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Alcohol 43 (2009) 347–358

ALCOHOL

Opioid antagonists block the acquisition of ethanol-mediated conditioned tactile preference in infant rats

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Received 18 December 2008; accepted 20 June 2009

Abstract

It has been difficult to find conditioned preference for tactile cues paired with ethanol intoxication in rats. Toward understanding the ontogeny of ethanol reinforcement, we aimed at establishing a simple and reliable procedure for (1) assessing primary appetitive conditioning to ethanol in infant rats and (2) discerning the role the opioid system plays in ethanol-mediated conditioning at this age. Experiment 1 determined the parameters (i.e., dose, interval of conditioning) for assessing ethanol-mediated conditioning. Pups were then trained with differential Pavlovian conditioning (Experiments 2 and 3) in which ethanol intoxication (1.0–2.0 g/kg, intragastrically or intraperitoneally delivered) was paired with a tactile stimulus (sandpaper) while an alternative texture signaled the absence of ethanol's effects. Unpaired control conditions were also used. Tactile preferences were assessed after two conditioning sessions. Paired rats spent significantly more time on sandpaper than unpaired controls, an effect that was greater after intragastric administration of 1.0 than 2.0 g/kg ethanol. This effect was replicated in Experiments 4a and 4c and found to be inhibited by pretreatment with general (naloxone [NAL]) or specific (D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 [CTOP] and naltrindole) opioid antagonists. Blood ethanol levels at conditioning were not altered by NAL (Experiment 4b). The study outlines a procedure that reveals appetitive conditioning to ethanol by infant rats. The results are discussed in terms of a potential ethanol-induced activation of the endogenous opioid system during the onset of the intoxication process. © 2009 Published by Elsevier Inc.

Keywords: Ethanol; Appetitive conditioning; Infant rat; Opioid system; Naloxone; Naltrindole

Introduction

Early exposure to ethanol increases the likelihood of later alcohol abuse and dependence, as found in both human (Alati et al., 2006; Baer et al., 2003) and animal studies (Chotro et al., 2007). It has been suggested (Pautassi et al., 2009; Spear and Molina, 2005) that ethanol-mediated motivational learning may underlie this association. The young organism would learn that the taste and flavor of ethanol—or any other stimuli paired with its administration—predicts the appetitive, positive reinforcing properties of the drug. Later re-exposure to these stimuli would increase the probability of ethanol seeking and self-administration. Hence, it is important to analyze how infant rats learn about ethanol's motivational properties and the neurobiology underlying this phenomenon (for a review on the relevance of ontogenetic analysis for understanding ethanol-related problems, see Ref. Pautassi et al., 2009).

It has been difficult to detect first-order, ethanol-mediated appetitive conditioning in the heterogeneous, nonselected adult or infant rat. The majority of studies have found avoidance of tactile or taste cues previously paired with ethanol's effects (Cunningham et al., 1993; Hunt et al., 1991; Molina et al., 1996; Pautassi et al., 2002; Schechter and Krimmer, 1992). To further explore ethanol's appetitive motivational properties, and the mechanisms modulating them, it seems important to develop rat models of primary positive ethanol reinforcement during early ontogeny.

High doses of ethanol usually support conditioned taste aversion in infant rats (Pautassi et al., 2002, 2005). Recent reports, however, also indicate that experience with ethanol doses of 2.0 g/kg or higher can result in appetitive learning in infant and adolescent rats (Molina et al., 2006, 2007; Pautassi et al., 2008) when assessed in terms of second-order conditioning (Molina et al., 2006, 2007) or revaluation procedures (Pautassi et al., 2006, 2007). Furthermore, infant rats administered 2.5 g/kg ethanol exhibit drug-induced motor activation, which is often regarded as an

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0741-8329/09/$ – see front matter © 2009 Published by Elsevier Inc.
doi: 10.1016/j.alcohol.2009.06.001

index of the appetitive reinforcing effects of drugs of abuse (Arias et al., 2008). Hence, it is also conceivable that higher ethanol doses can produce appetitive reinforcement. It is important to point out that none of these examples (i.e., Molina et al., 2006, 2007; Pautassi et al., 2006, 2007) used first-order conditioning procedures. First-order appetitive conditioning to ethanol's pharmacologic effects has been found in a very limited set of studies and usually has required additional experimental manipulations, such as pre-exposure to the pharmacologic properties of ethanol (20 days or more: Bienkowski et al., 1996; Reid et al., 1985; intraoral ethanol infusions: Pautassi et al., 2008) or a concurrent stress (Matsuzawa et al., 1998, 1999). One explanation for the disparity between the expression of ethanol-mediated learning in primary conditioning (expressed as aversions, Asin et al., 1985; Molina et al., 1996; Pautassi et al., 2002) and other conditioning methods (second-order conditioning or devaluation, Molina et al, 2006, 2007; Pautassi et al., 2006, 2007) may be that in methods of primary conditioning in rats the conditioned stimulus (CS) has been introduced during a late phase of ethanol intoxication (Molina et al., 1996; Pautassi et al. 2002). In contrast, the second-order conditioning and devaluation methods have included pairing of the early effects of ethanol with their respective CS or unconditioned stimulus (US). Hence, it could be postulated that the early and late phases of the blood ethanol curve may be associated, respectively, with appetitive and aversive effects of ethanol (Conrod et al., 1998, 2001).

