Pharmacology Biochemistry & Behavior, Vol. 26, pp. 207–210. Copyright © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Effect of the Chronic Ingestion of Chlorimipramine and Desipramine on the Hole Board Response to Acute Stresses in Male Rats[1]

E. L. RODRÍGUEZ ECHANDÍA,[2] S. T. BROITMAN AND M. R. FÓSCOLO

Laboratorio de Investigaciones Cerebrales (LINCE-CONICET) Facultad de Ciencias Médicas Universidad Nacional de Cuyo, 5500 Mendoza, Argentina

Received 11 June 1986

RODRÍGUEZ ECHANDÍA, E. L., S. T. BROITMAN AND M. R. FÓSCOLO. *Effect of the chronic ingestion of chlorimipramine and desipramine on the hole board response to acute stresses in male rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 207–210, 1987.—The effect of the chronic ingestion of chlorimipramine (CI) or desipramine (DS) on the alterations of hole board behavior caused by a model stress (2 IP injections of physiological saline) and by a short restraint stress (5 min) is analyzed in this study. The experimental groups ingested about 3 mg/kg/24 hr CI or DS for 15 days. Then some experimental and control rats were assigned to control of drug effects on baseline activity. The remaining rats were submitted to saline stress (Experiment I) or restraint stress (Experiment II). The baseline scores of hole board locomotion, head dipping, grooming and defecation were not affected by DS treatment but locomotion slightly increased in the CI treated group. Saline stress impaired significantly head dipping and caused excessive grooming in control rats. The CI treatment induced almost full protection against these behavioral effects of saline stress but DS treatment was ineffective. Restraint stress was found to cause a pronounced inhibition of head dipping as well as a great increase of the scores of grooming in the control group. The CI treatment clearly attenuated these effects of restraint but DS treatment was not effective. The results suggest that (1) male rats treated chronically with CI tolerated both acute stresses better than untreated rats, and that (2) a similar treatment with DS did not provide protection against the effect of such stresses on hole board responding. Inasmuch as CI and DS have different relative potency at noradrenergic and serotonergic systems, it is speculated that this might be in part responsible for their differences as stress protectors.

Chlorimipramine Desipramine Tricyclics Acute stresses Restraint stress Saline stress Behavior Rats

DECREASES in some spontaneous behaviors in rats, i.e., locomotion, exploration and rearing, can occur as an aftereffect of exposure to a variety of stressful stimuli [18, 26, 30]. Other behaviors, e.g., grooming, can be stimulated by many stressors [1, 8, 20]. These effects are related apparently to stress-induced changes in monoaminergic [2, 9, 12, 20], peptidergic [1] and hormonal mechanisms [3–5, 6, 8, 10].

Though the relationships between stress and depressive illness remain unclear, it has been suggested that antidepressant drugs act to increase resistance to stress [16,25]. In fact there are neurochemical and behavioral similarities between chronic treatment with antidepressants and repeated exposure to stress in rats [24].

The chronic treatment with the secondary amine tricyclic, desipramine (DS), has been reported to attenuate the behavioral response to some acute and chronic stressors [7, 23, 26]. Though DS was not found to prevent the plasma corticosterone rising in response to restraint stress [26], it can attenuate the corticosteroid response in other stress models [23]. As pointed out by Stone [25], it remains to be investigated (1) whether antidepressant drugs can facilitate adaptation to a wide variety of unrelated stressors, and (2) whether different kinds of antidepressants exert similar protective action against stress.

Chronic treatment with DS causes a preferential subsensitivity of noradrenaline systems in the brain [7,28], while the tertiary amine tricyclic, chlorimipramine (CI), induces a down regulation of serotonergic (5-HT) systems [11, 14, 20]. Mild stresses were shown to cause depression of hole board behavior in rats [18,20]. The aim of the present study was to

[1]Supported by LINCE-CONICET Program.

[2]Requests for reprints should be addressed to Dr. E. L. Rodríguez Echandía, Laboratorio de Investigaciones Cerebrales, Casilla Correo 425, Mendoza, Argentina.

