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Acute sensitivity and acute tolerance to ethanol in preweanling rats with or without prenatal experience with the drug

Carlos Arias [a,*], Juan Carlos Molina [a,b], Estela C. Mlewski [b], Ricardo Marcos Pautassi [a], Norman Spear [a]

[a] Center for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000, USA
[b] Instituto de Investigación Médica M. y M. Ferreyra (INIMEC-CONICET), Córdoba, C.P 5000, Argentina

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Abstract

The present study examined behavioral sensitivity and acute tolerance to ethanol in infants with or without a moderate prenatal ethanol experience. During gestational days 17–20 dams received 0.0 or 2.0 g/kg ethanol. On postnatal day 13 pups were administered 0.0, 0.5 or 2.5 g/kg ethanol prior to assessment of locomotion. One third of the pups were evaluated at 5–10, 30–35 and 60–65 min after ethanol administration; another third was tested only during the last two post-administration periods; and the remaining third was tested only at 60–65 min. At 30–35 min blood ethanol levels were similar to those attained at 60–65 min. The main results of the study were: (a) The 2.5 g/kg ethanol dose induced biphasic motor effects: stimulation 5–10 min after drug administration and sedation after 30–35 or 60–65 min. (b) Infants exhibited acute tolerance to ethanol's sedative effects. (c) Although pups prenatally treated with ethanol exhibited heightened locomotor activity levels, acute sensitivity and tolerance were not affected by prenatal treatment. In summary, infants are sensitive to biphasic motor consequences of ethanol and readily exhibit acute tolerance to ethanol's sedative effects. In addition, moderate prenatal ethanol exposure was sufficient to induce hyperreactivity in the offspring without affecting habituation.

Keywords: Prenatal ethanol; Ethanol acute sensitivity; Ethanol acute tolerance; Locomotor activity; Habituation; Infant and rat

There is a considerable body of experimental evidence indicating that prenatal exposure to ethanol can critically modulate subsequent ethanol intake. This association between prenatal ethanol exposure and later affinity for the drug has been detected in various strains of rats and mice when using a variety of modes of ethanol exposure during pregnancy (Chotro et al., 2007). Heightened ethanol consumption resulting from gestational exposure to ethanol has been observed through tests conducted during different ontogenetic periods, including infancy, adolescence and adulthood (Chotro et al., 2007; Spear and Molina, 2005). Recent epidemiologic studies have found results analogous to those reported in this preclinical research. Even when controlling genetic and environmental factors known to modulate ethanol affinity, prenatal exposure to ethanol still significantly predicts later ethanol consumption and onset of ethanol-related disorders (Alati et al., 2006; Baer et al., 1998, 2003; Yates et al., 1998).

Although mechanisms underlying the association between fetal exposure to ethanol and subsequent predisposition to accept the drug remain a matter of debate, animal models have identified factors that can mediate this association. When rats are exposed to high ethanol doses throughout the last 2 weeks of the gestation, long-lasting effects upon the activity of the hypothalamic-pituitary-adrenal axis (HPA) are consistently observed (Taylor et al., 1982, 1981; Weinberg et al., 1996). This prenatal treatment induces heightened responsiveness to various stressors, a phenomenon that could eventually predispose the organism to use or abuse ethanol as a means of alleviating stress-related negative states (e.g. anxiety or depression). This neuroendocrinological alteration seems to be accompanied by changes in neurotransmitter systems that not only participate in stress regulation but also modulate ethanol's positive as well as negative (anti-anxiety) reinforcing properties (Bailey

* Corresponding author. Center for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000, USA.
E-mail address: afelicidade@yahoo.es (C. Arias).

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et al., 2001; Druse et al., 1990; Sari and Zhou, 2004). Furthermore, chronic exposure during gestation to large ethanol doses has also been observed to modify acute sensitivity to ethanol's effects. For example, this treatment enhances tolerance to hypothermic effects of relatively high ethanol doses (Abel et al., 1981; Anandam et al., 1980; Lancaster and Spiegel, 1989) and can sensitize the organism to the stimulant effects of ethanol (Becker et al., 1993; Rockman et al., 1989).

