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Altered Stressor-Induced Changes in $GABA_A$ Receptor Function in the Cerebral Cortex of Adult Rats Exposed In Utero to Diazepam

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KELLOGG, C. K., M. K. TAYLOR, M. RODRIGUEZ-ZAFRA AND G. L. PLEGER. *Altered stressor-induced changes in $GABA_A$ receptor function in the cerebral cortex of adult rats exposed in utero to diazepam.* PHARMACOL BIOCHEM BEHAV **44**(2) 267–273, 1993.—Prenatal administration of the anxiolytic drug diazepam (DZP), 2.5 mg/kg) to the pregnant rat over gestational days 14–20 altered function and stressor-induced responsiveness of the $GABA_A$ receptor in the cerebral cortex of exposed animals as adults. In Experiment 1, the impact of 15 min of restraint on chloride-facilitated benzodiazepine binding was evaluated in male and female rats at 70–90 days of age. Early exposure to DZP led to an enhanced potency of chloride on binding in both males and females. In Experiment 2, GABA stimulation of ^{36}chloride uptake was measured in male rats at 35 or 70 days of age following 10 min of forced swimming at ambient temperature. In control animals, stressor-induced changes in receptor function were not evident until 70 days, and in DZP-exposed rats the stressor had no effect on receptor function at either age. These changes in $GABA_A$ receptor responsiveness induced by early exposure to DZP may underlie the disrupted behavioral responses to environmental challenge that have been previously reported.

$GABA_A$ Cerebral cortex Stress Diazepam

THERE is accumulating evidence that exposure to the benzodiazepine (BDZ) diazepam (DZP) during late gestation in the rat leads to altered responses to environmental challenges (stressors) in animals as adults. For example, such exposure has been shown to alter stressor-induced changes in plasma levels of corticosterone and prolactin (26), interfere with stressor-induced changes in norepinephrine turnover in the hypothalamus (26) and dopamine turnover in the prefrontal cortex (4), and alter specific behavioral responses to challenge (12). The primary site of action of BDZ compounds is on the polymolecular γ-aminobutyric acid–BDZ–chloride channel receptor complex, the $GABA_A$ receptor (7). This complex is present in the rat brain during late gestation as evidenced by the presence of BDZ binding sites throughout the fetal brain (22). Further, the $GABA_A$ receptor in the cerebral cortex appears linked to a chloride channel by late gestation (11). That the consequences of early developmental exposure to DZP include alterations in multiple stress responses suggests that the action of the drug in utero, acting via the $GABA_A$ receptor, may have interfered with the organization of neural systems underlying integrated responses to stressors.

Recent reports strongly support a participatory role for the $GABA_A$ receptor in an adult organism's response to challenge. Environmental challenges induce changes in $GABA_A$ receptor function in the cerebral cortex, changes that involve modification of the associated chloride channel, as indicated by stressor-induced changes in *t*-butylbicyclophosphorothionate (TBPS) binding and chloride enhancement of BDZ binding (8,28), as well as changes in GABA-gated chloride flux (24). Further, specific stressor-induced changes in GABA receptor function reflect prior experience of the organism (18).

The focus of the present research was to determine, therefore, whether late gestational exposure to DZP led to altered responsiveness of the $GABA_A$ receptor to a stressor. In studies of GABA-mediated chloride uptake, we observed an enhanced sensitivity to GABA (12) and bicuculline (2) in the adult cerebral cortex following late gestational exposure to DZP. Others reported decreased GABA-gated chloride uptake and decreased TBPS binding in adult mice following prenatal exposure to lorazepam (3,14). However, these changes were all observed in animals in a resting (basal) state. The responsiveness of the receptor complex to environmental challenge has not been evaluated. In the present study, we evaluated the influence of stressors on function of the $GABA_A$ receptor complex using two measures. In the first experiment, the effect of restraint on chloride-facilitated BDZ binding was eval-

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uated in the cerebral cortex of both male and female adult rats. Some of the effects of prenatal exposure to DZP are sexually dimorphic (6,12), and the first experiment was designed with that observation in mind. In the second experiment, the effect of forced swimming on GABA-mediated chloride uptake was evaluated in male rats at 35 and 70 days of age. Previous work has demonstrated that $GABA_A$ receptor function in naive, male rats is not influenced by environmental challenges at a late juvenile age (28 days) (19). We therefore compared function of the $GABA_A$ receptor at early vs. late adolescent ages in the present study. The results of both studies indicate that prenatal exposure to DZP does lead to altered responsiveness of the $GABA_A$ receptor complex in the cerebral cortex of rats as adults.

