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Naloxone and Baclofen Attenuate Ethanol's Locomotor-Activating Effects in Preweanling Sprague–Dawley Rats

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Heterogeneous rat strains appear to be particularly sensitive to the sedative effects of ethanol as adults and insensitive to ethanol's stimulant effects. Recently, the authors found that ethanol induces stimulant effects in preweanling Sprague–Dawley rats. In adult mice, these effects seem to be governed by the mesocorticolimbic dopaminergic pathway, which can be modulated by means of GABA B agonist (baclofen) or opioid antagonist (naloxone) treatments. This study tested whether these pharmacological treatments might reduce the activating effect of ethanol in preweanling Sprague–Dawley rats. Twelve-day-old pups given naloxone (Experiment 1A) or baclofen (Experiment 1B) before ethanol administration were tested in terms of locomotor activity in a novel environment. Naloxone and baclofen significantly reduced the stimulating effect of ethanol but had no effect on locomotor activity patterns in water-treated controls. Blood ethanol levels were not affected by naloxone or baclofen (Experiment 2). During the preweanling period, opioid and GABA B receptors seem to be involved in the stimulating effect of ethanol.

Keywords: baclofen, naloxone, ethanol, stimulation, infant rat

Ethanol effects on locomotor activity vary as a function of many factors, such as animal species, ethanol dosage, and time of assessment during the course of the state of acute intoxication (Eckardt et al., 1998). In general, rodents display suppression of motor activity after moderate or high acute ethanol administrations. Locomotor stimulant effects of ethanol have been reported more frequently in heterogeneous mice than in rat strains, but there are also marked differences in terms of sensitivity to these effects across inbred mouse strains (e.g., Dudek & Phillips, 1990; Dudek, Phillips, & Hahn, 1991). In genetically heterogeneous rats, ethanol rarely induces locomotor stimulation (e.g., Imperato & Di Chiara, 1985), and generally a dose–response sedationlike effect is observed (e.g., Chuck, McLaughlin, Arizzi-LaFrance, Salamone, & Correa, 2006; Correa, Arizzi, Betz, Mingote, & Salamone, 2003; Erickson & Kochhar, 1985). It is interesting that low ethanol doses (normally below 1g/kg) consistently induce stimulating motor effects in novel environments in subpopulation of rats with heightened baseline motor activity ("high responders"; Gingras & Cools, 1996) or in rat strains genetically selected for increased ethanol consumption, such as alcohol-preferring (P), Sardinian alcohol-preferring (sP), University of Chile B (UChB) or Alko Alcohol (AA) rats (Agabio et al., 2001; Colombo et al., 1998; Paivarinta & Korpi, 1993; Quintanilla, 1999; Rodd et al., 2004; Waller, Murphy, McBride, Lumeng, & Li, 1986). The fact that rat strains genetically bred for ethanol ingestion typically show behavioral stimulation after ethanol administration suggests an association between genetic predisposition to consume ethanol and susceptibility to the stimulant effects of the drug.

Research conducted with rat strains bred for enhanced ethanol intake has revealed important knowledge about neurobiological mechanisms that may regulate ethanol intake. Various neurochemical systems, (e.g., dopaminergic, serotonergic, GABAergic, opiodergic, cannabinoid, and peptidergic) seem to critically modulate ethanol consumption in alcohol-preferring rat lines (Bell, Rodd, Lumeng, Murphy, & McBride, 2006; Overstreet, Rezvani, Cowen, Chen, & Lawrence, 2006; Sommer, Hyytia, & Kiianmaa, 2006). Studies conducted with such inbred strains also have indicated a

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role for peripheral acetaldehyde metabolism on ethanol consumption. For example, alcohol-avoiding UChA rats possess a less efficient mitochondrial aldehyde dehydrogenase than that in alcohol-preferring UChB rats (Quintanilla, Israel, Sapag, & Tampier, 1996). This enzyme leads to higher accumulation of ethanol-derived acetaldehyde in UChA than in UChB rats and significantly predicts a low consumption phenotype (Quintanilla, Perez, & Tampier, in press). Acute and chronic sensitivity to ethanol's effects also differ across rat lines genetically selected for high or low ethanol consumption. Rat lines that drink excessive amount of ethanol are, as mentioned, more sensitive to the stimulating effects of ethanol. In addition, acute tolerance is developed faster in UChB rats than in UChA rats (Quintanilla et al., in press). Acute tolerance has been associated with a reduced sensitivity to ethanol's aversive effects and with increased ethanol intake in UChB rats (Quintanilla et al., in press). Sardinian and P alcohol-preferring rats also develop chronic tolerance faster than Sardinian or alcohol-nonpreferring (nP) rats (Bell et al., 2006; Colombo, Lobina, Carai, & Gessa., 2006).