The mechanisms responsible for the appetitive effects of ethanol are still not fully understood. It is clear, however, that several families of opioid receptors (including μ, κ, and δ) have been implicated in ethanol intake and reinforcement. In adult rodents, voluntary intake of ethanol is reduced by general opioid antagonists (e.g., naloxone [NAL] and naltrexone; Bienkowski et al., 1999; Davidson and Amit, 1997; Froehlich et al., 1991) and by selective kappa-receptor agonists and delta- and mu-receptor antagonists (Hyytia and Kiianmaa, 2001; Krishnan-Sarin et al., 1995; Lindholm et al., 2001). Ethanol-mediated conditioned reinforcement also involves the opioid system. NAL in conjunction with moderate (1.5 g/kg) or high (3.0 g/kg) doses of ethanol has been shown to increase conditioned taste aversion (Broadbent et al., 1996). Injections of a general opioid antagonist before testing blocks conditioned place preference (CPP) and facilitate extinction of both CPP and operant responding for ethanol in rodents (Bechtholt and Cunningham, 2005; Bienkowski et al., 1999; Cunningham et al., 1995, Kuzmin et al., 2008).

Yet, in adult rats, NAL seems to have little or no effect on conditioned place aversion (CPA) produced by ethanol. Borman and Cunningham (1997) found that NAL (0, 1.5, or 10 mg/kg) did not alter the expression of CPA induced by ethanol (1.8 g/kg, intraperitoneally [i.p.]). When given during acquisition, NAL seemed to directly support CPA and to enhance ethanol-mediated CPA. These results, however, do not discount the possibility that, in the rat, the opioid system may be involved in ethanol's appetitive reinforcing properties. The learning procedure used by Borman and Cunningham (1997) tested only aversive conditioning. A model of first-order appetitive reinforcement to ethanol would help the investigation of the control of the opioid system over the positive reinforcing properties of ethanol. For infants, the data on opioid involvement in ethanol's motivational properties are not as abundant as for adults. Nizhnikov et al. (2006a, b) have demonstrated that, when testing neonatal rats with an age-specific conditioning procedure (i.e., "surrogate nipple technique," Petrov et al., 2003) kappa- and mu-opioid receptor antagonists disrupt appetitive conditioning to ethanol administered i.p., intracisternally, or orally. It has also been shown that although infant rats (postnatal days [PDs] 7–8) that were exposed to ethanol exhibited increased acceptance of ethanol, this effect is blocked by co-administration of NAL with ethanol during the early exposure (Chotro et al., 2007). NAL treatment also blocks the heightened palatability to ethanol that follows prenatal exposure to the drug (Arias and Chotro, 2005) as well as the motor-activating effects induced by high doses of ethanol (2.5 g/kg; Arias et al., 2009).

The present study aimed at establishing a simple, yet reliable procedure for assessing (1) primary appetitive conditioning to ethanol in infant rats and (2) the possible involvement of the opioid system in ethanol's appetitive motivational effects. A first experiment determined parameters for assessing ethanol-mediated appetitive conditioning. After establishing ethanol-induced conditioned preference, subsequent experiments replicating this conditioning tested variables likely to affect its expression, including manipulations of the opioid system.

In detail, Experiment 1 used specific combinations of ethanol dose, postadministration time (PAT), and route of drug administration to identify a time course of blood ethanol levels (BELs) that would allow testing for ethanol-mediated motivational learning. Ethanol content in blood was tested at several time points in pups given 1 or 2 g/kg ethanol, delivered either i.p. or intragastrically (i.g.). Experiment 2 analyzed the expression of ethanol-mediated first-order tactile conditioning in infant rats. Animals were trained in a conditioning procedure in which a tactile stimulus was paired with a specific phase of ethanol intoxication (derived from the results of Experiment 1), whereas an alternative stimulus (CS−) signaled the absence of ethanol's effects. Experiment 3 tested whether exposure to the CS− at training is a necessary element for acquisition of ethanol-mediated motivational learning. Finally, the role of the endogenous opioid system on the acquisition of ethanol-mediated motivational learning was assessed (Experiment 4), by training pups in tactile conditioning after administration of ethanol alone or in conjunction with general (NAL, Experiment 4a) or specific opioid antagonists (D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 [CTOP] or naltrindole [NALT], Experiment