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Effects of a Novel Cognition-Enhancing Agent on Fetal Ethanol-Induced Learning Deficits

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Abstract

Background—Drinking during pregnancy has been associated with learning disabilities in affected offspring. At present, there are no clinically effective pharmacotherapeutic interventions for these learning deficits. Here, we examined the effects of ABT-239, a histamine H₃ receptor antagonist, on fetal ethanol-induced fear conditioning and spatial memory deficits.

Methods and Results—Long-Evans rat dams stably consumed a mean of 2.82 g ethanol/kg during a 4-hour period each day during pregnancy. This voluntary drinking pattern produced a mean peak serum ethanol level of 84 mg/dl. Maternal weight gain, litter size and birth weights were not different between the ethanol-consuming and control groups. Female adult offspring from the control and fetal alcohol-exposed (FAE) groups received saline or 1 mg ABT-239/kg 30 minutes prior to fear conditioning training. Three days later, freezing time to the context was significantly reduced in saline-treated FAE rats compared to control. Freezing time in ABT-239-treated FAE rats was not different than that in controls. In the spatial navigation study, adult male offspring received a single injection of saline or ABT-239 30 minutes prior to 12 training trials on a fixed platform version of the Morris Water Task. All rats reached the same performance asymptote on Trials 9 to 12 on Day 1. However, 4 days later, first-trial retention of platform location was significantly worse in the saline-treated FAE rats compared control offspring. Retention by ABT-239-treated FAE rats was similar to that by controls. ABT-239's effect on spatial memory retention in FAE rats was dose dependent.

Conclusions—These results suggest that ABT-239 administered prior to training can improve retention of acquired information by FAE offspring on more challenging versions of hippocampal-sensitive learning tasks. Further, the differential effects of ABT-239 in FAE offspring compared to controls raises questions about the impact of fetal ethanol exposure on histaminergic neurotransmission in affected offspring.

Keywords

Fetal Alcohol Spectrum Disorder; Histamine H₃ Receptor; Ethanol; Morris Water Task; Contextual Fear Conditioning; Learning

Drinking during pregnancy has been associated with long-lasting intellectual impairments in affected children (Hamilton et al., 2003; Hanson et al., 1978; Kodituwakku, 2009; Manji et al., 2009; Shaywitz et al., 1980; Streissguth et al., 1990;) and in animal models of prenatal ethanol exposure (Berman and Hannigan, 2000; Kelley et al., 1988; Lochry and Riley, 1980; Sutherland et al., 2000; Weeber et al., 2001). The mechanisms by which fetal alcohol exposure (FAE) causes long-lasting impairments in learning and memory are not well understood. Consequently, progress toward developing pharmacotherapeutic agents that could ameliorate FAE-induced learning deficits has been limited until recently (Krahe et al., 2009; Medina et al., 2006; Thomas et al., 2007; Vaglenova et al., 2008;).

Our previous studies of Sprague-Dawley rats using a 5% ethanol liquid diet paradigm as a model of moderate drinking during pregnancy indicated that FAE offspring exhibit performance deficits on increasingly challenging memory tasks (Savage et al., 2002; Sutherland et al., 2000; Weeber et al., 2001). Collaborative studies in littermates of the rats tested in these learning paradigms suggested that the memory impairments are, in part, linked to neurophysiological alterations that diminish activity-dependent enhancement of hippocampal synaptic neurotransmission in affected offspring. Specifically, FAE-reduced hippocampal long-term potentiation (LTP) in vitro (Swartzwelder et al., 1988) and in vivo (Sutherland et al., 1997), and tetanus stimulus-induced potentiation of [³H]-D-aspartate (D-ASP) release, a marker for glutamate release, in hippocampal slices (Savage et al., 1998, 2002). Diminished activity-dependent potentiation of D-ASP release is one of the most striking functional deficits we have observed in prenatal ethanol-exposed rat offspring (Savage et al., 1998). This deficit was observed in offspring whose mothers' peak serum ethanol concentration was only 30 mg/dl (Savage et al., 2002). More recently, we established that one of the neurochemical mechanisms contributing to this deficit in FAE offspring is decreased metabotropic glutamate receptor-mediated potentiation of glutamate release at the entorhinal cortical—dentate granule cell synapse (Galindo et al., 2004). The identification of a fetal ethanol-induced deficit at presynaptic glutamatergic nerve terminals prompted the question of whether agents that potentiate glutamate release, particularly under activity-dependent conditions, could ameliorate FAE-induced learning deficits.

