Alcohol 49 (2015) 707–712

Contents lists available at ScienceDirect

Alcohol

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

Anxiety-like behaviors at the end of the nocturnal period in sP rats with a "history" of unpredictable, limited access to alcohol

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ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form
22 April 2015
Accepted 24 April 2015

Keywords:
Time schedule of alcohol drinking
Limited and unpredictable access to alcohol
Experimental model of binge drinking
Anxiety-like behaviors
Positive allosteric modulator of the $GABA_B$ receptor, GS39783
Sardinian alcohol-preferring (sP) rats

ABSTRACT

Recent research found that exposure of selectively bred, Sardinian alcohol-preferring (sP) rats to multiple alcohol concentrations (10%, 20%, and 30%, v/v), under the 4-bottle "alcohol vs. water" choice regimen, in daily 1-h drinking sessions with an unpredictable time schedule, promoted high intakes of alcohol (≥2 g/kg) when the drinking session occurred over the final hours of the dark phase of the light/dark cycle. The present study investigated whether these high intakes of alcohol (a) were associated with alterations in rats' emotional state (Experiment 1) and (b) were pharmacologically manipulable (Experiment 2). In both experiments, over a period of 12 days, sP rats were initially exposed daily to a 1-h drinking session during the dark phase; time of alcohol exposure was changed each day and was unpredictable to rats. The day after this 12-day drinking phase, rats were (a) exposed to the Social Interaction (SI) test at the 1st or 12th hour of the dark phase with no alcohol available (Experiment 1) or (b) treated with the positive allosteric modulator of the $GABA_B$ receptor, GS39783 (0, 25, 50, and 100 mg/kg, intragastrically [i.g.]), and exposed to a drinking session at the 12th hour of the dark phase (Experiment 2). In Experiment 1, rats exposed to the SI test during the 12th hour spent approximately 35% less time in "social" behaviors than rats exposed to the SI test during the 1st hour. No difference in "social" behaviors was observed between alcohol-naive sP rats exposed to the SI test at the 1st and 12th hour. In Experiment 2, all doses of GS39783 selectively reduced alcohol intake. These results suggest that (a) expectation of alcohol availability likely exacerbated the anxiety-like state of sP rats and (b) the $GABA_B$ receptor is part of the neural substrate underlying these exceptionally high intakes of alcohol in sP rats.

Introduction

A recent study found that selectively bred, Sardinian alcohol-preferring (sP) rats were highly sensitive to the time schedule in which alcohol was accessible in daily drinking sessions of limited duration, with the concurrent availability of multiple alcohol concentrations (Colombo et al., 2014). Specifically, alcohol intake increased progressively as the time of alcohol access moved from the earliest to the latest hours of the dark phase of the 12:12-h light/dark cycle. When the drinking session occurred during one of the latest hours of the dark phase (11th and 12th hour), alcohol intake (a) was exceptionally elevated (averaging ≥2 g/kg), (b) was more than double that recorded over the first hours (1st and 2nd h) of the dark phase, (c) resulted in blood alcohol levels averaging approximately 100 mg% (meeting the criterion posed for binge drinking in humans (NIAAA, 2004)), and (d) produced clear signs of alcohol intoxication (e.g., impaired performance at a Rota-Rod task) (Colombo et al., 2014). Notably, sensitivity to the time schedule was (1) limited to the dark phase, as it did not extend to the light phase of the light/dark cycle, and (2) specific to alcohol, as it did not generalize to a highly palatable chocolate-flavored beverage (Colombo et al., 2014).

A critical feature of this experimental design was that time of alcohol exposure changed daily in a semi-random order (meaning that, over 12 consecutive days, rats experienced all 12-h time periods of the dark phase as periods of access to alcohol), becoming unpredictable to rats (Colombo et al., 2014). Unpredictability of time of alcohol availability was an important component of this experimental procedure, as it substantially contributed to the

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http://dx.doi.org/10.1016/j.alcohol.2015.04.010
0741-8329/

increase in alcohol intake recorded when the drinking session occurred over the final portion of the dark phase.

We hypothesized that the unpredictable schedule of alcohol access, together with the expectation of alcohol availability, may have – as time elapsed – generated a progressively increasing emotional "distress." The observed increase in alcohol intake suggests that rats might have coped with this negative affective state by seeking the anxiolytic effect of alcohol. Experiment 1 of the present study was therefore designed to evaluate whether sP rats with an alcohol "history" comprising repeated, unpredictable exposures to multiple alcohol concentrations in daily drinking sessions of 1 h displayed different levels of anxiety-related behaviors when exposed to the social interaction (SI) test at the first or last hour of the dark phase. The SI test is based on the propensity of adult male rats, unknown to each other, to engage in SI behaviors (e.g., sniffing, following, walking over, crawling over and under, grooming the partner) when exposed to an environment in which neither has established territory; the time spent by pairs of rats in these "social" behaviors provides an inverse measure of the rat anxiety-like state: the longer the time spent in social behaviors, the lower the "anxiety" of the rats (File, Kenny, & Ouagazzal, 1998; File, Lippa, Beer, & Lippa, 2004).

