Exposure to low and moderate doses of alcohol on late gestation modifies infantile response to and preference for alcohol in rats

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Summary. Several studies in rats have found that maternal administration of low or moderate doses of ethanol result in fetal perception of the chemosensory and toxic effects of ethanol. This prenatal experience with the drug enhances the palatability of ethanol's flavor and increases ethanol consumption during infancy and adolescence. The acquired preference for ethanol seems to be a conditioned response established prenatally, by the association of ethanol's sensory and reinforcing aspects, the latter mediated by the opioid system. These results are in accordance with data of studies in humans, and should be taken into account for clinical studies analyzing the relationship between prenatal ethanol exposure and later ethanol abuse problems.

Key words: ethanol, odor, taste, preference, consumption, rat, fetus.

Riassunto *(Esposizione a basse e moderate dosi di etanolo in gravidanza. Modificazioni nel comportamento verso l'alcol nel ratto)*. Numerosi studi sugli animali hanno dimostrato che la somministrazione di basse o moderate dosi di alcol alla madre costituisce una esperienza prenatale che aumenta la gradevolezza dell'etanolo nella prole e favorisce il consumo di etanolo durante infanzia e adolescenza. La preferenza per l'etanolo sembra essere una risposta condizionata che si instaura in fase prenatale sia per gli effetti sensoriali associati all'etanolo che per gli aspetti di rinforzo mediati dal sistema oppioide. Questi studi sono in accordo con i dati ottenuti nell'uomo e possono essere presi in considerazione per gli studi clinici che riguardano la relazione tra esposizione prenatale e problemi di abuso alcolico nel corso della vita.

Parole chiave: etanolo, odore, gusto, preferenza, consumo, ratto, feto.

INTRODUCTION

Studies in both humans and animals have extensively demonstrated the deleterious effects on the fetus of maternal alcohol ingestion. Although deficits produced by prenatal exposure to high ethanol levels are most severe and have been documented most significantly in children with fetal alcohol syndrome (FAS), there are data showing that children prenatally exposed to lower levels of alcohol frequently exhibit similar problems, especially those related to neurobehavioral impairment [1-3].

In recent years a growing amount of research has been published in relation to the effects of moderate drinking in humans. But, how is defined moderate drinking? Some authors have described moderate ethanol consumption as the one with a low risk for generating ethanol related problems [4]. Although the amount of factors that influence the effect of ethanol intake makes very difficult to establish a quantitative limit, in general for humans a moderate ethanol intake has been set between 1 and 1.99 drink/day for men and no more than 1 drink/day for women [5, 6]. Considering that one drink corresponds approximately to 0.5 fl oz ethanol, this consumption is equivalent to 24-28 g/day for men and 12-14 g/day for women [4]. The inferior limit for the moderate consumers' category has been established on 4 drink/week [7]. In clinical studies on the effects of ethanol consumption during gestation a moderate drinking pattern is considered as an intake of 7 to 14 drink/week. In two longitudinal clinical studies [8, 9] in which the effects of prenatal ethanol on neurobehavioral development were analyzed, concluded that there is no clear threshold for these effects. For some behaviors, such as mental development, even the smallest dose of ethanol (0.02 to 3.49 drink/week) seems to have effects on the fetus, although most neurobehavioral outcomes have higher thresholds [5]. So far, it is not clear whether exists a limit of ethanol intake during gestation that does not produce any effect on the development of the fetus. The difficulty for conclusions on this respect comes from the impossibility to control all the factors intervening in clinical studies. Some authors indicate that for concluding whether or not prenatal ethanol exposure affects a given cognitive function, certain factors should be considered such as the test used and the age of the tested subject [5]. Other factors such as acute stress that may help the expression of deficits produced by ethanol prenatal exposure

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that in normal conditions are not evidenced should be also taken into consideration [5]. Results of longitudinal studies also indicate a clear relationship between moderate ethanol intake during pregnancy and ethanol abuse problems when measured at age 14 [10] and 21 [11]. They have found that binge ethanol drinking during pregnancy is better predictor for young alcohol involvement and alcohol-related problems than other important factors such as family history for alcoholism, nicotine exposure, and other ambient variables like parent's consumption of drugs. These studies conclude that even modest levels of fetal alcohol exposure have to be considered in studies of the etiology of alcoholism and family history. Within the complex relationship between prenatal ethanol exposure and subsequent alcohol abuse several factors may influence the initiation of ethanol consumption during early stages of development. Besides the already known teratological effects of prenatal ethanol, there are other ways by which ethanol exposure *in utero* may promote subsequent ethanol directed behaviors. The use of animal models provides useful means for analyzing each of these ways in isolation as well as their interactions in controlled conditions.

