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Increased palatability of ethanol after prenatal ethanol exposure is mediated by the opioid system

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Abstract

Previous studies have shown that prenatal exposure to a moderate dose of ethanol (2 g/kg) during the last days of gestation of the rat (17–20) not only increases postnatal intake of the drug but also enhances the palatability of ethanol's taste when measured with a taste reactivity test. Prenatal administration of the opioid antagonist naloxone, together with ethanol, reduces ethanol intake. The aim of the present study was to analyze whether this decreased intake of ethanol after the administration of naloxone is accompanied by a reduction in ethanol's palatability. Results show that preweanling rats exposed prenatally to ethanol alone displayed more ethanol intake and more ingestive responses in reaction to its taste than non-exposed pups. Simultaneous prenatal administration of naloxone with ethanol prevented both the increased intake of ethanol and the higher amount of appetitive responses to its taste. These results indicate that the opioid system plays an important role in the effect of enhanced palatability of ethanol's taste after its prenatal exposure. Results also support the hypothesis of a conditioned response established in utero as a consequence of the association between ethanol's chemosensory and reinforcing aspects, the latter mediated by the opioid system.

Keywords: Rat; Ethanol; Prenatal exposure; Infant; Taste reactivity test; Naloxone; Intake; Conditioned response

The rat fetus has the capacity for perceiving the chemosensory properties of the amniotic fluid and other substances present in their prenatal milieu (Pedersen et al., 1986, 1983; Smotherman and Robinson, 1990). This fetal capacity has a direct relation to postnatal responses towards those substances (Teicher and Blass, 1977). For instance, it has been demonstrated that olfactory cues guiding rat neonates in their first nipple attachment are substances contained in the amniotic fluid. Contamination of the amniotic fluid with a flavored substance has been found to modify that early suckling behavior (Blass and Pedersen, 1980), and also can increase intake of that substance later in life (Smotherman, 1982a). It also has been shown that the rat fetus has the capacity for acquiring conditioned responses to chemosensory stimuli and that this prenatal associative memory can be expressed during infancy and adolescence (Abate et al., 2002; Chotro and Arias, 2003; Molina and Chotro, 1991; Stickrod et al., 1982).

The opioid system seems to be implicated in learning processes modulating the acquisition of taste or odor preferences during early infancy (Kehoe and Blass, 1986). Some authors suggest that this neurochemical system has a distinctive role in neonatal rat learning, temporally limited to a sensitive period that ends on postnatal day 9, in which pups learn easily odor preferences (Roth and Sullivan, 2001, 2003). Although it is not specified by those authors, it is conceivable that this sensitive period may also include the last prenatal period. In fact during the last days of gestation it has been observed that, as previously mentioned, chemosensory preferences can be acquired (Abate et al., 2001; Chotro and Arias, 2003; Molina and Chotro, 1991; Smotherman, 1982b; Stickrod et al., 1982) and also that the opioid system is involved in prenatal learning processes (Arnold et al., 1993; Chotro and Arias, 2003; Robinson et al., 1993a). It has been demonstrated that in the rat fetus, the opioid receptors subtypes μ and κ are functional and are capable of modulating fetal behavior during the last days of gestation (Smotherman et al., 1993). It also has been shown that the activity of the opioid system (specifically, μ opioid receptors) can be conditioned prenatally after pairing a

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chemosensory CS with a US that promotes the release of endogenous opioids, and that the rat fetus is capable of exhibiting a conditioned opioid response when the CS is again presented (Arnold et al., 1993). The administration of opioid antagonists, either nonselective or selective for each receptor subtype, has been found to be effective in modifying those fetal responses known to be regulated by the opioid system (Robinson et al., 1993b; Smotherman and Robinson, 1994).

Prenatal exposure to ethanol has been shown to affect subsequent recognition of the drug's odor and/or taste (Chotro and Molina, 1990; Chotro and Spear, 1997). Several studies have demonstrated that the rat fetus not only can perceive ethanol's chemosensory properties but also can form an association involving the taste and odor of this drug (Chotro et al., 1991; Chotro and Molina, 1990; Molina and Chotro, 1991). The fetus may also learn about the toxic aspects of the drug. When ethanol is administered to the pregnant rat, fetuses are exposed to the chemosensory as well as the toxic aspects of ethanol. The drug is rapidly distributed to fetal tissues reaching levels in fetal blood equal to those in maternal circulation (Szeto, 1989). Alcohol also accumulates in the amniotic fluid, and previous data have demonstrated that 60 min after the administration of a relatively low ethanol dose to the pregnant dam, the concentration of the drug in the amniotic fluid is enough to be perceived by the rat fetus (Chotro and Molina, 1990; Szeto, 1989). This prenatal experience with ethanol chemosensory aspects together with its toxic properties has been found to promote subsequent changes in responsiveness to the drug. For example, repeated administrations of low to moderate ethanol doses (1 or 2 g/kg) to the pregnant rat during gestational days (GD) 17–20 have been found to increase ethanol consumption in infant rats (Arias and Chotro, 2005; Chotro and Arias, 2003) (Dominguez et al., 1996, 1998; Molina et al., 1995).