Preweanling heterogeneous rats share some of the phenotypic traits observed in alcohol-preferring rat lines. Voluntary ethanol consumption is higher in 8- and 12-day-old infant rats than in later stages of development (Sanders & Spear, 2007; Truxell & Spear, 2004; Truxell, Molina, & Spear, 2007). During the first and second postnatal weeks, infants are highly sensitive to appetitive reinforcement by ethanol (Arias & Chotro, 2006; Cheslock et al., 2001; Chotro & Arias, 2007; Molina, Pautassi, Truxell, & Spear, 2007; Petrov, Varlinskaya, & Spear, 2001) and seem more resistant to the aversive consequences of the drug (Arias & Chotro, 2006; Hunt, Spear, & Spear, 1991). Acute tolerance to motor impairment effects of ethanol is more pronounced in preweanling rats than in adult heterogeneous rats (Arias, Molina, Mlewski, Pautassi & Spear, 2008; Silveri & Spear, 1998). Furthermore, we recently reported that preweanling heterogeneous rats are also sensitive to ethanol's activating effect (Arias, Molina, et al., 2008; Arias, Mlewski, Molina, & Spear, in press). Moderate to high ethanol doses (1.25 or 2.5*g*/kg vol/vol) increased locomotor activity in 8- and 12-day old pups. The stimulant effect of ethanol was observed during the initial stage of the acute intoxication, whereas sedationlike effects were clearly observed in later stages of the intoxication process (30–35 or 60–65 min). It is notable that this time course of ethanol's effects on motor activity in preweanling rats coincides with the time course of its biphasic motivational effects (Molina et al., 2007). During the rising phase of the blood ethanol curve, relatively high ethanol doses exerted locomotor-activating effects (Arias, Molina, et al., 2008), as well as appetitive reinforcement (Molina et al., 2007). When blood ethanol levels (BELs) reached peak values (approximately 200 mg%), ethanol induced sedationlike effects (Arias et al., 2008) and promoted aversive reinforcement (Molina et al., 2007). These results seem to argue in favor of the hypothesis that there is a common mechanism underlying motor and motivational effects of the drug in preweanling rats; overall, these antecedents suggest that specific stages of development can be utilized as a model for the study of mechanisms underlying motivational effects of ethanol.

Two nonexclusive hypotheses suggest that ethanol-induced stimulation is mediated by the mesocorticolimbic dopaminergic pathway (Di Chiara, Acquas, & Tanda, 1996; Xiao, Zhang, Krnjevic, & Ye, 2007). In vitro studies have shown that ethanol directly excites dopaminergic neurons in the ventral tegmental area (VTA: Appel, Liu, McElvain, & Brodie, 2003; Brodie & Appel, 2000; Brodie, Pesold, & Appel, 1999). On the other hand, other authors have proposed an indirect mechanism involving GABA B and μ-opioid receptors (Gianoulakis, 2001, 2004). Ethanol excites dopamine neurons in the ventral tegmental area, an effect mediated by the activation of μ-opioid receptors (Xiao et al., 2007). There is also evidence that opioid and GABAergic manipulations attenuate the stimulating effect of ethanol. Naltrexone reduced activating effects of ethanol in mice, apparently by blocking activation of μ-opioid receptors (Pastor, Miquel, & Aragon, 2005; Pastor, Sanchis-Segura, & Aragon, 2005; Sanchis-Segura, Grisel, et al., 2005; Sanchis-Segura, Pastor, & Aragon, 2004). Peripheral or local (in VTA) administration of baclofen (a GABA-B agonist) also reduced ethanol-mediated locomotor stimulatory effects in adult mice (Boehm, Piercy, Bergstrom, & Phillips, 2002; Chester & Cunningham, 1999). Furthermore, opioid and GABA-B systems seem to regulate also the motivational effects of ethanol in conditioned place preference procedures with mice (Bechtholt & Cunningham, 2005; Cunningham, Henderson, & Bormann, 1998), suggesting that stimulant and reinforcing properties of ethanol may share a similar neurochemical mechanism.

The goal of the present study is to analyze in preweanling (12-day-old) heterogeneous rats the participation of the opioid and GABAergic system in the stimulant effect of ethanol, which may eventually aid in understanding mechanisms supporting ethanol's reinforcing effects during this ontogenetic period. In the present study, before ethanol administration, 12-day-old pups were given naloxone (0.0, 0.5, 1.0, or 2.0 mg/kg; Experiment 1A) or baclofen (0.0, 1.0, 1.5, or 2.5 mg/kg; Experiment 1B). Five minutes after ethanol administration, infants were tested in terms of locomotor activity in a novel environment. In Experiment 2, blood ethanol concentrations were recorded with the aim of controlling possible drug effects on ethanol pharmacokinetics.

