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Effects of desipramine and alprazolam on forced swimming behaviour of adult rats exposed to prenatal diazepam

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Abstract

Pregnant rats were treated with a single daily s.c. injection of diazepam (2 mg/kg) over gestation days 14–20. This treatment led to a reduction in GABA receptor complex function since adult male offspring showed a strong decrease in electrographic hippocampal responses to alprazolam and a strongly increased response to picrotoxin after intra-locus coeruleus injection of the two compounds. No difference in immobility time in the forced swimming test and in spontaneous motor activity was observed between prenatally vehicle- and diazepam-exposed offspring. Conversely, prenatal exposure to diazepam potentiated the anti-immobility effect of subchronic desipramine (10 mg/kg i.p.) and made active a dose of desipramine (5 mg/kg i.p.) that was ineffective in prenatally vehicle-exposed rats. This effect was observed only in pretested rats. Prenatal exposure to diazepam blocked the anti-immobility effect of subchronic alprazolam (15 mg/kg i.p.) in both non-pretested and pretested rats. Spontaneous motor activity was strongly reduced in all groups. These findings suggest that a persistent reduction in GABA receptor complex function, induced by prenatal exposure to diazepam, does not alter the mobility of adult progeny in the forced swimming test, but it may have consequences when drugs acting on the GABA receptor complex are used.

Keywords: Prenatal diazepam; Forced swimming test; Desipramine; Alprazolam

1. Introduction

Several reports indicate that exposure to benzodiazepines during late gestation induces numerous permanent alterations in the neurochemistry (Kellogg and Retell, 1986; Miller et al., 1989; Rothe and Langer, 1988), physiology (Simmons et al., 1984) and behaviour (Cagiano et al., 1990; Livezey et al., 1986; Marczinski and Urbancic, 1988) of adult progeny. In particular, prenatal exposure to diazepam seems to decrease the density of benzodiazepine receptors (Livezey et al., 1986) and to increase the sensitivity to convulsants that specifically act on the GABA/benzodiazepine receptor channel in the rat brain (Bitran et al., 1991).

There is evidence that a reduction in the function of the GABA receptor complex is related to the anti-immobility effect of tricyclic antidepressants in the forced swimming test (Suranyi-Cadotte et al., 1985; Suzdak and Gianutsos, 1985), suggested as a valid animal model to evaluate antidepressant drugs (Willner, 1990). According to this hypothesis, tricyclic antidepressants, in addition to inhibiting monoamine uptake, would also act as GABA receptor complex functional antagonists (Squires and Saederup, 1988); moreover, pre-exposure of rats to a stressful situation, which decreases the number of benzodiazepine receptors in the brain (Medina et al., 1983), potentiates the anti-immobility effect of tricyclic antidepressants in the forced swimming test (Fernandez-Teruel et al., 1990). However, the GABA receptor complex may also be directly implicated in the antidepressant action because alprazolam, an anxiolytic, antidepressant triazolobenzodiazepine (Giusti et al., 1991; Rickels et al., 1985), reduces the immobility time in the forced swimming test (Flugy et al., 1992).

The locus coeruleus is considered an important structure for the mobility of the rat in the forced

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swimming test and for the anti-immobility effect of desipramine and alprazolam (Weiss, 1991; West, 1990). In fact, the output from the locus coeruleus, which is modulated by GABA/benzodiazepine receptors, is able to influence both spinal motoneurons (motility) and neuronal activity in the hippocampus, which in turn is involved in attentional immobility (Klemm and Dreyfus, 1975).

In the present study we investigated whether a permanent reduction in the function of the GABA receptor complex, induced by prenatal exposure to diazepam, altered the anti-immobility effect in the forced swimming test of desipramine and alprazolam in adult offspring. Since pre-exposure to stressful swimming influences the sensitivity to antidepressants (Borsini et al., 1989) and, since prenatal exposure to diazepam alters the central and peripheral response to stress (Simmons et al., 1984), non-pretested and pretested rats were studied. In order to obtain a measure of the functional state of the GABA receptor complex, the effects of a local intra-locus coeruleus injection of the specific agonist alprazolam and of the specific antagonist picrotoxin on the electrical activity of the hippocampus were also analysed.