The participation of the opioid system in the reinforcing properties of ethanol has been well demonstrated in humans and in animals. In fact, low blood ethanol levels have been found to stimulate the activity of the opioid system (Acquas et al., 1993), and the administration of μ-receptor agonists has been found to increase ethanol intake (Stromberg et al., 1997). The administration of nonselective opioid antagonists such as naloxone or naltrexone has been shown to reduce ethanol intake in adult rats and also to modify reactivity to its taste (Coonfield et al., 2002; Critcher et al., 1983; Hill and Kiefer, 1997; Stromberg et al., 1998a,b, 2002). It has been suggested that opioid antagonists reduce ethanol intake not only by decreasing the level of reward after ethanol ingestion but also by changing its palatability in a negative manner (Coonfield et al., 2002). However in all those studies in which a reduction of ethanol intake was observed under naltrexone, ethanol consumption returned to control levels when the naltrexone treatments were stopped (Coonfield et al., 2004; Goodwin et al., 2001; Hill and Kiefer, 1997). This may indicate that, at least with the doses used in those studies, naltrexone did not induce by itself a conditioned taste aversion to ethanol. Yet, there are studies showing that naloxone and naltrexone induce place aversion and taste aversion at moderate to high doses (Lett, 1985; Mucha, 1989; Mucha and Herz, 1985; Parker and Rennie, 1992; Stolerman et al., 1978).

Considering the role of the opioid system on perinatal learning processes as well as on the reinforcing aspects of ethanol, it has been proposed that the above mentioned increase in ethanol consumption observed after prenatal exposure to the drug could be a conditioned preference mediated by the opioid system. That is, the fetus perceives ethanol chemosensory aspects which could act as a conditioned stimulus (CS) and also experiences the reinforcing properties of the drug (unconditioned stimulus, US), the latter mediated by the opioid system. This hypothesis is supported by recent data showing that the effect of augmented ethanol intake was not observed in pups whose mothers were administered naloxone together with ethanol (Chotro and Arias, 2003). This result can be explained by the blockage of the reinforcing properties of ethanol, what would prevent the occurrence of the prenatal association. In that same study it was observed that postnatal re-exposure to ethanol and naloxone decreased ethanol intake in pups prenatally exposed to the drug, possibly by extinguishing the conditioned response.

In a more recent study the hedonic nature of the prenatal ethanol experience has been further investigated using a taste reactivity test in 14 day old rats (Arias and Chotro, 2005). It was found that pups exposed to ethanol in utero, when compared to non-exposed controls, not only consumed more ethanol but also displayed more appetitive responses (mouthing and paw licking) and less aversive behaviors (defined as general activity and wall climbing) in reaction to ethanol taste. Pups prenatally exposed to ethanol generalized those responses to a sucrose+quinine compound (SQ), which shares palatability attributes with alcohol (Kiefer et al., 1988), and they also showed higher intake of this solution than controls. Those results suggest that in the infant rat the palatability of the taste of ethanol was enhanced after exposure to the drug during the last days of gestation. They also agree with results of previous studies indicating that prenatal ethanol exposure induces a preference for this drug (Chotro and Arias, 2003; Chotro et al., 1996; Dominguez et al., 1996, 1998; Molina et al., 1995).

Accordingly, the aim of the present study was to analyze whether the administration of an opioid antagonist together with ethanol during the last gestational days affects the palatability of this drug. The response to the SQ compound taste, a stimulus that shares orosensory but not toxic properties with ethanol, was also analyzed. The hypothesis guiding the present study was that infant rats exposed prenatally to ethanol acquire in utero a conditioned preference for the ethanol taste and that naloxone administered together with ethanol prenatally will block or reduce the conditioning and therefore will decrease not only postnatal ethanol intake but also the palatability of ethanol's taste.

1. Materials and methods

Subjects for this experiment were 168 preweanling rats, 88 males and 80 females, obtained from 19 Wistar female rats.