A variety of novel cognition-enhancing agents have been identified whose mechanisms of action may, in part, include the heterologous regulation of neurotransmitter release. Among these mechanisms is the antagonism of presynaptic histamine H₃ receptors (see review by Esbenshade et al., 2008). Presynaptic H₃ receptors inhibit the release of a number of central nervous system neurotransmitters including histamine (Arrang et al., 1983), serotonin, norepinephrine, dopamine (Schlicker et al., 1988, 1989, 1993), acetylcholine (Clapham and Kilpatrick, 1992), and glutamate (Brown and Haas, 1999; Garduno-Torres et al., 2007; Molina-Hernandez et al., 2001). Conversely, H₃ receptor antagonists enhance the release of histamine (Giannoni et al., 2009), acetylcholine (Bacciottini et al., 2000; Fox et al., 2005) and dopamine (Fox et al., 2005) in vivo, and electrically evoked D-ASP release in frontal cortical and hippocampal slices in vitro (El-Emawy and Savage, unpublished observations). Further, H₃ receptor antagonists ameliorate performance deficits in a variety of rodent learning paradigms (Bernaerts et al., 2004; Fox et al., 2002, 2003, 2004, 2005; Huang et al., 2004; Komater et al., 2005; Orsetti et al., 2002).

To date, the mechanism underlying the procognitive effects of H₃ receptor antagonists has primarily been associated with an enhancement of acetylcholine release. However, the putative action of these agents at glutamatergic synapses coupled with the deficits we have observed in presynaptic mechanisms of synaptic potentiation at the perforant path—dentate granule cell synapse in FAE offspring (Galindo et al., 2004)—prompted us to investigate whether these agents could ameliorate the behavioral deficits we have observed in FAE offspring. The present study had 2 experimental objectives. First, we wanted to establish

whether the learning deficits observed previously in Sprague-Dawley rats whose mothers consumed a 5% ethanol liquid diet (Sutherland et al., 2000; Weeber et al., 2001) also occur in Long-Evans rat offspring whose mothers voluntarily drank 5% ethanol in saccharin water. Long-Evans rats were selected for use in this new FAE paradigm based on the preponderance of their use in learning and memory studies (Andrews et al., 1995) and their better visual acuity in spatial navigation tasks (Prusky et al., 2002). The voluntary drinking paradigm was selected because it has the dual advantage of not requiring multiple control groups to control for paired-feeding of liquid diet as well as for allaying concern that the consumption of liquid diets may be stressful (Rasmussen et al., 2000), a potential confound in FAE studies. The second objective was to examine the effects of a systemic injection of ABT-239, a nonimidazole antagonist of H₃ histamine receptors (Esbenshade et al., 2005), on FAE-induced deficits in more challenging versions of contextual fear conditioning and spatial navigation tasks.

MATERIALS AND METHODS

Materials

All reagents were acquired from Sigma-Aldrich Corp., (St. Louis, MO) unless indicated otherwise in parenthetical text.

Voluntary Drinking Paradigm

All procedures involving the use of live rats were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Three- to four-month-old Long-Evans rat breeders (Harlan Industries, Indianapolis, IN) were single-housed in plastic cages at 22 °C and kept on a “reverse” 12-hour dark/12-hour light schedule (lights on from 2100 to 0900 hours) with Harlan Teklad rat chow and tap water ad libitum. After 1 week of acclimation to the animal facility, all female rats were provided 0.066% (w/v) saccharin in tap water for 4 hours each day from 1000 to 1400 hours. On Days 1 & 2, the saccharin water contained 0% ethanol and on Days 3 & 4 it contained 2.5% ethanol (v/v). On Day 5 and thereafter, the saccharin water contained 5% ethanol (v/v) (see Fig. 1C). Daily 4-hour consumption of ethanol was monitored for at least 2 weeks and then mean daily ethanol consumption was determined for each female breeder. Those females that drank > 1 standard deviation above or below the group mean were removed from the study. The remainder of the females were assigned to either a saccharin control or 5% ethanol prenatal drinking group and matched such that the mean prepregnancy ethanol consumption by each group was similar.

Subsequently, females were placed with proven male breeders until pregnant as evidenced by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on gestational Day 1 (GD1), rat dams were provided saccharin water containing either 0% or 5% ethanol for 4 hours a day. The volume of 0% ethanol saccharin water provided to the controls was matched to the mean volume of saccharin water consumed by the 5% ethanol drinking group. Daily 4-hour ethanol consumption was recorded for each dam. To minimize handling-induced stress, rat dams were weighed once a week during pregnancy and daily weight gains extrapolated to determine daily ethanol consumption. At birth, all litters were weighed and culled to 10 pups. Offspring were weaned at 28 days of age and maintained in gang cages of 2 males or 3 females until used in the behavioral experiments.

Maternal Serum Ethanol Levels

A separate set of twelve rat dams was used to determine serum ethanol concentrations. These dams were run through the same voluntary drinking paradigm as described earlier,

except that at the end of the 4-hour ethanol consumption period on each of 3 alternate days during the third week of gestation, each rat dam was briefly anesthetized with isoflurane. One hundred liters of whole blood was collected from the tail vein and immediately mixed with 0.2 ml of 6.6% perchloric acid, frozen and stored at -20°C until assayed. Serum ethanol standards were created by mixing whole blood from untreated rats with known amounts of ethanol ranging from 0 to 240 mg ethanol/dl and then mixing 100 μl aliquots of each standard with perchloric acid and storing the standards frozen with the samples. Serum ethanol samples were assayed using a modification of the method of Lundquist et al. (1959).

