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Long-lasting behavioral changes induced by pre- or neonatal exposure to diazepam in rats

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Abstract

Prenatal treatment with small doses of diazepam may counteract the effect of physical stress in rats, normalizing the time course of neonatal reflexes and the behavioral responses in adulthood. However, prenatal administration of diazepam in greater doses may induce desensitization of γ-aminobutyric acid A ($GABA_A$) receptors and induce hypersensitivity to convulsants. This study was designed to examine in rats the influence of prenatal or neonatal diazepam treatment on development of neonatal reflexes, as index of brain maturation, and susceptibility to pentylenetetrazol-induced convulsions in adulthood. Pregnant Wistar rats were injected with diazepam 2.5 mg/kg/day, intraperitoneally (i.p.) on days 14–21 of pregnancy. Offspring showed a delay in the appearing of neonatal reflexes (cliff aversion, forelimb placing, forelimb grasping and bar holding) except for righting and startle reflexes. At 50 days of age, male rats showed a greater sensitivity to pentylenetetrazol compared to controls. In contrast, females showed a decreased susceptibility to convulsions. The appearance of reflexes in pups exposed to diazepam during neonatal life appeared to be similar to that of controls. Only the appearance of cliff aversion and startle reflexes appeared to be delayed in animals exposed neonatally to diazepam as compared to controls. In adulthood, female rats exposed in early neonatal life to diazepam again showed a resistance to pentylenetetrazol-induced convulsions as compared to male animals. These data suggest that prenatal exposure to diazepam induces long-lasting behavioral changes, which may be influenced by sex-dependent factors.

Keywords: Diazepam; Neonatal reflex; Pentylenetetrazol; Convulsion; $GABA_A$ receptor

1. Introduction

It has been shown that prenatal exposure to diazepam may counteract behavioral changes induced by physical stress applied during gestation. In particular, low doses (0.1–1 mg/kg) of diazepam normalize the delayed appearance of neonatal reflexes and the behavioral deficits in adulthood, namely increased immobility in the despair test and reduced retention of a passive avoidance task (Drago et al., 1999). However, evidence has been provided that prenatal exposure to benzodiazepines may affect the development of benzodiazepine binding sites, inducing persisting effects on the γ-aminobutyric acid (GABA) system that sometimes can be revealed by behavioral effects on motricity, learning capacity, social behavior, drug-induced convulsions (Gai and Grimm, 1982; Livezey et al., 1986; Cagiano et al., 1990; Cannizzaro et al., 1995a,b, 1998). These effects are generally related to prenatal administration of rather great doses of diazepam.

Data on neurochemical development are found to be generally consistent with the behavioral modifications. There is evidence that enhancement of benzodiazepine binding by GABA varies with age. However, treatment with diazepam may induce desensitization of $GABA_A$ receptor that is expressed during prenatal life (Laurie et al., 1992; Poulter et al., 1992, 1993; Ma et al., 1993) and this could be implicated in altered behavior and hypersensitivity to convulsants in adulthood.

The behavioral alteration induced by exposure to diazepam during gestation has been clearly demonstrated in adult rats. Since it is not known whether prenatal diazepam may also affect neonatal behavior, this study was designed to examine in rats the influence of prenatal or neonatal diazepam treatment on the expression of neonatal reflexes.

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These reflexes are considered as an index of brain maturation (Fox, 1965) and the delayed appearance of them may indicate an alteration of embryological mechanisms responsible for the correct development of the central nervous system. Changes in the expression of neonatal reflexes may also represent a predictive factor for other behavioral modifications to be shown in adulthood. In addition, pentylenetetrazol-induced convulsions in adulthood was tested in male and female animals. Although prenatal exposure to diazepam is known to augment the susceptibility to convulsants in adult animals, this phenomenon may be influenced by sex-dependent factors (Kokka et al., 1992).

In the present experiments, postnatal exposure to diazepam has also been applied in order to identify the effects of the drug mediated by a direct influence on the ontogenesis of $GABA_A$/benzodiazepine receptors. In fact, the density of these receptors increases with age from the birth to reach adult values at day 14 of life (Kapur, 2000; Taketo and Yoshioka, 2000).