2. Materials and methods

2.1. Animals and prenatal diazepam exposure

Pairs of primiparous female Wistar rats (Morini Bo, Italy), approximately 100 days of age, were mated in our laboratory with one male, 120 days of age, of the same strain. All rats were allowed free access to food and water and were housed at constant room temperature (20.0 ± 2.0°C, 55 ± 10% humidity) with a 12-h light-dark cycle (8 a.m. to 8 p.m.). The day on which sperm was detected in the vaginal smear was designated gestational day 1. Pregnancy was determined by weighing and palpation. On gestational day 13, pregnant females were randomly assigned to experimental and control groups. From gestational day 14 through to gestational day 20, the rats received a single daily s.c. injection of diazepam at 2 mg/kg or vehicle solution (40% v/v propylene glycol, 10% v/v ethyl alcohol in water). A separate group of pregnant females was left uninjected from gestational day 14 to gestational day 20. On the day of birth all litters were culled to 10 pups. Diazepam-exposed pups were not fostered at birth to untreated dams since it has been shown that the consequences of in utero diazepam exposure are not influenced by fostering (Bitran et al., 1991). All pups were weighed once weekly and weaned 28 days after birth. No differences in mortality or weight gain were observed in the rats treated with diazepam compared to vehicle or uninjected rats. Pups were separated by sex, and male rats were housed in groups of three per cage and randomly allocated to each experimental group. Three days before the forced swimming session began, the male rats (approximately 90 days of age and 275–300 g of weight) were handled. Vehicle solution and diazepam injections were given at 12:00 h; all experiments were conducted between 12:00 and 14:00 h. The sessions were started 1 h after the last dose of subchronic i.p. administration (24, 5 and 1 h before testing) of vehicle, desipramine (5–10 mg/kg) and alprazolam (5–15 mg/kg). This schedule of treatment was chosen because more pronounced effects were shown after three administrations of drugs in pretested rats (Borsini et al., 1989; Flugy et al., 1992).

2.2. Forced swimming test

On the first day male rats were placed for 15 min in a Plexiglas cylinder (40 cm high 18 cm inside diameter) containing from 5 l to 6 l, in relation to the size of the rat, of clean water maintained at 21 ± 1°C from which they could not escape. During the first 5 min, immobility time was recorded by a trained observer. A rat was judged to be immobile when it remained floating in the water, making only the movements necessary to keep its head above water. After 15 min the rats were removed and dried under a lamp (pretest group). Other rats were handled and placed under the lamp but not subjected to the pretest session (non-pretest group). Twenty-four hours later the rats were transferred into the cylinder and the immobility time was recorded for 5 min.

Compared to the immobility time of uninjected rats, prenatal vehicle injection did not affect immobility time. Thus, rats from uninjected and vehicle-injected offspring were combined into a single control group for statistical comparison with diazepam-injected offspring.

2.3. Spontaneous motor activity

Spontaneous motor activity was recorded with an Opto-Varimex device (Columbus Instruments International Corp., Columbus, OH, USA). It consists of a rectangular box, 43.2 × 44.6 cm, whose adjacent perpendicular sides have 15 infrared emitters, each beam is 0.32 cm in diameter and is separated from the next one by a distance of 2.65 cm. The interrupted beams indicate the position of the animal. With the aid of a computerized system, the following parameters were calculated: (a) the total distance covered by the animal, and (b) the time during which the animal remained still. The movements were recorded by the computerized system on paper. The duration of the test was 10 min; each rat began the experiment 1 min after being placed in the box.

2.4. Electrophysiological observations

Electroencephalographic recordings were obtained in both vehicle- and prenatal diazepam-exposed progeny through pairs of bipolar electrodes stereotaxically inserted in the locus coeruleus and in the target structure of its noradrenergic projection, namely the hippocampus. Surgical procedures were carried out under urethane anaesthesia in a DKI 1404 stereotaxic frame. Electroencephalographic activity was recorded through acute implantation of bipolar stainless steel co-axial electrodes (external diameter 0.5 mm, tip 25–50 μm). A Hamilton microsyringe, stereotaxically inserted into the locus coeruleus, was used for local administration of compounds. An eight-channel polygraph (Grass model 7B) was used for amplification and recording. The hippocampal bioelectrical activity was analysed in control conditions and following intra-locus coeruleus administration of vehicle, alprazolam (5 μg) or picrotoxin (1–2 μg).

