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Chronic Treatment With Desipramine Induces an Estrous Cycle-Dependent Anxiolytic-Like Action in the Burying Behavior, But Not in the Elevated Plus-Maze Test

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FERNÁNDEZ-GUASTI, A., L. MARTÍNEZ-MOTA, E. ESTRADA-CAMARENA, C. M. CONTRERAS AND C. LÓPEZ-RUBALCAVA. *Chronic treatment with desipramine induces an estrous cycle dependent anxiolytic-like action in the burying behavior, but not in the elevated plus-maze test.* PHARMACOL BIOCHEM BEHAV **63**(1) 13–20, 1999.—The effect of chronic desipramine (DMI, 2.5 mg/kg × 21–26 days) treatment in female rats in two anxiety paradigms was assessed: the burying behavior (BB) and the elevated plus-maze (EPM) tests. In the BB test DMI produced a significant decrease in burying in ovariectomized rats, an effect considered as anxiolytic-like. In cycling females, DMI also reduced the cumulative BB most notably in proestrus rats. However, in diestrus rats no anxiolytic-like actions were observed. In addition, DMI increased BB latencies in proestrus and estrus rats. In the EPM test, DMI produced anxiolytic-like actions only in ovariectomized rats, while no significant actions were found in cycling females. Finally, the chronic treatment with DMI produced a general reduction in the ambulatory behavior of rats in all estrous cycle phases. Results are discussed on the basis of the differences between both anxiety paradigms and the probable relationship between the steroids secreted during proestrus and chronic DMI treatment. © 1999 Elsevier Science Inc.

Desipramine Burying behavior Elevated plus-maze Female estrous cycle Proestrus

SEVERAL compounds that are currently prescribed for various forms of depression have as an additional therapeutic effect the alleviation of anxiety (26,31,32,39,40). Desmethylimipramine or desipramine (DMI) is one of the tricyclic antidepressants most commonly used. When administered acutely, this compound produces an increased synaptic availability of norepinephrine, due to reuptake blockade. In addition, DMI decreases the effectiveness of electrical stimulation of the locus coeruleus by suppressing the firing of CA_3 pyramidal neurons in the hippocampus through the activation of alpha-2 adrenoceptors (13). With a chronic treatment, DMI has additional effects: it decreases the density of beta-adrenergic receptors (48), it increases the levels of 5-HT and dopamine (34), reduces the number of 5-HT_2 receptors in frontal cortex (43), and produces a functional upregulation of 5-HT_{1A} receptors (38).

Interestingly, important associations between DMI and the $GABA_A$ receptor complex have also been established. In 1984, Suranyi-Cadotte et al. (51) found that rats treated for 21 days with desipramine had a marked reduction in the number of benzodiazepine binding sites in the rat forebrain (52). In addition, Bouthillier and de Montigny (7) demonstrated that long-term, but not acute treatment with DMI, decreases the response of hippocampal neurons to iontophoretically applied

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flunitrazepam. Consistent with these data, in vitro studies have shown that DMI and its metabolite were able to antagonize the $GABA_A$ receptors (50). As to DMI's antidepressive action, it has been reported that picrotoxin and pentylenetetrazol are able to synergize DMI's action after chronic and subchronic treatments (22). Finally, Cannizzaro et al. (10,11) found that prenatal exposure to diazepam augments the antidepressive effect of DMI in adult rats.

In 1990 we demonstrated that the effect of compounds acting at the GABA–benzodiazepine complex, like diazepam, varies according to the estrous cycle phases (19). Thus, in the proestrus, this benzodiazepine produced stronger actions as compared with other phases like metestrus. This observation suggested that diazepam might be interacting with the steroid hormones present during the proestrus phase, most likely progesterone. The anxiolytic-like action of progesterone has been proposed since 1940 and confirmed by several authors, either by exogenous hormone administration (20,46) or by selecting endocrine phases characterized by high levels of this steroid such as the late proestrus (3,20) or midpregnancy (2,45). Furthermore, it has been shown that the anxiolytic-like action of progesterone or its derivatives depends on the stimulation of the GABA–benzodiazepine system (4,21). Interestingly, Martínez-Mota and Contreras (unpublished data) have found more robust antidepressive-like actions of DMI, in the forced swimming test, in proestrus females compared to the other estrous cycle phases. These actions were seen after a chronic 21-day treatment with DMI 2.5 mg/kg. This treatment schedule has been found to increase the discharge frequency of neurons in the lateral septum (42), an area commonly related to the modulation of anxiety (28).