METHOD

Animals and Procedure

Adult (200–300 g) female rats (Long Evans, Blue Spruce; Harlan-Sprague Dawley, Altamont, NY) were mated overnight with males of the same strain. Gestation day 0 was designated as the day that sperm were detected in vaginal smears. Pregnant rats were weighed on gestational day 13 and randomly assigned to experimental or control groups. Experimental animals were injected subcutaneously with DZP (2.5 mg/kg; injectable preparation, Hoffmann-LaRoche, Inc., Nutley, NJ) once daily over gestational days 14–20. This dose of DZP has been widely used in our work (9,10) and has been shown to yield plasma levels of DZP and metabolites comparable to levels achieved in humans following exposure during pregnancy and labor (25). Diazepam persists in the brains of rat pups at low concentration up to 10 days postnatal age following prenatal exposure to the dose used in this study. However, no drug is detectable in brains of exposed pups at 21 days postnatal age (25). The amount of DZP administered was based upon the pregnant dam's weight on day 13 and held constant over the injection period. Control rats either received a comparable volume of vehicle (40% propylene glycol, 10% ethyl alcohol) or were left untreated. All injections were given at a volume of 0.5 ml/kg.

Litters were culled to 12 pups on the day of birth (designated postnatal day 0). Exposed pups were not fostered at birth to untreated dams because extensive work from this laboratory has shown that the consequences of in utero DZP exposure are not influenced by fostering. Offspring were weaned on day 28 and housed by sex and exposure. All animals were maintained in a colony room on a 12L : 12D cycle (lights on at 0600 h) at a constant temperature (24°C) and had ad lib access to food and water. In any one group, animals from a minimum of three litters were included. All experiments were initiated between 0800 and 1000 h. In the first experiment, male and female animals were studied at 70–90 days of age. While no effort was made to use females on a designated day of their estrous cycle, an effort was made to initiate studies on females within the first 2–4 h after the onset of light when, regardless of stage of the estrous cycle, progesterone levels are low (27). Further, in a related study vaginal smears were made of female subjects (exposed in utero to DZP or vehicle) for 3 days prior to testing, as well as on the test day, and the results indicated that in a random selection of females from the colony room approximately 75% were in diestrus on the test day. Hence, on any one day a high percentage of female rats removed from the colony room will be in the same stage of their estrous cycle. Because progesterone metabolites can influence $GABA_A$ receptor function and the anxiolytic efficacy of DZP (1,5), it is important to try and limit any variability that may arise from different hormonal states. In the second study, male rats only were studied at 35 or 70 days of age.

Environmental Challenge

Restraint. In Experiment 1, adult male (n = 3) and female (n = 4) rats were placed in an acrylic restrainer appropriate for animals 250–300 g (Harvard Apparatus, South Natick, MA). After a 15-min period, animals were quickly removed from the restrainers and rapidly killed by cervical luxation. To obtain basal measures, animals (three males and four females) remained in the colony room until just prior to sacrifice.

Forced swimming. To study the effects of stressors on GABA receptor function in relation to pubertal age, forced swimming was selected as the stressor because the same chamber could be used for animals of varying size and weight. The chamber was 17.5 in. in diameter and the water depth was 22 in. Temperature was maintained at 23–25°C. All animals were placed in the water for 10 min, removed, and immediately killed as indicated above. Animals that remained in the colony room until just prior to sacrifice were utilized to obtain basal measures. Three animals were included in each group at each age; within a group, all animals came from a different litter. The index of GABA receptor function used in this study was GABA-stimulated chloride uptake. A previous report indicated that forced swimming enhanced both the efficacy and potency of GABA on chloride uptake (24). All animals were prehandled daily for 5 days prior to testing because previous observations indicated that stressor-induced changes in this index would not be apparent if animals were not prehandled.