ANIMAL STUDIES

Experimental studies in animals have indicated that prenatal ethanol may alter neurochemical systems that are involved in the motivational and reinforcing aspects of the drug's consumption. Other way by which ethanol exposure could be modifying subsequent patterns of response to the drug is related to fetal perception of its chemosensory characteristics (odor and probably taste) *in utero*. Respect to this, studies in humans have shown that human fetuses are capable of detecting chemosensory stimuli in the amniotic fluid [12]. Additional support for this has been recently presented in a study showing that flavors contained in maternal diet during pregnancy are later preferred by the infant when compared to other novel flavors [13]. In another study in which newborn babies (24-48 hours postpartum) were tested in their behavioral response to ethanol odor, it was reported that neonatal responses to ethanol odor were affected by maternal ethanol ingestion during gestation. It was observed that babies born from mothers who reported moderate ethanol intake during pregnancy displayed higher reactivity levels to ethanol odor than babies from mothers classified as infrequent drinkers [14]. Newborns from both groups of mothers were also tested in their reaction to lemon odor and, in this case, no differences in behavioral reaction were observed between subjects. In all these studies it seems clear that the fetus recognizes chemosensory aspects of substances presented in their amniotic environment, including ethanol, and that this prenatal experience may change its response to them.

Acute prenatal ethanol exposure

Similarly to what has been described in humans, in studies with animals a direct relationship between prenatal ethanol exposure and changes in postnatal response to ethanol's odor and taste has been reported. This was clearly observed in a series of studies in which ethanol was directly administered into the amniotic fluid of each rat fetus, during only 10 min just before birth. This acute and brief alcohol exposure was enough to produce in the newborn rat an increase in motor activity in response to ethanol odor as well as an increase in ethanol intake and preference for ethanol odor in infant rats [15]. In addition, it was found an interaction between this short prenatal experience with only the chemosensory aspects of alcohol and postnatal conditioned learning involving ethanol odor or taste. Conditioned preferences for alcohol odor were obtained in infant rats after receiving paired presentations of alcohol and intraoral infusions of a sucrose solution. This conditioned preference for alcohol was enhanced in pups prenatally exposed to the drug [16]. In this same study it was also observed that the prenatal alcohol experience attenuated a conditioned aversion towards alcohol odor. Further studies showed that these effects were more clearly observed when alcohol was presented 10 min prior to cesarean delivery than with a longer delay, 30 min, or a shorter one, 3 min [16-18]. These last studies indicated that the effect observed was the result of an association between the prenatal ethanol chemosensory cues and tactile stimulation occurring during cesarean delivery. Due to the fact that in all these studies alcohol intoxication of the fetuses was explicitly avoided it can be concluded that those results are mainly related to processing of alcohol's chemosensory characteristics. Moreover, similar results were found when a non-alcohol stimulus, such as lemon, was presented to the rat fetus [16, 19].

These results are not unexpected when taking into account that, similarly to what has been described for human fetuses [12], in the rat fetus, during the last days of gestation certain olfactory subsystems are already functional [20]. Clear evidence of fetal perception and recognition of substances present in the amniotic fluid has been reported [21-23]. This fetal perception of the chemosensory properties of the amniotic environment before birth seems to be directly related to postnatal responses towards those substances. For example, it has been demonstrated that olfactory cues guiding rat neonates in their first nipple attachment are substances contained in the amniotic fluid [24]. Contamination of the amniotic fluid with a particular flavor has been found to modify that early behavior [25], and also may result in an increased consumption of that substance later in life [26]. It has been also shown that from gestational day 17 (GD 17) the rat fetus has the capacity for acquiring and displaying basic forms of non-associative and associative learning, such as stimulus sensitization and habituation or conditioned responses to tactile and chemosensory stimuli [27-33].