As previously mentioned, DMI has been reported to posses anxiolytic-like actions in some animal models of anxiety (5,33,41,52). It is important to note that chronic treatments are necessary in order to observe these effects (52). Moreover, DMI and other tricyclic antidepressants are capable of producing both anxiogenic and anxiolytic actions, depending mainly on the treatment schedule used, i.e., acute vs. chronic (5,44).

In the present study, we analyze if chronic treatment with DMI produces anxiolytic-like actions in female rats tested in two behavioral paradigms of anxiety: the burying behavior (BB), and the elevated plus-maze (EPM) tests. These paradigms were selected on the bases of their advantages to reveal anxiolytic-like effects under various pharmacological (24,47, 53,54) and physiological conditions (3,17,20,45). We also decided to establish if the anxiolytic-like actions of DMI vary, depending on the estrous cycle phase. To be able to assess possible motor alterations, a general ambulatory behavior test was included.

METHODS

Animals

Adult female Wistar rats (180–200 g b.wt.) were used in this study. All animals were individually housed in a room under inverted and controlled light:dark cycle conditions (lights on at 2200–1000 hs). Animals had ad libitum access to water and Purina rat chow throughout the experiments.

Procedure

All behavioral tests were performed during the dark phase of the circadian cycle. Animals were tested first in the BB paradigm at least 1 h after the onset of darkness. Four hours after the BB, the EPM test was run.

Anxiety Tests

Burying behavior (BB) test. The BB test has been previously described in detail (54). Briefly, for this test a cage measuring 27 × 16 × 23 cm, the same dimensions as those of the home cages, was employed. The experimental cage contained an electrified prod (7 cm long) that emerges from one of its walls 2 cm above the bedding material consisting of fine sawdust. Every time the animal touched the prod it received an electric shock of 0.3 mA. The source of the shock was a constant current shocker (La Fayette Instruments Co., model 5806). The prod remained electrified through out the test. Immediately after the placement of the animal in the cage, its behavior was registered for 10 min. Once the animal received the first shock it typically moved towards the prod; the animal then sprayed and pushed a pile of bedding material ahead with rapid alternating movements of its forepaws. The parameters registered in this anxiety test were the cumulative burying behavior (cumulative time, in seconds, that the animals spent burying the prod) and the burying behavior latency (time in seconds from the first shock to the burying behavior display) (54).

Elevated plus-maze (EPM) test. The EPM test has been described in detail elsewhere (24). Briefly, the experimental device consisted of an elevated (40 cm above the floor), plus-shaped maze placed in a room illuminated by two 40-W red bulbs. The four arms were 50 cm long and 10 cm wide. The opposing arms were surrounded by white 40 cm high opaque plastic walls (closed arms), while the other arms lacked walls (open arms). The animal was removed from its home cage and placed in the center of the maze facing a closed arm. An observer, blindfolded to the treatment, was situated 2 m from the center of the maze. An entry into an arm was determined when the animal placed all four paws on one arm. The cumulative time spent in the open arms, the number of entries made into the open arms, and total number of crossings were recorded over a 10-min session. Data were expressed as percentage of the total time spent in open arms, total number of entries to open arms (these two parameters considered to reflect anxiety levels), and total arm entries (considered to reflect exploratory behavior).

Ambulatory behavior test. Immediately after the burying behavior test, general ambulation was registered in all animals. Ambulatory behavior was recorded in a box measuring 43 × 36 × 19 cm, that was placed over a sensitive plate (48 × 40 cm) of an activity meter (Stoelting Co., Chicago, IL, USA) connected to a counter (Stoelting Co., USA). Each animal was placed in the cage, and the number of counts recorded after a 10 min period. The data are expressed as mean number of counts in 10 min. In other studies, it has been demonstrated that the previous exposure to the BB test does not affect the ambulatory behavior of rats (18,37).