Experiment 1

The first experiment tested whether naloxone (in Experiment 1A) or baclofen (in Experiment 1B) would reduce the stimulant effect of ethanol on PD 12. Nonspecific opioid antagonists attenuate motivational and stimulant effects of ethanol in mice (Camarini, Nogueira Pires, & Calil, 2000; Pastor, Miquel, et al., 2005; Pastor, Sanchis-Segura, et al., 2005). In preweanling rats, naloxone (1.0 or 10 mg/kg) reduced conditioned acceptance of ethanol in 8-day-old pups (Chotro & Arias, 2007). Ethanol reinforcement in newborns (Nizhnikov, Varlinskaya, & Spear, 2006) and ethanol-mediated appetitive learning in fetuses (Arias & Chotro, 2005; Chotro & Arias, 2003) is modulated by the activation of the endogenous opioid system. Baclofen also attenuates ethanol-mediated locomotor activation in adult mice (Boehm et al., 2002; Chester & Cunningham, 1999). Ethanol-mediated conditioned place preference in mice was also reduced by central (Bechtholt & Cunningham, 2005) but not peripheral administration of baclofen (Chester & Cunningham, 1999).

Naloxone (Experiment 1A: 0.0, 0.5, 1.0, or 2.0 mg/kg) or baclofen (Experiment 1B: 0.0, 0.5, 1.5, or 2.5 mg/kg) was given to 12-day-old pups before ethanol administration (0.0 or 2.5 g/kg). At this age, this ethanol dose induces clear locomotor stimulation

(Arias et al., in press). Thirty minutes after ethanol administration, locomotor activity was registered.

Method

Subjects

Seventy-two Sprague–Dawley pups (36 females, and 36 males), representative of nine litters were utilized in each of Experiments 1A and 1B. Animals were born and reared at the vivarium of the Center for Developmental Psychobiology, Binghamton University, under conditions of constant room temperature (22 ± 1.0 °C), on a 12-hr light–dark cycle. Births were examined daily, and the day of parturition was considered as Postnatal Day 0 (PD0). All litters were culled to 10 pups (5 females and 5 males whenever possible) within 48 hr after birth. All procedures were in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and the guidelines indicated by the Binghamton University Institutional Animal Care and Use Committee (IACUC) review committee.

Procedures

Naloxone, baclofen, and ethanol treatments. On PD 12, pups were separated from their mothers and randomly assigned to one of the eight independent groups defined by orthogonal combination of ethanol (0.0 or 2.5 g/kg) and naloxone (Experiment 1A: 0.0, 0.5, 1.0, or 2.0 mg/kg) or baclofen (Experiment 1B: 0.0, 1.0, 1.5, or 2.5 mg/kg) treatments. Pups from a given litter were evenly distributed across drug condition, and in no case was more than 1 subject from a given litter assigned to the same group. Pups were placed in a holding maternity cage (45 × 20 × 20 cm) partially filled with clean wood shavings. The floor of the cage was maintained at 33 °C (± 1 °C) through the use of a heating pad. Thirty minutes later, body weights were individually recorded (±0.01 g) and pups received a subcutaneous injection of either naloxone (Experiment 1A: 0.0, 0.5, 1.0, or 2.5 mg/kg) or baclofen (Experiment 1B: 0.0, 1.0, 1.5, or 2.5 mg/kg). The vehicle was an isotonic saline solution. The volume injected in each pup was 1.0% of their body weight. Concentrations of drug solution were as follows: naloxone: 0.05, 0.10, and 0.20 mg/ml for the 0.5, 1.0, and 2.0 mg/kg doses, respectively; baclofen: 0.10, 0.15, and 0.25 mg/ml for the 1.0, 1.5, and 2.5 mg/kg doses, respectively. Naloxone dosage was based on doses previously found effective for attenuating the stimulatory effect of ethanol in adult mice (e.g., Camarini et al., 2000). In previous studies using infant rats, 1 mg/kg reduced ethanol intake (Chotro & Arias, 2003) and ethanol's reinforcing properties (Chotro & Arias, 2007). Baclofen dosage was selected in preliminary studies from doses effective in reducing ethanol's activating effects in preweanling rats (data not shown). In mice, peripheral administration of similar baclofen doses successfully reduced ethanol's stimulating effects (e.g., Shen, Dorow, Harland, Burkhart-Kasch, & Phillips, 1998). After receiving the injection, pups were placed again in couples in the holding chamber.