Animals were born and reared in a temperature-controlled vivarium at the University of the Basque Country (Spain). The colony room was maintained on a 12-h light/12-h dark illumination cycle, with light onset at 8 a.m. Female rats were time-mated to provide subjects for this study and were housed individually in standard maternity cages with continuous access to food (Panlab, Spain, maternity formula) and water. Dams remained undisturbed until the initiation of the ethanol treatment on GD 17 (GD 0=presence of sperm in vaginal smear). In all experiments the European regulations for care and treatment of experimental animals were followed, and all procedures were controlled and approved by the Diputación Foral de Gipuzkoa (Spain), in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

1.1. Maternal ethanol and naloxone administration

Pregnant dams received through gestational days 17–20 one daily intragastric administration of either 2 g/kg ethanol dose or water. This ethanol dose resulted from the administration of a volume equivalent to 0.015 ml of a 16.8% *v*/*v* ethanol solution per gram of body weight. Dams administered the 0 g/kg dose received similar volume of only the vehicle (water). Immediately after this administration, half of the dams of each treatment received a subcutaneous injection of 10 mg/kg of naloxone (Sigma-Aldrich, Spain), volume of administration was 2 ml/kg of a 5 mg/ml naloxone solution; whereas the remaining dams received a similar volume of a saline solution. Time of administration was similar for all days (10–11 a.m.). The day of birth was designated as postnatal day 0 (PD 0).

1.2. Taste reactivity test

On PD 14, 2 h before the test, pups were separated from the mother and placed in holding chambers (15 × 8 × 15 cm) maintained at 28–30 °C with heating pads. All pups were intraorally cannulated using a procedure described in previous studies (Chotro and Alonso, 2003; Hall and Rosenblatt, 1977). Cannulae are made with 5 cm sections of polyethylene tubing (PE 10, i.d. = 0.28 mm), one end of the section is heated to form a small flange. A thin wire attached to the non-flanged end of the cannula is placed on the internal surface of the pup's cheek and the wire is then pushed through the oral muccosae until the flanged end of the cannula is positioned over the internal surface of the cheek while the remainder of the cannula exits from the oral cavity. The entire procedure takes less than 5 s per pup and induces minimal stress. These cannulae were later used to infuse the solutions during the test. Before the test, pups' bladders were voided by gentle brushing of the anogenital area. Then, body weights were registered and subjects were placed into heated individual chambers. Pups from each prenatal treatment were assigned randomly to one of three test conditions defined by the solution administered during this test (ethanol 6% *v*/*v*, E; sucrose 0.1 M + quinine 0.0001 M, SQ; or water, W).

Pups were tested in a trapezoid-shaped chamber with its front wall (29 cm wide) made of clear glass and the remaining walls (back 18 cm, sides 11.5 cm) and floor made of mirror, so as to allow observation of the pups' oro-facial expression and body movements in any position. The chamber was 12.5 cm high and was divided into two equal sections. Two pups at a time were evaluated, one per section of the chamber.

Pups remained for 2 min in the test chamber before the beginning of the intraoral infusions of the solutions; this was considered as baseline period. Intraoral infusions were performed using an infusion pump (KD Scientific) connected to the oral cannula of each pup by a polyethylene catheter (Clay Adams, PE 50). Total volume administered to each subject's mouth during this test was equivalent to 2.5% of their body weight and was infused in five pulses every 2 min, the volume of each pulse was 0.5% of their body weight. The pulse duration was 15 s with intervals between pulses of 105 s (duration of the test: 10 min preceded by a 2 min baseline). The rate of infusion was dependent on the pup's body weight. Pups could either consume or reject the infused solution. During this period pups were videotaped from the front glass wall for subsequent analysis of their behavior. At the end of the session post-infusion weights were registered and pups were placed into heated holding cages separated from the remaining untested pups. Once the entire litter was tested pups were returned to the mother.

Based on previous studies using taste reactivity tests with infant rats (Chotro et al., 1996; Hall and Bryan, 1981; Vigorito and Sclafani, 1988), and with adult rats (Grill and Norgren, 1978; Parker, 1988, 1995), as well as on our own observation of the pups' reaction to the tastants used in the present study, the following behavioral measures were selected as dependent variables under analysis: general activity and wall climbing, considered here as aversive reactions; Passive drips, as a mild aversive reaction; and mouthing and paw licks, as ingestive/appetitive responses. Other behaviors described in the literature, such as chin-rubbing, gaping, paw treading, head shaking or forelimb-flailing, were not observed in the present and previous studies from our laboratory with infant rats in response to the substances here tested. Consumption of the substances administered was also measured, and was determined by the percentage of body weight gain using the following formula:

$$[(\text{postinfusion weight} - \text{preinfusion weight}) / \text{preinfusion weight}] \times 100.$$