Toxic effects and behavioral changes

When ethanol is administered to the pregnant rat, the drug is rapidly distributed to fetal tissues reaching levels in fetal blood equal to those in maternal circulation

[34]. 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 sufficient to be perceived by the rat fetus [15, 34]. Thus, maternal administration of alcohol during the last days of gestation results in fetal exposure to both, the ethanol toxic and chemosensory properties.

In studies in which low (1 g/kg) or moderate (2 g/kg) dose of alcohol were administered to the pregnant rat during the last days of gestation, *i.e.* gestational days (GD) 17 to 20, no significant teratological effects were detected. In those studies ethanol exposure failed to affect several maternal-fetal and perinatal physical parameters such as placenta weight, umbilical cord length, offspring's body weight, weight and size of olfactory bulbs, cerebral hemispheres, and cerebellum [35]. However, in some cases these same alcohol doses were found to induce an increase in baseline motor activity [17, 36]. This hyperactivity effect was significantly reduced in the presence of alcohol odor, but not when other novel odors, such as lemon, were presented [35]. Nevertheless, this seems to be a very weak effect and not very consistent since no differences in baseline motor activity were found in other studies with neonates prenatally exposed to 1 g/kg alcohol [37] or in 14 day old pups treated *in utero* with 1 or 2 g/kg alcohol [38].

What has been consistently found in those studies is that ethanol exposure promotes subsequent changes in responsiveness to the drug. For instance, repeated administrations of low to moderate ethanol doses (1-2 g/kg) to the pregnant rat during GD 17-20 promoted changes in behavioral and autonomic responses to ethanol odor interacting with postnatal re-exposure to the drug [39]. In that study, pups exposed to alcohol pre and postnatally showed a stronger orienting response towards alcohol odor, which is a marked bradycardia, when compared to pups not exposed to alcohol prenatally. This same prenatal experience with alcohol has been also found to change fetuses responsiveness to the toxic as well as to the sensory aspects of alcohol. In a study in which fetuses' behavior was evaluated during GD 20, it was found that maternal ethanol intoxication with 1 or 2 g/kg induced a drastic reduction in fetal movements [36]. In general, alcohol administration during pregnancy results in a decrease in fetal motor activity and breathing movements. This has been documented in humans [40], in sheep [41], as well as in rats [42]. What was more surprising from that study was that, besides this acute effect of alcohol intoxication, it has been also observed that rat fetuses, whose mothers were repeatedly administered with low doses of alcohol during the previous three days of gestation showed a more marked decrease in motor activity while intoxicated when compared to fetuses never exposed to the drug before [36]. This indicated that the fetus became sensitized to the sedative effects of ethanol, what results surprising since repeated exposure to ethanol usually results in the opposite process, *i.e.* tolerance. Another interesting result from this study was that those same fetuses, when tested sober, displayed more mouthing to the taste of alcohol and also it was observed a trend to display less facial wiping to this taste, when compared to fetuses with no previous experience with ethanol. No differences between ethanol exposed and non-exposed fetuses were observed when the response to saline or lemon was tested, confirming that ethanol experience was affecting specifically the response to the flavor of the drug.