Thirty minutes after naloxone or baclofen administration, pups received an intragastric (i.g.) administration of 0.0 or 2.5 g/kg ethanol (volume administered was equivalent to 0.015 ml per gram of body weight of a 21% ethanol solution; the vehicle was distillated water). We performed i.g. administrations using a 10-cm length of polyethylene tubing (PE-10 Clay Adams, Parsippany, NJ) attached to a 1-ml syringe with a 27 gauge × 1/2-in. needle. This tubing was gently introduced through the mouth and slowly pushed into the stomach. The entire procedure took less than 20 s per pup.

Locomotor activity assessment. Five minutes after ethanol administration, locomotor activity was evaluated in a novel environment consisting of a square Plexiglas container (10 × 10 × 12 cm). The floor of this apparatus was lined with absorbent paper. A new piece of paper was used for each animal. A circuit board (2 cm wide) surrounded the four sides of each chamber. This board had six infrared photo emitters and six infrared photoreceptors. The photo beams crossed the chamber generating a matrix of nine cells that allowed measurement of overall amount of activity. Custom-made software served to analyze the number of beams crossed by each subject every 10th of a second. Each activity test had a total duration of 8 min, and data were collected in 1-min bins. In preliminary studies, this measure (number of beams broken per minute) was highly and significantly correlated with time spent wall climbing and walking in 12-day-old Sprague–Dawley rats during a 5-min test ($rxy = 0.84$, $p < .001$, $n = 15$). Body weight was not significantly correlated with number of beams broken ($rxy = -0.11$, $p = .700$).

Data Analysis

The factorial design of the present experiment was defined by the following variables: naloxone (Experiment 1A: 0.0, 0.5, 1.0, or 2.0 mg/kg) or baclofen (Experiment 1B: 0.0, 1.0, 1.5, or 2.5 mg/kg) treatment and ethanol treatment (0.0 or 2.5 g/kg). Activity data were analyzed by means of a 2 (ethanol treatment) × 4 (naloxone or baclofen treatment) × 8 (minute of testing) mixed analysis of variance (ANOVA). In these analyses, the 8 min of the locomotor activity test served as a repeated measure. The dependent variable under examination was general activity as operationalized through the number of infrared beams interrupted by each pup per minute. We further analyzed significant effects or interactions indicated by the ANOVAs through post hoc tests (least significant difference test with a Type I error set at 0.05).

Results

Experiment 1A

Figure 1 depicts locomotor activity scores across the 8 min of testing as a function of ethanol and naloxone treatments. Ethanol exerted a clear stimulating effect that was attenuated by baloxone. The ANOVA indicated a significant main effect of minute, $F(7, 448) = 58.29$, $p < .0001$. The overall ANOVA also indicated that the following interactions were significant: Ethanol Treatment × Minute, $F(7, 448) = 14.51$, $p < .0001$; and Ethanol Treatment × Naloxone Treatment, $F(3, 64) = 3.42$, $p < .05$.

We conducted additional follow-up one-way ANOVAs including ethanol treatment as the only independent factor, with activity scores obtained in each testing minute as the dependent variable. These analyses indicated that pups treated with ethanol showed higher activity scores at Minutes 1, 2, 3, and 5 than water-treated animals: $F(1, 70) = 9.10$, $p < .005$; $F(1, 70) = 17.21$, $p < .0001$; $F(1, 70) = 12.08$, $p < .001$and $F(1, 70) = 4.96$, $p < .05$,

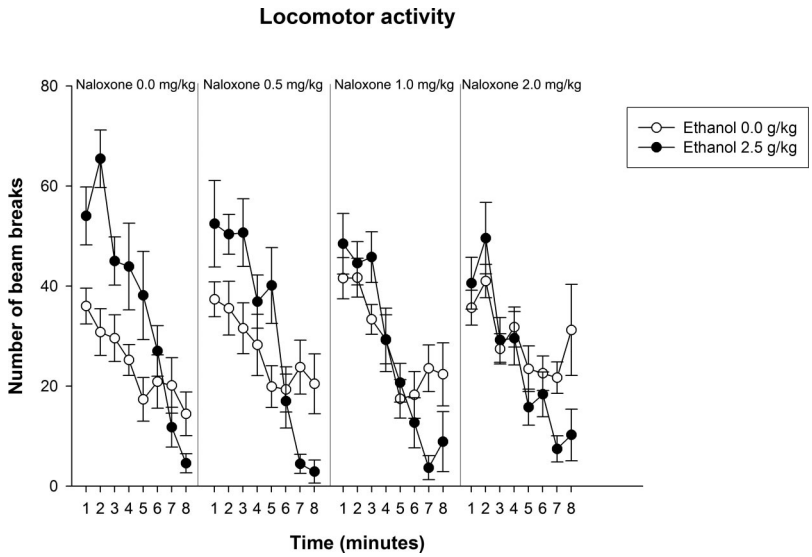

Figure 1. Locomotor activity scores across the 8 min of testing as a function of ethanol (0.0 or 2.5 g/kg) and naloxone (0.0, 0.5, 1.0, or 2.0 mg/kg) treatments. Vertical lines represent standard errors of the means.