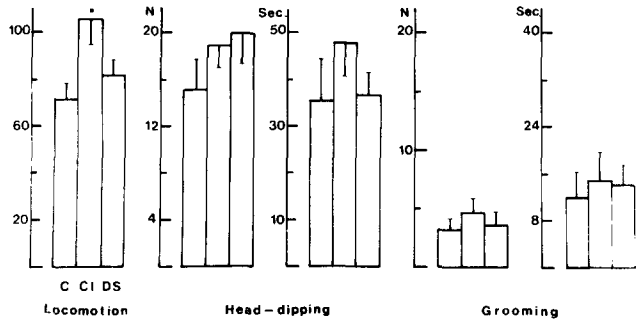

FIG. 1. Baseline scores of hole board locomotion, frequency (N°) and time spent (sec) head dipping and frequency (N°) and time spent (sec) grooming in 5 min trials (means±SEM). C: control columns. CI: chlorimipramine group. DS: desipramine treated group. $*p<0.05$ vs. control group (Duncan's test).

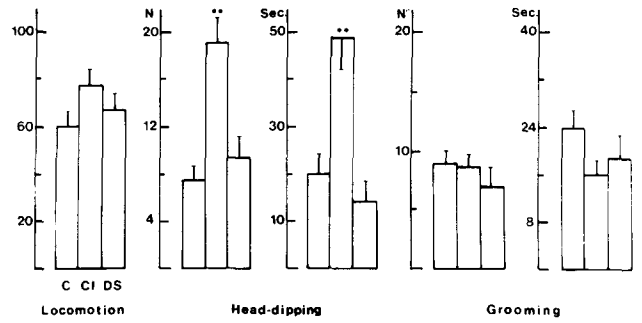

FIG. 2. Effect of saline stress on hole board activity in C, CI and DS treated groups (means±SEM). For explanation see Fig. 1. $**p<0.01$ vs. control group (Duncan's test).

analyze the effect of the chronic administration of CI and DS on the impairment of hole board behavior caused by a model of mild stress (2 IP injections of saline solution) and by a short restraint stress in male rats.

METHOD

Subjects and Drug Treatments

Male holtzman rats (about 200 g) were reared in groups of 5 and maintained under controlled temperature (23±2°C) and lighting (light on 0600 to 2000 hr). The experimental rats were provided with tap water containing 25 mg/kg of CI (n=36) or DS (n=37). The controls (n=38) received tap water devoid of drugs. Water intake was recorded daily and between-groups differences were not observed (results not shown). The mean amount of drug ingestion was 3.3±0.31 mg/kg/day for CI and 2.9±0.29 for DS ingesting rats. Fifteen days after beginning the treatment rats were divided into 3 groups. The first group was assigned to control drug effects on baseline activity (Experiment I). The other groups were submitted to Experiments II and III. Drug treatments were maintained up to the end of the experiments.

Experiment I

To determine eventual effects of drug treatments on baseline activity a group of CI (n=10), DS (n=11) and control rats (n=11) was tested in the hole board for the first time without further treatment. Data from this experiment were used as controls of Experiments II and III.

Experiment II

A second group of rats (CI n=13, DS n=13 and C n=14) was submitted to the stressor effect of 2 IP injections of physiological saline (0.4 ml/100 g) at 30 min intervals (CI-Saline, DS-Saline and C-Saline groups). These were tested in the hole board for the first time 30 min after stress.

Experiment III

The remaining rats (CI n=13, DS n=13 and C n=13) were submitted to restraint stress. The wire immobilization cages (15 cm length, 8 cm height and 7 cm width) had movable lids; when they were locked, the rats were blocked in a supine position and could not turn themselves around. They remained immobilized in that position for 5 min (CI-Restraining, DS-Restraining and C-Restraining groups). These groups were tested for the first time in the hole board immediately after stress.

