Alcohol 48 (2014) 367–374

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

New evidence of ethanol's anxiolytic properties in the infant rat

Roberto Sebastián Miranda-Morales*, Michael E. Nizhnikov, Dustin H. Waters, Norman E. Spear

Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902-6000, USA

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form
16 December 2013
Accepted 28 January 2014

Keywords:
Anxiolysis
Ethanol motivational properties
Conditioned aversion
Light/dark test
Infant rat

ABSTRACT

Ethanol induces appetitive, aversive, and anxiolytic effects that are involved in the development of ethanol use and dependence. Because early ethanol exposure produces later increased responsiveness to ethanol, considerable effort has been devoted to analysis of ethanol's appetitive and aversive properties during early ontogeny. Yet, there is a relative scarcity of research related to the anxiolytic effects of ethanol during early infancy, perhaps explained by a lack of age-appropriate tests. The main aim of this study was to validate a model for the assessment of ethanol's anxiolytic effects in the infant rat (postnatal days 13–16). The potentially anxiolytic effects of ethanol tested included: i) amelioration of conditioned place aversion, ii) ethanol intake in the presence of an aversive conditioned stimulus, iii) the inhibitory behavioral effect in an anxiogenic environment, and iv) innate aversion to a brightly illuminated area in a modified light/dark paradigm. Ethanol doses employed across experiments were 0.0, 0.5, and 2.0 g/kg. Results indicated that a low ethanol dose (0.5 g/kg) was effective in attenuating expression of a conditioned aversion. Ethanol intake, however, was unaffected by simultaneous exposure to an aversive stimulus. An anxiogenic environment diminished ethanol-induced locomotor stimulation. Finally, animals given 0.5 g/kg ethanol and evaluated in a light/dark box showed increased time spent in the illuminated area and increased latency to escape from the brightly lit compartment than rats treated with a higher dose of ethanol or vehicle. These new results suggest that ethanol doses as low as 0.5 g/kg are effective in ameliorating an aversive and/or anxiogenic state in preweanling rats. These behavioral preparations can be used to assess ethanol's anxiolytic properties during early development.

Published by Elsevier Inc.

Introduction

A number of studies have shown that infant rats exhibit a particular sensitivity to ethanol's motivational effects (reviewed in Abate, Pueta, Spear, & Molina, 2008; Chotro, Arias, & Laviola, 2007; Pautassi, Nizhnikov, & Spear, 2009; Spear & Molina, 2005). Aside from their importance in helping our understanding of the ontogeny of response to ethanol, preweanling rats have proven valuable for assessing ethanol-related effects. These infants acquire ethanol-induced first- and second-order conditioning, readily express ethanol-mediated taste conditioning, are sensitive to ethanol-induced locomotor activation and ethanol-mediated operant responding, and consume relatively high amounts of the drug without initiation procedures (Pautassi et al., 2009). In addition, infant rats are sensitive to the biphasic motivational properties of ethanol. Conditioned preferences or aversions are established as a function of ethanol dose and post-administration time (Molina, Pautassi, Truxell, & Spear, 2007). Appetitive reinforcing effects are present after the administration of a low and even a relatively high dose of ethanol (0.5 or 2.0 g/kg, respectively) during the ascending limb of blood ethanol concentration (BEC). When BECs reach approximately 210 mg% (with 2 g/kg ethanol intoxication; post-administration time: 37.5 min), aversive rather than appetitive motivational properties of ethanol are prevalent.