As stated before, the behavioral measures were analyzed from the videotapes and scored every 30 s. General activity was rated in the seven categories described by Hall and Bryan (1981): "0=no movement, except for occasional twitches; 1=slight movement, usually of the head or paw, sustained for 5 s; 2=substantial movement of the head and paws, including grooming, but no locomotion; 3=locomotion involving forelimbs and often including rooting and probing but with hind limbs motionless and usually serving as a pivot; 4=clear and sustained locomotion about the test container; 5=vigorous

locomotion, often including rolling, kicking, and wall climbing; 6=an extreme, but occasionally observed, form of rating five in which the pup tumbled about its container for most of the 30 s interval, locomoting, rolling, probing, wall climbing and jumping." For statistical analysis, the four 30 s periods following the onset of each infusion were collapsed into 1 trial (one per infusion trial plus one for baseline) so, scores for general activity ranged between 0 and 24.

A pup was considered wall climbing when standing on its rear limbs with its forepaws against the wall of the testing chamber. Time in seconds wall climbing was also registered for each pup every 30 s during baseline and test intervals. Passive drips was defined as the time in seconds the pup remained still with it four limbs on the floor, just letting the liquid drip from the mouth in the absence of any mouth movement; obviously this behavior was only observed during the infusion portion (15 s) of each of the five test trials. Mouthing was defined as any obvious movement of the mouth and jaws, and total time (in seconds) mouthing and paw licking was also registered during testing trials. Mouthing and paw licking were independent measures, i.e. mouthing movements displayed during paw-licking were not measured as mouthing. Taking into account results of a previous study in which similar effects of the prenatal treatment were observed on mouthing and paw licking (Arias and Chotro, 2005), as well as another study by Parker (1995), both variables were combined into a new one called ingestive responses. Since mouthing, paw licking and passive drips are behaviors directly related to the intraoral infusion of the liquids, no data were obtained during baseline periods for these variables, therefore baseline data were only analyzed for general activity and wall climbing. As was the case for general activity, all these behaviors were scored every 30 s, and for statistical analysis the four 30 s periods immediately following the onset of each infusion were collapsed in one trial (2 min). So, for general activity and wall climbing there was 1 baseline trial followed by 5 infusion trials and for passive drips, and ingestive responses (mouthing+paw licking) only 5 testing trials. All behavioral observations were performed by a trained researcher blind to the experimental conditions.

1.3. Intake test

On PD 15 all subjects were evaluated in terms of consumption of the same solution they had received on the PD 14 test. Two hours before the test, pups were separated from their mothers, cannulated and kept in a heated holding chamber.

Almost all procedures followed for this intake test were similar to the ones described for the taste reactivity test. The differences were that the substances were infused in a continuous flow into their mouths (not in pulses as was the case for the previous test), total volume administered of each solution was equivalent to 5.5% of their body weight, the duration of the test was 15 min, and that pups were tested in clear Plexiglass chambers (15 × 8 × 15 cm) maintained at 28–30 °C with heating pads. Once the test concluded, pups were weighed and, when the entire litter was tested, all were returned to the maternity cage. Intake was also expressed as percentage of body weight gain.

2. Data analysis

Data from each solution tested (E, SQ and W) were analyzed separately. As mentioned before, in order to simplify the statistical and visual presentation of the data, the scores of four 30 s bins that followed the onset of each infusion were collapsed in one trial. For general activity and wall climbing, the initial four 30 s trials were also grouped in one baseline trial which was analyzed, separately from the remaining infusion trials, with 2 (Prenatal ethanol) × 2 (Prenatal naloxone) ANOVAs for each solution tested. General activity, wall climbing, passive drips, and ingestive responses data collected during the 5 test trials were analyzed with 2 (Prenatal ethanol) × 2 (Prenatal naloxone) × 5 (Trial) mixed ANOVAs, for each tested solution. Body weights of the pups on PD 14 and intake data obtained during days 14 and 15 were also analyzed with 2 (Prenatal ethanol) × 2 (Prenatal naloxone) ANOVAs for each of the three solutions tested. When required, the appropriate post-hoc comparisons were performed. A rejection criterion of $p < 0.05$ was adopted for all the analyses presented in this study (Table 1).

3. Results

3.1. General activity

Baseline general activity was not affected by the prenatal treatments in any of the three tests (Fig. 1). When analyzing the reaction to ethanol, only a significant effect of Trial was observed, $F(4,232) = 5.87$, $p < 0.001$. Further analyses indicated that general activity on trial 5 was significantly lower than on the previous trials. On the SQ test, the ANOVA indicated no significant effects; and on the water test an effect of Trial was evidenced $F(4,184) = 10.08$, $p < 0.001$, further t-test analysis indicated that general activity on trial 5 was significantly lower than in the previous trials and also that trials 3 and 4 differed from trial 1.