2.5. Drugs

Desipramine (desipramine hydrochloride) and picrotoxin (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in 0.9% NaCl solution; diazepam was given as Valium (Roche, Milan, Italy); alprazolam (Upjohn Co., Kalamazoo, MI, USA) was suspended in a 1% aqueous solution of carboxymethylcellulose. All drugs were administered in a constant volume of 2 ml/kg body weight of animal. Control treatments were vehicle solutions. For intra-locus coeruleus administration volumes of 1 μl were injected.

2.6. Statistical analysis

Data for the immobility time in the forced swimming test and for spontaneous motor activity were first evaluated with Bartlett's test to verify the hypothesis of homoscedasticity. Since the chi-squared value was not significant in any case this hypothesis was considered valid for data of single groups obtained from 3–6 separate experiments run on different days. The statistical evaluation of data related to immobility time in the forced swimming test, of alprazolam-treated rats, and to spontaneous motor activity was based on a one- and two-factor ANOVA. We did not make a post-hoc test in view of the limited number of factors and levels analysed each time. For statistical evaluation of data related to immobility time in the forced swimming test of desipramine-treated rats we used a multiple regression analysis with 7 independent variables: desipramine, prenatal treatment and pretest plus 3 variables obtained from their combination two by two plus 1 variable obtained from their combination three by three. We therefore used a backward method for the choice of variables (Mineo, 1970), and the following variables were selected: pretest, pretest × desipramine, pretest × desipramine × prenatal treatment (Table 2). For electrophysiological data the F of Snedecor followed by Student's t-test was applied.

3. Results

Prenatally diazepam-exposed rats did not differ from control animals in immobility time either in pretest or test swimming sessions (Fig. 1) or in spontaneous motor activity (Table 1).

Desipramine significantly reduced immobility time in the forced swimming test in pretested control rats at a dose of 10 mg/kg i.p. Prenatal exposure to diazepam potentiated the anti-immobility effect of desipramine (10 mg/kg i.p.); moreover, the anti-immobility effect of desipramine was achieved at a dose lower (5 mg/kg i.p) than that effective in control rats. Desipramine (5–10 mg/kg i.p) did not influence immobility time in non-pretested rats prenatally exposed to vehicle or diazepam (Fig. 2 and Table 2).

Table 1
The effects of subchronic desipramine and alprazolam on spontaneous motor activity of adult rats prenatally exposed to vehicle and diazepam

Treatment	Distance travelled (cm/10 min)	Resting time (s/10 min)
Prenatal vehicle		
Vehicle	564 ± 117	263 ± 33
Desipramine (10 mg/kg i.p.)	82 ± 36 [a]	462 ± 90 [a]
Alprazolam (15 mg/kg i.p.)	133 ± 41 [a]	461 ± 32 [a]
Prenatal diazepam		
Vehicle	584 ± 118	301 ± 64
Desipramine (10 mg/kg i.p.)	99 ± 33 [a]	504 ± 29 [a]
Alprazolam (15 mg/kg i.p.)	110 ± 35 [a]	506 ± 43 [a]

Each number is the mean ± S.E.M. for 10 rats selected from 3–4 litters. Data were obtained from 3 separate experiments run on different days.
[a] $P < 0.001$ (ANOVA).

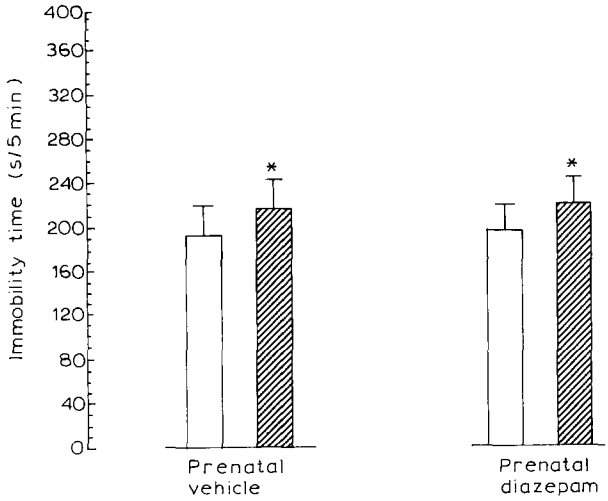

Fig. 1. Immobility time in non-pretested (open bar) and pretested (closed bar) adult rats prenatally exposed to vehicle or diazepam. Each bar represents the mean ± S.E.M. for 20 rats selected from 6–8 litters. Data were obtained from 6 separate experiments run on different days. * $P < 0.01$ (ANOVA).