respectively. At Minutes 7 and 8, infants that had been given ethanol showed lower activity scores than water controls did, $F(1, 70) = 32.24$, $p < .0001$; and $F(1, 70) = 15.42$, $p < .0005$, respectively. However, the stimulating effect of ethanol was modulated by naloxone. Further analysis of the significant interaction between ethanol treatment and naloxone treatment revealed that pups treated with ethanol and vehicle (Group EtOH-0.0) had significantly higher locomotor activity scores than those treated with water and vehicle (Group water-0.0) or those given ethanol and the higher naloxone doses (Group EtOH-1.0 and Group EtOH-2.0, respectively). In all cases, locomotor activity scores from pups given ethanol and Naloxone (Groups EtOH-0.5, EtOH-1.0, or EtOH-2.0) did not differ from their respective water-treated controls (Groups Water-0.5, Water-1.0, and Water-2.0, respectively).

In summary, a relatively high ethanol dose increased locomotor activity in 12-day-old rats. This effect was attenuated by administration of a nonspecific opioid antagonist, naloxone, which did not affect locomotor activity in water-treated animals.

Experiment 1B

Figure 2 represents activity scores obtained during the locomotor activity test as a function of ethanol (0.0 or 2.5 g/kg) and baclofen (0.0, 1.0, 1.5, and 2.5 mg/kg) treatments. As was the case

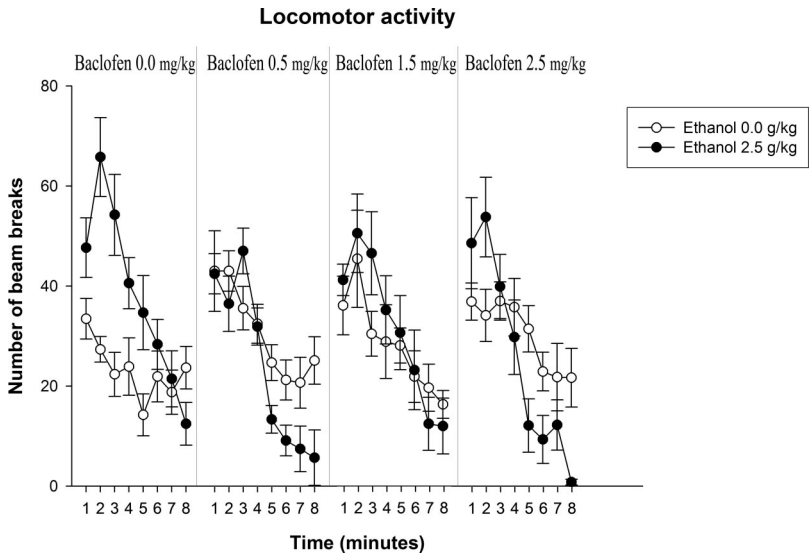

Figure 2. Locomotor activity scores across the 8 min of testing as a function of ethanol (0.0 or 2.5 g/kg) and baclofen (0.0, 0.5, 1.5, or 2.5 mg/kg) treatments. Vertical lines represent standard errors of the means.

in Experiment 1A, ethanol increased locomotor activity, an effect evident only in pups not given baclofen, the EtOH-0.0 condition. Baclofen attenuated the stimulant effect of ethanol. The ANOVA indicated significant effects of minute, $F(7, 448) = 59.51$, $p < .0001$; as well as the following interactions: Ethanol Treatment × Minute, $F(7, 392) = 11.63$, $p < .0001$; and Ethanol Treatment × Baclofen Treatment, $F(3, 64) = 3.28$, $p < .05$.

Follow-up one-way ANOVAs were conducted including ethanol treatment as the only between-groups factor, with activity score from each testing minute as the dependent variable. These analyses indicated that pups treated with ethanol showed higher activity levels at Minutes 2 and 3, $F(1, 70) = 7.93$, $p < .01$; and $F(1, 70) = 14.25$, $p < .0005$, respectively. In contrast, at Minute 8, pups treated with ethanol showed lower activity scores than water-treated infants, $F(1, 70) = 18.97$, $p < .0001$.