Changes in ethanol preference

Repeated prenatal alcohol experience has been also found to produce an increase in ethanol consumption when tested during postnatal stages. This enhanced alcohol intake effect has been consistently found in several studies in which pregnant females were administered either 1 or 2 g/kg alcohol during the last days of gestation [35, 43-45]. Rat neonates exposed prenatally to those alcohol doses subsequently recognized alcohol odor when presented alone or in compound with amniotic fluid, and reduced their general activity in the presence of the drug [35]. Those same subjects were tested as infants on postnatal day 15, in terms of ethanol intake, and it was reported that only pups prenatally exposed to the lower ethanol dose, *i.e.* 1 g/kg, showed an increased ethanol intake when compared to saline pre-exposed pups. These pups showed also a higher consumption of a compound of quinine and sucrose which has been previously proved to be perceived by the rat as very similar to the taste of alcohol, measured at behavioral and physiological levels [46, 47]. On the other hand, subjects who had been exposed prenatally to the moderate alcohol dose, 2 g/kg, showed intermediate ethanol intake scores, not differing from pups whose mothers were administered with water during pregnancy or pups experiencing ethanol but in a lower concentration. In addition, no differences were observed between both prenatal alcohol treatments when intake of other substances were tested, such as water, a sucrose solution, or a quinine solution [44]. With similar prenatal treatments, however, in a recent study slightly different results were observed [43]. It was found that preweanlings exposed *in utero* to the 2 g/kg alcohol dose were the ones showing a strong increase of alcohol intake when compared to controls or to pups treated with the lower alcohol dose. In that study it was also observed that the effect of enhanced consumption of alcohol could be observed also during postweaning periods, but in that case the expression of the effect was affected by gender of the subjects.

Gender effect

Males treated prenatally with the higher alcohol dose increased significantly their alcohol intake on postnatal day 28, while the increase in alcohol consumption was observed more clearly in those female subjects exposed to the lower dose. The effect observed in adolescent rats indicates a long-term retention of the prenatal experience with ethanol, what corroborates

the importance of the near-term fetus' knowledge of the chemosensory aspects of its amniotic milieu [24]. Also may indicate that this experience is strong enough to be retained after the numerous changes in the internal and external context that overcomes the developing subject. For instance, the drastic changes from prenatal to postnatal environment, during infancy all those changes related to the development of the visual and auditive perception, such as eye and ear opening, and later from preweanling to postweaning stages. The gender-specific effect found at that age could be related to a different sensibility to ethanol by females in comparison to male subjects. Although this effect related to gender has not been observed in preweanling rats, in adolescent rats there has been reported differential ethanol intake as a function of sex [48].

Increase in ethanol consumption

The effect mainly observed after prenatal exposure to low or moderate alcohol doses is an increase in ethanol consumption. This increased alcohol intake could be indicating a preference for it, and the main hypothesis for explaining this preference is an appetitive conditioning occurring during fetal stages. The conditioned stimulus (CS) in this case could be the chemosensory characteristics of the drug and the unconditioned stimulus (US), the reinforcing aspects of alcohol probably mediated by the opioid system.

As mentioned before, in infant rats the taste and/or smell of ethanol can act as a CS, which paired with appetitive or aversive reinforcers may result in conditioned preferences or aversions, respectively [49-51]. It has been also shown that ethanol can work as a US promoting aversions when paired with an unfamiliar flavored substance, either pre- or postnatally [37, 52]. In studies of conditioned place preference, low ethanol doses have been found to act as positive reinforcers, especially at younger ages [53, 54]. Furthermore, in some cases ethanol can act as a CS and a US at the same time. For example, 10 or 11-day old infant rats administered intragastrically with a 3 g/kg dose of ethanol, but not with a 1.5 g/kg dose, showed a strong aversion to ethanol odor or taste [49-51]. As has been mentioned before, fetuses have also the capacity for perceiving alcohol chemosensory aspects *in utero* and also of associative learning about stimuli present in their environment. As stated before, conditioned responses to alcohol odor have been observed after the association of alcohol chemosensory aspects and stimulation occurring during cesarean delivery [16, 18, 55].

Reinforcing aspects

Respect to the reinforcing aspects of alcohol consumption, considerable evidence has been accumulated supporting the role of the endogenous opioid system in the mediation of them. Indeed, low blood ethanol levels have been found to stimulate the activity of the opioid system [56] and also the administration of μ-receptor agonists has been found to increase ethanol intake [57]. On the other hand, the administration of non-selective opioid antagonists naloxone or naltrexone has been shown to reduce ethanol intake in rats [58, 59]. In human subjects treatment with naltrexone has been successfully used for reducing ethanol craving and clinical relapse in recovering alcoholics [60]. Several investigations indicate that the reinforcing properties of ethanol are regulated by the activity of the μ- and δ-opioid receptors, although more recent studies using selective antagonists conclude that voluntary consumption of ethanol is primarily modulated by μ-opioid receptors [57, 61].