Analysis of $GABA_A$ Receptor Function

Chloride-enhanced BDZ binding. Following decapitation, the cortex was dissected from the rest of the brain. Chloride enhancement of BDZ binding was measured according to procedures previously used in this laboratory (18,19). Briefly, tissue was homogenized in 50 vol Tris-HCl buffer (50 mM, pH 7.4) and centrifuged. The resulting pellet was washed four times in 50 mM Tris-citrate buffer (pH 7.4) containing 100 mM NaCl. The tissues was washed a final time in Tris-citrate buffer without NaCl. The binding assay was initiated immediately following preparation of the tissue. Binding was carried out at 0°C in a total volume of 1 ml containing: 0.65 ml Tris-citrate buffer, 0.05 ml [^{3}H]flunitrazepam ([^{3}H]Flu; 1 nM final concentration), 0.1 ml tissue, displacer, and salt to final volume. Basal binding was measured in the absence of NaCl. Facilitation of [^{3}H]Flu binding was measured over seven concentrations of NaCl (12.5–500 mM). Nonspecific binding was determined by the addition of 10 μM clonazepam. The reaction was terminated by filtration over GF/B filters using a Brandel Harvester. Chloride enhancement of binding was expressed as percent facilitation over binding in the absence of NaCl. The efficacy of chloride on BDZ binding (the percent maximal facilitation) and the potency of chloride (the inverse of the EC_{50}) were determined for each experiment from computer-derived curves (20). Basal [^{3}H]Flu binding (measured in the absence of NaCl) was expressed as nmoles/mg protein. Protein was measured using a Lowry procedure (13).

GABA-stimulated chloride uptake. Prior to dissection of the cortex from the rest of the brain, meninges were removed. The cortex was separated from underlying white matter. Tissue was homogenized in HEPES-Tris buffer (pH 7.4) and

synaptoneurosomes prepared as described by Schwartz et al. (23). GABA stimulation of chloride uptake was measured as described by Kellogg and Pleger (11). Briefly, uptake was measured in a total volume of 0.5 ml containing 0.5 μCi ^{36}chloride, varying concentrations (10) of GABA (1–1,000 μM), and HEPES-Tris buffer. The reaction was initiated by the addition of preincubated (30°C) freshly prepared synaptoneurosomes to preincubated reaction tubes. The protein per incubation tube (in mg) was 1.89 ± 0.05 at 35 days and 1.80 ± .005 at 70 days. The reaction was terminated after 10 s by adding cold HEPES-Tris buffer containing picrotoxin (100 μM) and immediately filtering through GF/C filters presoaked in 0.05% polyethylenimine. GABA-mediated chloride uptake was expressed as percent stimulation over basal uptake (uptake in the absence of GABA). The efficacy of GABA (maximal percent stimulation) and the potency of GABA (the inverse of the EC_{50}) were determined for each experiment from computer-derived curves (20). Basal chloride uptake (in the absence of GABA) was expressed as nmoles/mg protein.

Statistical Analysis

The data were analyzed using a commercial statistical package (BIMED BMDP, 1990) for one- or two-way analysis of variance (ANOVA) and covariance (ANCOVA) for repeated measures. Significance was determined at $p < 0.05$. For graphic representation, the data were curve fit (Kaleidagraph) using a nonweighted, nonlinear program based upon a four-parameter logistic equation.

