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Perinatally Protein-Deprived Rats and Reactivity to Anxiolytic Drugs in the Plus-Maze Test: An Animal Model for Screening Antipanic Agents?

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LAINO, C. H., N. E. CORDOBA AND O. A. ORSINGHER. *Perinatally protein-deprived rats and reactivity to anxiolytic drugs in the plus-maze test: An animal model for screening antipanic agents?* PHARMACOL BIOCHEM BEHAV **46**(1) 89–94, 1993.—Adult rats submitted to a protein deprivation schedule at perinatal age (from 14th day of fetal life until 50 days of age) and then recovered on balanced chow (D rats) were assayed in the elevated plus-maze test for anticonflict effects of diazepam and drugs with therapeutic efficacy in panic disorders as compared with controls (C rats). Diazepam and alprazolam showed a similar anticonflict effect in D rats than in C rats. In contrast, buspirone, which was ineffective in C rats at a wide dosage range, showed a significant anticonflict effect on D rats at 0.3 mg/kg. Neither propranolol, desipramine, nor phenelzine treatment (10 mg/kg/day during 3–7 days) induced anticonflict effect in C rats. Conversely, these treatments fostered a significant and selective anxiolytic effect on D rats. Such results underscore long-lasting alterations caused by early undernutrition, namely, changes in reactivity to the drugs assayed. In addition, perinatally deprived rats may represent a useful animal model for studying potential antipanic agents.

Perinatal undernutrition Panic disorders Elevated plus-maze test Diazepam
Buspirone Alprazolam Phenelzine Propranolol Desipramine

PROTEIN deprivation at perinatal age has long-lasting effects on morphological, neurochemical, neurological, and behavioral parameters that persist in adulthood even following prolonged periods of nutritional recovery (25). Alterations in different neurotransmitter systems (40), which may account for changes in the reactivity to central agonists on different effects, such as hypothermic (7), hypnotic (12), anxiolytic (1,8), and stimulant (6,18), have also been reported. As concerns anxiolytic drugs, a smaller anticonflict effect of benzodiazepines was detected in early malnourished rats tested on different tasks (2,3,8,11).

Anxiolytic as well as anxiogenic agents have been validated in the elevated plus-maze. This test has the advantage of measuring spontaneous behavior in absence of noxious stimuli and food or water deprivation (29). However, drugs regularly used in the treatment of panic disorder, such as tricyclic antidepressants, monoamine oxidase inhibitors, or propranolol, have regularly been found to exert no anticonflict effects in this task (4,14,15). Panic disorder is a type of anxiety characterized by unexpected and recurrent panic attacks, with or without agoraphobia (DSMIIIR, 1987), on which therapy with most common benzodiazepines (diazepam, chlordiazepoxide) becomes ineffective (33). Patients suffering from panic attacks showed a generalized activation of the noradrenergic system, both at central and peripheral levels, with somatic and autonomic symptoms (9,41). On the other hand, perinatally deprived rats showed alterations in noradrenergic neurotransmission (13,21,22,24) that resembled those of patients under panic attacks. Consequently, we assessed in adult rats submitted to a protein deprivation schedule at perinatal age (from 14th day of fetal life until 50 days of age) and then recovered on balanced chow (D rats) by means of the elevated plus-maze test the anticonflict effect of different drugs used in the treatment of panic disorders, such as alprazolam (31,34,35), buspirone (32), propranolol (17,20), desipramine, and phenelzine (39,42) as compared with the effect of diazepam.

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METHOD

Animals

A protein deprivation schedule as previously described (24), with minor modifications, was used. Briefly, pregnant female Wistar rats, from our own colony, were divided into two groups at 14 days of pregnancy, housed in individual polyethylene cages, and fed isocaloric diets containing 24 or 8% casein for controls (C) and deprived (D) rats, respectively. Both diets were supplemented with DL-methionine (4 g/kg). At birth, litters were culled to eight pups. After weaning (30 days), pups continued consuming the same diet as dams until the end of the deprivation period (50 days of age). Thereafter, both groups were given balanced laboratory chow for at least 40 days prior to assays. Under this deprivation schedule, there were no interlitter differences between control and experimental groups in terms of length of gestation, number of pups per litter (regularly 8–12), and male to female ratio (close to 1). Twenty-four hours after birth, undernourished litters weighed significantly less than controls. Pups weighed 5.6 ± 0.2 and 3.9 ± 0.2 g for C and D rats, respectively ($p < 0.05$, Student's t-test). Average mortality as a result of this procedure was lower than 10%. Control and deprived groups comprised rats from different litters to avoid sibling replication. Preliminary trials showed that male and female rats had the same reactivity to the drugs assayed in the plus-maze test. Yet, in the present experiment female rats were employed due to a lower variability of results obtained, most likely on account of their lower size, which allowed a better displacement in the maze. Body weights at the time of experiments were 227 ± 2 and 191 ± 2 g for C and D rats, respectively ($p < 0.05$, Student's t-test). Animals were maintained in groups of six per cage at 22 ± 2°C on a 12 L : 12 D cycle (light on at 7:00 a.m.), with food and water ad lib and handled twice a week for cage cleaning.