Since loading of rat pups may be very low in studies where drugs are supplied via lactation, one should be aware that results from experiments where drug treatment is given to lactating animals might be difficult to interpret. However, this may be true also for prenatal exposure to drugs that may penetrate poorly through the placental barrier. Thus, any difference between prenatal and neonatal drug exposure remains uncertain.

2. Materials and methods

2.1. Animals

A total of 16 pregnant female rats of the Wistar strain (purchased from Morini, Italy), weighing 280 ± 10 g, were used throughout the experiment. Animals were housed in single cages and were kept under standard conditions at room temperature of 20 °C and with constant light–dark cycle (lights on between 08.00 and 20.00 h). All animals had free access to water and standard commercial food. After a week of habituation in the facilities, they were randomly assigned to one of four experimental groups (four animals per group).

After parturition pups were countered and weighed before behavioral tests daily at 15.00 until weaning. At weaning, males were separated from females and housed five per cage until they were 50 days. Groups of nine animals were randomly selected for the experiment on pentylenetetrazol-induced convulsions. All experiments were conducted blind to treatment in conformity with the European Communities Council Directive 86/609/EEC.

2.2. Drug treatment

Diazepam (Roche, Italy) was dissolved in 5:1 benzyl alcohol/water and administered intraperitoneally (i.p.) at the dose of 2.5 mg/kg/day in a total solution volume of 1 ml according to two different treatment schedules: prenatally (between days 14 and 21 of pregnancy) and neonatally

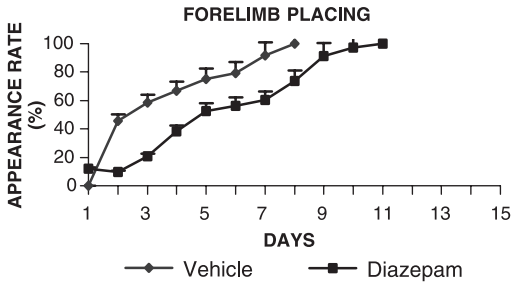

Fig. 2. Effects of prenatal exposure to diazepam (2.5 mg/kg/day injected i.p. on gestational days 14–21) on the appearance rate of forelimb placing reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 46$ for vehicle- and 52 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,97) = 398.5$, $p < 0.05$.

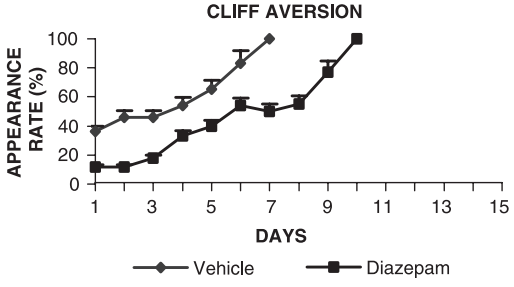

Fig. 1. Effects of prenatal exposure to diazepam (2.5 mg/kg/day injected i.p. on gestational days 14–21) on the appearance rate of cliff aversion reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 46$ for vehicle- and 52 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,97) = 341.4$, $p < 0.05$.

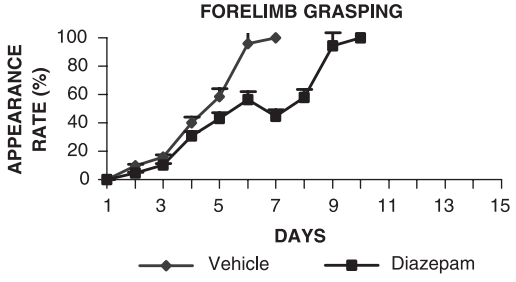

Fig. 3. Effects of prenatal exposure to diazepam (2.5 mg/kg/day injected i.p. on gestational days 14–21) on the appearance rate of forelimb grasping reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 46$ for vehicle- and 52 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,97) = 312.9$, $p < 0.05$.

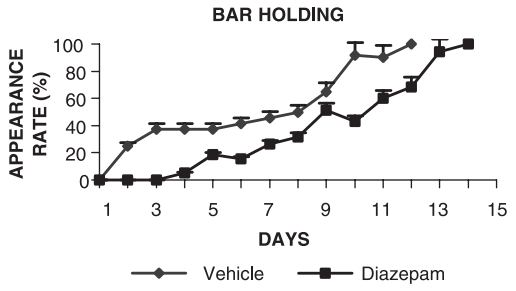

Fig. 4. Effects of prenatal exposure to diazepam (2.5 mg/kg/day injected i.p. on gestational days 14–21) on the appearance rate of bar holding reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 46$ for vehicle- and 52 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,97) = 479.5$, $p < 0.05$.