Experimental Procedure

Two main groups were included: ovariectomized, and intact female rats. Ovariectomy was performed under pentobarbital anesthesia. Through a ventral incision, the ovaries were recognized and removed; 2 weeks after ovariectomy the chronic treatment was begun. In the non-ovariectomized group, 8 days after the beginning of the chronic treatment with DMI or saline, vaginal smears were daily taken to establish the estrous cycle phase. Thus, the estrous cycle was registered during at least 13 consecutive days. Subgroups were established according to the estrous-cycle phases; these were identified based on the vaginal-cytology as follows: proestrus

(round, nucleated cells), estrus (cornified cells), metestrus (round, nucleated cells, cornified cells and leukocytes), and diestrus (predominance of leukocytes) (20). All animals were injected intraperitoneally (IP) with either 2.5 mg/kg DMI (Sigma Chemicals, St. Louis, MO, USA) in a volume of 0.2 ml or saline (0.2 ml). As a rule, animals received DMI treatment during 21 days. However, some animals could have received one to five more injections when searching for a specific cycle phase. The number of females included for each group varied between 10 and 23.

Animals were tested first in the BB paradigm 2 hs after the last injection of saline or DMI and, at least, 1 hs after the onset of darkness. Four hours after the BB, the EPM test was run.

Pilot Study

A pilot study was conducted in order to establish if the baseline behavior of rats and the action of the prototypical anxiolytic drug diazepam (1.0 mg/kg) in the EPM test were altered by previous exposure (4 hs before) of rats to the BB test. For this study, male Wistar rats (300–350 g) were individually housed with free access to food and water. Rats were divided into two groups: (a) animals tested in the BB paradigm and 4 hs later in the EPM ($n = 15$), and (b) animals tested only in the EPM at the time equivalent to that of group "a" ($n = 10$). Rats received either a saline or diazepam injection (IP 30 min prior to the EPM test).

Statistics

The Mann–Whitney *U*-test was used to compare DMI treated animals in each estrous cycle phase with their proper control group (saline treated animals). The comparisons of the proportion of animals displaying burying behavior were done using the Fisher *F*-test.

For the analysis of the possible interaction between DMI's treatment and the various estrous cycle phases, a two-way ANOVA test was performed.

RESULTS

The results of the pilot study, in which male rats were tested in the EPM with and without a previous experience on the BB, showed that the baseline behavior of rats was not different under the two experimental conditions [% time in open arms: 1.42 ± 0.39 (control) vs. 5.33 ± 1.98 (with BB), NS; open arm entries: 0.82 ± 0.23 (control) vs. 2.05 ± 0.54 (with BB), NS; total arm entries: 9.17 ± 0.08 (control) vs. 8.94 ± 1.27 (with BB), NS]. In addition, the anxiolytic-like effect of the prototypical anxiolytic drug, diazepam (1.0 mg/kg), on the EPM test was not different in rats exposed to both anxiety tests from those who were tested directly in the EPM test [% time in open arms: 12.10 ± 3.16 (control) vs. 13.66 ± 2.93 (with BB), NS; open arm entries: 4.50 ± 1.10 (control) vs. 5.57 ± 1.47 (with BB), NS; total arm entries: 9.12 ± 1.54 (control) vs. 12.0 ± 3.35 (with BB), NS].

Figure 1 shows the action of the long-term treatment with DMI on the EPM and the BB test in ovariectomized rats. In the EPM test, results show that DMI treatment produced significant increases in the percent of time that animals spent in the open arms, number of entries to open arms, and general exploratory behavior (expressed by the total number of crossing to open and closed arms). In the BB test, DMI produced a significant decrease in the cumulative BB, with a concomitant increase in the BB latency that did not reach statistical significance.