One-Trial Contextual Fear Conditioning

Female adult rat offspring, 90 to 150 days of age, from the 2 prenatal treatment groups received intraperitoneal injections of either saline or 1 mg ABT-239/kg 30 minutes prior to 1-trial contextual fear conditioning. Rats were fear-conditioned as described by Weeber et al. (2001) except that the training apparatus used was a Habitest System (Coulbourn Instruments, Allentown, PA) equipped with a Precision Regulated Shocker and a 30 cm wide $\times$ 25 cm deep $\times$ 30 cm high test cage. Briefly, after a 3-minute acclimation period in the chamber, a thirty-second-long 90 dB tone (the conditioned stimulus, CS) was initiated. Twenty-eight seconds into the CS, a 1 mA foot shock (the unconditioned stimulus) was administered for a period of 2 seconds through the stainless steel rods which make up the floor of the chamber. The rat was allowed 2 minutes after foot-shock to continue exploring the cage and then returned to its home cage. Fear-conditioned learning was assessed 72 hours later. The rat was returned to the conditioning chamber for a 5-minute testing session. The tone and foot shock were not delivered during this test session. The rat's behavior was videotaped and contextual fear-conditioned learning and memory to the context were assessed using a time-sampling procedure by 2 individuals blinded to the subject's group identity. Every 5 seconds, the rat was scored as either moving or "frozen" (the absence of movement other than respiratory movements). Data were expressed as percent freezing time.

Morris Water Task

Male adult rat offspring, 130 to 160 days of age, from the 2 prenatal treatment groups received an intraperitoneal injection of either saline or 1 mg ABT-239/kg 30 minutes prior to the first training trial on Day 1. The test apparatus was a plastic circular swimming pool, 175 cm in diameter with 72-cm high walls. Three unique visual cues were placed on each of the 4 walls above and beyond the tank wall. The distance between the pool edge and the walls was approximately 1 m. The pool was filled with water maintained at 22°C and made opaque by the addition of approximately 50 g of nontoxic white paint (Tempura). The wall height above the water line was maintained at 36 cm. A rectangular Plexiglas platform covered with a textured clear plastic material was maintained in the southwest quadrant of the pool with its top surface 0.5 cm below the surface of the water. The rat's swim path was tracked using a digital video camera fixed to the ceiling of the room and subsequently analyzed offline. Day 1, the "Training Day", consisted of twelve trials divided into 3 blocks of 4 trials. On a typical trial, the rat was released from 1 of 4 release points around the perimeter of the pool which were sampled randomly without replacement. The rat swam until it mounted the escape platform or until 60 seconds had elapsed since release, at which time the animal was guided by the experimenter to the platform. After 5 seconds on the platform, the rat was dried and returned to a holding cage for a 10-minute inter-trial interval. The home cages were placed beneath a heat lamp to reduce core temperature loss. Upon completion of the twelve training trials on Day 1, the rats were returned to their home cage in the animal colony room. No training was conducted on Days 2 through 4. Day 5, the "Test Day", was conducted with the platform in the same location as on the Training Day with the only additional methodological constraint that the test trials were selected randomly and alternating from either the north or east reference points, the 2 points of release furthest

from the southwest platform location, which increased the likelihood of detecting deficits in performance during the critical early test trials. All individual trials were videotaped for offline analyses. For each trial, the digitally tracked swim paths were used to compute escape latency.

Data Analysis

Statistical comparisons of each voluntary drinking paradigm measure were made using a Student's two-tailed *t*-test. Comparison of the effects of ABT-239 on saccharin control and ethanol-exposed offspring in each behavioral paradigm was made using a two-way analysis of variance. Subsequent one-way ANOVAs were used to test for significant differences among treatment groups. All statistical procedures were performed using SigmaPlot, Version 11.0 (Systat Software Inc., San Jose, CA).

RESULTS

Voluntary Drinking Paradigm

In a series of preliminary studies, we examined ethanol consumption as a function of ethanol concentration in 0.066% saccharin water and as a function of time after introduction of the drinking tube. Figure 1A illustrates the amount of ethanol consumed by nonpregnant female rats using different ethanol concentrations ranging from 2% to 6.2% ethanol in saccharin water. At the 2% concentration, rat dams consumed all of the ethanol provided in the 50-mL volume drinking tube. At higher concentrations of ethanol, the amount of ethanol consumed on a gram per kilogram body weight basis was not significantly different (Fig. 1A) suggesting that Long-Evans female rats will voluntarily consume a certain amount of ethanol independent of the ethanol concentration in drinking solution. Based on these results, we selected the 5% ethanol concentration for all subsequent studies. In a second preliminary study, we examined the hourly consumption of 5% ethanol. Ethanol consumption was greatest during the first hour after the drinking tubes were introduced at 1000 hours, and then diminished to a relatively steady state level during the second and subsequent third to sixth hours (Fig. 1B). Based on this study, we chose a 4-hour daily ethanol consumption period as a standard for all subsequent studies.