Hole Board Test

The hole board was an open field (1.0 m^2) marked off in 20×20 cm squares with 16 holes (each 2 cm in diameter), spaced 20 cm apart from one another. The field was placed 20 cm above the floor and was illuminated by a 40 W lamp suspended over the center of the arena 3.0 m from the floor. Each rat was placed in the center of the field for a 5 min trial between 1200 and 1400 hr. The behaviors measured were: locomotion (number of squares crossed or entered defined as the whole of the animal entering the square), exploration (frequency and time spent head dipping), grooming (frequency and time spent) and incidence of defecation. Immediately after testing all rats were weighed; between-groups differences in weight were not detected (results not shown).

Statistics

Data within each experiment were analyzed by the one way analysis of variance (ANOVA) and the Duncan's multiple range test. Between-experiments comparison of data was done by the two way analysis of variance (ANOVA II) and the Scheffe's *t*-test for multiple comparisons. Data are presented as means±SEM. A level of probability less than 0.05 was considered significant.

RESULTS

Experiment I

The baseline scores of locomotion, head dipping and grooming of the CI, DS and C-groups selected for this experiment are illustrated in Fig. 1. DS treatment did not affect baseline activity. However, CI slightly increased locomotion

(CI vs. C: Duncan test $p<0.05$) without significantly affecting exploration and grooming. All groups showed similar incidence of defecation (C: 2.6±0.75, CI: 2.5±0.72, DS: 2.8±0.97).

Experiment II. Effect of CI and DS on the Behavioral Responses to Saline Stress

As reported elsewhere [18,20] saline stress decreased head dipping (C of Experiment I vs. C-Saline: Scheffe's test $p<0.05$ for frequency and time spent) and caused excessive grooming in control rats (C of Experiment I vs. C-Saline: Scheffe's test $p<0.05$ for frequency and time spent). The CI treatment attenuated the behavioral effects of this stress but DS treatment was ineffective.

Figure 2 shows that scores of locomotion of C-Saline, CI-Saline and DS-Saline groups were similar. Between-groups differences in the scores of head dipping were found, $F(2,30)=8.03$, $p<0.005$ for frequency, and $F(2,30)=5.24$, $p<0.002$ for time spent. This was due to the CIM-Saline group (Fig. 2) showing higher scores of exploration than D-Saline and C-Saline groups (Duncan's test $p<0.01$ for frequency and time spent). Between-groups differences in the frequency and time spent grooming did not attain statistical significance. The CI-Saline group, however, showed a tendency for lower scores of time spent grooming than C-Saline group (Fig. 2). The saline stress did not affect the scores of defecation in either group (results not shown).

Experiment III. Effect of CI and DS on the Behavioral Responses to Restraint Stress

Restraint stress was found to produce a pronounced impairment of exploration (C of Experiment I vs. C-Restraining group: Scheffe's test $p<0.001$ for both frequency and time spent head dipping) as well as a great stimulation of grooming in the control rats (C of Experiment I vs. C-Restraining group: Scheffe's test $p<0.001$ for frequency and time spent). The CI treatment attenuated these effects of restraint but the DS treatment was ineffective.

Between-groups differences in the scores of locomotion reached significance, $F(2,28)=1.16$, $p<0.01$. This was due to the CI-Restraining group (Fig. 3) which reached higher scores than DS-Restraining and C-Restraining groups (Duncan's test $p<0.05$). Between-groups differences in the scores of head dipping were also apparent, $F(2,28)=6.18$, $p<0.01$ for frequency, and $F(2,28)=4.77$, $p<0.02$ for time spent. This was due to the CI-Restraining group (Fig. 3) which showed greater exploration than the other groups (Duncan's test $p<0.01$ for frequency and time spent head dipping). Comparison of Figs. 1 and 3 shows that the scores of exploration of the CI-Restraining group approached the scores of C of Experiment I. Between-groups differences in the scores of grooming were also significant, $F(2,28)=3.70$, $p<0.05$ for frequency, and $F(2,28)=3.62$, $p<0.05$ for time spent. The CIM-Restraining group showed lower frequency and time spent grooming (Fig. 3) than DS-Restraining and C-Restraining groups (Duncan's test $p<0.01$). Between-groups differences in the scores of defecation were not significant (results not shown).