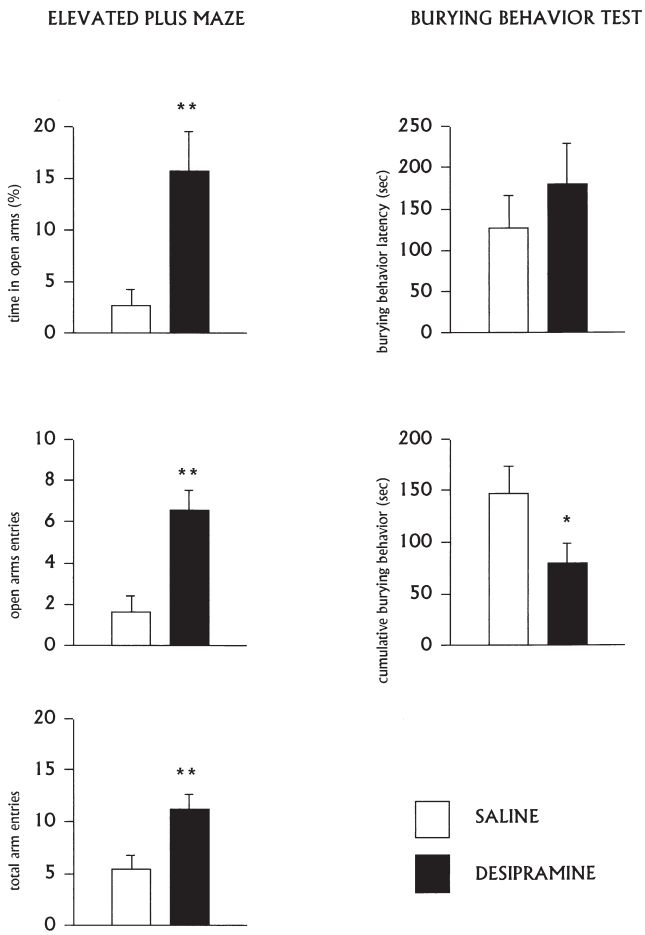

FIG. 1. Effect of chronic desipramine (DMI, 2.5 mg/kg × 21 days) or saline on ovariectomized rats in two animal models of anxiety: elevated plus maze and burying behavior tests. Mann–Whitney *U*-test: $*p < 0.05$; $**p < 0.02$.

The actions of DMI on the percentage of animals that expressed BB in the different phases of the estrous cycle are shown in Fig. 2 (panel A). In this graph, it is clear that DMI produced a significant decrease in the proportion of animals that display BB only in the proestrous phase. The analysis of the parameters registered in the BB test according to the different estrous cycle phases are shown in Fig. 2 (panels B and C). Only those animals who expressed burying behavior were considered for the statistical analysis. Thus, this figure compares the burying behavior latency (panel B) and the cumulative time spent burying (panel C) between DMI- and saline-treated female rats along the four estrous cycle phases. The results of the two-way ANOVA test for the BB latency were: treatment $F(1, 106) = 18.92$, $p < 0.001$; estrous cycle phases $F(3, 106) = 0.22$, $p < 0.87$, and in the interaction between both sources of variance $F(3, 106) = 0.85$, $p < 0.85$. A generalized increase in BB latency was observed in DMI-treated rats at all the estrous cycle phases, that was statistically significant only for proestrus and estrus rats when compared to their control saline-treated groups. Figure 2B shows the cumulative BB after chronic DMI in female rats during the various stages of the estrous cycle. Clearly, no differences in the saline-treated groups in this parameter were observed along the endocrine