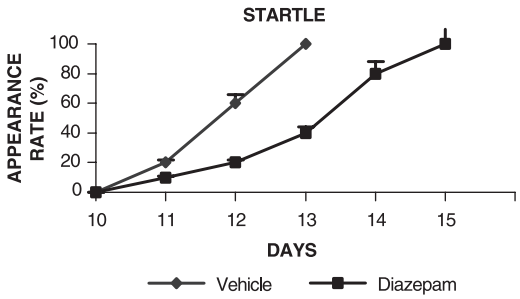

Fig. 6. Effects of neonatal exposure to diazepam (2.5 mg/kg/day injected i.p. since day 1 after parturition until weaning of the litter, to lactating mothers) on the appearance rate of startle reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 48$ for vehicle- and 42 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,89) = 341.5$, $p < 0.05$.

(between day 1 of parturition and the weaning, to lactating mothers). Control rats were injected with the vehicle alone (1 ml, i.p.), with the same experimental procedure and periods.

2.3. Neonatal behavior test

Neonatal reflexes were studied by applying a battery of tests adapted from Fox (1965) and Wahlsten (1974) to all rat pups daily since day 1 of life. The appearance day of the following reflexes was scored for 15 days: cliff aversion (the rat withdraws from the edge of a flat surface when its snout and forepaws are placed over a cliff 60 cm high); startle (the rat shows a whole-body startle response when a loud snap of the fingers occurs 10 cm away); righting (the rat is capable of rapidly returning to its feet when placed on its back); forelimb placing [the rat places its forepaw on cardboard which is stroked against the dorsal surface of the paw (the rat is grasped between the thumb and the forefinger of the observer)]; forelimb grasping [the rat grasps strongly the barrel of the 16-gauge needle (diameter 1.0 mm) when it is touched against the palm of each forepaw (the rat is grasped between the thumb and the forefinger of the observer)]; bar holding [the rat holds itself from a wooden stick (diameter 2.0 mm) for 5 s (the rat is grasped between the thumb and the forefinger of the observer)]. Results were expressed as percentage of offspring showing the above reflexes over the total number of animals in each litter group (Drago et al., 1984).

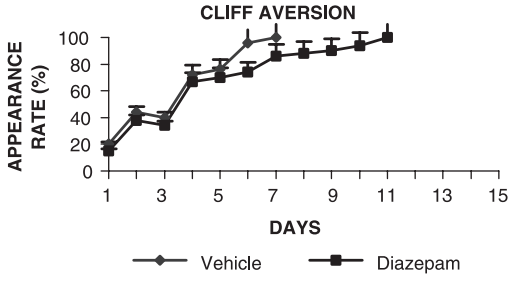

Fig. 5. Effects of neonatal exposure to diazepam (2.5 mg/kg/day injected i.p. since day 1 after parturition until weaning of the litter, to lactating mothers) on the appearance rate of cliff aversion reflex in neonate rats. Values are mean ± S.E.M. of percentage of animals exhibiting the reflex over the total of each of four litters ($n = 48$ for vehicle- and 42 for diazepam-treated animals) plotted vs. days of observation. Two-way ANOVA revealed a significant drug effect with $F(1,89) = 414.5$, $p < 0.05$.

2.4. Pentylenetetrazol-induced convulsions

At 50 days of age, male and female rats pre- or neonatally exposed to diazepam were tested in a model of drug-induced seizures. Pentylenetetrazol (Sigma, USA) of 60 mg/kg was dissolved in saline and injected i.p. They were observed for 30 min beginning after injection. Latency was recorded as the time elapsing from drug administration

Table 1
Effect of prenatal exposure to diazepam (2.5 mg/kg/die, i.p. since days 14 to 21 of gestation) on susceptibility to convulsions induced by pentylenetetrazol (60 mg/kg) in male and female adult rats

	Latency to first jerk (s)	Total duration of convulsions (s)
Males		
Vehicle	70.0	56.2 ± 4.9
Diazepam	28.0[a]	89.1 ± 8.0[a]
Females		
Vehicle	62.5	53.1 ± 5.6
Diazepam	75.0[b]	30.0 ± 2.9[c]

Values are expressed in medians (latency to first jerk) and in mean ± S.E.M. (total duration of convulsions). The number of animals was nine per each experimental group.