More important for the aims of the present study was the significant interaction between ethanol and Baclofen treatments, which was further analyzed by means of post hoc tests. These analyses revealed that pups given ethanol and vehicle (Group EtOH-0.0) showed significantly higher levels of activity than the corresponding water-treated controls (Group Water-0.0) and than those treated with ethanol and 1.0 or 2.5 mg/kg baclofen (Groups EtOH-1.0 and EtOH-2.5). In addition, pups treated with ethanol and baclofen (Groups EtOH-1.0, EtOH-1.5 and EtOH-2.5) did not significantly differ from their corresponding water-treated control (Groups Water −1.0, Water −1.5, and Water-2.5).

In the present experiment, ethanol again increased locomotor activity in preweanling rats. This effect was clearly attenuated by peripheral administration of baclofen before testing. Baclofen did not significantly affect locomotor activity in rats not given ethanol, the water-treated subjects.

Experiment 2

In Experiment 2, we aimed to determine blood ethanol concentrations (BECs) in 12-day-old pups given naloxone (Experiment 2A) or baclofen (Experiment 2B) before ethanol. The goal of the experiment was to test the effect of naloxone or baclofen treatments on ethanol absorption and metabolism; in adult rats, absorption of ethanol has been found to be affected significantly (Linseman & Le, 1997). To test such an effect in the present circumstances, we injected 12-day-old pups either with naloxone (Experiment 2A: 0.0, 0.5, 1.0, or 2.0 mg/kg) or baclofen (Experiment 2B: 0.0, 0.5, 1.5, or 2.5 mg/kg) before ethanol administration (2.5 g/kg). BELs were determined by taking blood 10.5 min after ethanol administration, the time point that coincides with the middle of the activity test conducted in Experiment 1.

Method

Subjects

Forty-eight Sprague–Dawley pups (12 females and 12 males for Experiment 2A, and 12 females and 12 males for Experiment 2B) representative of six litters were utilized. Animals were born and reared at the vivarium of the Center for Developmental Psychobiology at Binghamton University. Housing conditions were the same as those described in Experiment 1.

Procedures

On PD 12, pups were separated from their mothers and randomly assigned to a given naloxone (Experiment 2A: 0.0, 0.5, 1.0, or 2.0 mg/kg) or baclofen (Experiment 2B: 0.0, 0.5, 1.5, or 2.5 mg/kg) treatment. Pups were kept under the same conditions as in the previous experiments. Naloxone, baclofen, and ethanol administration procedures and parameters were also the same as those described in Experiment 1.

Pups were sacrificed 10.5 min after receiving their ethanol dose, a time point that coincides with the middle of the activity test conducted in Experiment 1. Trunk blood was obtained after decapitation. Blood samples were collected with a heparinized capillary tube. They were immediately centrifuged (6.000 rpm; Micro-Hematocrit Centrifuge, Hawksley & Sons LTD, Sussex, England) and stored at −70 °C. BECs were determined using an AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA). Calculations of BECs were made by oxidating ethanol to acetaldehyde in the presence of ethanol oxidase. The apparatus measures the rate of oxygen required by this process, which is proportional to ethanol concentration. BECs were expressed as milligrams of ethanol per deciliter of body fluid (mg/dl = mg%).

Data Analysis

The design of the present experiments included variation in naloxone treatment (0.0, 0.5, 1.0, or 2.0 mg/kg) for Experiment 2A, and baclofen treatment (0.0, 0.5, 1.5, or 2.5 mg/kg) for Experiment 2B. BECs were analyzed by means of one-way between-factor ANOVAs. Significant effects were further analyzed through post hoc tests (Fisher test with a Type I error set at 0.05).

Results

Experiment 2A

The corresponding ANOVA did not reveal a significant effect of naloxone treatment (see Table 1). According to this experiment, BELs were not affected by the opioid antagonist.

Table 1
Blood Ethanol Concentration (mg%) in 12-Day-Old Pups 10.5 Min After Ethanol Administration (2.5 g/kg) as a Function of Naloxone (0.0, 0.5, 1.0, or 2.0 mg/kg) or Baclofen (0.0, 1.0, 1.5, or 2.5 mg/kg)

Drug and dose (mg/kg)	Blood ethanol concentration ($M \pm SE$)
Naloxone	
0.0	157.17 ± 6.14
0.5	161.93 ± 9.57
1.0	146.55 ± 6.04
2.0	161.48 ± 5.69
Baclofen	
0.0	161.50 ± 3.99
1.0	167.16 ± 5.07
1.5	167.18 ± 5.07
2.5	158.35 ± 2.17

Note. All *n*s = 6. mg% = milligrams of ethanol per deciliter of body fluid.

Experiment 2B

BELs corresponding to pups given baclofen before ethanol administration are presented in Table 1. The ANOVA found no effect of Baclofen treatment on BECs.