Motivational properties of ethanol are not restricted to its appetitive or aversive effects. The drug also produces anti-anxiety effects similar to those found in anxiolytic drugs. For instance, Wilson, Burghardt, Ford, Wilkinson, and Primeaux (2004) compared the anxiolytic effects of the benzodiazepine agonist diazepam and ethanol in rats. This study found that both ethanol and diazepam (a GABA-A agonist) caused dose-dependent increases in time spent in the open arms of an elevated plus maze and also reduced burying behavior in the prod-burying task (Wilson et al., 2004). These potentially negative reinforcing effects of ethanol seem to play an important role in modulating patterns of ethanol use and abuse (Koob et al., 2004). Effects of ethanol on anxiety have been repeatedly reported in clinical literature (Kushner, Abrams, & Borchardt, 2000) as well as in animal literature (Wilson et al., 2004). Nevertheless, this claim rarely has received

* Corresponding author. Instituto de Investigación Médica M. y M. Ferreyra (INIMEC-CONICET-UNC), Córdoba, CP 5000, Argentina. Tel.: +54 0351 4681465x218; fax: +54 0351 4695163.
E-mail addresses: smiranda@immf.uncor.edu, mirandaseba@gmail.com (R. S. Miranda-Morales).

0741-8329/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.alcohol.2014.01.007

careful experimental attention in developing animals. To our knowledge, few experimental preparations have been designed to specifically test negative reinforcement from ethanol in infant rats. The adult literature dealing with this phenomenon is based on techniques not suitable for tests early in life.

A few studies nevertheless provide information that can usefully be applied to tests of negative reinforcement in preweanling rats. For instance, Molina, Serwatka, Enters, Spear, and Spear (1987) found, in infant rats, that although ethanol impaired acquisition or expression of conditioned aversion to a visual cue paired to footshock, ethanol had no effect on conditioned aversion to an olfactory stimulus similarly paired with footshock. In both experiments, rats exposed to ethanol were given similar CS–US pairings (CS: conditioned stimulus; US: unconditional stimulus) to induce conditioning. The authors suggest that alcohol may impair some aspects of learning but spare others, depending perhaps on the particular sensory modality to be conditioned (i.e., visual vs. olfactory). McKinzie, Lee, Bronfen, Spear, and Spear (1994) indicated that ethanol is also capable of impairing retrieval processes of aversive memories. It is notable that in these studies, animals were conditioned and/or tested 30 min after administration of ethanol, when peak ethanol blood levels are achieved and when it often is easier to find aversive rather than appetitive ethanol hedonic effects.

More recently, through a devaluation procedure, Pautassi, Sanders, Miller, Spear, and Molina (2006) tested ethanol's anxiolytic effects in preweanlings by measuring ethanol's devaluation of an aversive memory. In this study, after conditioning of an aversion to an odor (CS; lemon odor), infant rats were exposed to the US (citric acid), paired with administration of ethanol. Pairing the US with moderate to low ethanol doses ameliorated the otherwise strong aversion to the CS. This effort seemed to depend on the temporal course of the intoxication. A reduction in the magnitude of the aversive response was observed only if the US and the post-absorptive effects of ethanol were paired 5 min (but not 25 min) after ethanol administration (Pautassi et al., 2006). Similarly, Pautassi, Nizhnikov, Molina, Boehm, and Spear (2007) studied ethanol's effects on distress calls induced in the preweanling rat by intraoral infusion of citric acid. Upon comparison with the effects of midazolam (MDZ; a fast-acting GABA-A agonist known to have anxiolytic effects), they found similar calming effects of 0.5 g/kg ethanol and 0.09 mg/kg MDZ. Surprisingly, ethanol but not MDZ was capable of attenuating a conditioned aversion through the devaluation procedure, indicating that these effects of ethanol may not be GABA-mediated and instead, appetitive effects of ethanol could underlie this devaluation effect (Pautassi et al., 2007). These results imply value in seeking a more effective model for assessing anxiolytic effects in early ontogeny. The present experiments tested four apparently anxiolytic effects of low and moderate ethanol doses (0.5 and 2.0 g/kg). The first experiment tested ethanol's amelioration of a conditioned avoidance. The second experiment assessed ethanol intake in the presence of an aversive conditioned stimulus. The third experiment tested effects of ethanol on exploration of a novel anxiogenic environment, to determine the value of ethanol-induced locomotor stimulation as an index of ethanol anti-anxiety effects. The fourth experiment examined behavior in a modified light/dark box, using a test adapted from those used previously for adult rats.