It has also been observed that prenatal exposure to moderate ethanol doses (1 or 2 g/kg, peak blood concentrations ranging between 40 and 120 mg/dl) during the last 4 days of gestation (gestational days 17–20; GDs 17–20) affects later ethanol intake patterns. This moderate prenatal ethanol treatment results in high ethanol intake during infancy (Arias and Chotro, 2005a,b, 2006; Chotro and Arias, 2003; Dominguez et al., 1998; Molina et al., 1995; Pueta et al., 2005) as well as during adolescence (Chotro and Arias, 2003). In these studies, processing of ethanol-related chemosensory information by late-term fetuses seems to predispose the organism to accept ethanol odor and taste (Chotro et al., 2007; Spear and Molina, 2005). Yet, the participation of other intervening factors in this effect cannot be completely rule out. With this ethanol exposure there is no evidence of morphological alterations (Dominguez et al., 1996) or deficits in associative learning capabilities assessed through Pavlovian conditioning procedures (Nizhnikov et al., 2006; Pueta et al., 2005) attributable to the teratogenic properties of ethanol. However, these animals show a tendency towards hyper-reactivity when they are confronted with novel stimuli (Chotro and Spear, 1997; Dominguez et al., 1996). Recent experimental evidence also suggests that moderate fetal exposure to ethanol during late gestation sensitizes the neonate to positive reinforcing effects of low ethanol doses (0.25–0.75 g/kg; Nizhnikov et al., 2006) and the fetus to sedative effects of higher doses (1–2 g/kg; Chotro and Spear, 1997). It is less clear that such hyper-reactivity and heightened behavioral sensitivity to ethanol persists into subsequent stages of development, including time points where heightened ethanol affinity has been observed to be a function of prenatal ethanol experience.

It is likely that behavioral sensitivity to a given drug will vary as a function of post-administration time. This variation can occur due to pharmacokinetic processes (absorption, distribution and elimination) as well as development of acute tolerance within the process of intoxication or intervening factors such as habituation to the testing environment. Acute tolerance refers to the development of resistance to a drug's physiological or behavioral effects within a single bout of intoxication. This particular tolerance is not explainable through metabolic adaptive mechanisms and has been detected during infancy and adolescence in the rat (Silveri and Spear, 2001; Spear and Varlinskaya, 2005). To our knowledge, the effect of low-to-moderate ethanol during late gestation on acute tolerance has not been investigated. Altered reactivity as well as the possibility that prenatal or perinatal ethanol exposure may impair attention and non-associative learning processes (Hunt and Phillips, 2004; Westergren et al., 1996), imply experimental challenges for analyzing corresponding effects on acute sensitivity and tolerance to ethanol. As will be explained in detail, the present study is based on an experimental strategy that permits examination of the relative weights of acute ethanol effects, learning processes such habituation, and possible interactions between these factors.

The present study examines whether behavioral sensitivity to ethanol doses known to exert biphasic (appetitive and aversive) motivational effects (Molina et al., 2007a) would be affected by prenatal exposure to ethanol. Prior to behavioral assessment, a pharmacokinetic study determined blood ethanol concentrations in pups that differed in their prenatal history with ethanol (Experiment 1). This pharmacokinetic study pursued two goals. The first, in accord with previous metabolic studies conducted with infant rats (Kelly et al., 1987), was to determine blood ethanol concentrations (BECs) at the post-administration times when the animals would be tested for spontaneous motor activity. The intention was to evaluate whether the behavioral effects of a given dose of ethanol vary despite persistence of similar levels of intoxication as operationalized through blood ethanol levels (i.e. acute tolerance). The second goal was to examine whether BECs accrued with different ethanol doses and at different post-administration times would differ as a function of prenatal ethanol treatment. This is necessary in view of recent studies reporting minimal but still significant changes in ethanol pharmacokinetics as a function of brief or chronic ethanol exposure during gestation (Bhalla et al., 2005; Nizhnikov et al., 2006).

Based on the pharmacokinetic profile obtained in Experiment 1, we conducted a second study focused on acute locomotor effects of ethanol (0.5 and 2.5 g/kg) at different post-administration times as a function of prenatal ethanol exposure. One set of animals was tested during the rising phase of blood ethanol concentrations (5–10 min), when achieving peak blood ethanol levels (30–35 min) and during a later phase of the intoxication (60–65 min, when these levels nevertheless remained at peak levels). A second group of animals was evaluated only during the last two post-administration times, while the remaining group was evaluated during only the last period. This design, which we will term "inverted ladder" (see Fig. 2 and Experiment 2: Methods), should allow determination of the weight of the acute effects of varying levels of intoxication (as a function of dose and post-administration time) while controlling for non-associative learning processes (e.g. sensitization or habituation) that might occur during test. In turn, these learning processes might be modulated not only by state of intoxication, but also by prenatal ethanol exposure to the drug.

1. Experiment 1

This experiment was conducted to determine infantile BECs resulting from the intragastric (i.g.) administration of 0.5 and 2.5 g/kg ethanol. Blood ethanol levels were determined at 7.5, 32.5 and 62.5 min post-administration time. According to prior studies it could be expected that after i.g. administration of 2.5 g/kg ethanol, infantile BECs at 7.5 min post-administration time will be well below peak blood ethanol levels (Kelly et al., 1987; Molina et al., 2007a; Pautassi et al., 2006). Peak blood ethanol levels in these circumstances are encountered between