In the rat fetus, two opioid receptor subtypes, μ (mu) and κ (kappa), are functional and are capable of modulating fetal behavior during the last days of gestation [62, 63]. It has been also demonstrated that the activity of the opioid system (specifically μ-opioid receptors) can be conditioned prenatally after pairing a chemosensory CS with a US that promotes the release of endogenous opioids, and that subsequently the rat fetus is capable of exhibiting a conditioned opioid response when the CS is again presented [64]. The administration of opioid antagonists, either nonselective or selective for each receptor subtype, has been found to be effective modifying those fetal responses known to be regulated by the opioid system [28, 62].

The hypothesis of a conditioned preference established *in utero* as a consequence of the association between ethanol chemosensory and reinforcing aspects, the latter mediated by the opioid system, was supported by data showing that the effect of augmented ethanol intake was not observed in pups whose mothers were administered naloxone together with ethanol. Similarly, postnatal re-exposure to ethanol flavor and naloxone decreased subsequent ethanol intake in pups prenatally exposed to the drug [43]. The administration of the non-selective opioid antagonist to the pregnant rat seemed to alter the enhanced ethanol intake effect obtained after maternal ethanol intoxication. Therefore, it can be assumed that the activity of the opioid system was closely involved in the establishment of that effect. The increased ethanol intake cannot be merely explained in terms of habituation of neophobia or increased familiarity with the stimulus, since subjects with the same prenatal experience with ethanol flavor but differing in terms of naloxone treatment, displayed significantly different levels of ethanol consumption. It could be argued that naloxone treatment may have changed the palatability of ethanol when administered together. Indeed, opioid antagonists have been shown to alter the palatability or hedonic value of ethanol and other substances, when measured with a taste reactivity test [65-67]. To our knowledge, however, there are no evidences that this change in the taste value of ethanol is related to a change in the perception of the chemosensory aspects of this drug. Therefore, in that study, subjects (fetuses or infants) receiving ethanol together with naloxone could have experienced a qualitatively different cue, but different in terms of affective properties and not in terms of chemosensory properties of ethanol. By administering naloxone, the intention was to "block" the reinforcing properties of ethanol. But, it is possible that instead of becoming a neutral cue etha-

nol has become a cue with negative value. If that were the case, an alternative explanation should be considered for the reduced consumption observed in pups treated with ethanol and naloxone. When fetuses were exposed to both substances, if naloxone were turning ethanol in an aversive stimulus, then the reduced ethanol intake on postnatal stages could be the result of an aversive conditioning acquired *in utero*. While, when naloxone was administered postnatally together with the taste of ethanol, if ethanol would acquire a negative value, instead of an extinction effect we would have a counter-conditioning situation. In any case, results of that study seem to support the idea that the increased ethanol consumption observed in pups exposed prenatally to this drug is a conditioned appetitive response established *in utero* as a consequence of the association between the chemosensory properties of ethanol and its reinforcing aspects mediated by the endogenous opioid system. Additional support for these conclusions is provided by numerous evidences of fetal capacities for acquiring conditioned responses to stimuli presented in its environment and the implication of the opioid system in the establishment and expression of conditioned responses [28, 29, 64]. Also in agreement with those results are studies in which prenatal ethanol exposure resulted in increased mouthing in response to ethanol taste in rat fetuses and neonates [35, 36], an index that has been related to enhanced palatability of substances [68, 69]. In addition, the μ-opioid system has been found to be responsible for increasing mouthing and licking responses in rat fetuses and, in general, to regulate fetal oral appetitive responses [28, 29].

