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Research report

Naloxone blocks ethanol-mediated appetitive conditioning and locomotor activation in adolescent rats

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ABSTRACT

Age-related differences in ethanol sensitivity could put adolescents at risk for developing alcohol-related problems. Little information exists, however, about adolescent sensitivity to ethanol's appetitive effects and the neurobiological mechanisms underlying ethanol reinforcement during this developmental stage. The present study assessed the role of the opioid system in adolescent rats in an appetitive second-order schedule of ethanol reinforcement and ethanol-induced locomotor stimulation. On postnatal day 32 (PD32), animals were pretreated with the general opioid antagonist naloxone (0.0, 0.75, 1.50, or 2.5 mg/kg) and then given pairings of ethanol (0.0 or 2.0 g/kg, intragastrically) with intraoral pulses of water (conditioned stimulus 1 [CS_1], first-order conditioning phase). CS_1 delivery occurred 30–45 min after ethanol administration when the effect of ethanol was assumed to be appetitive. On PD33, adolescents were exposed to CS_1 (second-order conditioning phase) while in a chamber featuring distinctive exteroceptive cues (CS_2). Preference for CS_2 was then tested. Adolescents given CS_1–ethanol pairings exhibited greater preference for CS_2 than controls, indicating ethanol-mediated reinforcement, but only when not pretreated with naloxone. Blood alcohol levels during conditioning were not altered by naloxone. Experiment 2 revealed that ethanol-induced locomotor activation soon after administration, and naloxone dose-dependently suppressed this stimulating effect. The present study indicates that adolescent rats are sensitive to ethanol's reinforcing and locomotor-stimulating effects. Both effects of ethanol appear to be mediated by endogenous opioid system activation.

1. Introduction

Until recently, research on underage drinking has been largely neglected [1], partially attributable to the belief that adolescent ethanol intake is a transient phenomenon that lessens as the subject engages in family and work-related duties [1]. Epidemiological and preclinical studies, however, show that the effects of adolescent drinking can be long lasting. Adolescent ethanol consumption is highly prevalent (with alcohol initiation in approximately 60% of teens aged 15–16 years in the United States [2]), and the risk of developing alcohol abuse and dependence is significantly higher in those who begin drinking before the age of 21 [3,4].

The use of animal models has shed some light on the determinants of ethanol intake in adolescents. When compared with adult counterparts, adolescent rats are less sensitive to several effects of ethanol that presumably serve as deterrents to escalated ethanol use [5]. These effects include ethanol-induced locomotor impairment [6], sedation [7], and ethanol-induced hangover [8]. Adolescents are also more resistant than adults to the suppressive effects of high-dose ethanol on social behaviors [9]. However, adolescents are more sensitive than adults to the cognitive impairments associated with ethanol intoxication [10] and show increased social facilitation after low-dose ethanol [9].

A few studies have suggested that adolescents may show a distinctive response pattern to ethanol's appetitive and aversive motivational effects compared with older subjects. Ethanol-mediated conditioned place preference (CPP) is readily found in mice [11] and in genetically selected rats [12,13], but is rare in heterogeneous, non-selected adult rats [14]. The expression of CPP by ethanol in heterogeneous adult rats has been reported only after very extensive training and pre-exposure procedures (15 days or more [15–17]), concurrent presentation of other reinforcers [18] or exposure to electric shock [19]. In contrast, Philpot et al. [20] observed ethanol-mediated CPP in rats after only four training trials conducted shortly after weaning on postnatal day 25 (PD25; 0.2 g/kg) and also during late adolescence on PD45 (0.5 and

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1.0 g/kg). Adolescent and adult rats also appear to differ in their sensitivity to ethanol's aversive effects. Adult, but not adolescent, rats expressed conditioned taste aversion (CTA) after pairings of ethanol (1.0 and 1.5 g/kg) and a sapid stimulus [21]. Adolescents expressed CTA, albeit at a higher dose. These studies suggest that adolescents may be relatively insensitive to ethanol's aversive effects and more likely than adults to acquire ethanol-mediated appetitive learning.