3.2. Wall climbing

Wall climbing during the three tests is depicted in Fig. 2. The ANOVAs with the baseline wall climbing data indicated no significant effects in any of the three tests. During the

Table 1
Pup's mean and standard deviations of weights on PD 14 and PD 15 as a function of prenatal exposure to ethanol (0 or 2 g/kg) and naloxone (saline, Sal or naloxone, Nal)

Group	PD14		PD15		n
	Mean	SD	Mean	SD	
0-Nal	25.68	2.35	27.31	2.28	36
0-Sal	26.79	3.26	28.48	3.45	40
2-Nal	26.33	2.7	28.01	2.52	46
2-Sal	25.78	1.64	27.49	1.56	46

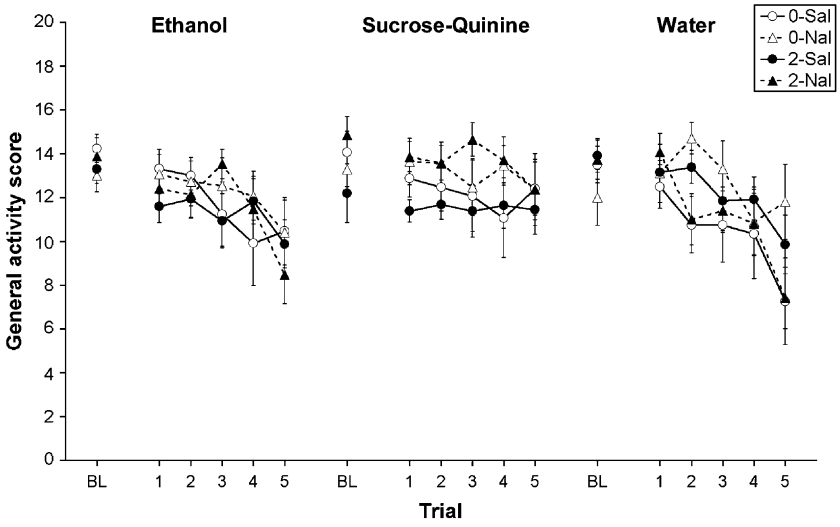

Fig. 1. Mean general activity data during baseline (BL) and the 5 testing trials, in response to ethanol, sucrose–quinine, or water, as a function of prenatal exposure to ethanol (0 or 2 g/kg) and naloxone (saline, Sal or naloxone, Nal). Vertical bars represent standard error of the mean.

ethanol test only an effect of trial was observed $F(4,232)=2.73$, $p<0.05$. On this test pups showed more wall climbing on the first two trials than in the last one. When data from the SQ test were analyzed, it was found a significant effect of Trial, $F(1,52)=5.59$, $p<0.05$, and the significant interaction Prenatal ethanol × Prenatal naloxone × Trial, $F(4,208)=2.53$, $p<0.05$. Tukey HSD post-hoc tests revealed that group 2-Sal displayed less wall climbing in response to SQ than group 0-Sal and than group 2-Nal. The remaining three groups did not differ significantly. When pups were tested with water, the ANOVA indicated a significant effect of Trial, $F(4,184)=2.97$, $p<0.05$, and *t*-test revealed that on the last trial pups displayed less wall climbing than in the first three trials.

3.3. Passive drips

As can be observed in Fig. 3, in response to either ethanol, SQ or water pups displayed a gradual increase of passive drips across trials. This was confirmed by the ANOVAs indicating only significant effects of Trial. For the ethanol test, $F(4,232)=27.26$, $p<0.001$, for the SQ test, $F(4,208)=14.56$, $p<0.001$, and for the water test, $F(4,184)=14.64$, $p<0.001$.

3.4. Ingestive responses

Fig. 4 illustrates ingestive responses of the different groups during each of the three tests. Analysis of the data from the ethanol test indicated significant effects of Prenatal ethanol, $F(1,58)=12.73$, $p<0.001$; of Trial, $F(4,232)=5.70$, $p<0.001$, and a significant interaction between Prenatal ethanol and Prenatal naloxone, $F(1,58)=4.96$, $p<0.05$. Tukey HSD post-hoc test revealed that group 2-Sal responded to ethanol with more ingestive responses than the remaining three groups, which did not differ significantly between them.

When SQ was tested, the ANOVA indicated significant effects of Prenatal ethanol, $F(1,52)=13.37$, $p<0.001$, Trial, $F(4,208)=16.89$, $p<0.001$, and the following interactions: Prenatal ethanol × Prenatal naloxone, $F(1,52)=6.67$, $p<0.05$, Prenatal naloxone × Trial, $F(4,208)=2.48$, $p<0.05$, and the interaction between the three factors, $F(4,208)=3.22$, $p<0.05$.