Alprazolam significantly reduced immobility time in both non-pretested ($F = 20.994$; $P = 0.00023$) and pretested control rats ($F = 33.649$; $P = 0.000017$) at a dose of 15 mg/kg i.p. Prenatal exposure to diazepam counteracted the anti-immobility effect of alprazolam (15 mg/kg i.p.) in non-pretested and pretested rats (Fig. 3). Spontaneous motor activity was strongly reduced in both desipramine (10 mg/kg i.p.)- and alprazolam (15 mg/kg i.p.)-treated rats (Table 1).

Electrophysiological data showed that intra-locus coeruleus alprazolam induced, in control rats, a strong inhibitory effect in hippocampal activity. From a background activity with sharp waves of 150 ± 15 μV the amplitude decreased, after alprazolam, to 30 ± 5 μV,

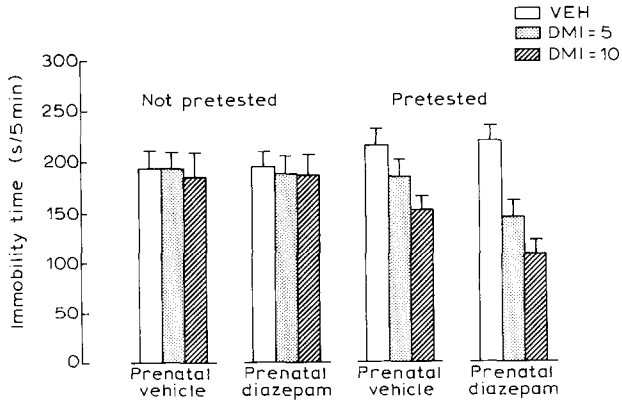

Fig. 2. The effect of vehicle, desipramine 5 mg/kg, and desipramine 10 mg/kg administered i.p. 24, 5, 1 h before the pretest or test sessions on the immobility time of adult rats prenatally exposed to vehicle or diazepam. Each bar represents the mean ± S.E.M. for 12 rats selected from 4–5 litters. The data were obtained from 4 separate experiments run on different days. For statistical data see Table 2.

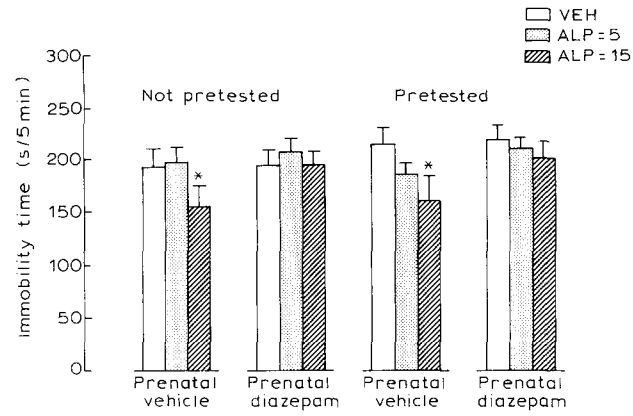

Fig. 3. The effect of vehicle, alprazolam 5 mg/kg, and alprazolam 15 mg/kg administered i.p. 24, 5, 1 h before the pretest or test sessions on the immobility time of adult rats prenatally exposed to vehicle or diazepam. Each bar represents the mean S.E.M. for 12 rats selected from 4–5 litters. The data were obtained from 4 separate experiments run on different days. * $P < 0.001$ (ANOVA).

with a synchronizing effect and a complete abolition of sharp waves ($P < 0.001$). In contrast, in prenatally diazepam-exposed rats, at the dose administered, alprazolam was no longer effective in modifying hippocampal activity, with an amplitude of 130 ± 12 μV in the pre-alprazolam condition and of 138 ± 14 μV during alprazolam infusion (not significant). Opposite effects were observed with picrotoxin administration. In the control rats, intra-locus coeruleus picrotoxin at 1 μg failed to modify hippocampal activity. At the threshold dose of 2 μg picrotoxin induced an increased amplitude (of about 200%) in hippocampal activity with the appearance of spikes at a frequency of 38 ± 4/min. In prenatally diazepam-exposed rats the threshold dosage for hippocampal hyperactivity was decreased to 1 μg, spike amplitude was augmented up to 600% ($P < 0.001$) and spike frequency was increased to 58 ± 5/min ($P < 0.001$) (Fig. 4).