RESULTS

Effect of Restraint on Chloride-Enhanced BDZ Binding

Chloride enhancement of BDZ binding in the cerebral cortex from both males and females is illustrated in Fig. 1. Basal [^{3}H]Flu binding (at 1 nM) in the absence of NaCl is indicated in Table 1 and did not differ as a function of environmental condition or prenatal exposure for either male or female rats. In all groups, however, [^{3}H]Flu binding varied significantly with chloride concentration [males, $F(6, 48) = 39.62$, $p < 0.0001$; females, $F(6, 72) = 112.79$, $p < 0.0001$]. Observation of the data presented in Fig. 1 indicates that the effect of chloride on BDZ binding differed markedly between adult male and female rats. Chloride appeared to be more potent in tissue from male rats and the response to the stressor was more robust in male rats. To more precisely define the effects of prenatal exposure or condition on chloride-enhanced binding, the efficacy and potency of chloride (calculated for each experiment) were analyzed for both male and female rats using two-way ANOVA. The data are presented in Table 1. Analysis indicated that in male rats the percent maximal stimulation (efficacy) varied significantly as a function of condition, $F(1, 8) = 13.23$, $p < 0.01$, but not as a function of prenatal exposure. Hence, collapsing across exposure group the percent maximal stimulation was higher in restrained animals. The potency of chloride (the inverse of EC_{50}), however, varied significantly as a function of prenatal exposure, $F(1, 8) = 9.02$, $p < 0.02$. In addition, there was a significant interaction between exposure and condition, $F(1, 8) = 6.27$, $p < 0.05$, in male rats. Thus, the EC_{50} for chloride (collapsing across condition) was less in animals prenatally exposed to DZP than in controls. Looking more closely, however, the EC_{50} for chloride was markedly less in animals exposed in utero to DZP than in controls only in the basal state. In response to restraint, the EC_{50} for chloride tended to decrease (albeit minimally) in control male rats relative to the basal state (a typical response to stressors), whereas in prenatally exposed male rats the EC_{50} increased from the basal state in response to restraint.

In female rats, analysis also indicated that the efficacy of chloride (maximal percent facilitation) varied significantly as a function of condition, $F(1, 12) = 12.14$, $p < 0.005$, but did not vary as a function of prenatal exposure. The potency of chloride (the inverse of EC_{50}), however, varied significantly as a function of both condition, $F(1, 12) = 17.33$, $p < 0.001$, and exposure, $F(1, 12) = 4.47$, $p < 0.05$. Collapsing across exposure groups, the EC_{50} for chloride increased following restraint; thus, the potency decreased. Collapsing across conditions, the EC_{50} for chloride was lower in prenatally exposed rats than in controls. There was no significant interaction between exposure and condition in females. Thus, the potency of chloride was decreased by restraint in both control and prenatally exposed female rats; however, DZP-exposed rats demonstrated a greater potency of chloride in both basal and stressor-induced conditions. Female rats then appeared to respond to the stressor somewhat differently from males; in response to a stressor, both efficacy and potency tend to increase in control male rats, whereas in females, while the efficacy of chloride was increased by the stressor the potency was markedly decreased. In both sexes, the major effect of prenatal exposure to DZP was to increase the sensitivity of the BDZ recognition site to chloride (increase the potency of chloride).

Effect of Forced Swimming on GABA-Mediated Chloride Uptake

The ability of GABA to enhance chloride uptake at both 35 and 70 days of age in male rats is illustrated in Fig. 2. In all groups, the percent stimulation varied significantly with GABA concentration, $F(8, 64) = 407.48$, $p < 0.0001$. Basal chloride uptake (in the absence of GABA) did not vary with condition or exposure and was 22.79 ± 0.31 nmoles/mg protein at 35 days and 26.00 ± 0.37 nmoles/mg protein at 70 days. It also seems quite clear from the data shown in Fig. 2 that environmental condition affected GABA-mediated chloride uptake only in 70-day-old control (vehicle exposed) animals. To verify this observation, the maximal stimulation by GABA was analyzed by one-way ANOVA for each prenatal exposure group at the two ages. As indicated in Table 2, the percent maximal stimulation varied significantly as a function of condition (swim vs. basal) in the adult vehicle-exposed group, $F(1, 4) = 7.48$, $p < 0.05$, but not in the adult DZP-exposed group, $F(1, 4) = 0.09$, $p > 0.5$. At 35 days, the percent maximal stimulation measured in the basal state in both vehicle- and DZP-exposed rats was higher than that measured at 70 days in the same condition in both exposure groups. There was no effect of forced swimming on maximal facilitation measured at 35 days. The EC_{50} for GABA stimulation of chloride uptake did not differ as a function of condition or exposure and was similar at both ages. These results indicate, therefore, that the ability of a stressor to modify GABA-mediated chloride uptake appears late in adolescent development in the rat and that prenatal exposure to DZP interferes with the appearance of this stressor-related responsiveness of the cortical $GABA_A$ receptor.