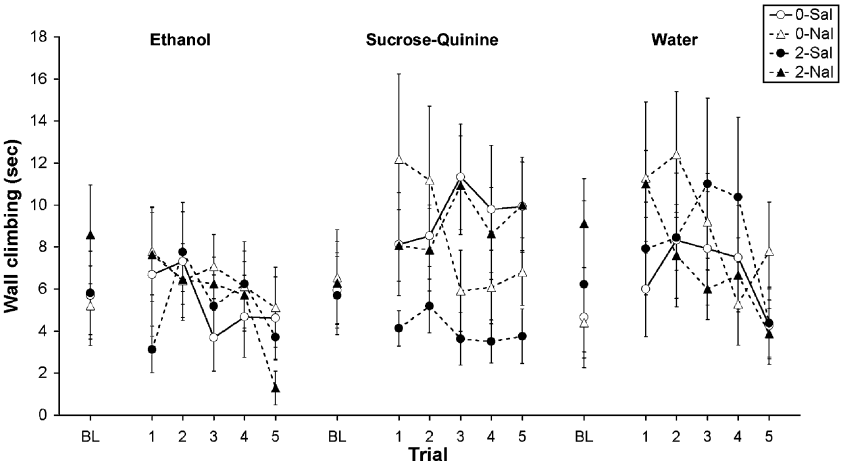

Fig. 2. Mean wall climbing data (s) during baseline (BL) and the 5 testing trials, in response to ethanol, sucrose–quinine, or water, as a function of prenatal exposure to ethanol (0 or 2 g/kg) and naloxone (saline, Sal or naloxone, Nal). Vertical bars represent standard error of the mean.

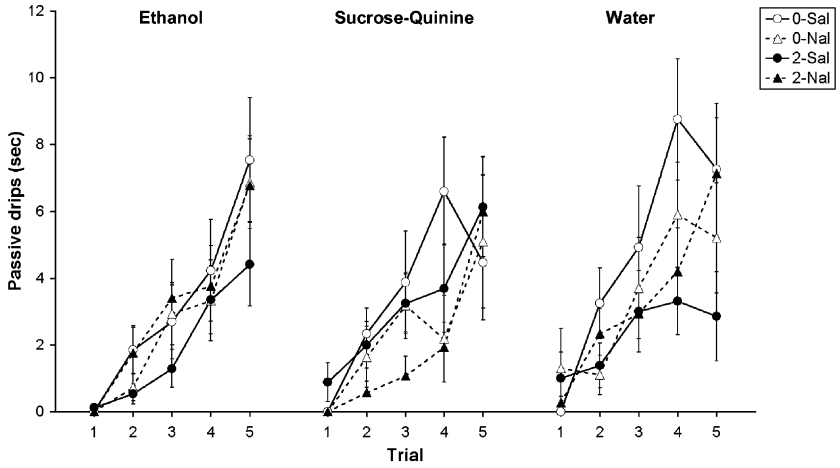

Fig. 3. Mean passive drips (s) data for pups exposed prenatally to 0, 1, or 2 g/kg of ethanol, during the 5 testing trials, in response to ethanol, sucrose–quinine, or water, as a function of prenatal exposure to ethanol (0 or 2 g/kg) and naloxone (saline, Sal or naloxone, Nal). Vertical bars represent standard error of the mean.

Tukey post-hoc test revealed that Group 2-Sal displayed more ingestive responses to SQ than the remaining groups, but only on the first trial.

In response to water there was only a significant effect of Trial, $F(1,184)=4.77$, $p<0.005$, and t-tests indicated that in trial 1 pups spent more time responding than in trials 3, 4 and 5; and in trial 2 more than in trials 4 and 5.

3.5. Intake

Intake data from day 14 are represented on Fig. 5. As can be observed, intake of SQ or water was not affected by the prenatal treatments on either testing day. This was confirmed by the ANOVAs indicating no significant effects of any variable for these tests. The analysis of the data of ethanol intake on PD 14 indicated a significant effect of Prenatal ethanol, $F(1,58)=4.70$, $p<0.05$, while the interaction Prenatal ethanol × Prenatal naloxone was close to significance, $F(1,58)=3.61$, $p=0.06$. Orthogonal planned comparisons indicated that group 2-Sal consumed significantly more ethanol than groups 2-Nal and 0-Sal.