Finally, we characterized voluntary drinking in adult female rats for a minimum of 2 weeks prior to pregnancy and then throughout gestation. Rats were gradually introduced to 5% ethanol in a 3-step process over a period of the first 5 days of drinking, after which they sustained a relatively stable level of daily 4-hour ethanol consumption over the remainder of the first 2 weeks of drinking (Fig. 1C). To minimize the effects of variable drinking on offspring outcome measures, females who drank >1 standard deviation above or below the mean of the entire group during the 2-week pregnancy period were removed from the study. In the 4 rounds of breeding represented in this report, a total of 3 females drank >1 standard deviation above the group mean. About 12% of the females drank <1 standard deviation below the group mean during the prepregnancy drinking.

Figure 1C also illustrates ethanol consumption during pregnancy. Rat dams in the 5% ethanol group quickly returned to their prepregnancy drinking level and stably consumed a mean of 2.82 g of ethanol/kg body weight, roughly 16 ml of 5% ethanol, over the 4-hour interval each day. The peak mean serum ethanol level, determined in a separate set of dams, was 84.0 ± 5.5 mg ethanol/dl (Table 1). No significant differences in maternal weight gain during pregnancy, litter size or offspring birth weights were observed (Table 1). In addition, offspring body weights at the time of behavioral assessment were not different among prenatal treatment groups for either males or females (Table 1).

ABT-239 Dosing Considerations

A 1 mg ABT-239/kg dose was selected for our initial behavioral studies based on prior observations that it was an optimal test dose for increasing acetylcholine release in vivo and enhancing performance in other experimental models of cognitive deficits (Fox et al., 2005). Further, this ABT-239 dose does not alter foot shock sensitivity threshold or locomotor activity (Fox et al., 2005), parameters which could have confounded the interpretation of freezing to context or swimming behaviors. This dose produces a free drug concentration in brain of approximately 25 nM 40 minutes after an intraperitoneal injection (estimated from pharmacokinetic data in Fox et al., 2005 and pharmacodynamic data in Esbenshade et al., 2005). This concentration would occupy approximately 90% of H₃ receptors in brain. ABT-239 has a thousand-fold greater affinity for histamine H₃ receptors compared to H₁, H₂ and H₄ receptors and receptor occupancy at other mono-aminergic receptors is negligible at this dose (Esbenshade et al., 2005).

One-trial Contextual Fear Conditioning

Figure 2 illustrates the effects of prenatal ethanol exposure and ABT-239 on 1-trial contextual fear conditioning in adult female offspring. Female offspring were selected for this initial study based on our previous 1-trial contextual fear conditioning study using female Sprague-Dawley rat offspring (Weeber et al., 2001). Freezing to context in saline-treated saccharin control female offspring averaged about 57% of the total time in the context chamber (Figure 2). Freezing time in the ABT-239-treated saccharin control rats was similar. Saline-treated FAE offspring froze for only about 31% of the 5-minute sampling time. In contrast, freezing time in ABT-239-treated FAE was about 49%. A two-way ANOVA revealed a significant difference between prenatal treatment groups, but neither the drug treatment effect nor the interaction between prenatal group and drug treatment was significant. A subsequent one-way ANOVA indicated that freezing time in the saline-treated FAE offspring was significantly lower than the other 3 treatment groups.

Morris Water Task

Figure 3 illustrates the effects of FAE and ABT-239 on acquisition and retention of a fixed platform location in the Morris Water Task. Male offspring were selected for this initial study based on our previous Morris Water Task studies using male Sprague-Dawley rat offspring (Savage et al., 2002; Sutherland et al., 2000). In all 4 experimental groups, latency to find the platform improved over the first 8 trials and reached a performance asymptote during the 9th through 12th trials of training on Day 1 (Figure 3A). A repeated measure ANOVA indicated that neither FAE nor ABT-239 treatment 30 minutes prior to the first training trial on Day 1 affected acquisition rate. Four days after the training trials, saline-injected control rats required an average of 6 seconds more than their Day 1 performance asymptote to find the fixed platform location on the first attempt. Subsequently, their escape latencies were similar to performance asymptote on Day 1. Similar results were observed in the ABT-239-treated saccharin control rats. Saline-injected FAE rats required an average of an additional 21 seconds above their Day 1 performance asymptote to find the platform on the first test trial on Day 5 (Figure 3A), a significantly longer period of time compared to the control groups. Escape latency in saline-injected FAE rats remained elevated on Test Trial 2 compared to the other groups but the difference was no longer significant. By Test Trial 3 and in subsequent test trials, retention was similar among the 4 experimental groups. In ABT-239-treated FAE rats, escape latencies were similar to the control groups during all test trials (Figure 3A). When retention of the fixed platform location was expressed as the difference in escape latency on the first test trial on Day 5 minus the performance asymptote on Day 1 (average of trials 9 through 12), the difference measure was 3 times greater in the saline-treated FAE group compared to the other 3 groups (Figure 3B), indicating diminished retention of the fixed platform location. A two-way ANOVA revealed a significant