According to the present experiments, BELs were not affected by the nonspecific opioid antagonist, naloxone, or by the GABA-B agonist, baclofen. The attenuation of the ethanol's stimulating effects induced by these drugs observed in Experiments 1A and 1B cannot be explained by interference of naloxone or baclofen in ethanol absorption and metabolism.

General Discussion

The present study tested whether naloxone or baclofen would attenuate the stimulating effect of ethanol in preweanling rats. In Experiments 1A and 1B, a relatively high ethanol dose (2.5 g/kg) increased motor activity in 12-day-old pups, consistent with previous observations in our laboratory (Arias, Molina, et al., 2008; Arias et al., in press). Naloxone (Experiment 1A) and bBaclofen (Experiment 1B) significantly reduced the stimulating effects of ethanol without affecting locomotor activity in water-treated animals, ethanol absorption, or metabolism (Experiment 2).

These results indicate that ethanol-induced activation in preweanling rats is modulated by opioid and GABA-B receptors. These findings are congruent with previous studies conducted with adult mice, in which systemic baclofen (e.g., Shen et al., 1998) or naloxone (e.g., Pastor, Miquel, et al., 2005) attenuated the enhanced locomotor effects induced by ethanol. Baclofen also attenuated ethanol's activating effects in rats bred for increased ethanol consumption (Quintanilla et al., in press). Similarly, local administration of a GABA-B agonist (Boehm et al., 2002) also reduced ethanol's activating effects.

The present ameliorating effect of an opioid antagonist on ethanol-induced activity has not consistently been observed in other animal models. Naloxone did not attenuate ethanol's activating effects in mice bred for high (FAST) or low (SLOW) sensitivity to the locomotor stimulant effects of ethanol or in other outbred mice strains, such as Swiss mice (Gevaerd, Sultowski, & Takahashi, 1999). However, with different testing conditions, opioid antagonists successfully attenuated ethanol-mediated locomotor activation in Swiss mice (Camarini et al., 2000; Pastor, Miquel, et al., 2005). Holstein, Pastor, Meyer, and Phillips (2005) suggested that procedural variations may partially account for the discrepancies in these studies. According to Holstein et al. (2005), time of assessment after ethanol administration may help determine the effects of opioid antagonists on locomotor effects of ethanol. Naloxone seems to be less effective in reducing ethanol-mediated stimulation when mice are tested during the early, rising phase of the blood ethanol curve than when tested in later stages of the acute intoxication process (see Holstein et al., 2005). In other words, the opioid antagonist may enhance the sedative effects of ethanol rather than reduce the stimulation effects. Recently, we reported that preweanling rats displayed clear increments in locomotor activity when tested soon (5–10 min) after ethanol administration (2.5 g/kg), whereas 30 min after drug treatment, ethanol suppressed motor activity (Arias, Molina, et al., 2008; Arias et al., in press). In the present study, we tested animals during the initial stage of acute intoxication (5–13 min after ethanol administration), a postadministration interval in which the present ethanol dose (2.5 g/kg) mainly exerts stimulation in infant rats (Arias et al., in press). Hence, it seems more likely that, in the present study, naloxone blocked the stimulatory effect rather than enhancing ethanol-induced sedation. On the other hand, systemic administration of baclofen decreased ethanol's activating effect in several mice strains (including FAST mice) even when the drug was paired with the initial phase of the intoxication (Shen et al., 1998).

The stimulant effects of ethanol and other drugs of abuse seem to be associated with the mesocorticolimbic dopaminergic pathway (Di Chiara et al., 1996). It has been hypothesized that ethanol directly excites dopaminergic neurons that project to the nucleus accumbens (Brodie et al., 1999). On the other hand, other authors have suggested that ethanol increases the firing rate of dopaminergic neurons through an indirect mechanism involving the endogenous opioid system (Gianoulakis, 2004; Herz, 1997). Dopaminergic neurons in the VTA are excited by ethanol by means of an inhibitory effect of ethanol over GABAergic interneurons in the VTA, an inhibitory action of ethanol apparently modulated by μ-opioid receptors (Xiao et al., 2007). Accordingly, ethanol-induced activation in mice was reduced by local baclofen administration in the VTA (Boehm et al., 2002) and also by systemic administration of a μ-receptor antagonist (Pastor, Sanchis-Segura, & Aragon, 2005). Although naloxone is considered a nonspecific opioid antagonist, low doses of this drug similar to those used in the present study seem to have more specificity for μ receptors (e.g., Takemori & Portoghese, 1984). The fact that in our study naloxone and baclofen reduced ethanol's activating effects fits well with the hypothesis postulating that GABA B and μ-opioid receptors in VTA modulate the excitatory effects of ethanol on dopaminergic neurons in VTA.