DISCUSSION

The present results indicate that late gestational exposure to DZP alters the stressor-induced responsiveness of $GABA_A$ receptor in the cerebral cortex of exposed animals as adults. Previous studies have shown that in response to an environ-

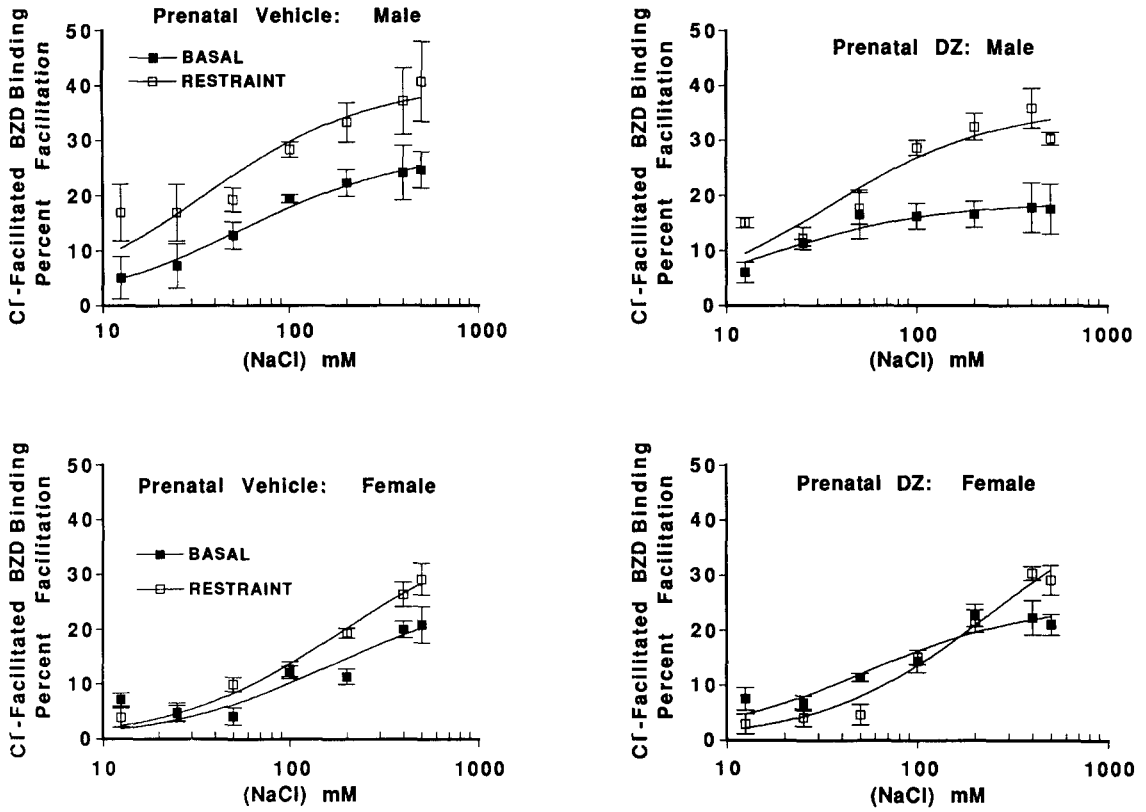

FIG. 1. Chloride-facilitated [^{3}H]flunitrazepam ([^{3}H]Flu) binding to membranes from the cerebral cortex of male and female rats at 70–90 days of age as a function of prenatal exposure to diazepam (DZ) over gestational days 14–20. Control animals were either exposed in utero to the vehicle or prenatally uninjected. Basal responses were measured in animals immediately after removal from the animal quarters and stressor-induced changes in the response were measured following 15 min of restraint. Data presented as mean ± SEM of percent facilitation of binding in the presence of NaCl over binding in the absence of NaCl. BZD, benzodiazapine.