difference between prenatal treatment groups, but neither the drug treatment effect nor the interaction between prenatal group and drug treatment was significant. A subsequent one-way ANOVA indicated that retention in the saline-treated FAE group was significantly different than the other 3 treatment groups (Figure 3B).

The dose-dependent effect of ABT-239 on the retention of spatial memory in the Morris Water Task was examined in a subsequent experiment using a separate set of experimental offspring. Three lower ABT-239 doses (0.03, 0.1 and 0.3 mg/kg) were selected in an attempt to identify a minimally effective ABT-239 dose for improving retention. A 3 mg/kg dose was also included to assess whether a greater improvement in retention would be observed in FAE offspring, compared to the 1 mg/kg dose. A between-subjects design was utilized, each test subject was used only once, and all ABT-239 doses were tested in a randomized manner on a given training day. At the lowest ABT-239 dose tested (0.03 mg/kg), the retention deficit was similar to that observed in saline-treated FAE rats (Figure 4). The 0.1 and 0.3 mg/kg ABT-239 doses produced some modest improvement in retention in FAE rats. At 1.0 and 3.0 mg/kg ABT-239, retention in FAE rats was similar to saline-treated saccharin controls. As observed in the first experiment, ABT-239 did not improve retention at any dose in the saccharin control rats. A two-way ANOVA revealed a significant difference between prenatal treatment groups, but neither drug treatment nor the interaction between prenatal group and drug treatment was significant. Subsequent one-way ANOVAs by ABT-239 dose revealed significantly diminished retention in the saline-treated and 0.03 mg/kg ABT-239-treated FAE offspring.

DISCUSSION

There are 2 salient observations in this study. First, Long-Evans rat dams who voluntarily consume 5% ethanol in saccharin water 4 hours a day throughout pregnancy produce offspring with learning deficits (Figs. 2,3) similar to those observed in Sprague-Dawley rats using a 5% ethanol liquid diet paradigm (Sutherland et al., 2000; Weeber et al., 2001). Importantly, these 2 different prenatal ethanol exposure paradigms produce similar peak maternal serum ethanol concentrations (Sutherland et al., 1997; Table 1). Second, the FAE-induced learning deficits observed in the present report are amenable to therapeutic intervention using ABT-239, an agent effective in other animal models of cognitive deficits (Fox et al., 2004, 2005). These observations suggest that this voluntary ethanol drinking paradigm may be useful as a preclinical screen for evaluating the efficacy of pharmacotherapeutic agents for treatment of learning disabilities associated with fetal ethanol exposure.

Rat dams in this voluntary drinking paradigm consumed a mean of 2.82 g ethanol/kg/d throughout pregnancy. The resultant mean peak serum ethanol level obtained in this paradigm was 84.0 mg/dl, an ethanol level that can be achieved by a 120 lb. woman consuming 2 to 3 standard drinks over a period of 1 hour (<http://www.ou.edu/oupd/bac.htm>). This quantity of ethanol, about 0.56 g of ethanol per day in our rat dams, is equivalent to less than 4 kcal/d or less than 5% of the daily caloric intake of rat dams (Altman and Dittmer, 1974). The daily consumption of this relatively small quantity of “empty calories” did not impair maternal weight gain during pregnancy, litter size or offspring birthweight. Further, offspring body weights at the time of behavioral testing were not different (Table 1).

The voluntary ethanol drinking paradigm employed in this study has a number of advantages over the ethanol liquid diet we have employed in the past. First, all rat dams in the voluntary drinking paradigm consume the same diet as their source of calories and nutrients which removes the potential confound of subtle variations in nutrients and minimizes the potential confound associated with the stress of forced consumption of an unfamiliar food source

(Rasmussen et al., 2000). A second, related point is that this paradigm eliminates the requirement of an ad libitum chow group to control for the effects of paired-feeding of a liquid diet, reducing the number of experimental groups from 3 to 2. This reduces the number of animals required and other experimental costs; important issues when considering drug dose–response studies or other resource-intensive experimental designs. Third, the 4-hour intermittent exposure pattern of ethanol consumption entrained the drinking patterns of rat dams such that the day-to-day and intra-dam ethanol consumption levels were similar for a voluntary drinking paradigm and less variable than what we have observed using a 5% ethanol liquid diet paradigm in the past (data not shown). One might predict that limiting the variability in ethanol consumption would help diminish variations in outcome measures in FAE offspring.