4. Discussion

Our findings confirm that prenatal exposure to diazepam leads to a persistent alteration in the function

Table 2
Variables chosen, and their statistical significance, for the multiple regression analysis used for the evaluation of the effect of desipramine in the forced swimming test

Variables	$R2$	$n1$	$n2$	F	P-level
All variables	0.7468797				
DMI × Prenatal T.	0.7468769	1	113	0.0012	0.9721
Prenatal T.	0.7468759	2	113	0.0009	0.9992
Pretest × Prenatal T.	0.7464596	3	113	0.0625	0.9795
DMI	0.7418143	4	113	0.5653	0.6883
Pretest	0.6568187	5	113	8.0411	0.0001

DMI = desipramine, T. = treatment.

of the GABA/benzodiazepine/chloride channel receptor in the brain of adult progeny (Bitran et al., 1991; Livezey et al., 1986). In fact, the rats prenatally exposed to diazepam displayed a strong decrease in the electrographic hippocampal response to alprazolam and a strongly increased response to picrotoxin after intra-locus coeruleus injection of the two compounds. However, this alteration did not seem to influence the immobility time in pretest and test sessions of forced swimming or the spontaneous motor activity, since there were no differences between vehicle- and diazepam-exposed rats.

In agreement with our previous results (Flugy et al., 1992) subchronic treatment with desipramine significantly reduced immobility time in pretested control rats at a dose of 10 mg/kg i.p.; at this dose desipramine was inactive in non-pretested control rats. Prenatal exposure to diazepam significantly potentiated the action of 10 mg/kg i.p. of desipramine and made 5 mg/kg i.p. effective in pretested rats. These effects seem to be specific, since spontaneous motor activity was similarly reduced in prenatally vehicle- and diazepam-exposed rats. It seems, therefore, that the biochemical events occurring during the pretest session are important for revealing in the test session the increased sensitivity to desipramine of prenatally diazepam-exposed rats.

There is evidence that monoaminergic systems are critically involved in the anti-immobility effect of the tricyclic antidepressants (Delini-Stula et al., 1988; Duncan et al., 1985; Wieland and Lucki, 1990); moreover, tricyclic antidepressants might act as functional GABA receptor complex antagonists since they down-regulate benzodiazepine and GABA receptors, decrease the GABA-stimulated ^{36}Cl-uptake and block the inhibitory effect of GABA at the [^{35}S]ter-butyl-bicyclo[2.2.2.] phosphorothionate binding site (Squires and Saederup, 1988; Suranyi-Cadotte et al., 1985; Suzdak and Gianutsos, 1985). These findings are in agreement with the evidence that acute stress, which decreases the number of benzodiazepine receptors in the rat brain (Andrews et al., 1992; Medina et al., 1983), increases the anti-immobility effect of desipramine in the forced swimming test (Fernandez-Teruel et al., 1990) and that, in contrast, diazepam, given before stress, prevents the decrease in the number of benzodiazepine receptors (Andrews et al., 1992; Medina et al., 1983) and antagonizes the anti-immobility effect of desipramine (Borsini et al., 1989; Flugy et al., 1992). Prenatal exposure to diazepam, at the dose used in this study, reduced the GABA receptor complex function (Livezey et al., 1986) and altered the central and peripheral response to stress (Simmons et al., 1984). In addition, the present study showed a strong increase in the locus coeruleus response to picrotoxin in rats prenatally exposed to diazepam. These findings suggest that the increased sensitivity to desipramine of prenatally diazepam-exposed rats could reflect a reduced basal activity of GABAergic neurons and an alteration in the dynamic state and in the functional activity of central monamin-