Elevated Plus-maze Test

The elevated plus-maze consisted of two open arms (50 × 10 cm) and two enclosed arms of the same size with 40-cm high walls, devised so that one side of the plus-maze was opposite to an identical side (i.e., either open or enclosed) with a 10-cm square in the middle. The device was wooden and raised to a height of 50 cm from the floor (28). Each rat was placed in the middle of the maze facing a closed arm for a 5-min session, during which the number of arm entries and the time spent on each arm were recorded. An index of anxiety was obtained by expressing the time spent on the open arms as a percentage of time spent on both open and closed arms. Total number of arm entries was registered as an index of motor activity. Measurements were taken between 10:00 a.m and 4:00 p.m. by an observer seated 1 m away from the center of the device. Animals were tested only once, that is, naive C and D rats were used in each experimental group.

Drugs

Buspirone HCl (Lab Gador, Buenos Aires, Argentina), diazepam (Valium, Roche, Buenos Aires, Argentina), desipramine HCl (Lab Montpellier, Buenos Aires, Argentina), DL-propranolol (Sigma Chemical Co., St. Louis, MO), alprazolam (Lab Andrómaco, Buenos Aires, Argentina), and phenelzine sulfate (Lab Prest, Buenos Aires, Argentina) were used. Diazepam was suspended in distilled water containing 2% Tween-80. The remaining drugs were dissolved in 0.9% NaCl solution.

Procedure

During acute experiments, C and D rats were injected IP with diazepam (0.3 and 1.0 mg/kg), alprazolam (0.03, 0.1, and 0.3 mg/kg), buspirone (0.3 and 1.0 mg/kg), or vehicle (1 ml/kg) 30 min before trials. Desipramine, phenelzine, or propranolol (10 mg/kg) were injected IP daily during 3 or 7 days. Controls received saline (1 ml/kg/day). Animals were tested 1 h after the last injection of desipramine or 24 h following phenelzine or propranolol treatment.

Statistics

Differences between groups were analyzed by two-way analysis of variance (ANOVA) and subsequent posthoc comparisons were performed using Fisher's least significant difference test with a probability of type I error set at 0.05.

RESULTS

In saline-treated groups, D rats showed a tendency to spend less time in the open arms than C rats, although there were not significant differences (Figs. 1–6).

Administration of diazepam (0.3 and 1.0 mg/kg) dose dependently increased the percentage of time spent on the open arms in C and D rats, $F(2, 38) = 9.7$, $p < 0.01$ (Fig. 1). ANOVA demonstrates a significant diet effect, $F(1, 38) = 4.7$, $p < 0.05$. However, no significant drug × diet interaction was noticed. Open/total ratio after diazepam administration was lower in D rats than in C rats at the dose of 1.0 mg/kg.

Dose–response curves of the anxiolytic effect of alprazolam are shown in Fig. 2. Analysis of the data indicated a significant drug effect, $F(3, 111) = 14.5$, $p < 0.01$, but no significant diet effect, $F(1, 111) = 0.090$, $p > 0.05$. The lower dose of alprazolam (0.03 mg/kg), which showed no anticonflict effect in C rats, significantly increased the open/total ratio in D rats. However, no drug × diet interaction was observed.

