Pharmacology, Biochemistry and Behavior 101 (2012) 417–426

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Amphetamine modifies ethanol intake of psychosocially stressed male rats

Larissa A. Pohorecky *, April Sweeny

Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08854, USA

ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form 10 January 2012
Accepted 14 January 2012
Available online 20 January 2012

Keywords:
Rank status
Ethanol preference
Saccharin intake
Anhedonia
Dominance
Social isolation

ABSTRACT

Studies of socially housed rodents have provided significant information regarding the consequences of exposure to stressors. Psychosocial stressors are known to alter the ingestion of ethanol and the activity of the dopaminergic neuronal system. Since both stressors and ethanol are known to affect the function of dopaminergic neurons, we employed amphetamine to assess the role of this neural system on the ingestion of ethanol by psychosocially stressed male rats. Male rats housed two per cage were designated as dominant or subdominant rats based on evaluations of agonistic behavior and body weight changes. The dyad-housed rats and a group of single-housed rats were sequentially assessed for ethanol intake after injections of saline or amphetamine (0.3, 0.9 or 2.7 mg/kg i.p.) both prior to dyad housing and subsequently again during dyad-housing. Prior to dyad housing ethanol intake of future subdominant rats was higher than that of future dominant rats. Dyad-housing significantly increased ethanol intake of dominant rats. Pre-dyad the highest dose of amphetamine potently depressed ethanol ingestion. Sensitivity to amphetamine's depressant effect on ethanol intake was higher at the dyad test in all subjects, most prominently in single-housed rats. In contrast to the single-housed rats, the dyad-housed rats displayed saccharin anhedonia. It can be concluded that dopaminergic system modulates, at least partially, the psychosocial stress-induced changes in ethanol intake. Furthermore, the level of ethanol ingestion at the pre-dyad test was predictive of future hierarchical status.

1. Introduction

The stress response involves the coordinated physiological and behavioral response of an organism to a stressful challenge. While the stressful challenge (stressor) can be environmental or physiological, the adaptive coping mechanisms are both rapid in the case of acute stressors, and long term in the case of chronic stressors. Experimental evidence indicates that rodents display two general types of coping strategies when faced with a psychosocial stressor. These two extensively investigated strategies have been designated as "active" coping and "passive" coping (e.g., Benus et al., 1991; Koolhaas et al., 1999). Because of the distinct characteristics of "active" coping, generally associated with dominant (Dom) status, and of "passive" coping style, associated with non-Dom status (Benus et al., 1991; Koolhaas et al., 1999), it is likely that sensitivity to drugs may also differ with a subject's rank status.

The influence of stress, on the ingestion of ethanol (EtOH), by humans and experimental animals, is now well recognized and is documented by several reviews (Björkqvist, 2001; José et al., 2000; Pohorecky, 1990; Pohorecky, 1991; Uhart and Wand, 2009). The specific effects of psychosocial stress on the ingestion of EtOH have been evaluated employing a number of animal models. Studies on the effect of social isolation (single-housing, SiH) on the ingestion EtOH have provided inconsistent findings. While some stress researchers reported that isolation-stressed rodents ingested more EtOH compared to group-housed rodents (Ehlers et al., 2007; Juárez and Vázquez-Cortés, 2003; Parker and Radow, 1974; Roske et al., 1994), others reported a decline (Adams and Oldham, 1996) or no effect on EtOH intake (Doremus et al., 2005; Thorsell et al., 2005). Stress researchers have also employed the resident–intruder model to evaluate the stressfulness of defeat on the ingestion of EtOH. These investigators reported a decline in EtOH intake in defeated rats (Funk et al., 2005; van Erp and Miczek, 2001) but not in mice (Croft et al., 2005; Keeney and Hogg, 1999). One of the most successful, and ethologically relevant, approaches to study the consequences of chronic social stress is a model based on hierarchical stress. Such studies reported that EtOH intake was enhanced in group-housed *non-Dom* rats compared to the Dom rats (Blanchard et al., 1987; Blanchard et al., 1993; Ellison, 1987; Pohorecky, 2008; Pohorecky, 2010; Weisinger et al., 1989; Wolffgramm and Heyne, 1991). The triad-housing model developed in our laboratory allows for the rapid development of a stable and robust social hierarchy among co-housed male rats (Blakley and Pohorecky, 2006; Pohorecky et al., 1999; Pohorecky et al., 2004). This model involves the co-habitation of two or more male animals with continuous visual and olfactory contact. Employing this model we have reported that the change in intake of EtOH produced by distinct acute novel stressors depended on the rank/housing status of the subjects (Pohorecky, 2008). For