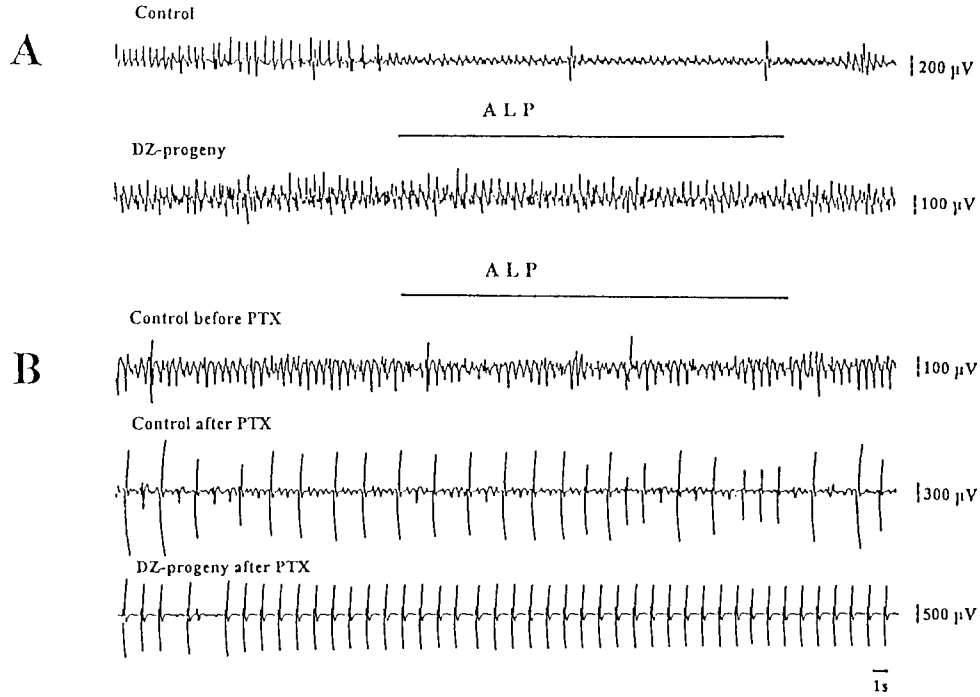

Fig. 4. Electrographic recordings of hippocampal activity in prenatally vehicle- and diazepam-exposed progeny after intra-locus coeruleus injection of vehicle, alprazolam (5 μg) (A) or picrotoxin (2 μg) (B). ALP = alprazolam; DZ = diazepam; PTX = picrotoxin.

ergic neurons. The final result may be a modified interaction between the biochemical changes due to the stress of the pretest session and the neuronal effects exerted by desipramine.

Subchronic treatment with alprazolam significantly decreased immobility time in both non-pretested and pretested rats. Prenatal exposure to diazepam blocked the anti-immobility effect of alprazolam. The noradrenergic projections from the locus coeruleus have a critical influence on the mobility of rats in the forced swimming test; exposure to the stress of forced swimming increases locus coeruleus activity and greatly decreases the concentration of noradrenaline in many regions of the brain. The final result is a decrease in the mobility of the rat (Weiss, 1991). The activation of the GABA receptor complex has a direct inhibitory influence on the locus coeruleus (Guyenet and Aghajanian, 1979) and an indirect facilitatory influence on cortical noradrenergic trasmission, probably by increasing the availability of noradrenaline at its receptors (Dennis et al., 1985). Alprazolam shows a great ability to decrease locus coeruleus activity (Grant et al., 1984), acting as potent and selective GABA/benzodiazepine/receptor agonist (Giusti et al., 1991). Thus, the decrease in locus coeruleus activity and the increase in cortical noradrenergic transmission may account, at least in part, for the enhancement of mobility in the forced swimming test induced by alprazolam in the rat. Prenatal exposure to diazepam, decreasing GABA receptor function, would result in an ineffective action of alprazolam on the locus coeruleus and on cerebral noradrenergic transmission which, in turn, would be ineffective in preventing the stress-induced increased immobility. This hypothesis is substantiated by our observation that rats prenatally exposed to diazepam displayed a strong decrease in the electrographic hippocampal response after intra-locus coeruleus injection of alprazolam.

In summary, the present study shows that the prenatal exposure of male rats to diazepam did not influence the immobility time in the forced swimming test during adulthood, but altered the anti-immobility effect of desipramine and alprazolam. A decrease in the function of the GABA receptor complex in prenatally diazepam-exposed rats is suggested by the increased locus coeruleus response to picrotoxin and by the decreased locus coeruleus response to alprazolam. These findings indicate that persisting neuronal alterations induced by prenatal exposure to diazepam may not manifest themselves in behavioural impairments, but they may have serious permanent consequences on the behavioural effects of different drugs acting on the GABA receptor complex. In addition, the experimental model reported here may be useful in contributing to our understanding of the role of the GABA receptor complex in the action of different antidepressant drugs.

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