Pharmacology, Biochemistry and Behavior 97 (2011) 462–469

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats

Ricardo Marcos Pautassi [a,c,*], Michael E. Nizhnikov [b], Ma. Carolina Fabio [a,c], Norman E. Spear [b]

[a] Instituto de Investigación Médica M. y M. Ferreyra (INIMEC-CONICET), Córdoba, C.P 5000, Argentina
[b] Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902-6000, USA
[c] Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba, C.P 5000, Argentina

ARTICLE INFO

Article history:
Received 8 June 2010
Received in revised form 4 October 2010
Accepted 6 October 2010
Available online 14 October 2010

Keywords:
Ethanol
Appetitive conditioning
Preweanling rat
Acetaldehyde
Motor activation
D-penicillamine

ABSTRACT

Ethanol's motivational consequences have been related to the actions of acetaldehyde, a metabolic product of ethanol oxidation. The present study assessed the role of acetaldehyde in the motivational effects of ethanol on preweanling rats. In Experiment 1 pups (postnatal days 13–14, PD 13–14) were given systemic administration of D-penicillamine (DP, a drug that sequesters acetaldehyde: 0, 25, 50 or 75 mg/kg) before pairings of 1.0 g/kg ethanol and a rough surface (sandpaper, conditioned stimulus, CS). At test, pups given sandpaper–ethanol pairings exhibited greater preference for the CS than unpaired controls, but this preference was not expressed by pups given DP. Pre-training administration of 25 or 50 mg/kg DP completely blocked the expression of ethanol-mediated appetitive conditioning. D-penicillamine did not alter blood ethanol levels. Subsequent experiments revealed that ethanol-induced activation was blocked by central (intra-cisterna magna injections, volume: 1 μl, dose: 0 or 75 μg) but not systemic treatment with DP (0, 25, 50 or 75 mg/kg; ip). These results indicate that: (a) preweanling rats are sensitive to the reinforcing effect of ethanol, and (b) that this effect is associated with the motor activating effect of the drug. These effects seem to be mediated by the first metabolite of ethanol, acetaldehyde.

1. Introduction

Although controlled alcohol drinking is the norm in most people, for many casual drinking leads to an uncontrolled pattern of consumption (i.e., alcohol dependence). Among the several consequences of alcohol (chemically known as ethanol), its motivational effects are those primary involved in facilitating the transition from drug use to abuse and dependence (Koob and Le Moal, 2001). Exposure to ethanol early in life is yet another factor that facilitates this transition (Yates et al., 1998).

Ethanol exerts appetitive, aversive and negative reinforcing effects that can be captured, analyzed and pharmacologically dissected through the use of animal models (Pautassi et al., 2009), such as the conditioned place preference procedure (CPP). After a few pairings between an initial neutral surface (conditional stimulus, CS) and ethanol's effects (unconditional stimulus, US), animals exhibit preference for the ethanol-paired CS when tested in a preference test (Ciccocioppo et al., 1999). Another benchmark for ethanol's appetitive reinforcement involves assessing the locomotor activity evoked by the drug (Arias et al., 2008, 2009; Faria et al., 2008). Although still under discussion, the rationale is that ethanol's appetitive effects and ethanol-induced locomotor activity share a common neurobiological mechanism, the activation of an opioid-modulated, mesocorticolimbic dopaminergic system (Arias et al., 2009; Wise and Bozarth, 1987).

Ethanol-mediated CPP and ethanol-induced locomotor activity have proven useful in pinpointing several neurotransmitters (e.g., dopaminergic and opioidergic) and brain areas (e.g., ventral tegmental area, VTA) associated with ethanol's hedonics and have underscored the important role that procedural (route of administration; Nizhnikov et al., 2009) and environmental factors (stress; Matsuzawa et al., 2000) play in the expression of ethanol's appetitive effects. They have also provided data for a theoretical account suggesting that many of ethanol's motivational consequences can be attributed to the first metabolite of the drug, acetaldehyde (ACD). The initial response to the hypothesis of ethanol as a "pro-drug" was extremely controversial (Deitrich, 2004). The wealth of data accumulated since then, however, has been solid enough to suggest at least a mediational role for ACD in the effects of ethanol (Sheng Deng and Deitrich, 2008).