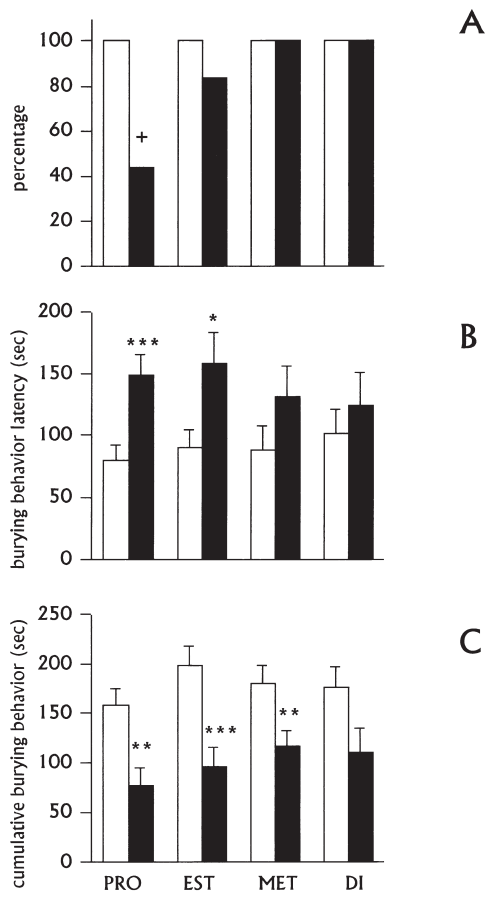

FIG. 2. Effect of chronic desipramine (DMI, 2.5 mg/kg × 21–26 days, solid bars) or saline (clear bars) in rats tested on the burying behavior paradigm. Percentage of animals displaying burying behavior (A), burying behavior latency (B), and cumulative burying behavior (C). Fisher F-test: $+p < 0.05$; Mann–Whitney U-test: $*p < 0.05$; $**p < 0.02$; $***p < 0.002$.

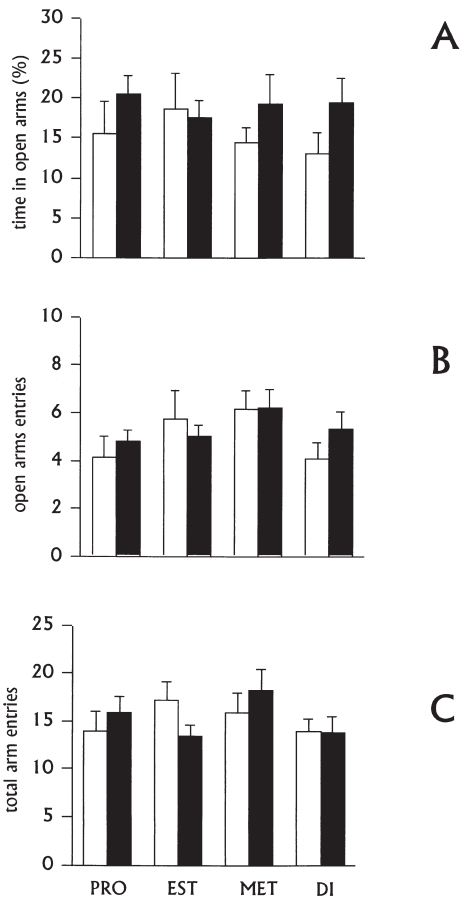

FIG. 3. Effect of chronic desipramine (DMI, 2.5 mg/kg × 21 days, solid bars) or saline (clear bars) on rats tested on the elevated plus-maze. Percentage of time in open bars (A), open arm entries (B) and total arm entries (C). Mann–Whitney U-test not significant.

cycle. The results of the two-way ANOVA test were: treatment $F(1, 106) = 29.62$, $p < 0.001$; estrous cycle phase $F(3, 106) = 1.09$, $p = 0.35$, and the interaction between these two parameters $F(3, 106) = 0.43$, $p = 0.73$. The comparisons between the treated vs. their proper control (saline-treated) group revealed a statistically significant reduction in burying behavior in the proestrus, estrous, and metestrus phases.

Figure 3 shows the effect of chronically injected DMI or saline on the elevated plus-maze test in female rats for the different phases of the estrous cycle. Two-way ANOVA after DMI treatment revealed no change in any of the parameters registered. Time in open arms: treatment $F(1, 112) = 4.20$, $p = 0.04$, phase $F(3, 112) = 0.13$, $p = 0.13$, interaction treatment × phase $F(3, 112) = 0.38$, $p = 0.76$. Number of entries to the open arms: treatment $F(1, 112) = 1.10$, $p = 0.29$, phase $F(1, 112) = 2.05$, $p = 0.11$, interaction treatment × phase $F(1,112) = 3.11$, $p = 0.55$. Total number of crossings: treatment $F(1, 112) = 0.002$, $p = 0.96$, phase $F(1,112) = 1.19$, $p = 0.31$, interaction treatment × phase $F(1,112) = 1.10$, $p = 0.29$.