Another approach for assessing ethanol's hedonic effects involves a second-order conditioning CPP procedure [22–24] that circumvents the traditional difficulty in establishing first-order CPP. In the second-order conditioning preparation, rats are given pairings of ethanol and a conditioned taste stimulus (CS_1) infused intraorally (e.g., very small amounts of water or sucrose). In a second phase, the intraorally infused CS_1 is paired with an environment (CS_2) featuring distinctive cues (e.g., sandpaper flooring and striped wallpaper). Ethanol-mediated conditioning is indexed by measuring preference or aversion for the CS_2 environment. Fourteen-day-old rats readily exhibited appetitive second-order conditioning when the intraoral CS_1 was paired shortly (5–20 min post-administration) after intubation with 0.5 or 2.0 g/kg ethanol or during a late phase (30–45 min post-administration) of the intoxication induced by 0.5 g/kg ethanol [22,23]. Second-order aversion, however, emerged when the CS_1 predicted the late effects induced by 2.0 g/kg ethanol [22,23]. A subsequent study [24] found age-related differences in the expression of ethanol-mediated second-order conditioning. Adolescent, but not adult, rats expressed second-order appetitive conditioning (0.5–2.0 g/kg). This outcome was unchanged if the CS_1 predicted the initial or late stage of the ethanol post-administration interval. At higher doses (3.0–3.25 g/kg), ethanol induced conditioned aversion, which was similar in adolescents and adults [25]. One caveat of these studies [24,25] was that the CS_1 paired with ethanol (sucrose, 10%, v/v) had motivational properties of its own that may have interacted with ethanol's effects in the establishment of conditioning.

Ethanol-induced psychomotor stimulation represents yet another, albeit indirect, measure of ethanol's motivational effects. Humans at risk for developing alcohol problems show greater ethanol-induced tachycardia than individuals not at risk for the disorder [26]. Interestingly, adolescent, but not adult, rats self-administer sufficient oral ethanol to produce tachycardia [27]. Heterogeneous adult rats are also insensitive to the locomotor-activating effects of ethanol and, unlike mice [28], typically show locomotor depression after ethanol administration [29]. However, acute ethanol administration induces locomotor activation in heterogeneous infant rats [30–33] and adult rats selectively bred to ingest large amounts of ethanol [34]. In a recent study, we observed ethanol-induced locomotor activation in adolescent rats (PD 28) after intubation with 2.5 g/kg, but not 0.5 g/kg, ethanol [35]. Moreover, females that had exhibited heightened sensitivity to ethanol's psychomotor effects on PD28 ingested significantly larger quantities of ethanol than counterparts less sensitive to ethanol-induced locomotor activation [35].

The adolescents' idiosyncratic variation in reactivity to ethanol may put them at risk for alcohol-related problems [5]. More work is needed, however, to test the sensitivity of adolescents to ethanol's hedonic effects and the neurobiological mechanisms underlying ethanol reinforcement during this stage. The opioid neurotransmitter system is known to be involved in ethanol reinforcement and intake. Administration of opioid receptor antagonists inhibits CPP induced by ethanol in adult mice [36] and infant rats [37] and disrupts appetitive conditioning induced by ethanol in neonatal rats [38]. The psychomotor stimulant effects of ethanol also appear to be opioid-dependent. General opioid antagonism inhibits tachycardia produced by alcohol ingestion in healthy subjects [39] and dose-dependently reduces ethanol-induced locomotor activation in adult mice [28] and infant rats [32]. The involvement of the endogenous opioid system in mediating ethanol's motivational effects in adolescents has yet to be explored.