* Corresponding author at: Center of Alcohol Studies, Rutgers University, 607 Allison Road, Piscataway, NJ 08854-1100, USA. Tel.: +1 732 445 3593; fax: +1 732 445 3500.
E-mail address: larissa@rci.rutgers.edu (L.A. Pohorecky).

0091-3057/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2012.01.010

example, enhancing anxiety levels by exposing rats to an elevated plus maze had a negative impact on the EtOH ingestion of triad-housed rats, but had no effect on EtOH intake of single-housed rats (Pohorecky, 2008). Additionally, studies with group-living non-human primates indicate that lower ranking animals ingested more EtOH compared to high-ranking animals (Crowley et al., 1990; Fahlke et al., 2002; Higley et al., 1996).

A significant body of evidence documents the effects of EtOH on the dopaminergic (DArgic) system in the brain. Acutely, EtOH has been shown to increase tonic dopamine (DA) release, while its effect on phasic release was less reliable (Robinson et al., 2009), and the firing rate of DA neurons in high EtOH preferring rats was higher compared to their non-EtOH preferring counterparts (Melis et al., 2009; Morzorati et al., 2010). Ingestion of EtOH also elevated the activity of DArgic neurons (Appel et al., 2003; Brodie et al., 1999; Morzorati et al., 2010), and DA levels in the nucleus accumbens (Di Chiara and Imperato, 1988). The DAgic system is believed to mediate, at least partially, both EtOH self-administration and the rewarding aspects of EtOH ingestion (Koob and Weiss, 1992; Melis et al., 2009).

Stressors also produce alterations in DArgic function (reviewed by Marinelli et al., 2006), and deficits in stress coping may reflect deficits in this system (Kapur and Mann, 1992). Significant evidence has also accrued on the effects of psychosocial stress on brain DA neurons. For instance, in monkeys the effect social stress on the DArgic systems was dependent on social rank. Morgan et al. (2002) have noted that cocaine functioned as a reinforcer for subordinate but not the dominant (Dom) monkeys, suggesting the differences in the DArgic systems to be rank dependent. In rodents defeat was associated with DArgic hyperactivity and with increased phasic DAgic signaling of the mesolimbic pathway (Anstrom et al., 2009). Resident rats have been reported to have higher extraneuronal levels of DA compared to the intruder rats after a resident intruder test (Ferrari et al., 2003), while levels of DA D2 receptor binding were higher in Dom than in subordinate cynomolgus macaques (Morgan et al., 2002), and the binding capacity of DA transporters was found to be lower in repeatedly defeated tree shrews (Isovich et al., 2000).

Previous evidence indicated that the depressant effect of EtOH on behavior was dependent on the subject's rank status. For example, triad-housed subdominant rats were more sensitive than Dom rats the behavioral depressant effects of EtOH (Blakley and Pohorecky, 2006). The present studies address the hypothesis that rank status may also affect sensitivity to other drugs, specifically the psychostimulant drug amphetamine (Amp). Amphetamine was selected based on the evidence that implicates the DA neuronal system in both the ingestion of EtOH and in social stress. Drugs that release DA in the brain such as Amp (e.g., Verheij and Cools, 2008 for a review), are known to increase the reward value of various stimuli (Di Chiara and Imperato, 1988; Tupala et al., 2004) and would be expected to also alter the intake of EtOH. The additional aim of our study was to further evaluate the role of the DArgic system in the behavioral consequences of psychosocial stress. Lastly, to evaluate the specificity of Amp's action on EtOH ingestion in psychosocially stressed rats, we also examined the intake of saccharin, a widely regarded rewarding substance for rodents.