Buspirone exhibited no anticonflict effect in C rats at any

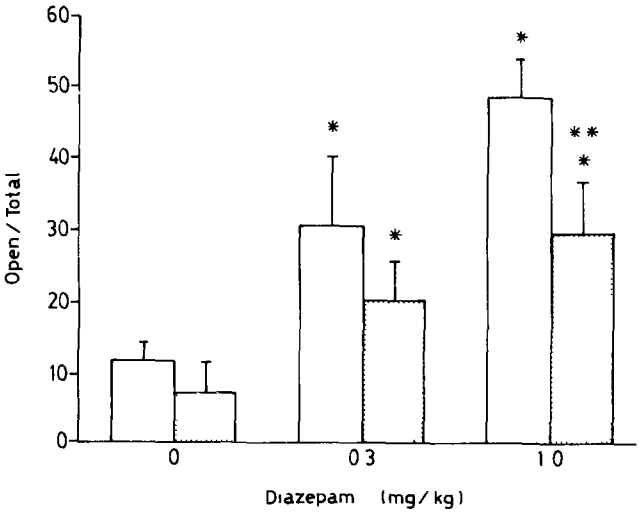

FIG. 1. Anticonflict effect of diazepam in C (open bars) and D (shaded bars) rats in the plus-maze test. Shown are the mean ± SEM of five to six rats. *$p < 0.05$ as compared with appropriate saline group. **$p < 0.05$ as compared with C rats.

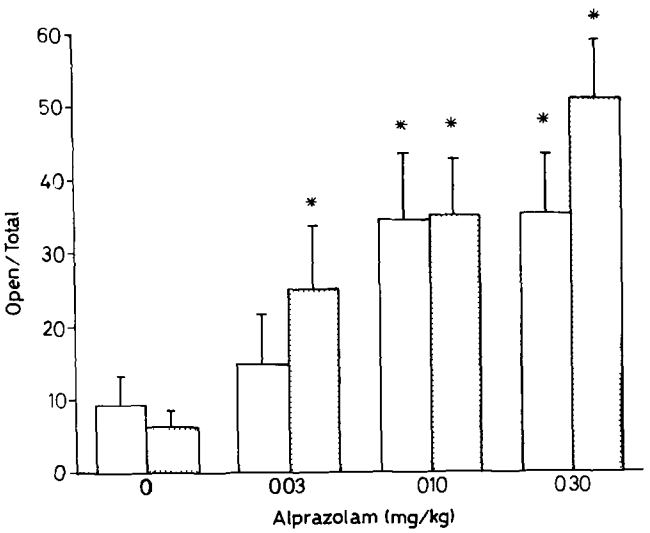

FIG. 2. Effect of various doses of alprazolam in C (open bars) and D (shaded bars) rats in the plus-maze test. Shown are the mean ± SEM of 8–17 rats. *$p < 0.01$ as compared with appropriate saline group.

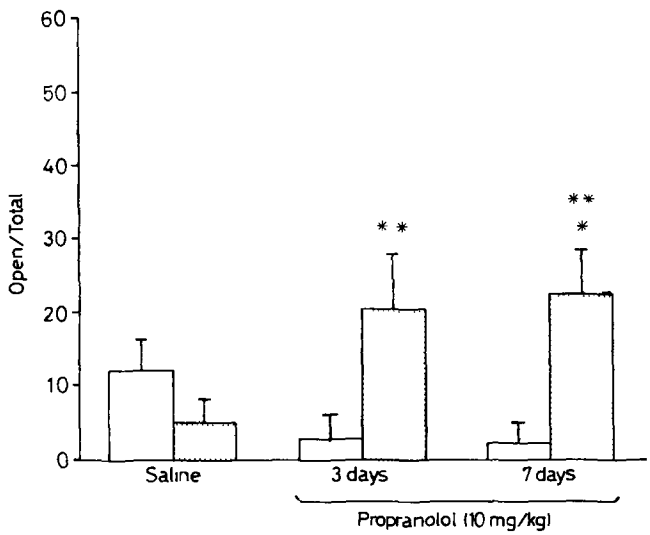

FIG. 4. Effect of propranolol in C (open bars) and D (shaded bars) rats in the plus-maze test after 3 or 7 days of treatment. Bars are the mean ± SEM of 7–12 rats. *$p < 0.05$ as compared with saline D rats. **$p < 0.05$ as compared with respective C rats.

of the doses used (Fig. 3). In D rats, 0.3 mg/kg increased significantly the rate of open/total time. ANOVA revealed a drug effect, $F(2, 66) = 7.1$, $p < 0.01$, and a drug × diet interaction, $F(2, 66) = 6.7$, $p < 0.01$.

Figure 4 illustrates the effect of 3- or 7-day propranolol treatment (10 mg/kg/day), during which a significant effect of the diet, $F(1, 64) = 7.8$, $p < 0.01$, together with a drug × diet interaction, $F(2, 64) = 4.5$, $p < 0.05$, was detected. Propranolol was not effective in C rats. In contrast, administration during 3 or 7 days significantly increased the anticonflict activity in D rats.