The pre-pregnancy drinking phase of this paradigm was important for several reasons as well. First, this approach more closely models the human condition. A female rat that drank before becoming pregnant but discontinues drinking while pregnant is perhaps the single best control group for assessing the effects of drinking during pregnancy. In addition, establishing individual drinking levels in each female rat prior to pregnancy allowed us to minimize the potential confound of variable voluntary drinking during pregnancy by removing the females who drank considerably (>1 standard deviation) more or less than the group mean. To our surprise, there was a relatively low level of variability in 4-hour voluntary drinking among female rats. Less than 12% of the female rats were removed from the study before breeding, with the majority of exclusions because of consumption more than 1 standard deviation below the group mean. In addition, the pre-pregnancy drinking data in the remainder of the females was used to balance average consumption between the saccharin control and 5% ethanol prenatal treatment groups, thereby diminishing the potential confound of variations in ethanol preference or tolerance among rat dams on offspring outcome measures.

Using this voluntary drinking paradigm with Long-Evans rats, we observed qualitatively similar deficits in 1-trial contextual fear conditioning in female offspring (Fig. 2) as were observed previously using a 5% ethanol liquid diet paradigm with Sprague-Dawley rats (Weeber et al., 2001). Likewise, as reported previously (Sutherland et al., 2000), we observed no differences in the acquisition of spatial information in male FAE rats compared to controls (Fig. 3A), but a significant deficit in retention of spatial information (Fig. 3B). In this study, careful selection of the intervals between training and testing in both behavioral paradigms served 2 useful purposes. First, making each behavioral task more challenging generally has increased the “signal effect” when assessing FAE-induced learning deficits. For example, we reported previously that when given 3 fear-conditioning trials in a single day followed by testing the next day, FAE offspring froze for the same total time as controls. When a single trial of fear conditioning was employed, a significant reduction in total freezing time was observed in FAE offspring. When testing occurred 3 days after a single trial of fear conditioning, as occurred in this study, freezing was reduced in control rats, but the difference in freezing between control and FAE groups was greater (Weeber et al., 2001; Fig. 2). In a similar fashion, a 4-day interval between training and testing in the Morris Water Task marginally diminished retention in control offspring but magnified the retention deficit in FAE rats (Fig. 3B). For both behavioral paradigms, enhancing the signal effect also facilitated the assessment of the effects of ABT-239 on these learning deficits in FAE rats.

In both of the learning paradigms utilized in our studies, a single 1 mg/kg injection of ABT-239 thirty minutes prior to training diminished the learning deficit on the testing days such that the performance in ABT-239-treated FAE rats was not different from control rat offspring (Fig. 2 and Fig. 3B). A subsequent ABT-239 dose–response study in the Morris

Water Task indicated that the procognitive effect in FAE rats was dose-dependent (Fig. 4). Beneficial effects were observed over a similar ABT-239 dose range as reported using a five-trial repeated acquisition inhibitory avoidance response task in SHR rats and in a social memory task in aged rats (Fox et al., 2005). We have recently reported social learning deficits and other behavioral alterations in Long-Evans FAE offspring using this voluntary drinking paradigm (Hamilton et al., 2010) and we are currently investigating whether ABT-239 can ameliorate social learning deficits in our FAE rat offspring. Future studies will also examine whether these learning deficits occur in both sexes and whether ABT-239 ameliorates these deficits in both sexes. From a clinical perspective, another important question is whether immature offspring respond to ABT-239 in a manner similar to what we have observed here using adult offspring. In addition, ABT-239's efficacy when administered at times other than immediately before training sessions requires investigation. For example, Orsetti et al., (2002) reported that the H₃ receptor antagonist thioperamide enhances place recognition memory in the scopolamine model of amnesia when administered immediately after the training trials but not when administered immediately before the test trials.

In contrast to FAE rat offspring, ABT-239 did not facilitate learning in our saccharin control offspring (Figs. 2, 3 and 4). Most previous studies assessing the procognitive effects of ABT-239 and other H₃ receptor antagonists have primarily focused on animal models of cognitive deficits. Examples include aged rats, inbred strains of rodents with poorer learning capacity, and acute drug-induced cognitive deficits using agents such as the muscarinic cholinergic receptor antagonist scopolamine or the NMDA receptor channel antagonist MK-801 (Bernaerts et al., 2004; Fox et al., 2002, 2003, 2004, 2005; Huang et al., 2004; Komater et al., 2005; Orsetti et al., 2002). To the limited extent that this question has been studied to date, these drugs do not appear to produce significant cognition-enhancing effects in normal, functionally intact young adult rodents (Fox et al., 2004). In this study, we made the learning paradigms more challenging by extending the intervals between training and testing such that one might have predicted better retention in ABT-239-treated saccharin controls compared to saline-treated controls. But neither contextual fear-conditioned learning (Fig. 2) nor retention in the Morris Water Task was enhanced in control rats at any of the doses tested (Fig. 4). These observations suggest that H₃ receptor antagonists, in particular and perhaps cognition-enhancing agents in general may have limited efficacy in normal, "functionally intact" brain. This concept may not be limited to behavioral effects as we have observed an analogous outcome in studies where the same 1 mg/kg dose of ABT-239 reversed LTP deficits in FAE rats but did not enhance LTP in saccharin control offspring (Varaschin et al., 2010).