[a] Significant difference vs. sex-matched vehicle-treated controls ($p < 0.05$, Mann–Whitney U-test for the latency to first jerk; $p < 0.05$, Dunnett's test for the total duration of convulsions).

[b] Significant difference vs. diazepam-treated males ($p < 0.05$, Mann–Whitney U-test).

[c] Significant difference vs. sex-matched vehicle-treated controls and diazepam-treated males ($p < 0.05$, Dunnett's test).

and the first motor jerk (first myoclonic attack). The total duration of convulsions (tonic seizures, myoclonus, and clonic convulsions) was also measured.

2.5. Statistical analysis

The statistical analysis of data was made using the two-way analysis of variance (ANOVA) on the percentage values and the post-hoc Dunnett's test for multiple comparisons. The Mann–Whitney *U*-test was used for non-parametric data from the pentylenetetrazol-induced convulsion experiments. A *P* level of 0.05 or less was considered as indicative of a statistically significant difference.

3. Results

The appearance rate of neonatal reflexes for pups exposed in utero to diazepam is depicted in Figs. 1–4. The time course of cliff aversion, forelimb placing, forelimb grasping and bar holding reflexes shows a delayed incidence of reflex appearance in neonates that were exposed prenatally to diazepam compared to controls (significant drug effect, two-way ANOVA, $p < 0.05$). No change was found for the righting and startle reflexes (data not shown). For all reflexes, except for forelimb grasping, a statistically significant difference was seen between diazepam- and vehicle-exposed animals starting on day 1 or 2 of observation ($p < 0.05$, Dunnett's test for multiple comparisons) that remained until the last day of observation.

The incidence of reflexes appearance in animals exposed to diazepam in early neonatal life was similar to that of control animals for all reflexes (data not shown). Only cliff aversion (Fig. 5) and startle reflexes (Fig. 6) in pups exposed neonatally to diazepam showed a significantly slower appearance compared to those of controls (significant drug effect, two-way ANOVA, $p < 0.05$).

Table 2
Effect of neonatal exposure to diazepam (2.5 mg/kg/die, i.p. to lactating mothers since day 1 after parturition until weaning of the litter) on susceptibility to convulsions induced by pentylenetetrazol (60 mg/kg) in male and female adult rats

	Latency to first jerk (s)	Total duration of convulsions (s)
Males		
Vehicle	65.0	62.0 ± 7.1
Diazepam	57.5	65.1 ± 6.9
Females		
Vehicle	63.0	73.0 ± 8.1
Diazepam	90.0[a]	37.1 ± 4.3[a]

Values are expressed in medians (latency to first jerk) and in mean ± S.E.M. (total duration of convulsions). The number of animals was nine per each experimental group.

[a] Significant difference vs. sex-matched vehicle-treated controls and diazepam-treated males ($p < 0.05$, Mann–Whitney *U*-test for the latency to first jerk; $p < 0.05$, Dunnett's test for the total duration of convulsions).

Table 1 shows the data (latency and total duration of convulsions) on the epileptic susceptibility of rats exposed prenatally to diazepam. Latency to pentylenetetrazol-induced seizures was lower and the total duration of convulsions was greater in males exposed prenatally to diazepam as compared to those of controls. Furthermore, prenatal exposure to diazepam was followed by increased latency to the first jerk in females compared to males. The total duration of convulsions appeared to be shorter in females exposed prenatally to diazepam as compared to males and to control rats of the same sex. In addition, females (but not males) exposed neonatally to diazepam showed to be less susceptible to convulsions compared to controls, in that they exhibited increased latency to the first jerk and reduced total duration of pentylenetetrazol-induced convulsions as compared to controls (Table 2).

