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Prenatal Lorazepam Exposure: 4. Persistent Alterations in Pentylenetetrazole-Induced Seizure Threshhold and GABA-Dependent Chloride Uptake After Prenatal Lorazepam Exposure

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KOFF, J. M. AND L. G. MILLER. *Prenatal lorazepam exposure: 4. Persistent alterations in pentylenetetrazole-induced seizure threshold and GABA-dependent cholride uptake after prenatal lorazepam exposure.* PHARMACOL BIOCHEM BEHAV **51**(4) 721-724, 1995. – Prenatal benzodiazepine exposure is associated with behavioral and neurochemical alterations in the early postnatal period. To determine the persistence of these effects, we evaluated pentylenetetrazole-induced seizure threshhold and GABA-dependent chloride uptake in mice at 6 and 12 months of age after prenatal lorazepam exposure. Seizure threshhold was reduced after acute lorazepam pretreatment in mice exposed to lorazepam prenatally, compared to control groups, at 6 and 12 months of age. Maximal GABA-dependent chloride uptake was also reduced in exposed mice at 6 and 12 months of age. These data indicate that behavioral and neurochemical alterations persist well into maturity after prenatal lorazepam exposure.

Prenatal Lorazepam $GABA_A$ receptor Benzodiazepine

PRENATAL exposure to benzodiazepines has been associated with a specific developmental syndrome in humans [e.g., (9)]. A considerable literature in animals also indicates behavioral alterations in offspring exposed to benzodiazepines prenatally in several species (7,14). Most studies address alterations in the immediate postnatal period or soon thereafter. However, some reports indicate that behavioral changes occur into maturity [e.g., (2)]. Our own studies indicate alterations in motor activity and pentylenetetrazole-induced seizure threshhold in mice at the onset of maturity at 6 weeks of age (3,4).

In addition, limited data suggest that prenatal benzodiazepine exposure is associated with neurochemical alterations in offspring. For example, studies from our laboratory (3,4) and those by Bitran and colleagues (2) indicate alterations in $GABA_A$ receptor binding and function after prenatal benzodiazepine exposure. These data concern both the immediate postnatal period and mature animals, but no study has attempted a more extensive time course for neurochemical alterations.

To evaluate in more detail the persistence of behavioral and neurochemical alterations in mice exposed to a benzodiazepine prenatally, we exposed mice to lorazepam in utero and evaluated pentylenetetrazole-induced seizure threshhold and GABA-dependent chloride uptake at 6 months and 1 year of age.

METHOD

Materials

Female CD1 mice were obtained from Charles River Laboratories (Wilmington, MA) and maintained on a 12 : 12D cycle

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with food and water ad lib. Osmotic pumps (Model 2001) were obtained from Alza (Palo Alto, CA). Lorazepam was a gift from Wyeth (Radnor, PA) and pentylenetetrazole and muscimol were obtained from Sigma (St. Louis, MO). [$^{36}Cl^-$] (spec. act. 12.5 mCi/mg) was obtained from DuPont-NEN (Boston, MA). All other reagents were purchased from standard commercial sources.

Lorazepam Exposure

Female CD1 mice were treated with lorazepam as previously described (4). Briefly, at day 14 of gestation, mice were implanted with SC osmotic pumps delivering 2 mg/kg/day of lorazepam in PEG 400 or vehicle. Control mice received no implanted pumps. Maternal and fetal lorazepam concentrations achieved by this method were confirmed in a prior study (4). Pumps were removed at day 20 of gestation.

Handling of Offspring

All offspring were reared by untreated control dams. There were no differences in litter size or weights among the treatment groups at 6 or 12 months, or between these age groups (data not shown). Mice were weaned at 21 days of age and males and females of each litter were maintained in separate cages.

Pentylenetetrazole-Induced Seizure Threshhold

Pentylenetetrazol-induced seizures were performed as previously described (13). Briefly, tail veins were cannulated using a 25-ga needle and pentylenetetrazol, 7.5 mg/ml, was injected into unrestrained mice at a rate of 0.6 ml/min. Injections were terminated at the onset of a tonic-clonic seizure as determined by two observers. At 6 months of age, mice were evaluated as above and after lorazepam, 10 mg/kg IP 20 min prior to pentylenetetrazole. At 12 months of age, evaluations were performed only after this dose of lorazepam.

GABA-Dependent Chloride Uptake

Uptake of [$^{36}Cl^-$] was performed as previously described (4). Briefly, cortical synaptoneurosomes were incubated for 10 min at 30°C. Tissue was then added to mitrotiter wells containing the GABA analogue muscimol, 0–50 μM and [$^{36}Cl^-$]. After 10 s, the reaction was terminated by filtration using buffer containing picrotoxin (6 μM). Chloride uptake was quantitated by scintillation spectrometry. Each assay was performed in triplicate using tissue pooled from three to four cortices.