The effects of DMI or saline on ambulatory behavior of both ovariectomized and intact female rats are shown in Table 1. DMI clearly reduced the number of counts in all females, independent of their endocrine stage.

DISCUSSION

In the present study, chronic treatment with DMI produces anxiolytic-like actions in ovariectomized female rats in two different anxiety paradigms: the burying behavior (BB) and the elevated plus-maze (EPM) tests. In cycling rats, these anxiolytic-like actions are also observed in the BB test at all estrous cycle phases but diestrus. However, the anxiolytic-like actions of DMI treatment in the EPM disappear in cycling rats.

Stress has been reported to be a potentially important factor that alters drug actions in the EPM test (30,16). For example, chlordiazepoxide anxiolytic-like effects on the EPM are abolished by administration of an electric shock immediately before testing (16). Based on this evidence, we conducted the pilot study to establish if previous exposure to the BB test has an effect on the behavior of animals tested in the EPM. The results obtained allows ruling out the possibility that the previous experience of rats in the BB test affects the results observed in the EPM test. Furthermore, the fact that in ovariectomized rats DMI is able to produce anxiolytic-like actions in both animal models of anxiety (present study) also discards the possibility that DMI actions could be affected by the previous exposure of rats to the BB test.

There is increasing evidence from animal models of anxiety (52) and clinical data (44) that the different classes of

TABLE 1
AMBULATORY BEHAVIOR

Condition	Treatment	No. of Counts (Mean ± SEM)	*n*
Ovarietctomized	Saline	271.54 ± 21.86	11
	DMI	193.27 ± 22.51†	11
Proestrus	Saline	289.71 ± 17.44	14
	DMI	219.28 ± 13.14†	14
Estrus	Saline	291.50 ± 28.11	15
	DMI	212.13 ± 16.26*	23
Metestrus	Saline	363.93 ± 32.24	14
	DMI	220.63 ± 21.70‡	11
Diestrus	Saline	312.13 ± 23.25	15
	DMI	216.10 ± 12.93‡	10

Mann–Whitney *U*-test: *$p < 0.05$; †$p < 0.02$; ‡$p < 0.002$.

antidepressants drugs, namely the tricyclic antidepressants, the monoamine oxidase inhibitors, and the selective serotonin reuptake inhibitors (SSRIs) possess anxiolytic-like activity. Present results obtained in ovariectomized rats, chronically treated with DMI, are in line with these findings. In this case, DMI is able to produce anxiolytic-like actions in the BB and the EPM test, both paradigms largely considered as reliable animal models of anxiety (30,53,54). As previously mentioned, antidepressants seem to require long-term administration to produce anxiolytic-like effects (5,44,52). Moreover, it has been reported that acute-treatment with DMI and other antidepressants produces anxiogenic-like actions (5,44). As an account for the paradoxical effects of DMI and other antidepressants (anxiolytic versus anxiogenic actions), it has been proposed that acute administration would produce increased synaptic availability of norepinephrine, due to reuptake blockade, and thus lead to an increased central arousal and peripheral symptoms that might result in anxiogenic-like actions (44). After chronic treatment, stabilization of noradrenergic activity would occur and the changes induced on other neurotransmitter systems (vide supra) altogether would account for the anxiolytic-like actions of DMI. It is interesting that, in the present study, the anxiolytic-like actions of DMI seem to be modifiable depending on the estrous cycle phase studied.