4. Discussion

The evidence for persisting effects after early exposure to benzodiazepine receptor ligands is impressive at first sight. Startle and some learning tasks are affected by prenatal diazepam; submissiveness behavior is affected by neonatal lorazepam; social behavior and convulsions are affected by the benzodiazepine receptor ligand with partial inverse agonistic properties, CGS 8216 (Gai and Grimm, 1982; Livezey et al., 1986; Cagiano et al., 1990; Cannizzaro et al., 1995a,b, 1998). Benzodiazepines inhibit chemically induced seizures in neonatal rats, but the developmental profile of sensitivity to the convulsants is disputed. Furthermore, benzodiazepines stimulate motor behavior in the neonatal rat (Pohorecky and Roberts, 1991). Other authors (Ryan and Pappas, 1986) have evidenced that pups prenatally exposed to diazepam had a weight deficit at birth and a significant delay on the hair growth. They also observed a significant dose-dependent reduction of liveliness either at birth or 1 week after. No differences were evidenced in terms of teeth eruptions.

The physical development of the rat is disturbed only by extremely high doses of benzodiazepines (Tucker, 1985). Indeed, the drug dose range seems to be crucial in relation to the type of biological effect. In rats, prenatal exposure to diazepam may affect behavioral and neuroendocrine parameters depending on the dose and the gestational period of treatment. In the present experiments, the dose of diazepam and duration of treatment were selected (2.5 mg/kg/die since days 14 to 21 of gestation) in the range that other authors have used in similar studies (Bitran et al., 1991; Kellogg et al., 1991; Inglefield et al., 1993).

Here we show that prenatal exposure to diazepam (2.5 mg/kg) reduces the appearance rate of neonatal reflexes in rats. Little effects were observed when diazepam was given neonatally (between day 1 of parturition and the weaning, to lactating mothers). In fact, the expression of only cliff aversion and startle reflexes appeared to be delayed. These

results may indicate that early exposure to a great dose of diazepam affect neural mechanisms responsible for the expression of neonatal reflexes. The effect is less evident when diazepam is administered in neonatal life, when probably the development processes concerning these mechanisms are almost completed. Also, diazepam-induced sedation may play a role in this effect of the drug. Furthermore, the poor changes observed after neonatal exposure to diazepam should depend on a scarce drug loading of the pups. This leaves any comparisons very uncertain and accordingly any difference between prenatal and neonatal drug exposure is difficult to interpret.

It has been shown that the doses of 0.1–1 mg/kg diazepam counteracted the neonatal and adult behavioral changes observed in rats exposed prenatally to physical stress (Drago et al., 1999). Interestingly, in this case, prenatal exposure to diazepam was followed by normalization of neonatal reflexes' appearance and of adult behavior (passive avoidance behavior and despair induced by constrained swim) that showed to be altered by prenatal application of stress. Thus, it is likely that only sustained doses of diazepam delay the appearance rate of neonatal reflexes in rats. Unfortunately, in the present experiments, only the 2.5 mg/kg dose was used, and it remains to be elucidated whether lesser doses do exert the same influence on the expression of neonatal behavior in rats. It should also be mentioned that diazepam is a long-lasting benzodiazepine with a plasma half-life of about 34 h (Herman and Wilkinson, 1996). Thus, despite treatment suspension in the prenatal exposure protocol, in the absence of a cross fostering to lactating untreated dams, pups have continued to be exposed to the drug, through the milk, for several days after delivery. Furthermore, since pharmacokinetic tolerance may have occurred in pups exposed prenatally to diazepam, a longer drug exposure could have affected diazepam disposition at the time of the test, then influencing the results. This may explain the difference in the appearance of neonatal reflexes in prenatally vs. neonatally exposed pups.

Since neonatal reflexes may be considered as an index of brain maturation (Fox, 1965), the present findings suggest that prenatal exposure to diazepam may affect embryological mechanisms responsible for the correct development of the brain. Different neurotransmitters have been shown to influence central nervous system development through an interaction with their own brain receptors (Lauder, 1993). In particular, $GABA_A$ receptor subunit expression may affect cell migration and differentiation, and synaptogenesis (Barbin et al., 1993; Behar et al., 1994). It has been also definitely demonstrated that the stimulation of $GABA_A$ receptor influences its subunits expression (Poulter et al., 1997). Furthermore, GABA may stop DNA synthesis in developing neurons (Lo Turco et al., 1995). This is a possible explanation of the influence of diazepam administration on brain development in animals and its behavioral effects after prenatal or neonatal administration.