In animals, the intraperitoneal (ip) administration of ACD exerts biphasic effects when measured in terms of motor activity (i.e., motor activation and depression; Font et al., 2005) and motivational learning (conditioned stimulus preference and aversion; Aragon et al., 1986; Quertemont and De Witte, 2001; although its role in mediating taste aversion has been disputed, see Quertemont and De Witte, 2001). These biphasic and somehow contradictory findings have been accounted for

* Corresponding author. Instituto de Investigación Médica M. y M. Ferreyra (INIMEC-CONICET), Friuli 2434, Córdoba, C.P 5000, Argentina. Tel.: +54 351 4681465; fax: +54 351 4695163.
E-mail address: rpautassi@gmail.com (R.M. Pautassi).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.10.005

by ascribing different consequences to ACD as a function of its site of action. ACD is peripherally produced in the liver by alcohol dehydrogenase (ADH). Whereas these blood acetaldehyde levels are believed to be aversive, the central effects of the metabolite are apparently highly reinforcing (Quertemont and Didone, 2006). Centrally administered ACD supports conditioned place preference (Quertemont and De Witte, 2001; Quintanilla and Tampier, 2003) and evokes motor activation (Arizzi et al., 2003). Moreover, it has been shown that rats selectively bred for displaying high levels of ethanol intake (P rats) will actively work to self-administer ACD into the posterior VTA (Rodd-Henricks et al., 2002). The existence of central acetaldehyde was initially dismissed because this compound rarely crosses the hematoencefalic barrier and the presence of ADH in the brain is low (Quertemont and Tambour, 2004). More recent data, however, undoubtedly suggested that ACD is produced in the brain through the catalase/H_2O_2 system (Aragon et al., 1992). Consistent with the putative reinforcing role of centrally-produced ACD, manipulations of brain catalase activity lead to changes in ethanol's hedonics and intake. Acute lead acetate administration was associated with increased catalase activity and augmented ethanol-induced locomotion in mice (Correa et al., 2005). Conversely, the inhibition of catalase activity via administration of 3-amino-1,2,4-triazole resulted in an attenuation of ethanol-induced taste aversion, decreased ethanol intake (Aragon et al., 1985, 1992) and facilitated ethanol-induced place preference (Font et al., 2008). Moreover, ethanol-induced motor effects are altered by the administration of a catalase inhibitor into the hypothalamic arcuate nucleus, a brain region with a high density of catalase (Sanchis-Segura et al., 2005).

Another pharmacological tool for assessing ACD's involvement in ethanol's hedonics is D-penicillamine (DP), a drug that turns off the pharmacological activity of this metabolite. DP is a thiol compound that sequesters the ACD produced by the oxidation of ethanol without altering the circulating levels of ethanol (Font et al., 2005). Systemic administration of DP blocks ethanol-induced locomotion, ethanol intake and conditioned place preference — but not aversion — in mice (Font et al., 2005, 2006a,b). Few studies have assessed the role of ACD in mediating ethanol's appetitive effects in rats, most likely because adult rats rarely exhibit signs of conditioned preference to ethanol (Pautassi et al., 2009). A recent study, however, found CPP by ethanol (1.0 g/kg, IG) in adult rats and blocked this effect by administering DP or 4-methylpyrazole, a peripheral competitive inhibitor of ADH (Peana et al., 2008). A follow-up study (Enrico et al., 2009) found that either ACD or ethanol (1 g/kg IG) stimulated the activity of dopamine neurons in the nucleus accumbens. Intraperitoneal administration of DP prevented this stimulation, suggesting that DP alters ethanol's hedonics by inhibiting the stimulatory action of ACD on the mesolimbic dopamine transmission.