TABLE 1

PRENATAL EXPOSURE TO DZP*: EFFECT ON RESTRAINT-INDUCED CHANGES IN [^{3}H]FLUNITRAZEPAM BINDING IN THE CEREBRAL CORTEX OF ADULT RATS

	Males		Females	
	Basal	Restraint	Basal	Restraint
Basal [^{3}H]Flu binding† (fmol/mg protein)				
Controls	487 ± 12	464 ± 59	492 ± 12	455 ± 27
DZP exposed	526 ± 40	515 ± 18	440 ± 32	454 ± 38
Chloride-enhanced [^{3}H]Flu binding % Maximal facilitation‡				
Controls	24.74 ± 3.70	37.72 ± 5.66	21.66 ± 2.29	28.32 ± 3.06
DZP exposed	17.25 ± 4.05	34.59 ± 2.72	23.32 ± 2.40	32.19 ± 1.45
EC_{50} (mM)§				
Controls	50.90 ± 8.71	44.04 ± 3.73	90.82 ± 8.45	143.84 ± 24.50
DZP exposed	17.65 ± 1.62	41.03 ± 7.32	51.46 ± 6.88	120.94 ± 12.28

Data expressed as mean ± SEM.

*2.5 mg/kg over gestational days 14–20.

†At 1 nM [^{3}H]Flu.

‡Significant effect of condition, males ($p < 0.05$) and females ($p < 0.005$).

§Males: Significant exposure × condition interaction ($p < 0.05$). Females: Significant effect of exposure ($p < 0.05$) and condition ($p < 0.001$).

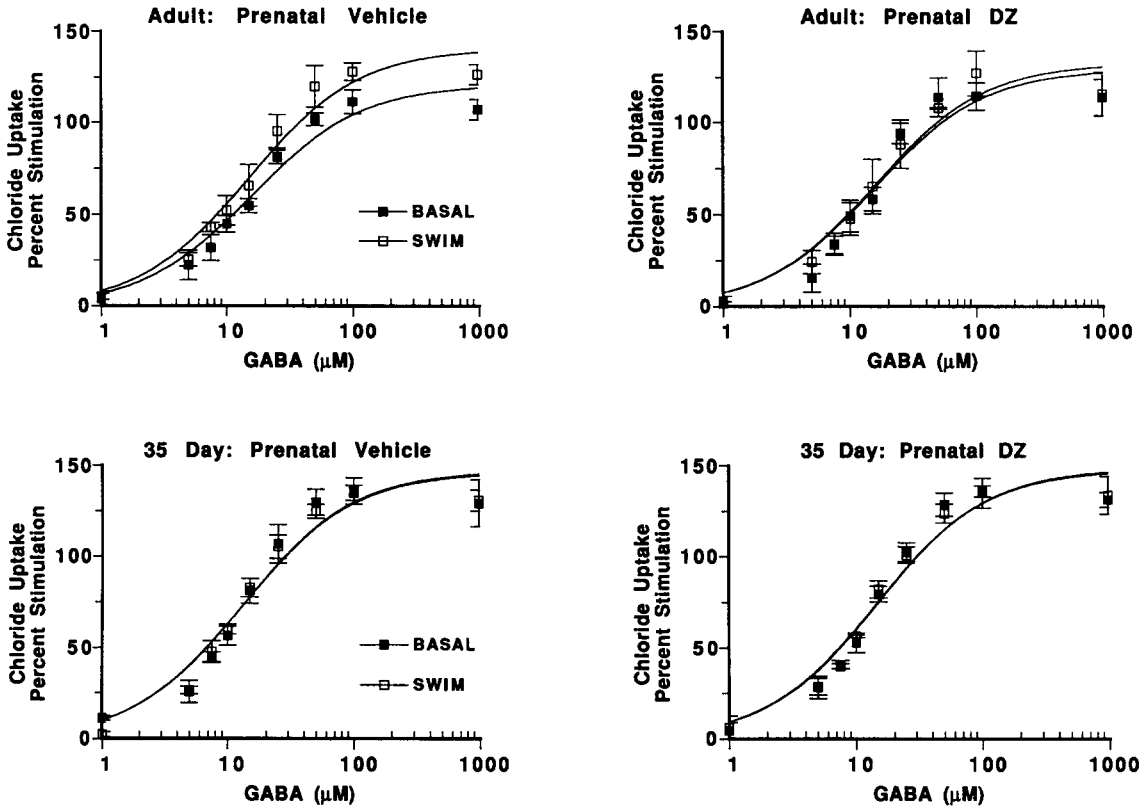

FIG. 2. GABA-stimulated ^{36}chloride uptake in synaptoneurosomal preparations of the cerebral cortex of male rats at 35 and 70 days of age as a function of prenatal exposure to diazepam (DZ) or vehicle over gestational days 14–20. Basal responses were measured in animals immediately after removal from the animal quarters and stressor-induced responses were measured following 10 min of forced swimming at ambient (23–25°C) temperature. Data presented as mean ± SEM of percent stimulation of chloride uptake in the presence of GABA over uptake in the absence of GABA.