TABLE 1

PENTYLENETETRAZOLE-INDUCED SEIZURE THRESHHOLD AFTER PRENATAL LORAZEPAM EXPOSURE

	Pentylenetetrazole (mg)	
Exposure	6 Months	12 Months
Control	0.97 ± 0.03	0.90 ± 0.05
Vehicle	1.06 ± 0.07	0.93 ± 0.06
Lorazepam	0.97 ± 0.08	0.86 ± 0.08

Pentylenetetrazole-induced seizures were performed as described in the text. Results are mean ± SEM, n = 3–5 litters for each group. There are no significant differences.

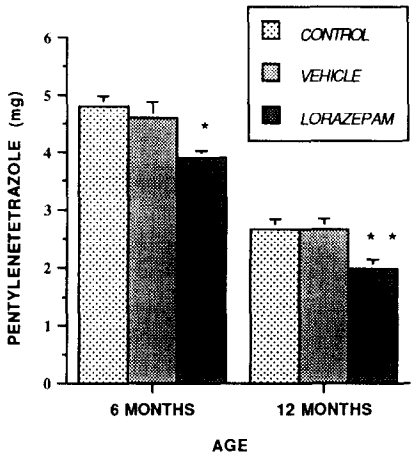

FIG. 1. Pentylenetetrazole-induced seizure threshhold after prenatal benzodiazepine exposure. Mice were exposed to lorazepam or vehicle prenatally as described in the text. At 6 or 12 months of age, mice then received lorazepam, 10 mg/kg IP, and after 20 min were infused with pentylenetetrazole, 4.5 mg/min until the onset of a tonic-clonic seizure. Results are mean ± SEM, n = 3–5 litters at each point. *p < 0.05 vs. control and vehicle at 6 months; **p < 0.05 vs. control and vehicle at 12 months.

Data Analysis

All data were analyzed by litter rather than by individual animals to avoid potential litter effects (5). For chloride uptake studies, data were fit to exponential functions and maximal uptake and EC_{50} values were determined from these functions. Statistical analyses employed analysis of variance with Dunnett's and Newman-Keuls tests. A value of $p < 0.05$ was considered significant.

RESULTS

Pentylenetetrazole-Induced Seizure Threshhold

At 6 months of age, seizure threshhold was similar after pentylenetetrazole among the three exposure groups (Table 1). However, after acute lorazepam pretreatment of exposed mice, seizure threshhold was significantly reduced in mice exposed to lorazepam compared to control and vehicle-exposed mice (Fig. 1). Similar results were observed after acute lorazepam pretreatment in exposed mice at 12 months of age. In addition, seizure threshholds after lorazepam pretreatment of exposed mice were significantly reduced in each exposure group at 12 months of age compared to 6 months.

GABA-Dependent Chloride Uptake

At 6 months of age, maximal uptake was significantly reduced in mice exposed prenatally to lorazepam compared to control and vehicle-exposed mice (Fig. 2, Table 2). Similar

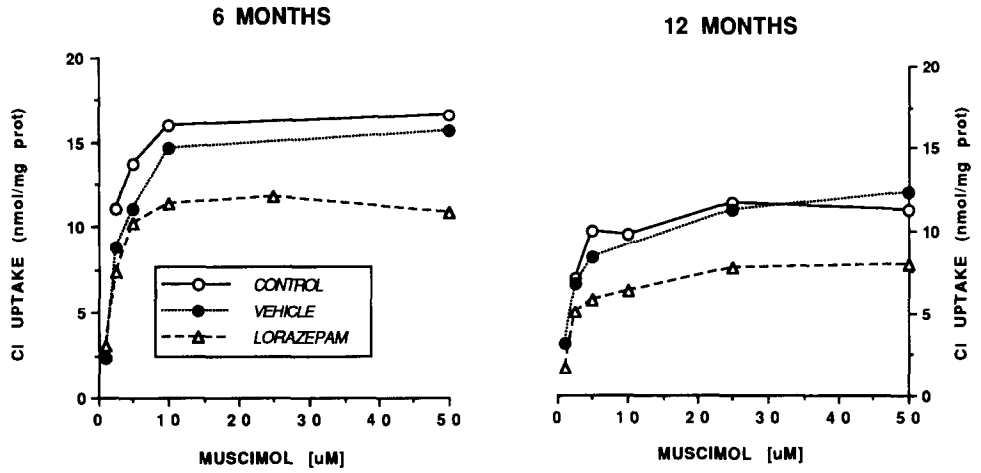

FIG. 2. GABA-dependent choride uptake after prenatal benzodiazepine exposure. Mice were exposed to lorazepam prenatally as described in the text. Muscimol-stimulated chloride uptake was performed as described. Results are from representative experiments for each age group.

results were observed in prenatally exposed at 12 months of age. Maximal uptake was significantly reduced in each prenatal exposure group at 12 months of age compared to 6 months. EC_{50} values were not significantly different in any exposure group at either age (Table 2).