To our knowledge, there is very little information on the role of ACD in the motivational effects of ethanol during early ontogeny of the rat. The relevance of studying this phenomenon is multiple. Unlike their adult counterparts, preweanling rats are highly sensitive to ethanol's appetitive effects, readily exhibiting first and second-order appetitive tactile conditioning to ethanol (Molina et al., 2006, 2007; Nizhnikov et al., 2009; Pautassi et al., 2008a,b) as well as ethanol-induced motor activation (Arias et al., 2008, 2009, 2010). These effects are observed after a wide range of doses (0.5–2.0 g/kg) and can be blocked by dopamine and opioid antagonists (Nizhnikov et al., 2009; Arias et al., 2009). Interestingly, the preweanling's sensitivity to ethanol reinforcement and psychomotor activation coincides with high avidity for ethanol intake. When assessed through the consumption-off-the floor procedure (Sanders and Spear, 2007), non-initiated preweanling rats achieve blood alcohol levels comparable to those found in adult alcohol-preferring (P) rats (Truxell and Spear, 2004; Truxell et al., 2007). It seems that the use of a preweanling animal model provides a useful preparation for analyzing determinants of ethanol's reinforcement and affinity (Pautassi et al., 2009). A feature of the rat's developing brain provides further rationale for assessing the ACD role in ethanol reinforcement during infancy. The levels of brain catalase exhibit an inverse relationship with age (i.e., greater levels at younger ages; Mavelli et al., 1982; Maestro and McDonald, 1987, 1989), thus suggesting that central production of ACD is higher in preweanling than in adult rats.

The present study assessed the role of acetaldehyde in the motivational effects of ethanol in preweanling rats, as measured through conditioned tactile preference and ethanol-induced motor activation. In Experiment 1 pups were given D-penicillamine (0, 25, 50 or 75 mg/kg) before pairings of 1.0 g/kg ethanol and a rough surface (sandpaper). Pups were then tested for sandpaper preference in a two-way preference test. Experiments 2 and 3 tested ethanol-induced activation in a novel environment at PD13 following systemic or central (intra-cisterna magna injections) administration of DP. The possibility of DP altering blood ethanol levels was also analyzed.

2. Materials and methods

2.1. General procedures

2.1.1. Subjects

Two-hundred and sixty-nine Sprague–Dawley rat pups were employed. These animals were derived from 37 litters born and reared in an AAALAC-accredited facility (vivarium of the Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY, USA). Number of animals and litter representation in each experiment was as follows. Experiment 1, 96 animals (14 litters); Experiment 2, 107 animals (15 litters); Experiment 3, 66 animals (8 litters). Births were examined daily and the day of parturition was considered as postnatal day 0 (PD 0). Pups were housed with the dam in cages with free access to water and food. The colony was kept at 22–24 °C and a 12-h light–dark cycle was used. At the start of the experiments (PD 13) animals had a mean body weight of 31.7 ± 0.5 g. The experimental protocol was approved by the Binghamton University Institutional Review Committee for the use of Animal Subjects and all procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Research Council, 1985).

2.1.2. Tactile conditioning and test procedures (Experiment 1, PDs 13–15)

Conditioning procedures closely followed those employed by Nizhnikov et al. (2009). Briefly, Experiment 1 consisted of two phases: conditioning (two daily conditioning trials, PDs 13–14) and a test session (PD 15).

Conditioning sessions: Pups were withdrawn from their mother for 60 min. Then, paired (P) pups were weighed and administered D-penicillamine (DP, 0, 25, 50 or 75 mg/kg, ip), followed 15 min later by an ethanol intubation (1.0 g/kg, IG). The pups were exposed to a tactile conditioned stimulus (CS, sandpaper; coarse: 50, Gatorgrit, USA) during ethanol post-administration time 20–30 min. Unpaired controls (UP) were given DP treatment and exposed to the rough CS at the same time paired animals did. UP animals, however, were not given ethanol until 90 min after the termination of CS exposure. To equate the level of maternal deprivation across groups, all animals were returned to the mother 120 min after delivery of ethanol in UP pups.

D-penicillamine doses were selected on the basis of studies showing their effectiveness in blocking the reinforcing effects of ethanol in adult mice (Font et al., 2006a) and adult Wistar rats (Peana et al., 2008). Ethanol dose, route and interval of conditioning were chosen on the basis of our previous study (Nizhnikov et al., 2009).

Test session: The test took place in a Plexiglas rectangular chamber ($28 \times 13 \times 15.5$ cm), illuminated by an overhead 40-W red bulb. Half of the floor of the chamber was covered with sandpaper, and the other half was covered with a smooth cardboard surface. Time spent over each section of the apparatus was recorded in a minute-by-minute basis by experimenters unaware of the treatment of each animal. The