mental challenge such as forced swimming there is an increase in the efficacy and potency of chloride on BDZ binding, an increase in TBPS binding, and an increase in the efficacy and potency of GABA on chloride uptake in the cerebral cortex of naive, male rats (8,24,28). Such changes have been interpreted to reflect adaptive responses of the organism.

In the present study, the efficacy and, to a lesser extent, the potency of chloride-facilitated BDZ binding were likewise potentiated by restraint in control male rats. Prenatal exposure to DZP, furthermore, altered the potency, but not the efficacy, of chloride-enhanced BDZ binding in male rats; the sensitivity of the BDZ recognition site to chloride was actually

TABLE 2

PRENATAL EXPOSURE TO DZP*: EFFECT ON STRESSOR-INDUCED† CHANGES IN GABA-STIMULATED CHLORIDE UPTAKE IN CORTICAL SYNAPTONEUROSOMES FROM MALE RATS AT EARLY AND LATE PUBERTAL AGES

	35 Days		70 Days	
	Basal	Swim	Basal	Swim
% Maximal uptake				
Control	135.78 ± 9.01	134.11 ± 3.30	110.21 ± 5.05	128.85 ± 4.57‡
DZP exposed	135.74 ± 4.58	135.89 ± 8.28	117.98 ± 8.29	122.32 ± 11.89
EC_{50} (μM)				
Control	12.21 ± 0.54	11.93 ± 1.32	14.16 ± 1.31	13.95 ± 1.79
DZP exposed	13.04 ± 1.41	12.83 ± 1.80	13.72 ± 1.00	14.97 ± 2.67

Data expressed as mean ± SEM.
*2.5 mg/kg administered to pregnant rat over gestational days 14–20.
†Fifteen minutes forced swimming at ambient temperature.
‡Significantly different from basal, control group ($p < 0.05$).

greater in the resting state in animals exposed in utero to DZP than in controls. Consistent with previous observations that prenatal exposure to BDZs does not alter binding characteristics of the BDZ recognition site (9,14), BDZ binding at 1 nM [^{3}H]Flu was not altered by prenatal exposure to DZP. The consequences of in utero exposure to DZP on chloride facilitation of BDZ binding in females were similar to those observed in males, that is, the potency of chloride was increased in both basal and stressor-induced conditions. However, in both control and DZP-exposed females the stressor-induced changes in this index of $GABA_A$ receptor function differed from control male rats: While the efficacy of chloride was increased, the potency of chloride decreased. The implication of this difference in the stressor-induced response of the complex in females to overall function of the receptor complex is unclear at this time. However, because the anxiolytic effect of BDZ compounds is influenced by hormonal state in females (1,5), the sensitivity of BDZ binding to chloride could change as a function of estrous cycle. While most females tested in this study were probably in the same stage of their estrous cycle, that stage was not determined.

In addition to altered chloride-facilitated BDZ binding, prenatal exposure to DZP also altered GABA-mediated chloride uptake. In young adult, control male rats, maximal stimulation of chloride uptake by GABA was enhanced following 10 min of forced swimming. While the magnitude of the response was less than previously reported (24), swimming in the present study was conducted at ambient (23–25°C) temperature, whereas in the previously reported study the temperature of the water was 15–17°C. The efficacy of GABA on chloride uptake in prenatally exposed adult male rats fell within the range of control animals in both basal and stressor-induced conditions; however, the difference between conditions was not significant in these animals. Thus, prenatal exposure to DZP prevented a differential response to the stressor.