We were also interested in evaluating whether the exceptionally high intake of alcohol recorded when the drinking session occurred during the latest hours of the dark phase was pharmacologically manipulable. To address this research question, we tested the effect of acute treatment with GS39783 on alcohol intake in sP rats with an alcohol "history" of repeated, unpredictable exposures to multiple alcohol concentrations in daily drinking sessions of 1 h and then exposed to alcohol during the 12th hour of the dark phase of an additional drinking session (Experiment 2). GS39783 is an *in vivo* effective, positive allosteric modulator (PAM) of the $GABA_B$ receptor ($GABA_B$ PAM) (Urwyler et al., 2003). During recent years, several behavioral studies have depicted an interesting pharmacological profile for GS39783: its acute or repeated administration (a) exerted anxiolytic-like effects in some rat and mouse models of anxiety (Cryan et al., 2004; Mombereau et al., 2004; see, however, Paterson & Hanania, 2010; Sweeney, O'Leary, & Cryan, 2013), and (b) suppressed several alcohol-related behaviors, including alcohol drinking in sP rats exposed to the 2-bottle "alcohol vs. water" choice regimen with unlimited access (Orrù et al., 2005) and operant, oral alcohol self-administration in alcohol-preferring sP, Indiana P, and Alko Alcohol (AA) rats (Maccioni et al., 2007, 2008, 2010, 2012, 2015). Notably, with regard to the aims of the present study, a recent investigation (Linsenbardt & Boehm, 2014) found that acute treatment with GS39783 suppressed binge-like drinking in C57BL/6J mice exposed to the "Drinking-in-the-Dark" procedure (i.e., daily exposure to a single alcohol bottle in daily 2- or 4-h drinking sessions, occurring early in the dark phase (see Thiele & Navarro, 2014)).

Materials and methods

All experimental procedures employed in the present study were in accordance with the Italian law on the "Protection of animals used for scientific reasons."

Animals

Male sP rats from the 85th generation were used. Independent groups of $n = 34$, $n = 48$, and $n = 32$ rats were used in Experiments 1A, 1B, and 2, respectively. Rats were singly housed in standard plastic cages with wood chip bedding; single housing started at the age of approximately 45 days. The animal facility was under an inverted 12:12-h light/dark cycle (lights on at 8:00 PM) at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Food pellets (2018 Diet; Harlan, San Pietro al Natisone, UD, Italy) and water were always available in the home cage.

Starting from the first day of single-cage housing, rats were extensively habituated to handling. Rats used in Experiments 1A and 1B were also familiarized with the SI-test arena (see below). To this end, they were individually exposed to the test arena in four 10-min sessions occurring on the days preceding the test session; environmental conditions of these familiarization sessions were identical to those of the test session (see below). Rats used in Experiment 2 were extensively habituated to intragastric infusions (by metal gavage).

Experimental procedure

Schematic representations of Experiments 1A and 2 are given in Fig. 1, top and bottom panels, respectively.

At the age of approximately 60 days, rats used in Experiments 1A and 2 were exposed to the home-cage, 4-bottle choice regimen between water and 10%, 20%, and 30% (v/v) alcohol with unlimited access (24 h/day) for 12 consecutive days (Phase 1). A 24-h period of alcohol withdrawal, with water as the sole fluid available, was interposed between Phases 1 and 2.

Rats were then exposed to the above 4-bottle choice regimen, with access limited to 1 h/day, for 13 consecutive days (Phase 2). The initial drinking session of Phase 2 ("acclimatization" session) differed from all subsequent sessions as rats were not yet accustomed to the limited-access regimen; it was predicted that their alcohol drinking could be influenced by this novel condition (namely, abrupt and unexpected removal of the alcohol bottles). Therefore, data from this initial drinking session were excluded from analysis. Time of access to alcohol was established semi-randomly so that, over 12 consecutive days, all 12 h of the dark phase were tested. The sequence used in Experiment 1A was: 10, 2, 8, 3, 7, 11, 5, 9, 12, 1, 4, and 6; the "acclimatization" session was replicated on the 11th day of the sequence. The sequence used in Experiment 2 was: 10, 4, 2, 8, 12, 6, 7, 11, 1, 9, 3, and 5. The "acclimatization" session was replicated on the 6th day of the sequence. Water was always available (24 h/day).

In both Phases 1 and 2, on a daily basis, bottles were refilled with fresh solution and their positions changed randomly to avoid development of position preference. Alcohol and water intake was expressed in g/kg pure alcohol and mL/kg water, respectively, and monitored by weighing the bottles with a 0.01-g accuracy (a) every day immediately before the start of the dark phase in Phase 1, and (b) immediately before and immediately after each daily drinking session in Phase 2. Possible fluid spillage was calculated by using multiple bottles filled with the different alcohol concentrations and positioned in empty cages interspersed in the cage racks; mean spilled volumes were subtracted before data analysis.

In Experiment 1A, the SI test was conducted on the day immediately after the last drinking session of Phase 2. Rats were divided into 17 pairs, matched for (a) body weight (no more than ±5%) and (b) alcohol intake and schedule sensitivity over Phase 2. Eight pairs of rats were exposed to the SI test during the 1st hour of the dark phase ("1st-hour" rat group); conversely, 9 pairs of rats were exposed to the SI test during the 12th hour of the dark phase ("12th-hour" rat group). Rats were not exposed to alcohol on the test day. In Experiment 1B, alcohol-naive rats were divided into 24 pairs, matched for body weight (no more than ±5%), and exposed to the SI test at the same exact age of rats of Experiment 1A. Twelve pairs of rats were exposed to the SI test during the 1st hour of the dark phase ("1st-hour" rat group). Conversely, 12 pairs of rats were exposed to the SI test during the 12th hour of the dark phase ("12th-hour" rat group). In both Experiments 1A and