General method

Subjects

Sprague–Dawley infant rats (postnatal days [PDs] 13–16) were used across all experiments (99 in Experiment 1; 112 in Experiment 2; 88 in Experiment 3; and 63 in Experiment 4). These animals were born and reared in the vivarium at the Center for Development and Behavioral Neuroscience (AAALAC-accredited facility, in Department of Psychology, Binghamton University, Binghamton, NY, USA). Births were examined daily, and day of birth was considered Postnatal Day 0 (PD 0). The colony was maintained at 22–24 °C under a 14 h/10 h light/dark cycle. The experiments were approved by the Binghamton University Institutional Review Committee for the Use of Animal Subjects and complied with the *NIH Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1996).

Drug preparation and administration procedures

For Experiments 1 and 2, the kappa receptor agonist U62,066E (Sigma–Aldrich; St. Louis, MO) was used as an aversive US. The drug was dissolved in saline (NaCl, 0.9% v/v) and administered at a dose of 0.0, 0.5, or 1.0 mg/kg. The injection volume was kept at 0.01 mL/g and saline was used as vehicle. U62,066E (hereafter referred as: U62) was administered via intraperitoneal (i.p.) injection. Injections took less than 5 s and were performed in the region situated between the diaphragm and the genitalia.

Across experiments, different concentrations of ethanol solutions were employed (190 proof ethanol, Pharmaco; Brookfield, CT; vehicle: distilled water) to establish doses of 0.0, 0.5, or 2.0 g/kg. The volume administered was equivalent to 0.015 mL/g of body weight of 0.0, 4.2, or 16.8% ethanol solution, respectively. Pups assigned to the control condition (0 g/kg) received only vehicle (water). Ethanol solutions were given by intragastric (i.g.) administration using a 10-cm length of polyethylene tubing (PE-10) attached to a 1 mL syringe with a 27 G × 1/2 inch 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. For Experiment 2, a 0.0 or 5.0% v/v ethanol solution was used for intake assessment.

Conditioned place aversion (CPA) test (Experiment 1)

Phase 1 (conditioning)

Pups were separated from their dams on PD 14, placed in pairs in a holding cage for 2 h, voided and weighed. The mean weight of all subjects was calculated and used as a benchmark for the volume of the i.p. injection of U62 (0.0, 0.5, or 1.0 mg/kg). Kappa activation is a stress-inducing event (Bruchas, Land, & Chavkin, 2010) and kappa agonists were found to be aversive (Anderson, Morales, Spear, & Varlinskaya, 2014; Pautassi, Nizhnikov, Acevedo, & Spear, 2012). For this reason, we decided to employ U62 as an unconditional aversive stimulus (US). For conditioning (pairing of rough texture with consequences of U62), 5 min after i.p. injection, animals were placed into a Plexiglas® container (9 × 15 cm) in which the bottom was lined with a piece of rough sandpaper (coarse: 50, Gatorgrit; Fairborn, OH) (conditioned stimulus: CS), where they remained for 15 min. Conditioning was conducted in a dim illumination environment (10 lux). Immediately after conditioning, pups were returned to the holding chambers. Two hours post-injection, pups were returned to their dams. On PD 15, pups received the same conditioning treatment as on PD 14.

Phase 2 (testing)

A 2-way tactile preference test was performed at PD 16. After a 2 h maternal separation, pups were intubated with 0.0, 0.5, or 2.0 g/kg of ethanol. This manipulation was intended to test ethanol-induced anxiolytic effects and amelioration of a conditioned aversion. Five minutes after intubation, pups were placed in a Plexiglas® box floored with sandpaper (the CS used during conditioning) on one side and a novel texture (a smooth cardboard-like floor; the