The results obtained with DMI or saline treatments in cycling rats show differences according to the estrous cycle phase and the animal model of anxiety used. Thus, in the BB test, almost a 60% of the proestrus rats does not show the BB response. Those showing the response (40%) as well as estrus rats exhibit an increase in BB latency (denoting decreased reactivity) and a reduction in BB (directly denoting anxiety levels). Endocrinologically, these stages are characterized by the presence of estrogen and increasing levels of progesterone (25). We and others have demonstrated that progesterone may induce anxiolytic-like responses in various anxiety paradigms including the burying behavior test (3,4,46). Thus, it could be proposed that DMI interacts with steroids to produce its action on burying behavior. Recent, nonpublished data from our laboratory show that DMI interacts with both estrogen and progesterone to induce anxiolytic-like effects. However, present findings showing clear effects of DMI in ovariectomized rats suggest that steroids might modulate DMI's anxiolytic-like effects rather than producing them. In accordance with this interpretation is the fact that DMI induces a trend towards an increase in burying behavior latency and to a reduction in the total burying behavior in all other phases of the estrous cycle.

The nature of the possible interaction between DMI and steroids, at present, remains unclear. It has been proposed that steroids may affect systems involved in the regulation of anxiety such as GABA–benzodiazepine and serotonin (3, 4,21). Most of the literature data shows that chronic DMI either antagonizes the $GABA_A$ receptor (50), or reduces its number (15). In addition, this antidepressant seems to synergize with picrotoxin and pentylenetetrazol to produce a decrease in immobility in the forced swimming test (22). Taken together, these data would argue against the idea of a positive interaction between DMI and an endogenous compound (possible progesterone) acting at the GABA/benzodiazepine system in the mediation of anxiolysis. However, there are also data supporting this positive interaction, i.e., Cannizzaro et al. (10) showed that after the stimulation of the benzodiazepine receptor the antidepressive action of DMI was potentiated. Suranyi-Cadotte et al. (51) reported that long-term desipramine treatment reduced the binding of the GABAergic compound [^{35}S] *t*-butylbicyclophosphorothionate (TBPS) in rat hippocampus. These authors propose that since TBPS inhibits GABAergic neurotransmission by blocking the GABA-gated chloride channels, the long-term effects of DMI might result in an increased capacity to generate chloride currents. This conclusion is in agreement with the ability of long-term desipramine treatment to increase GAD activity and $GABA_A$ receptors in rat hippocampus (49). Therefore, it is possible that DMI acts at the level of the chloride channel coupled to the GABA receptors to enhance GABAergic neurotransmission and thereby produce its anxiolytic-like activity. Further studies should be done in order to analyze if steroids can modify desipramine actions in GABA-gated chloride channels. Interestingly, Bitran et al. (2) showed that the efficacy of GABA-stimulated chloride transport was reduced in cortical tissue from ovariectomized females when compared to proestrus rats. This experiment supports the idea that the chloride channel associated to the GABA/benzodiazepine receptor complex can also be influenced by steroids. Further experiments should be undertaken to explore the possible association between the GABA/benzodiazepine system and DMI in the mediation of its anxiolytic-like effects.

An interesting observation from the present results is that, in the BB test, chronic DMI-treatment had different effects in ovariectomized rats and diestrus females. One could have expected that these two groups would behave similarly. However, there are differences between both groups that could account for the variation in the behavioral response. Thus, it has been found that ovariectomy induces a decrease in the density of dopamine receptors (6), a progressive increase in [^{3}H] flunitrazepam-specific binding associated with $GABA_A$ receptors (6), and some anatomical changes like a significant reduction in dendritic spine density in hippocampal pyramidal cells (27). Finally, ovariectomy affects corticosterone levels and adrenal weight (14). It is possible that some of these changes could modify DMI's activity and account for the differential response. However, specific experiments should be done in order to clarify this point.

It is important to mention that within the saline-treated animals no differences, neither in burying behavior latency nor in the time spent burying, were found along the various phases of the estrous cycle. This is in contrast to previous data from our and other laboratories (3,20) showing a reduction in BB at the proestrus phase. Nevertheless, the reasons for such difference could rely on the proestrus stage analyzed, i.e., late

versus early proestrus. In the present study, the hour of the day selected for our observations in the burying behavior test corresponded to the early proestrus, where no clear spontaneous anxiolytic-like actions have been found (20).