On PD 15 (Fig. 5), the ANOVA indicated a significant effect of Prenatal naloxone, $F(1,58)=4.06$, $p<0.05$ and a significant interaction between both prenatal treatments, $F(1,58)=4.10$, $p<0.05$. Post-hoc analyses (LSD Fisher test) of this interaction revealed that, again, group 2-Sal consumed significantly more ethanol than the remaining groups.

Body weights of pups that entered in any of the three tests were not affected by the prenatal treatments.

4. Discussion

In this study it was found that pups exposed prenatally to ethanol alone displayed more ingestive responses in reaction to the taste of this drug than non-exposed pups. The prenatal experience with ethanol also produced an increase in the consumption of this drug. Simultaneous administration of naloxone with ethanol during the last gestational days prevented both, the increased intake of the drug and the higher amount of ingestive responses to the taste of ethanol.

The enhanced ingestive responses to ethanol as well as the increased ethanol intake are congruent with results of previous

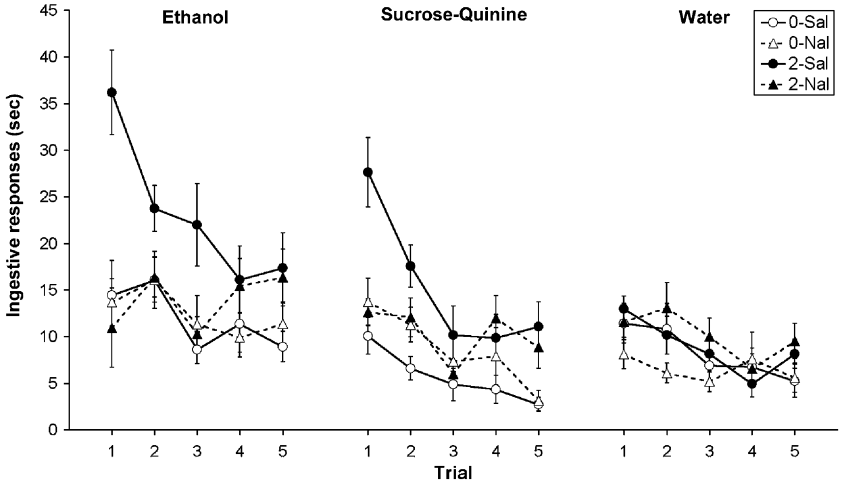

Fig. 4. Mean ingestive responses data (s) during the 5 testing trials, in response to ethanol, sucrose–quinine, or water, as a function of prenatal exposure to ethanol (0 or 2 g/kg) and naloxone (Nal or Sal). Vertical bars represent standard error of the mean.

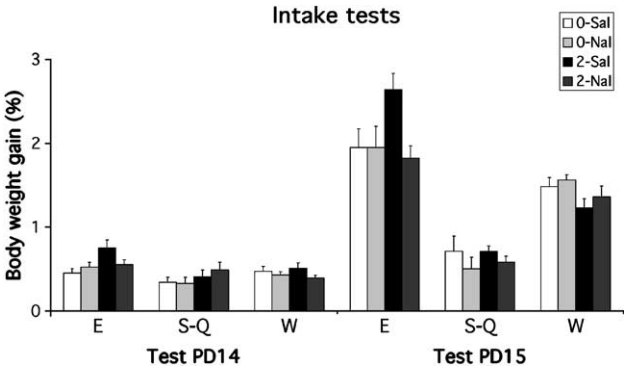

Fig. 5. Mean percentage of body weight gain as a function of the solution intraorally infused (ethanol, sucrose–quinine or water), prenatal ethanol treatment (0 or 2 g/kg), prenatal naloxone treatment (saline, Sal or naloxone, Nal), and day of testing (PD14 or PD15). Vertical bars represent standard error of the mean.

studies from this and other laboratories. The reduction in ethanol intake observed in those pups exposed to ethanol and naloxone also agree with a previous study in which pups exposed prenatally or postnatally to both substances reduced their ethanol consumption (Chotro and Arias, 2003). The new finding of this study, i.e. the decrease on ingestive responses to ethanol after combining ethanol and naloxone prenatally, confirms that the increased ethanol intake observed after prenatal ethanol exposure is the result of an enhanced palatability of ethanol's taste and that the opioid system is involved in this process. This result provides additional support to the hypothesis of an appetitive conditioning established in utero between the chemosensory and the rewarding properties of ethanol, mediated by the opioid system.