Figure 5 shows the effect of repeated desipramine administration (10 mg/kg/day). Statistical analysis evidenced significant diet effect, $F(1, 52) = 5.5$, $p < 0.01$, as well as drug × diet interaction, $F(2, 54) = 3.8$, $p < 0.05$. Desipramine, which did not modify C rats, induced a clear anticonflict effect in D rats as compared with their controls.

Repeated phenelzine administration (10 mg/kg) showed a pattern similar to that observed with desipramine treatment, that is, lack of effects in C rats and a time-dependent anticonflict activity in D rats, that reached statistical significance after 7 days of treatment. ANOVA indicated a significant drug, $F(2, 75) = 5.2$, $p < 0.01$, diet, $F(2, 75) = 7.1$, $p < 0.01$, ef-

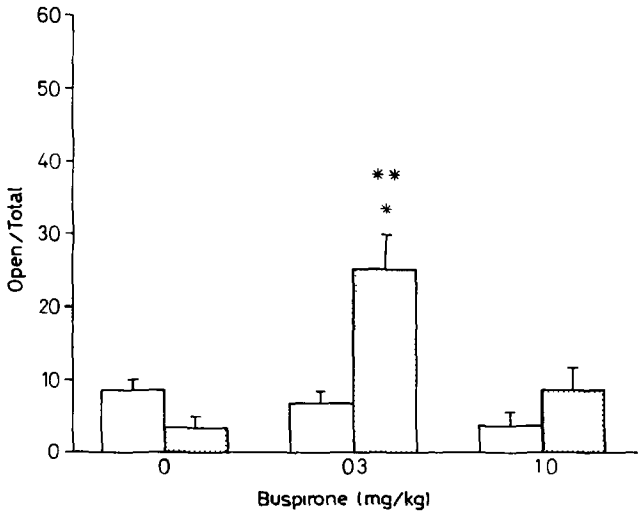

FIG. 3. Anticonflict effect of buspirone in C (open bars) and D (shaded bars) rats. Shown are the mean ± SEM of 16–26 rats. *$p < 0.01$ as compared with respective saline group. **$p < 0.01$ as compared with C rats.

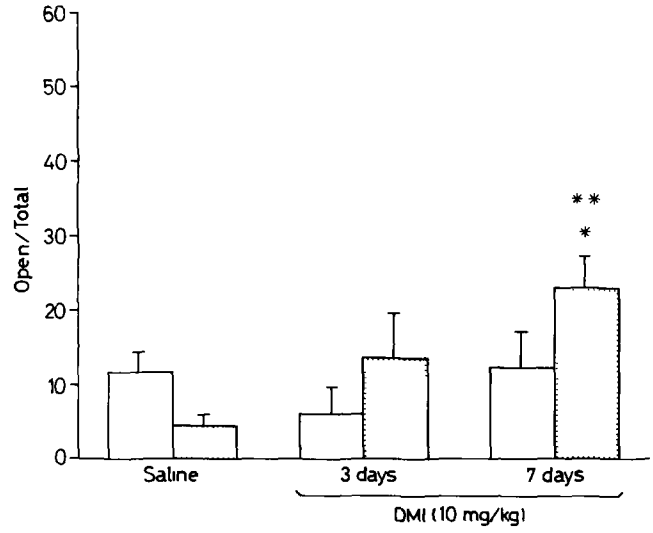

FIG. 5. Effect of desipramine in C (open bars) and D (shaded bars) rats in the plus-maze test. Each value represents the mean ± SEM of 8–14 rats. *$p < 0.05$ as compared with saline D rats. **$p < 0.05$ as compared with respective C rats.