Some studies have reported that diazepam and other benzodiazepines increase in rats the sensitivity to convulsants (Gavish et al., 1985; Bitran et al., 1991; Cannizzaro et al., 1995a; Koff and Miller, 1995). These authors, however, have evaluated the effects of prenatal administration of diazepam on postnatal behavior of males only and not of female animals. They have concluded that the treatment with benzodiazepines in prenatal life determines either a decrease in benzodiazepine binding sites or an altered (reduced) sensitivity of $GABA_A$ receptors in the brain. The present data confirm that prenatal exposure to diazepam is followed by an increased susceptibility of the convulsant, pentylenetetrazol. However, adult female rats exposed either prenatally or neonatally to diazepam are less sensitive to pentylenetetrazol compared to males and control animals. In contrast, male rats appeared to be more sensitive to convulsant when exposed to diazepam either prenatally or neonatally. The influence of drug exposure on the sensitivity to convulsants may be, hence, sex-dependent. Circulating female sex hormones, possibly neurosteroid metabolites of progesterone, are known to interact directly with the $GABA_A$ receptor complex. In fact, ovariectomy reduces the threshold to pentylenetetrazol-induced seizures of females to that of males (Kokka et al., 1992). Thus, female sex hormones are probably involved in sex differences in susceptibility to pentylenetetrazol-induced seizures. Interestingly, early exposure to diazepam during gestation alters the function of the $GABA_A$ receptor complex of adult progeny in a sexually dimorphic manner influencing behavioral responses of animals in tests of anxiety (Kellogg et al., 1991). Furthermore, the present results indicate that development processes concerning $GABA_A$/benzodiazepine receptor complex do not take place only during gestational period but also in early postnatal life. In a recent study, neonatal exposure to diazepam induced males and neonatally virilised females to show a clear increase in the absolute power of electroencephalopraphic (EEG) fast frequencies. In normal females and neonatally castrated males, the anxiolytic only produced a moderate EEG activation. The authors conclude that sexual dimorphism in diazepam action depends upon neonatal sexual differentiation (Fernandez-Guasti et al., 2003). These results are consistent with the present findings showing that male rats are more sensitive than female animals to pentylenetetrazol.

Furthermore, in order to interpret correctly the present results, it should be recalled that in 7- to 14-day-old rats, dentate granule cells express DZP-insensitive $GABA_A$ receptors (Kapur, 2000). From the present results, it cannot be concluded that the $GABA_A$/benzodiazepine receptor complex plays a pivotal role or just a supporting role during ontogeny. In particular, these results suggest a negligible control of $GABA_A$/benzodiazepine receptors over the development of neonatal reflexes. However, the role of $GABA_A$/benzodiazepine neurotransmission on brain development may be different in male and female subjects. A receptor

assay under the present experimental conditions is required to explore this possible explanation.

It is obviously hard to compare the present results to those obtained in human studies. Pregnancy may be accompanied by anxiety, necessitating therapeutic intervention by anxiolytic drugs like diazepam (Iqbal et al., 2002). It is known that children may show varying degrees of mental retardation when exposed to various dose levels of diazepam during prenatal life (Laegreid et al., 1989). However, other investigators have observed that the use of diazepam during labor is not harmful to the mother of her infant (Briggs et al., 1994). The dose range is important for diazepam effects on neonates. In fact, it should be noted that the dose used in the present study is much greater than the usual range-dose applied in clinical practice for the anxiolitic therapy. Lesser doses, such as 0.2 and 0.3 mg/kg, were not teratogenic and did not cause behavioral alterations although there was a better resistance to pain (Davies and Rosen, 1977). The same doses can affect uterine contractility during labor without changing uterine muscle tone (Toaff et al., 1977) and influence levels of β-endorphin and adreno-cortico-tropic hormone (ACTH) in human females during pre-abortion period (Pippingskold et al., 1991).

References

Barbin, G., Pollard, H., Gairsa, J.L., Ben-Ari, Y., 1993. Involvement of $GABA_A$ receptor α1β1 and α1β1γ2 subunits in the outgrowth of cultured hippocampal neurons. Neurosci. Lett. 152, 150–154.