DISCUSSION

These data indicate that prenatal benzodiazepine exposure is associated with persistent behavioral and neurochemical alterations. Pentylenetetrazole-induced seizure threshhold after acute lorazepam administration was significantly decreased at both 6 and 12 months of age after prenatal lorazepam exposure compared to controls. Maximal GABA-dependent chloride uptake was also significantly decreased at 6 and 12 months of age compared to controls. It is also notable that both seizure threshhold and chloride uptake were significantly reduced for each exposure group at 12 months compared to 6 months of age.

These data are consistent with prior studies from our laboratory. We previously reported no change in seizure threshhold in prenatally exposed mice at 6 weeks of age, but a reduction in threshhold determined after lorazepam pretreatment (3). This appeared to indicate a shift toward the "inverse agonist" spectrum of benzodiazepine activity after prenatal lorazepam exposure. Similar results were observed at 6 and 12 months of age in the present study, indicating persistence of the effects of prenatal lorazepam exposure through a substantial portion of the life span of the mouse. Similar changes in GABA-dependent chloride uptake after prenatal lorazepam exposure also ocurred at 6 weeks, 6 months, and 12 months, indicating persistent neurochemical alterations (4).

Results for both behavioral and neurochemical assays from other laboratories are conflicting. Prior studies of seizure threshholds have reported increases, decreases, and no change after benzodiazepine exposure in rats (2,6,12). The study reported by Bitran et al. (2) was similar to the present data in that no change was reported in pentylenetetrazole-induced seizure threshhold after prenatal diazepam exposure. Comparison among studies is confounded by use of different benzodiazepines, a variety of exposure protocols, and differing seizure threshhold methodologies. Similar problems arise in comparisons of neurochemical studies. Bitran et al. (2) reported no change in GABA-dependent chloride uptake in benzodiazepine-exposed rats, using different exposure and uptake methods, but found an enhanced inhibition of uptake by the GABA antagonist bicuculline. The present study did not address effects of prenatal benzodiazepine exposure on benzodiazepine stimulation of $GABA_A$ receptor function.

The observed decrements in seizure threshhold and in GABA-dependent chloride uptake suggest reductions in both benzodiazepine and GABA efficacy after prenatal exposure. In concert with the reduced binding of the putative chloride channel ligand [^{35}S]TBPS at 6 weeks of age (10), these data are most consistent with changes in coupling of $GABA_A$ receptor subunits. Such a mechanism would account for the lack of change in seizure threshhold for pentylenetetrazole alone, as well as alterations in benzodiazepine effects and in chloride uptake. The molecular substrate for this effect remains uncertain; changes might be present in multiple $GABA_A$ subunits,

TABLE 2

GABA-DEPENDENT CHLORIDE UPTAKE AFTER PRENATAL LORAZEPAM EXPOSURE

Exposure	6 Months	12 Months
Maximal uptake (nmol/mg prot.)		
Control	16.4 ± 1.2	11.1 ± 1.0
Vehicle	15.1 ± 1.1	10.8 ± 0.5
Lorazepam	12.6 ± 0.8*	7.7 ± 0.8*
EC_{50} (μM)		
Control	1.4 ± 0.3	1.3 ± 0.2
Vehicle	1.9 ± 0.3	1.7 ± 0.3
Lorazepam	1.3 ± 0.2	1.2 ± 0.2

GABA-dependent chloride uptake was performed as described in the text. Results are mean ± SEM, n = 3–5 litters for each group. *$p < 0.05$ compared to control and vehicle groups.

or in α or β subunits alone (11). Recent evidence from our laboratory indicating decreased mRNA for several beta subunits is consistent with this hypothesis (Pratt et al., submitted for publication).

An unexpected finding in this study is the reduction in both seizure threshhold and GABA-dependent chloride uptake at 12 months compared to 6 months of age. Although prior data are conflicting, most behavioral studies reported little or no change with increasing age, although seizure threshhold was not specifically evaluated (1). Prior studies from our laboratory indicated no change in maximal chloride uptake with age, although both 6 and 12 month points were not evaluated (1).

The persistence of behavioral and neurochemical alterations after prenatal benzodiazepine exposure suggests a permanent alteration in $GABA_A$ receptor structure or function. Whether such an alteration occurs at the genetic level remains uncertain. Our recent data demonstrate benzodiazepine-sensitive sites in the upstream regulatory region for the human alpha1 subunit of the $GABA_A$ receptor (Kang and Miller, submitted for publication). These sites, if present for other subunits and in other species, might be modulated by prenatal exposure to yield persistent effects. Additional molecular genetic studies may address this issue.

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