The present work assessed: (i) the sensitivity of adolescent, heterogeneous rats to ethanol's reinforcing and psychomotor stimulating effects and (ii) the participation of the opioid system in these effects of ethanol. Appetitive conditioning with ethanol was tested in Experiment 1 with a second-order conditioning procedure after administration of the general opioid antagonist naloxone. In addition to the important issue of testing the role of the endogenous opioid system, water instead of sucrose was used as the CS_1 paired with ethanol's effects, which was different from our previous second-order conditioning study [24]. This procedural refinement removes potential confounding factors associated with the use of non-neutral sapid CSs and establishes the generality of the effect. Experiment 2 tested ethanol-induced locomotor activation after various doses of naloxone. In adult rats [40], naloxone can alter ethanol's pharmacokinetics. Therefore, we also measured blood alcohol levels (BALs) in adolescents following naloxone or vehicle injection.

2. Materials and methods

2.1. General procedures

2.1.1. Subjects

A total of 175 adolescent Wistar rats (89 males and 86 females) were used. These animals were 28–30 days old at the start of the experimental procedures, had a mean body weight of 123.04 ± 1.7 g (females, 113.49 ± 1.62 g; males, 131.94 ± 2.18 g), and were born and reared in the vivarium of the Center for Development and Behavioral Neuroscience at Binghamton University. The number of animals and litter representation across experiments were the following: Experiment 1 (44 males and 41 females, representing 18 litters), Experiment 2 (45 males and 45 females, representing 11 litters). Different, naïve animals were used in each Experiment. Room temperature (22–24 °C) and lighting conditions (12 h/12 h light/dark cycle, with lights on at 8:00 a.m.) in the vivarium were automatically controlled. Births were checked on a daily basis, and the day of parturition was considered PD0. Litters were culled to 10 animals (five males and five females, whenever possible) on PD1, and the subjects were housed in standard maternity cages with *ad libitum* access to water and food. On PD21 (weaning), the animals were transferred in same-sex groups of five subjects to clean tubs lined with pine shavings. The experimental procedures complied with the Guide for the Care and Use of Laboratory Animals [41] and were approved by the Institutional Animal Care and Use Committee within a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

2.1.2. Surgery and cannulation procedures

In Experiment 1, the delivery of the water CS_1 during conditioning and testing was done through intraoral cannulae made of polyethylene tubing. Specifically, the free end of the cannula was slipped inside another polyethylene tube, which in turn was connected to a Gilmont syringe (Barnant Co., Barrington, IL) mounted in a rotary microsyringe infusion pump (Kashinsky, Binghamton, NY). Cannula implantation surgery was performed on PD30 under an air-sealed hood and lasted about 8 min per animal. The surgery began by individually transferring the animal to an induction chamber where it was anesthetized with 2.5% isoflurane vapor via oxygen carrier gas (55 psi). The effectiveness of the anesthesia was ensured by assessing muscle tone and pupil reflexes. The back of the neck and right cheek were then shaved, and Betaiodine antiseptic and ethanol were applied to the bare skin. The animals were then transferred to a surgery table and kept warm (32–34 °C) through the use of a heating pad. To maintain anesthesia, isoflurane was provided through a cone positioned next to the nose of the animal. A slit was then made in the cheek using a 14-gauge disposable needle (Harvard Instruments, Columbus, OH). A small section of polyethylene-10 tubing (10 cm; Clay-Adams, Parsippany, NJ) was run through the needle, which was subsequently removed. A 0.5 cm flange was then made on one end of the tubing. The tubing was gently pulled through the medial internal surface of the cheek. Consequently, the flanged end of the cannula was positioned over the oral mucosae while the remaining tubing exited from the mouth. The surgery continued with the insertion of another needle in the back of the neck. This second needle was guided under the skin to exit approximately 1 cm away from the site of the tube. The tube was then run through the needle until it appeared on the top of the neck. The second needle was then retracted, and the tubing was fastened with a fast-acting adhesive (Vetbond, 3 M, St. Paul, MN). The animal was then placed in an individual holding cage with free access to food and water. After surgery, the animal was treated with a topical antibiotic (Neosporin, Johnson & Johnson, New Brunswick, NJ).