Behar, T.N., Schaffner, A.E., Colton, C.A., Somogyi, R., Olah, Z., Lehel, C., Barker, J.L., 1994. GABA induced chemokinesis and NGF induced chemotaxis of embryonic spinal cord neurons. J. Neurosci. 14, 29–38.

Bitran, D., Primus, R.J., Kellogg, C.K., 1991. Gestational exposure to diazepam increases sensitivity to convulsants that act at the GABA/benzodiazepine receptor complex. Eur. J. Pharmacol. 196, 223–231.

Briggs, G.G., Freeman, R.K., Yaffe, S., 1994. Drugs in Pregnancy and Lactation. Williams and Wilkins, Baltimore.

Cagiano, R., De Salvia, M.A., Perischella, M., Renna, G., Tattoli, M., Cuomo, V., 1990. Behavioural changes in the offspring of rats exposed to diazepam during gestation. Eur. J. Pharmacol. 177, 67–74.

Cannizzaro, C., Cannizzaro, E., Gagliano, M., Mangiapane, N., 1995a. Behavioural responsiveness to picrotoxin and desipramine in adult rats prenatally exposed to different benzodiazepine receptor agonists. Eur. Neuropsychopharmacol. 5, 523–526.

Cannizzaro, C., Cannizzaro, E., Gagliano, M., Mineo, A., Sabatino, M., Cannizzaro, G., 1995b. Effects of desipramine and alprazolam on forced swimming behaviour of adult rats exposed to prenatal diazepam. Eur. J. Pharmacol. 273, 239–245.

Cannizzaro, C., Cannizzaro, E., Gagliano, M., Mineo, A., 1998. Locomotor and antidepressant-like effects of 5-HT(1A) agonist LY 228729 in prenatally benzodiazepine-exposed rats. Eur. Neuropsychopharmacol. 8, 27–32.

Davies, J.M., Rosen, M., 1977. Intramuscular diazepam in labour. A double-blind trial in multiparae. Br. J. Anaesth. 49, 601–604.

Drago, F., Continella, G., Alloro, M.C., Lo Presti, L., Scapagnini, U., 1984. Behavioral effects of phosphatidylserine after perinatal administration in rats. Int. J. Dev. Neurosci. 2, 347–353.

Drago, F., Di Leo, F., Giardina, L., 1999. Prenatal stress induces body weight deficit and behavioural alterations in rats: the effect of diazepam. Eur. Neuropsychopharmacol. 3, 239–245.

Fernandez-Guasti, A., del Rio Postilla, I.Y., Ugalde, E., Corsi-Cabrera, M., 2003. Diazepam and progesterone produce sexually dimorphic actions on the rat EEG: role of the neonatal sexual differentiation process. Psychoneuroendocrinology 28, 85–100.

Fox, M.W., 1965. Reflex ontogeny and behavioral development of the mouse. Anim. Behav. 13, 234–245.

Gai, N., Grimm, V.E., 1982. The effect of prenatal exposure to diazepam on aspects of postnatal development and behavior in rats. Psychopharmacology 78, 225–229.

Gavish, M., Avnimelech-Gigus, N., Feldon, J., Myslobodsky, M., 1985. Prenatal chlordiazepoxide effects on metrazol seizures and benzodiazepine receptors density in adult albino rats. Life Sci. 36, 1693–1698.

Herman, R.J., Wilkinson, G.R., 1996. Disposition of diazepam in young and elderly subjects after acute and chronic dosing. Br. J. Clin. Pharmacol. 42, 147–155.

Inglefield, J.R., Bitran, D., Olschowka, J.A., Kellogg, C.K., 1993. Selective effects on CRF neurons and catecholamine terminals in two stress-responsive regions of adult rat brain after prenatal exposure to diazepam. Brain Res. Bull. 31, 353–359.

Iqbal, M.M., Sobhan, T., Aftab, S.R., Mahmud, S.Z., 2002. Diazepam use during pregnancy: a review of the literature. Del. Med. J. 74, 127–135.

Kapur, J., 2000. Hippocampal neurons express GABA A receptor insensitive to diazepam in hyperexcitable conditions. Epilepsia 41 (6), S86–S89.