PRENATAL EXPERIENCE: PREFERENCE, AVERSION, PALATABILITY

There are studies providing data that call into question the interpretation of a preference for ethanol as a consequence of the prenatal experience with the drug. For example, in one study rat fetuses on GD 17-20 received paired presentations (through maternal intragastric administration) of ethanol and cineole, a substance that has been proved to reach the amniotic fluid and to be perceived by the near term fetus [70]. This paired condition was compared to another one in which fetuses were exposed to these same two substances but separated by a 4-hour interval, and also to a control group in which dams received only water. On postnatal day 16 all pups received repeated intraoral infusions of milk and once their mouthing responses were habituated they were tested in terms of their dishabituation when milk was presented contaminated with alcohol or cineole. It was observed that pups which have received prenatally cineole paired with ethanol responded to cineole with less mouthing than pups from the unpaired condition or than the water group. It was also reported that the prenatal experience did not affect the response to milk contaminated with ethanol [70]. These results were interpreted as an aversive response to cineole after its prenatal pairing with ethanol intoxication. An interpretation that was confirmed by the results of another study, with similar prenatal treatments, in which it was found that 15 day old pups prenatally exposed to cineole paired with ethanol consumed less cineole than pups receiving those same two substances, but 4 hours apart [37]. Although in that study pups from the paired condition did not consume significantly less cineole than the water control pups, these authors conclude that the difference in consumption between unpaired and paired groups is due to a conditioned aversion to cineole acquired by the pups which received cineole paired with ethanol intoxication *in utero*. Those same pups received after the intake test (cineole or water) an intragastric (i.g.) administration of either ethanol (1g/kg) or water in order to induce a conditioned aversion to cineole using ethanol as a US. The following day (PD 16) they were tested in a habituation-dishabituation test. As was the case of the previous described study, they were first habituated to milk and then presented with a milk-cineole mixture. The results showed that all pups receiving cineole and alcohol showed less mouthing to cineole than the remaining groups, however pups from the paired group mouth less than the unpaired group especially during the first testing trials. In summary, according to the results of these last described studies, low doses of alcohol may act as aversive US when administered prenatally or postnatally together with a olfactory CS [37, 70].

Nevertheless, in another recent study, it has been reported that neonate rats prenatally exposed to cineole paired with ethanol attached more time to surrogate nipples scented with cineole than controls, a response that could be considered evidence of a learned preference [71]. Yet, the authors of that study do not discard the hypothesis that rat pups could be attaching to the nipples in order to obtain calming effects and counteract anxiety promoted by the aversive memory generated prenatally. Although, in view of these results, they conclude that the affective valence of the experience occurring *in utero* remains unclear.

Another possible way of understanding this apparent contradiction in the pup's response to ethanol and/or in the unconditioned effects of prenatal ethanol exposure, is considering studies about drugs that are rewarding when tested in some paradigms but can generate a conditioned taste aversion (CTA) when associated with other flavors [72-74]. So, for instance, *in utero* ethanol could be generating a CTA when presented together with the taste of cineole, what will be reflected in reduced cineole consumption, but this may not affect the rat's auto-administration of the drug. Furthermore, in the case of ethanol, that has in addition to its pharmacological effects a particular taste and odor, its palatability can remain unchanged or even be enhanced after several exposure trials [75].

Increased intake of a flavored solution does not imply necessarily a preference for it [76, 77], on the other hand, the palatability of a substance can be increased without evident changes in a consumption test [65, 66]. Taking this into account as well as the apparent contradictory results of the above mentioned studies, in order to further investigate the hedonic nature of the prenatal

ethanol experience, it was analyzed whether the increased ethanol intake effect observed in pups prenatally exposed to the drug is accompanied by a change in palatability of the substance. This was measured using a taste reactivity test adapted for infant rats by Hall and Bryan [68] who found that rat pups could express differential behavioral responses - preference or aversion - to several tastes [78]. It was found that pups exposed to both ethanol doses (1 and 2 g/kg) *in utero* not only consumed more ethanol when tested on PD 15 but also displayed more appetitive responses (mouthing and paw licking) and less aversive behaviors (general motor activity and wall climbing) in reaction to the taste of ethanol than controls [38]. In that same study, pups were tested in their reaction to a sucrose-quinine compound, a mixture that is perceived by the rat as very similar to ethanol taste. If increased mouthing and paw-licks on one hand, and decreased general activity and wall climbing, on the other hand can be considered as behavioral appetitive manifestations for a substance intraorally infused [68, 74, 78], these last 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. This enhanced palatability effect has been also shown to be blocked or reduced when naloxone was administered to the pregnant dam together with ethanol [79]. In this last study, it was found that naloxone administered together with ethanol to the pregnant rat not only reduced ethanol intake in the infant offspring but also decreased the appetitive behaviors and increased the aversive reactions to the taste of ethanol observed in those pups whose mothers were administered only alcohol during gestation.