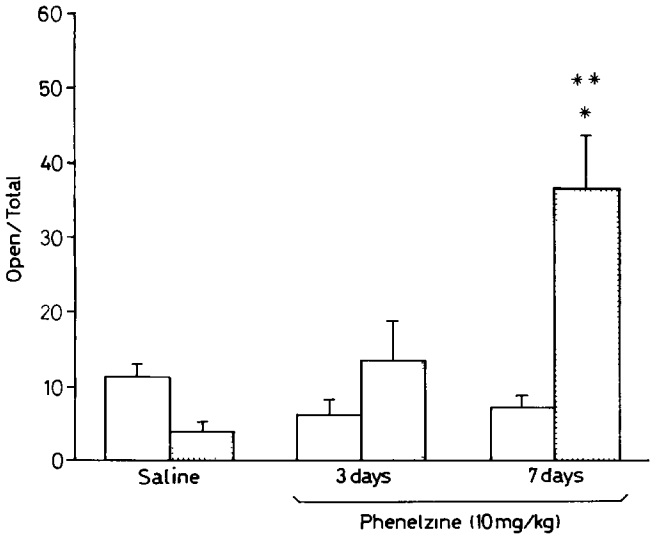

FIG. 6. Effect of phenelzine in C (open bars) and D (shaded bars) rats in the plus-maze test after 3 or 7 days of treatment. Bars are the mean ± SEM of 8–16 rats. *$p < 0.01$ as compared with saline D rats. **$p < 0.01$ as compared with respective C rats.

fect, and drug × diet interaction, $F(2, 75) = 7.1$, $p < 0.01$ (Fig. 6).

As seen in Table 1, the total number of arm entries made during the 5-min test period was unaffected by most treatments. However, buspirone slightly reduced the number of entries in C rats but not in D rats. Following desipramine administration, a decrease in this parameter was observed for C and D rats after 3 days but not after 7 days of treatment. Conversely, the higher dose of alprazolam used (0.3 mg/kg) slightly increased the number of entries in C and D rats. Diazepam, phenelzine, and propranolol did not alter this index at any doses used.

DISCUSSION

Perinatal undernutrition induces behavioral abnormalities, generally ascribed to increased sensitivity to stressful or aversive situations (5,23,36). In previous reports, we described that under basal conditions D rats spent less time than C rats in the open arms of the elevated plus-maze, a finding assigned as an index of basal anxiety and/or fear to experimental events (11,12). However, in the present experiments, although this phenomenon was observed in all saline-treated groups, the tendency was nonsignificant. Diazepam and alprazolam displayed equal anticonflict potency in normal rats both in Vogel's test and the plus-maze test (37). Early malnutrition induced lower reactivity to the anxiolytic effect of diazepam either with the Geller-Seifter, light-dark transitions (8), or saltsuppressed drinking tasks (2). Our experiments with diazepam and alprazolam showed no drug × diet interaction, suggesting a similar reactivity to these drugs in D and C rats. Explicit comparisons based upon the open/total ratio after 1 mg/kg diazepam showed a significant lower value in D rats as compared with C rats, whereas 0.3 mg/kg alprazolam showed a higher response in D than C rats.

Concerning buspirone, anxiogenic, anxiolytic, or no effects have been reported for the plus-maze test (26–30). Recently,

TABLE 1

EFFECTS OF DIFFERENT TREATMENTS ON THE TOTAL NUMBER OF ENTRIES MADE IN THE PLUS-MAZE TEST

	Doses (mg/kg/day)	Treatment	C rats	D rats
Saline	–	Acute	9.5 ± 2.5 (6)	5.8 ± 1.1 (6)
Diazepam	0.3	Acute	9.2 ± 1.2 (5)	9.0 ± 2.3 (5)
Diazepam	1.0	Acute	12.4 ± 1.1 (5)	8.2 ± 2.2 (5)
Saline	–	Acute	7.4 ± 0.9 (16)	5.4 ± 0.6 (16)
Buspirone	0.3	Acute	3.9 ± 0.5 (16)*	6.4 ± 0.8 (16)
Buspirone	1.0	Acute	4.0 ± 0.7 (11)*	4.4 ± 0.6 (11)
Saline	–	Acute	5.4 ± 1.0 (13)	6.1 ± 1.0 (13)
Alprazolam	0.03	Acute	6.6 ± 1.9 (8)	7.9 ± 1.1 (8)
Alprazolam	0.10	Acute	7.4 ± 1.1 (16)	7.2 ± 1.0 (17)
Alprazolam	0.30	Acute	8.3 ± 1.2 (13)*	10.6 ± 0.9 (9)*
Saline	–	3/7 days	4.9 ± 0.8 (16)	5.8 ± 0.9 (16)
Phenelzine	10	3 days	5.2 ± 1.2 (8)	7.9 ± 1.1 (8)
Phenelzine	10	7 days	5.9 ± 0.7 (10)	6.5 ± 0.7 (11)
Saline	–	3/7 days	7.1 ± 1.0 (14)	5.8 ± 0.8 (14)
Desipramine	10	3 days	2.9 ± 0.7 (8)*	2.7 ± 0.9 (8)*
Desipramine	10	7 days	5.0 ± 1.0 (10)	6.9 ± 1.2 (8)
Saline	–	3/7 days	4.9 ± 0.9 (12)	6.3 ± 1.0 (12)
Propranolol	10	3 days	4.6 ± 1.2 (7)	7.0 ± 1.5 (8)
Propranolol	10	7 days	3.5 ± 0.7 (8)	6.5 ± 1.1 (8)

Values are expressed as mean ± SEM. Chronic saline represents pooled values of 3 and 7 days. In parentheses, number of animals in each group is indicated.