Pups prenatally exposed to ethanol and saline also showed more ingestive responses and less aversive behaviors in response to the taste of SQ. Naloxone administered together with ethanol increased the aversive responses and decreased the appetitive ones to the taste of SQ. This reaction to a compound that shares with ethanol gustatory but not toxic properties, indicates that the information the pup is acquiring in utero is directly related to the orosensory aspects of ethanol. It could be argued that prenatal ethanol exposure increases acceptability of any substance. Nevertheless, previous results have demonstrated that similar prenatal treatments do not affect consumption of other tastes such as sucrose or quinine alone (Dominguez et al., 1998; Molina et al., 1995). Moreover, in the present study, no differences were observed between naloxone and saline treated groups in terms of water intake.

However, intake of SQ was not affected by the prenatal treatments. In previous studies it has been reported that pups exposed prenatally to this ethanol dose consumed more of the SQ compound (Dominguez et al., 1998; Molina et al., 1995). The difference between those studies and the present one, is that here pups were exposed to the taste of SQ the day before the intake test, i.e. during the taste reactivity test. That previous experience with the taste could have helped to better discriminate it from ethanol, reducing the generalization of the conditioned preference. This effect of enhanced discrimination after non-reinforced exposure to a taste has been previously observed with infant rats and similar gustatory stimuli (Chotro and Alonso, 2003).

In the present study prenatal ethanol administration did not reduce the expression of aversive responses to ethanol taste but did reduce it to the taste of SQ. Previously, we have reported a decrease in wall climbing and general activity in reaction to the taste of ethanol in pups exposed prenatally to a similar ethanol dose than the one employed in the present study (Arias and Chotro, 2005). Other authors also found a reduction of general activity in the presence of ethanol odor after prenatal ethanol exposure (Chotro et al., 1996; Dominguez et al., 1996). Results of the present and previous studies indicate that the prenatal experience with ethanol induces a preference for the taste of ethanol (Arias and Chotro, 2005; Chotro and Arias, 2003; Molina et al., 1995). This positive shift in the response patterns to ethanol favored the observation of appetitive behaviors and probably reduced the occurrence of aversive responses to ethanol. On the other hand, during the SQ test there was more wall climbing than during the ethanol test, which indicates that the taste of the SQ compound is perceived as more aversive than the taste of ethanol. This fact facilitated the observation of differences in wall climbing between groups 2-Sal and 0-Sal and therefore, the detection of the effect of the prenatal treatment with naloxone.

The increased ethanol intake and the enhanced acceptability of ethanol observed in the present study, as well as in a previous work (Chotro and Arias, 2003), cannot be explained in terms of non-associative learning processes, such as increased familiarity or reduced neophobia, because pups experiencing only ethanol in utero consumed significantly more ethanol than pups with the same ethanol exposure but in combination with naloxone.

It could be argued that pups treated prenatally with ethanol and naloxone reduced their ethanol intake because naloxone may have induce a conditioned taste aversion for ethanol. In fact, several studies have shown that naloxone and naltrexone can induce place aversion as well as conditioned taste aversion (Lett, 1985; Mucha, 1989; Mucha and Herz, 1985; Parker and Rennie, 1992; Stolerman et al., 1978). Yet, in one of these studies it was found that naltrexone was effective inducing a conditioned taste aversion to sucrose but did not produce aversive responses in a taste reactivity test, indicating that naltrexone did not produce a conditioned dislike for the flavor with which it was paired (Parker and Rennie, 1992). Respect to ethanol's flavor, several studies have shown that under the effects of naltrexone the palatability and consumption of ethanol are reduced in adult rats. However, when naltrexone treatment was interrupted ethanol intake returned up to control levels, suggesting that a conditioned taste aversion was not developed (Coonfield et al., 2004; Goodwin et al., 2001; Hill and Kiefer, 1997). Although in those studies the palatability of ethanol was not evaluated after been paired with naltrexone, it is conceivable that ethanol's taste has not become aversive either, particularly in view of the results of the above mentioned study by Parker and Rennie (1992). Likewise, in a study with infant rats it was observed that naloxone reduced

ethanol intake, though when later tested without naloxone pups showed intake levels comparable to those which have not experienced ethanol paired with naloxone (Chotro and Arias, 2003). Therefore, the reduced ethanol intake observed in ethanol–naloxone treated pups from the present study, respect to subjects treated only with ethanol, cannot be attributed to the acquisition of a conditioned taste aversion but to the lack of acquisition of a conditioned preference.

In sum, considering the present results as well as those of two previous studies from this lab (Arias and Chotro, 2005; Chotro and Arias, 2003) it can be concluded that the rat pup shows a clear preference for the flavor of ethanol after prenatal exposure to this drug, also that the opioid system is directly related to this appetitive response, which can be prevented by the administration of an opioid antagonist concurrently with ethanol. The results also confirm that the preference for ethanol is a conditioned response acquired in utero in which the taste of ethanol acts as the CS.

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