Kellogg, C.K., Primus, R.J., Bitran, D., 1991. Sexually dimorphic influence of prenatal exposure to diazepam on behavioral responses to environmental challenge and on gamma-aminobutyric acid (GABA)-stimulated chloride uptake in the brain. J. Pharmacol. Exp. Ther. 256, 259–265.

Koff, J.M., Miller, L.G., 1995. Prenatal lorazepam exposure: persistent alterations in pentylenetetrazole-induced seizure threshold and GABA-dependent chloride uptake after prenatal lorazepam exposure. Pharmacol. Biochem. Behav. 51, 721–724.

Kokka, N., Sapp, D.W., Witte, U., Olsen, R.W., 1992. Sex differences in sensitivity to pentylenetetrazol but not in $GABA_A$ receptor binding. Pharmacol. Biochem. Behav. 43, 441–447.

Laegreid, L., Olegard, R., Walstrom, J., Conradi, N., 1989. Teratogenic effects of benzodiazepine use during pregnancy. J. Pediatr. 114, 126–131.

Lauder, J.M., 1993. Neurotransmitters as growth regulatory signals; role of receptors and second messengers. Trends Neurosci. 19, 465–493.

Laurie, D.J., Wisden, W., Seeburg, P.H., 1992. The distribution of thirteen $GABA_A$ receptor subunits mRNAS in the rat brain: III. Embryonic and postnatal development. J. Neurosci. 12, 4151–4172.

Livezey, G.T., Marczynsky, T.J., Isaac, L., 1986. Prenatal diazepam: chronic anxiety and deficits in brain receptors in mature rat progeny. Neurobehav. Toxicol. Teratol. 8, 425–432.

Lo Turco, J.L., Owens, D.F., Health, M.J.S., Davies, M.B.E., Kreigstein, A.R., 1995. GABA and glutamate depolarise cortical progenitor cells and inhibit DNA synthesis. Neuron 15, 1287–1298.

Ma, W., Saunders, P.A., Somogyi, R., Poulter, M.O., Barker, J.L., 1993. Early co-expression of $GABA_A$ receptor subunit mRNAS in the developing rat spinal cord and dorsal root ganglia. J. Comp. Neurol. 338, 337–359.

Pippingskold, K., Lehtinen, A.M., Laatikainen, T., Hanninen, H., Korttila, K., 1991. The effect of orally administered diasepam and midazolam on plasma beta-endorphin, ACTH and preoperative anxiety. Acta Anaesthesiol. Scand. 35, 175–180.

Pohorecky, L.A., Roberts, P., 1991. Activity in a modified open-field apparatus: effect of diazepam and prenatal stress. Neurotoxicol. Teratol. 13, 129–133.

Poulter, M.O., Barker, J.L., O'Carrol, A.M., Lolait, S.J., Mahan, L.C., 1992. Differential and transient expression of $GABA_A$ receptor alpha-subunit mRNAS in developing rat CNS. J. Neurosci. 12, 2888–2900.

Poulter, M.O., Barker, J.L., O'Carrol, A.M., Lolait, S.J., Mahan, L.C., 1993. Co-existent expression of GABAA receptor beta 2, beta 3 and gamma 2 subunit messangers RNAS during embryogenesis and early postnatal development of the rat central nervous system. Neuroscience 53, 1019–1033.

Poulter, M.O., Ohannesian, L., Lammert, Y., Feltz, P., 1997. Evidence that $GABA_A$ receptor subunit mRNA expression during development is regulated by $GABA_A$ receptor stimulation. J. Neurochem. 68, 631–639.

Ryan, C.L., Pappas, B.A., 1986. Intrauterine diazepam exposure: effects on physical and neurobehavioral development in the rat. Neurobehav. Toxicol. Teratol. 8, 279–286.

Taketo, M., Yoshioka, T., 2000. Developmental change of GABA(A) receptor-mediated current in rat hippocampus. Neuroscience 96, 507–514.

Toaff, M.E., Hezroni, J., Toaff, R., 1977. Effect of diazepam on uterine activity during labor. Isr. J. Med. Sci. 13, 1007–1012.

Tucker, J.C., 1985. Benzodiazepines and the developing rat: a critical review. Neurosci. Biobehav. Rev. 9, 101–111.

Wahlsten, D., 1974. A developmental time trial for postnatal changes in brain and behavior of B6D2F2 mice. Brain Res. 72, 251–265.