The rationale for testing a cognition-enhancing agent whose primary action is to increase neurotransmitter release was based on prior observations of a FAE-induced deficit in metabotropic glutamate receptor-mediated potentiation of glutamate release in the dentate gyrus (Galindo et al., 2004). It is tempting to speculate that the primary action of ABT-239 in this study was to facilitate glutamate release in the dentate gyrus or other brain regions of affected FAE offspring. This interpretation is currently under investigation in our laboratory. However, there are other putative mechanisms of ABT-239 action that may have contributed to the observed procognitive effects. For example, the antagonism of histamine H₃ receptors on cholinergic nerve terminals facilitates acetylcholine release (Fox et al., 2005), which could facilitate glutamatergic neurotransmission. In addition, H₃ receptor antagonist-mediated inhibition of autoreceptors located on histaminergic nerve terminals promotes histamine release (Arrang et al., 1983), which may promote excitatory effects on glutamatergic neurons mediated via postsynaptic histamine H₁ and H₂ receptors (Haas, 1984; Managhan-Vaughn et al., 1998; Selbach et al., 1997). Further, histamine has been reported to have positive allosteric effects at the spermidine site on NMDA receptors (i.e.:

Bekkers, 1993; Sharonova et al., 1996; Vorobjev et al., 1993). The extent to which each of these additional mechanisms may have contributed to the procognitive effects in FAE offspring is not known, but it is likely that the manner by which H₃ receptor antagonists enhance synaptic transmission at glutamatergic synapses in the central nervous system is complex.

The differential effects of ABT-239 in FAE offspring compared to controls (Fig. 2 and 3B) raises other questions including whether prenatal ethanol exposure has altered histaminergic regulation of glutamatergic transmission in a manner that is amenable to therapeutic intervention. Specifically, has FAE elevated the inhibitory influence of H₃ receptors on glutamate release, and possibly acetylcholine release, in brain regions that subserve contextual fear and spatial learning? Further, stressors increase the activation of histaminergic neurons leading to increased histamine release in hippocampal formation and other cortical areas (Miklos and Kovacs, 2003; Yoshitake et al., 2003). This effect may be particularly important in FAE offspring given that the learning paradigms employed in our studies are moderately stressful and fetal ethanol-exposed rats can have heightened or prolonged responses to stress (Taylor et al., 1982; Weinberg, 1988). Thus, it is tempting to speculate that an activity-dependent increase in histamine release during behavioral training coupled with a heightened presynaptic H₃ receptor-mediated inhibition of glutamate and/or acetylcholine release in FAE offspring may be one mechanism contributing to learning deficits in our FAE offspring. In this case then, histamine H₃ receptor antagonists such as ABT-239 may blunt an activity-dependent histamine H₃ receptor-mediated inhibition of excitatory neurotransmission in the hippocampal formation and other brain regions involved in learning. This speculation is currently under investigation in our laboratory.

In summary, we have observed that FAE-induced learning deficits are amenable to a cognition-enhancing agent that acts as a H₃ receptor antagonist. The fact that this agent was without effect in control offspring highlights the importance of examining the therapeutic potential of agents in animal models that emulate the clinical disorder in question. Further, the differential effect in FAE offspring compared to controls also raises suspicion that prenatal ethanol exposure may have altered histaminergic regulation of excitatory neurotransmission in affected brain regions. The mechanistic basis for these alterations is not known at the present time, but may involve FAE-induced alterations in histamine neurotransmission, an area that has not been investigated in this field of research.

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Fig. 1.

Voluntary ethanol consumption in adult female Long-Evans rats. **(A)** Effect of different concentrations of ethanol (v/v) in 0.066% saccharin water on ethanol consumption. Data bars represent the mean $\pm$ SEM. of 8 female rats in each group consuming ethanol solutions 4 hours a day over a period of 2 weeks. **(B)** Hourly 5% ethanol consumption over a period of 6 hours. Data bars represent the mean $\pm$ SEM. hourly consumption of a 5% ethanol solution by 16 female rats, collected over a period of 2 weeks. **1C:** Daily 4-hour ethanol consumption over a 2-week period prior to pregnancy and during pregnancy. Data bars represent the mean $\pm$ SEM. grams of ethanol consumed each day by 116 rats prior to pregnancy and by 51 rats assigned to the 5% ethanol group during pregnancy.