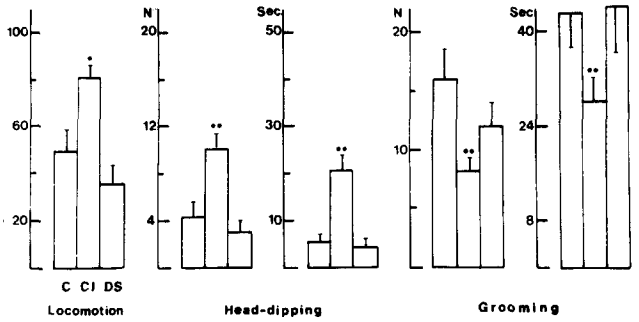

FIG. 3. Effect of restraint stress on hole board activity in C, CI and DS treated groups (means±SEM). For explanation see Fig. 1. *$p<0.05$ and **$p<0.01$ vs. control group (Duncan's test).

DISCUSSION

The results suggest that male rats treated chronically with 3 mg/kg/day CI tolerated the acute stressors better than untreated rats. CI induced almost full protection against the effects of the saline stress on hole board behavior. It also significantly protected against restraint stress. The fact that the chronic treatment with similar doses of DS did not affect hold board responding to stress is of interest and deserves discussion.

It is known that CI and DS are equipotent in their antidepressive action and are given in the same doses when used in the treatment of depression. It might be the case, however, that CI were more potent than DS in rats and that the doses chosen for DS were too low to provide stress protection. The fact that DS given chronically at high doses (20 mg/kg/day) failed to protect rats on either the reduction of exploration and the plasma corticosterone increase in response to restraint stress [26] is against this interpretation. Apparently, therefore, DS itself is not a drug suitable for prevention of some responses to acute stresses in rats. This does not preclude, however, that DS can prevent other responses to acute stress. In fact, chronic treatment with DS was shown to reduce the anorectic response to restraint and footshock in rats [26].

It has been suggested that various subgroups of stress responses might have different relationships with the neurotransmitter systems that are supposed to mediate stress effects [26]. Stress stimulates monoamine release in the aminergic terminals of the brain [2, 12, 15]. The fact that DS and CI have different relative potency at noradrenaline and 5-HT terminals might be in part responsible for their different action on stress responses. Since chronic treatment with DS caused a preferential subsensitivity of beta-adrenoceptors [29], its antistress action might be preferentially related to such effects on noradrenergic systems. Present experiments would suggest that DS-induced subsensitivity of noradrenergic systems would not affect significantly the hole board responses to acute stress in rats. This is of interest since other stress responses in rats have been associated mainly to noradrenergic activity. For instance, Porsolt [17] suggested that behavioral despair is most markedly affected by drugs acting on the noradrenergic system, whereas serotonergic systems would play a minor role.

The chronic treatment with secondary tricyclics such as CI causes a subsensitivity of 5-HT_2 receptors (see [27] for review) and a down regulation of the 5-HT systems [11, 18, 19]. It has been reported that chlordesipramine, the major metabolite of CI, is a potent noradrenaline uptake inhibitor [21]. In rats, however, CI would not be demethylated to its

secondary amine metabolite [12]. It is reasonable to speculate, therefore, that the inhibitory action of chronic CI on central 5-HT function may play an important role in preventing the stress-induced depression of hole board behavior. The following evidence is consistent with this speculation: (1) Stimulation of the 5-HT function by IP injections of the 5-HT uptake blocker, fluoxetine and the 5-HT precursor, 5-HTP were reported to cause hole board hypoactivity in rats. This effect can be attenuated by chronic treatment with CI or the acute administration of the 5-HT antagonist, methysergide [19]. (2) The 5-HT antagonists, methysergide and pizotifene, have been shown to prevent the excessive grooming observed in response to a mild stress [20]. This evidence does not preclude, however, that functional changes of other neurotransmitter systems may in part mediate the protective action of CI on the stress responses analyzed in this work.

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