons plus medulla or hypothalamus (the only two areas we have studied) nor dihydroxyphenylacetic acid levels in midbrain, hypothalamus, and frontal cortex were altered by chronic IMO (unpublished data).

In conclusion, catecholamines appear to exert an important and tonic role in the maintenance of activity in the holeboard and a tonic but minor role in the control of active behaviors in a forced swim situation. After chronic exposure to IMO, the role of catecholamines became more important only with regard to struggling behavior in the forced swim test. The specific catecholaminergic system and the type of receptors involved in these chronic-stress induced changes remain to be determined.

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