2. Methods

2.1. Subjects and housing

The subjects were male adult Long Evans rats weighing approximately 450 g at the start of the experiment (Harlan, Indianapolis, Indiana). Purina chow and water were available ad libitum throughout the study. The animal room was on a reverse light/dark cycle (12 h each, lights off at 12:30), and its ambient humidity and temperature (21 ± 1 °C) were strictly controlled. The housing cages were made of Plexiglas and had a wire mesh floor. One of the cage walls had either two (single cages) or four (dyad cages) 1-cm openings that accommodated the drinking spouts. The cages for the single-housed (SiH) rats were square (25 cm × 25 cm × 30 cm), and those for the dyad-housed rats were rectangular (26 cm wide × 82 cm long × 30 cm high). The dyad cages had a removable Plexiglas cage divider that partitioned the cage into two equal compartments. The bottom of the cage dividers consisted of a 6-cm high wire mesh screen that allowed rats to maintain sensory contact even when separated. These dividers were removed daily for a one-hour period that allowed the members of a dyad to interact and reinforce their social hierarchy. Rats were weighed daily during the periods when the intake of test solutions was being assessed, and at weekly intervals for the intervening periods. Our animal facility is certified by AAALAC, and the experimental protocols were approved by the Rutgers University Review Committee for the use of Animal Subjects, and all principles of laboratory care were strictly adhered to.

2.2. Agonistic behavior rating

On the day of dyad formation subjects were placed into a dyad cage with the cage dividers in place for a 5-minute adaptation period that allowed them to explore their new environment. This initial adaptation period allowed us to video record the physical appearance of the two co-housed subjects to facilitate subsequent identification. The cage divider was then removed and the ensuing social interactions were recorded over the next 10 min of dyad housing. Agonistic behaviors were scored using a modified and expanded version (Pohorecky et al., 1999; Pohorecky et al., 2004) of the method originally described by Peterson and Pohorecky (1989). Twenty-three different behaviors were scored as previously described in greater detail (Pohorecky et al., 2004), and subsequently were grouped into four major categories: self-centered (rearing, self-grooming, genital grooming), affiliative (approach, sniff body, sniff genitals, groom other, mount other), defensive (defensive upright, defensive back chick, immobility, vocalization, flight/attempt to jump out of the cage) and aggressive (piloerection, aggressive push-under, pounce on, nip other, cage mark, offensive block or pacing, offensive back chick, lateral threat, on top, roll–tumble) (Pohorecky et al., 2004; Pohorecky, 2006). The dyad subject that emitted 22 kHz ultrasonic calls was identified using a Mini Bat Detector (QMC Instruments Inc., London, UK), this rat generally remained immobile while vocalizing and its breathing rhythm appeared altered. Assignment of social status to the members of a dyad was subsequently based on the combined behavioral scores exhibited during the 10-min test, and the change in body weight determined 24 h after dyad formation, and could be further supported by the detection of the subject emitting ultrasonic and audible vocalizations (Pohorecky et al., 2004; Pohorecky, 2006). Overall, the Dom rats displayed most of the offensive behaviors, while the subdominant rats (Sdom) displayed most of the defensive behaviors. Additionally, there was a group of single-housed (SiH) rats that remained single-caged for the duration of the study. When the display of agonistic interactions was insufficiently low to determine differences rank status, the dyad was either discarded (1 case), or a given dyad member was exchanged to allow a clearer distinction of rank status (1 case). Testing was continued until thirteen stable dyad pairs were established on day 1 of the study. At intervals during the study agonistic interactions were verified to assess the stability of rank assignments.

2.3. Drinking protocols

2.3.1. Ethanol intake

A 1-hour limited access drinking session began at approximately 13:30 PM, following the daily social interaction period, and after replacement of the cage dividers. Two drinking bottles were attached to each cage compartment; one contained tap water and the other a