*$p < 0.05$ with respect to comparable group treated with saline.

Soderpalm et al. (38) described an anticonflict effect at a low dosage (about 12 μg/kg) and an opposite effect at higher doses (about 800 μg/kg). In view of the inverted U-shaped curves obtained, these authors suggest that anxiolytic or anxiogenic effects may result from predominance of pre- or postsynaptic activation of 5-hydroxytryptamine (5-HT) receptors in neuronal pathways involved in the regulation of anxiety. As regards early malnourished rats, it was described that ipsapirone, a buspirone analog, in a range of 0.5–5 mg/kg exerted an anxiogenic rather than anxiolytic effect in early malnourished rats (3). In our experiments, buspirone, which was ineffective in C rats, displayed in D rats a significant anxiolytic effect at 0.3 mg/kg but not at 1.0 mg/kg. Lower doses (4–64 μg/kg) were ineffective in both C and D rats (data not shown). Differences in the dosage used and/or methodological procedure may account for the discrepancies between our results and those obtained with ipsapirone in malnourished rats.

Consistent with different reports that described lack of anxiolytic effects of propranolol, desipramine, or phenelzine treatments in the elevated plus-maze, none of these drugs exert any anticonflict effect on C rats. Conversely, a significant anxiolytic activity was noted in D rats after 3 or 7 days of propranolol administration and after 7 days in desipramine and phenelzine groups, indicating a definite time-dependent effect for the last two treatments.

In general, the total number of entries made into the arms did not evidence any important changes for any of the treatments. Moreover, in the few changes observed there is not a clearcut correlation between modifications in motor activity and anxiolytic effects.

To summarize, the present data demonstrate that while drugs acting through the GABA–BDZ receptor complex, such as diazepam and alprazolam, showed a similar anticonflict effect in C and D rats drugs that interact with noradrenergic and/or serotonergic system (buspirone, propranolol, desipramine, and phenelzine) exerted in D rats a selective and significant anticonflict effect in the plus-maze test.

Concerning the pathophysiology of anxiety disorders, panic attacks have been associated with hyperreactivity of noradrenergic functions, while the supramolecular GABA–BDZ receptor complex may be involved in the etiology of generalized anxiety disorders (19). In this regard, a thorough study on patients suffering agoraphobia and panic attacks demonstrate that administration of yohimbine, an α_2-adrenergic blocking agent that increases noradrenergic activity, induces higher plasmatic levels of the noradrenaline (NA) metabolite 3,methoxy-4-hydroxyphenylglycol (MHPG), as well as increased blood pressure and pulse rate plus subjective and somatic symptoms when compared with healthy subjects (10). These alterations in the noradrenergic system are similar to changes observed in adult rats submitted to our deprivation schedule, in which an increased turnover rate of brain noradrenaline, without changes in noradrenaline content, suggests a higher than normal neuronal activity (26) that induces a reduction in brain adrenergic receptors density (21). At the peripheral level, D rats showed a significant subsensitivity to adrenergic agonists in the cardiovascular system as well as in other sympathetic innervated organs that may be attributed to an increased neuronal activity (13,22). Thus, these neuronal alterations may represent the pathophysiological basis of panic disorder in humans and may be related to the increased reactivity to stressful events observed in early malnourished animals. The fact that drugs with therapeutic efficacy in panic disorder showed a selective anticonflict effect in D rats seems to support this hypothesis.

Recently, Fontana and Commissaris proposed the conditioned suppressed drinking (CSD) conflict paradigm as an animal model to study antipanic agents. They observed that rats chronically treated with antidepressant agents tolerated a higher number of shocks in the CSD (16). As a whole, these results agree with our own because they showed effectiveness of antipanic agents under definite experimental conditions.

In conclusion, our data stressed long-lasting effects of early undernutrition, reflected by an altered reactivity to anxiolytic drugs in the elevated plus-maze test. In addition, perinatally deprived rats may represent an useful animal model for studying antipanic agents.

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