Considering then the responsiveness of the $GABA_A$ receptor complex in the cerebral cortex of adult male rats to stressors, prenatal exposure to DZP interfered with the ability of a stressor to induce changes in GABA-mediated chloride uptake, and in response to a stressor the potency of chloride at the BDZ binding site was decreased (from the basal state) in DZP-exposed animals, not a normal response in male rats. It is difficult at this time to interpret these changes in terms of overall function of the complex. However, previous studies indicated that, as assessed behaviorally, prenatal exposure to DZP leads to an impairment in the processing of information about the environment in male rats. Thus, in tests of social interaction in familiar or unfamiliar environments, prenatal exposure to DZP led to decreased interaction in the familiar environment and increased interaction in the unfamiliar environment (12). Roberts suggested that the role of inhibitory systems (and GABA is the major inhibitory neurotransmitter in mammalian brains) is to enable nervous systems to generate variability in behavior, thereby allowing organisms to adapt effectively to changes in their environment (21). The changes in $GABA_A$ receptor responsiveness induced by prenatal exposure to DZP, therefore, may underlie the disrupted behavioral responses to environmental challenge. Other studies have reported that late gestational exposure to the BDZ lorazepam via osmotic minipumps leads to a marked decrease in the maximal density of TBPS binding sites and decreased GABA-mediated chloride uptake in 6-week-old mice in the basal state (data from males and females combined) (3,14). In the present study, only the potency of chloride at BDZ binding sites was altered in the basal state of young, adult male rats following prenatal exposure to DZP. Basal measures of GABA-stimulated chloride uptake were not altered by the early exposure. The difference in impact of the early exposure on later receptor function observed in this vs. the other studies cited may reside in either the difference in species, difference in route of exposure, difference in the BDZ compound used for prenatal exposure, or the fact that the previously reported studies combined data from males and females in the evaluation. However, both the reported studies and the present study indicate that altered function of the $GABA_A$ receptor can be observed following early developmental exposure to a BDZ ligand.

The present study also demonstrated that functional responsiveness of the $GABA_A$ receptor in the cerebral cortex to environmental challenge emerges over pubertal developmental. Ten minutes of forced swimming did not alter GABA-mediated chloride uptake in the cerebral cortex of male control rats at 35 days as it did at 70 days. In fact, the percent maximal stimulation observed in the basal state at 35 days was similar to that reached following forced swimming at 70 days, indicating that a change in receptor function had taken place between 35 and 70 days. Previous studies had shown that this index of receptor function was not responsive to environmental challenges at a late juvenile age (28 days) (19). However, the present results indicate that even early after the onset of puberty changes in responsiveness of the complex to a stressor could not be detected. Interestingly, early adolescent animals also do not make the same behavioral responses to environmental challenge that adult rats make (16). In addition, while animals at 35 days show a change in social interaction when placed in an unfamiliar environment, their behavior in this environment is not yet modifiable by acute administration of DZP (17). Pubertal development therefore appears to be a major developmental period for the organization of mature adult-like responses to challenge. The factors that may contribute to the emergence of stressor-induced responsiveness of the $GABA_A$ receptor complex as an organism approaches young adulthood are not yet understood. However, there is extensive evidence, both in vitro and in vivo (2,15), that specific steroids can influence function of the $GABA_A$ receptor. Previous results from this lab demonstrated that gonadal status of male rats over pubertal development can influence the responsiveness of the $GABA_A$ receptor in adult rats (19). Other factors, such as a reorganization of neural circuitry underlying behavioral responses to stressors, may also contribute to the emergence over puberty of differential stressor-related responses at the cortical $GABA_A$ receptor. The present results do demonstrate though that as the receptor complex normally becomes responsive to stressors the consequences of prenatal exposure to DZP on $GABA_A$ receptor responsiveness become apparent. Thus, the impact of forced swimming on GABA-mediated chloride uptake was not evident in prenatally exposed male rats at either 35 or 70 days whereas it was evident in control rats at 70 days.

The prerequisite of pubertal development for the expression of many of the consequences of early developmental exposure to BDZ compounds has been observed in many different studies determining responses to environmental challenge (9). The early developmental impact of these drugs, apparently acting via the $GABA_A$ receptor complex, may interrupt the organization of integrated responses to stressors. If neural and behavioral effects become visible later in life when there is no longer any drug present, then the consequences of early

developmental exposure to a drug must result because the drug altered or reorganized some aspect (or aspects) of brain function. The impact of prenatal insults on underlying neural systems, therefore, may not be expressed until late in adolescent development, after the final organization of many of an organism's responses to challenge.

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