In sum, the administration of a low or moderate dose of ethanol to the pregnant rat during the last days of gestation clearly modifies the offspring's response to the flavor of ethanol. In most studies, this response seems to be a conditioned preference for ethanol, mediated by the opioid system, what results in an increased palatability of the drug and a high ethanol intake during the rat's infancy.

Several studies have demonstrated that the opioid system is implicated in learning processes modulating the acquisition of taste or odor preferences during early infancy [80]. Some researchers 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 and coincides with the emergence of walking [81]. One characteristic of this sensitive period is that pups tend to learn easily odor preferences [81-83]. Sullivan and collaborators have demonstrated that an odor paired with foot-shock on PD 7-8 generates a conditioned preference for that odor, while after the sensitive period, on PD 11-12, that same association generates a conditioned odor aversion [82-84]. It has been also reported that the administration of the opioid antagonist naltrexone disrupted the shock-induced odor preference in the younger pups but not the odor aversion in the older group [81]. 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 are readily acquired [26, 33, 37, 38, 43] and also that the opioid system is involved in prenatal learning processes [43, 64, 85]. Within this framework it results easy to explain the apparent paradox that has been raised in light of the results of recent studies: why doses of ethanol that promote conditioned aversions during postnatal stages result in clear preferences when administered during the gestational period? Indeed, conditioned aversions in adult rats have been observed using ethanol doses equivalent to those employed in all the above described studies, *i.e.* 1 and 2 g/kg, [53, 86, 87], but also with doses as low as 0.8 g/kg [86]. In preweanling rats, learned aversions were also reported after intoxication with ethanol doses ranging between 0.4 and 3 g/kg, but in all cases this was observed after PD 10 [49, 52, 88, 89]. Intriguingly, there are no studies showing that ethanol can act as an aversive stimulus for infant rats before PD 10. In studies in which the unconditioned effects of ethanol were analyzed in newborn rats, it was found that ethanol when administered i.p. in doses between 0.125 and 0.5 g/kg was reinforcing, as well as when using 0.75 g/kg dose, but the latter only in neonates prenatally exposed to ethanol [90, 91]. In a recent study, a preference for ethanol was found when rat pups were intoxicated with a relatively high ethanol dose (3 g/kg) before PD 9, while an aversion was observed when intoxication occurred after PD 9 [92]. So, the preference for ethanol observed in pups that were prenatally exposed to ethanol toxic and chemosensory aspects could be explained, if not completely at least in part, by those processes that have been described to occur during the sensitive period. In other words, in view of the unique role of the opioid system during this developmental period and that ethanol reinforcing properties are mediated by this neurochemical system [56], it seems probable that ethanol intoxication, particularly during this developmental stage, will be perceive by the fetus as a positive reinforcer. Additional support for this hypothesis is derived from the fact that, similarly to what has been observed in neonate rats, the prenatal administration of an opioid antagonist disrupts the acquisition of a preference for ethanol.

CONCLUSIONS

To sum up, animal studies about the effects of moderate or low doses of ethanol during the last gestational days indicate that, although no apparent teratological effects are evidenced with this treatment, the prenatal experience with ethanol may alter normal patterns of response to the drug. In this case, prenatal exposure to ethanol results in fetal learning about its sensory and toxic properties, which in turn is expressed during infancy, and even in adolescent periods as an increase in ethanol intake and a preference for its flavor. In general, the outcome of this animal research is congruent with data from human studies showing infantile recognition of and preference for substances previously experienced [14, 93, 94]. One potential implication of the data presented here involves the influence of early prenatal learning about ethanol on alcohol consump-

tion in humans. In our opinion, these results should be taken into account in studies in which the relation between prenatal exposure to the drug and later ethanol abuse problems is analyzed. Nevertheless, more research is necessary for a complete understanding of the consequences of ethanol exposure during gestation, as well as for the identification of the mechanisms by which this prenatal experience may lead to increase ethanol consumption.

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