The anxiolytic-like actions of DMI observed in the BB paradigm, in some but not all of the estrous cycle phases, contrast with the lack of action of this drug in the EPM test at all estrous cycle phases. The reasons underlying the different anxiolytic-like profile of DMI in the two anxiety paradigms used in the present study remain unclear. However, a possible explanation could be based on the data reported by Handley (29) showing that for the monoaminergic regulation of anxiety, the stimulus that triggers anxiety as well as the nature of the response are of particular relevance. Thus, in the BB test the animal is confronted by an electrified prod that is recognized as an aversive stimulus and in which the expression of an active behavior, such as burying, denotes the anxiety state. Conversely, in the EPM test, curiosity and caution are evoked by a novel situation and the animal, rather than expressing an active behavior to confront the aversiveness (in this case altitude) it chooses to explore the non aversive area (close arms). Broekkamp et al. (9) proposed that the different animal models of anxiety may reflect distinct types of anxiety disorders, which may be dissimilarly regulated. Hence, it is possible that hormones influencing DMI's anxiolytic-like actions could also modify the stress response to the different stimuli provided by distinct animal models of anxiety.

The fact that DMI's anxiolytic-like actions also vary depending on the endocrine state of the rat makes the GABAergic system a good candidate to participate in the mediation of its anxiolytic-like actions. However, because chronic treatment with DMI produces changes in neurotransmitter systems other than GABA, the participation of other systems in the mediation of DMI's anxiolytic-like actions should be considered. For example, an interaction between DMI and the serotonergic system known to participate in the regulation of anxiety (23), could be presumed. Thus, DMI does not act on 5-HT_{1A} receptors (35) that are importantly involved in anxiety (18), but promotes a decrease in the number of 5-HT_{2A} receptors in the cerebral cortex (43), which participation in anxiety seems, at present, controversial (8). However, it is important to mention that these changes in 5-HT_{2A} receptors seem to be related to DMI's antidepressive-like actions (8). Finally, the possible relationship between the noradrenergic and GABAergic systems in the control of anxiety should not be discarded (12,36,37).

From the present data it could be claimed that the effects on burying behavior observed after chronic DMI treatment are due to the reduction of general activity. However, several data argue against this deduction. Firstly, the actions of DMI on ambulatory behavior are seen in all the estrous cycle phases and not only in proestrus. Second, treatment with DMI does not affect the exploratory behavior tested as the number of total crossings in the elevated plus-maze. Finally, chronic DMI treatment had no actions in a motor coordination test using the rota rod (data not shown). Therefore, the present results suggest that the effects observed with chronic DMI are specific upon some parameters denoting reactivity and anxiety.

It is important to mention that Beardslee et al. in 1990 (1) reported that chronic treatment with imipramine, desipramine, and pargyline to female rats failed to induce anxiolytic-like effects on any measures of defensive burying. These results are in contrast to present data. Although, some important variations between both studies could explain the differential results, such as the dose and the duration of DMI treatment (5 mg/kg for 8 weeks vs. 2.5 mg/kg for 3–4 weeks), the rat's strain (Sprague–Dawley versus Wistar) (53), the shock intensity (3 vs. 0.3 mA) (54), and the experimental cage measures ($27 \times 16 \times 23$ cm vs. $40 \times 30 \times 40$ cm) (53), the reasons underlying the difference are most likely to be related to the endocrine stage of cycling females. Thus, in the study performed by Beardslee et al. the estrous cycle was not recorded and, hence, their sample most likely included in both proestrus and diestrus rats (1). This last variation could mask the effect of DMI on the various parameters of burying behavior recorded, because, in the present study, this drug has a robust effect in rats in proestrus and almost lacks an action in diestrus animals. Additionally, it is worth mentioning that in that study only five female rats were included in the DMI experiment. This low number of subjects could also contribute to obscure the actions of this treatment.

In conclusion, the present study shows that the chronic treatment with DMI produces anxiolytic-like actions in the burying behavior and in the elevated plus-maze tests. These anxiolytic-like actions seem to be modifiable, depending on the endocrinological state of female rats. In addition, there are important differences between the two animal models of anxiety. Specifically, the elevated plus-maze test appears to be less sensitive to the anxiolytic-like actions of DMI and only in absence of ovarian hormones makes it possible to observe DMI's anxiolytic-like activity.

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