Fig. 2.

Effect of ABT-239 on prenatal ethanol-induced deficits in 1-trial contextual fear conditioning. Saccharin or 5% ethanol-exposed offspring received a single injection of either saline or 1 mg/kg ABT-239 30 minutes prior to the conditioning trial. Fear-conditioned learning was measured 72 hours later. Data bars represent the mean $\pm$ SEM. of 14 to 20 rats in each prenatal drug treatment group. Two-way ANOVA revealed a significant difference between prenatal exposure groups ($F = 15.5$; $p < 0.001$), but neither drug treatment ($F = 3.75$; $p = 0.056$) nor the interaction of prenatal group and drug treatment ($F = 3.18$; $p = 0.078$) was significantly different. A subsequent one-way ANOVA indicated that freezing in the saline-treated, 5% ethanol group was significantly less than the other 3 groups ($F = 9.38$; $p < 0.001$). Asterisk denotes $p < 0.05$, Holm-Sidak post hoc test.

Fig. 3.

Effect of ABT-239 on prenatal ethanol-induced deficits in spatial memory retention. Saccharin or 5% ethanol-exposed offspring received a single injection of either saline or 1 mg/kg ABT-239 30 minutes prior to the twelve-trial training session on Day 1. 3A: Escape latency data from the twelve training trials on Day 1 followed by 6 test trials on Day 5. Data points represent the mean of 14 to 19 rats in each group. Standard error bars are only provided for selected trials to illustrate variability but minimize clutter in the data presentation. A one-way ANOVA indicated that the escape latency data in the saline-treated fetal alcohol-exposed (FAE) group was significantly greater than the saccharin control groups and the ABT-239 treated FAE group ($F = 3.446$, $p = 0.022$). The asterisk denotes $p <$

0.05, Holm-Sidak post hoc test. 3B: Summary of the platform location retention data. Retention was defined as the difference in escape latency during the first test trial on Day 5 minus the mean of the escape latency across the last 4 training trials (Trials 9 to 12) on Day 1. Data bars represent the mean $\pm$ SEM. of 14 to 19 rats in each prenatal drug treatment group. Two-way ANOVA revealed a significant difference between prenatal exposure groups ($F = 4.49$; $p = 0.038$), but neither drug treatment ($F = 2.52$; $p = 0.117$) nor the interaction of prenatal group and drug treatment ($F = 2.39$; $p = 0.127$) was significantly different. One-way ANOVA indicated that spatial memory retention in the saline-treated 5% ethanol group was significantly worse than the other 3 groups ($F = 3.27$; $p = 0.027$). Asterisk denotes $p < 0.05$, HolmSidak post hoc test.

Fig. 4.

Effect of different ABT-239 doses on fetal alcohol-exposed -induced deficits in spatial memory retention. Retention data is expressed as in Fig. 3B. Data bars represent the mean $\pm$ the SEM. retention of 9 to 13 rats in each group. The dotted line denotes retention in the saline-treated saccharin control group. One-way ANOVAs indicated that spatial memory retention in the 0 (saline-treated) and 0.03 mg/kg ABT-239-treated 5% ethanol groups was significantly worse than their respective saccharin control groups (0 mg/kg ABT-239: $F=6.05$, $p=0.017$; 0.03 mg/kg ABT-239: $F=3.27$, $p=0.038$). Asterisk denotes $p<0.05$, Holm-Sidak post hoc test.

Table 1

Effects of Daily 4-Hour Consumption of 5% Ethanol on Female Dams and Their Offspring

	Saccharin control	5% Ethanol group
Maternal weight gain during pregnancy	127 ± 3 ^a (44)	121 ± 4 (51)
Daily 4-hour 5% ethanol consumption	NA	2.82 ± 0.13 ^b (51)
Maternal serum ethanol concentration	NA	84.0 ± 5.5 ^c (24)
Litter size	12.5 ± 0.1 ^d (41)	12.4 ± 0.3 (42)
Pup birthweight	6.17 ± 0.13 ^e (41)	6.13 ± 0.11 (42)
Adult male offspring weight at testing	341 ± 6 ^f (61)	337 ± 4 (120)
Adult female offspring weight at testing	224 ± 9 ^f (34)	222 ± 10 (32)

NA, not applicable.

^aMean ± SEM grams increase in body weight from GD 1 through of GD21.^bMean ± SEM grams ethanol consumed/kg body weight per day.^cMean ± SEM mg ethanol/dl serum, collected at the end of a 4-hour drinking period.^dMean ± SEM number of live births/litter.^eMean ± SEM grams pup birthweight.^fMean ± SEM grams body weight at time of behavioral testing.

(n) – Group sample size.