Naloxone and baclofen were systemically injected. Hence, we cannot discard the possibility that these drugs exerted peripheral effects that may interact with stimulation induced by ethanol. GABA B receptors have been identified in the spinal cord and in the dorsal vagal complex (Towers et al., 2000; Varga & Kunos, 1992). In fact, ethanol is a potent inhibitor of the depressor baroreflex response, an effect mediated by ethanol's effects upon GABA A and GABA B receptors in the dorsal vagal complex (Varga & Kunos, 1992; Zhang, Abdel-Rahman, & Wooles, 1989). It is unclear, however, whether this ethanol effect on brainstem can affect locomotor activity in preweanling rats. Administration of baclofen locally in the VTA of adult mice attenuates locomotor activity induced by ethanol (Boehm et al., 2002), which supports the hypothesis that a central effect of ethanol modulated by GABA B receptors in the VTA mediates the stimulating effects of the drug. In the peripheral nervous system, there are also opioid receptors on which ethanol can act (e.g., Bedingfield, King, & Holloway, 1999; Narita, Miyoshi, & Suzuki, 2007). Indeed, peripheral opioid receptors seem to modulate partially the sedative and aversive effects of ethanol. Methylnaltrexone, an opioid antagonist that acts mainly peripherally, decreased the sedation as well as the aversive postabsorptive consequences of ethanol (Bedingfield et al., 1999). Therefore, it could be expected that, if naloxone blocks these peripheral opioid receptors, the sedative effects of ethanol should be attenuated, an effect that is not compatible with the results of Experiment 1A. At least in the case of naloxone, it seems more likely that this nonselective opioid

antagonist blocked the stimulating effect of ethanol by means of a central mechanism.

Infants were not habituated to the environment before drug manipulations. Hence, it is possible that the behavioral activating effects of ethanol are dependent on the novelty of the testing environment. Recent results from our laboratory support this hypothesis: In 12-day-old infant rats, we have observed that extensive previous exposure to the testing environment (one session per day during 3 days before testing) attenuates ethanol's activating effects. This result is in agreement with what has been observed in adult rats (Cools & Gingras, 1998; Gingras & Cools, 1996). Novelty seems to enhance the activating effects of ethanol as well as of other drugs of abuse (e.g., amphetamines, cocaine, morphine; see Carey, DePalma, & Damianopoulos, 2003; Kalinichev, White, & Holtzman, 2004). It is necessary to emphasize that the present study was designed to evaluate whether pharmacological manipulations that attenuate ethanol's activating effects in various animal models also play a role during early ontogeny of the rat. We selected an experimental paradigm (without previous habituation) in which stimulatory effects of ethanol are evident and that is intimately associated with the expression of ethanol's positive motivational effects during infancy. The dose of ethanol as well as the time of evaluation were selected in accordance with these issues. As mentioned, during the rising phase of the blood ethanol curve, ethanol exerts appetitive effects in preweanling rats (Molina et al., 2007). The basic profile of the results suggests that the stimulatory effects of ethanol observed under the present experimental conditions are dependent on the GABA and the opioid system; a neuropharmacological pattern that is similar to that reported in mice (Boehm et al., 2002; Pastor, Miquel, & Aragon, 205; Pastor, Sanchis-Segura, & Aragon, 2005) and genetically selected rats (Quintanilla et al., in press). Furthermore GABA B agonists and opioid antagonists modulate ethanol consumption and reinforcement in these animal models (e.g., Bechtholt & Cunningham, 2005; Quintanilla et al., in press). Taking into account the pattern of results of the present study, as well as those involving ethanol intake and reinforcement, there is a solid basis from which to investigate the participation of these neurobiochemical systems on ethanol intake and appetitive learning during early ontogeny.

In summary, ethanol's activating effects in preweanling heterogeneous rats seem to be modulated by opioid and GABA B receptors, suggesting that the mesolimbic dopaminergic pathway could be involved in these effects. The present results, together with other recent evidence (e.g., Arias & Chotro, 2006; Chotro & Arias, 2007; Molina et al., 2007; Sanders & Spear, 2007; Truxell & Spear, 2004; Truxell et al., 2007), indicate that the preweanling period in the rat may represent a valuable framework for the study of mechanisms underlying motivational and motor properties of ethanol and the possible association between these processes. In this regard, we recently observed that during the second postnatal week of life, when ethanol induces clear stimulating effects, the opioid system can modulate ethanol intake (Chotro & Arias, 2003) and conditioned acceptance of the drug (Chotro & Arias, 2007). This result suggests that a common mechanism may underlie ethanol's activating and motivational effects in preweanling rats. However, more research is needed to test the specific role of various neurochemical and metabolic systems in these effects during the early ontogeny of the rat.

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