European Journal of Pharmacology 740 (2014) 53–57

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural pharmacology

Assessment of pregnenolone effects on alcohol intake and preference in male alcohol preferring (P) rats

Amir H. Rezvani*, Edward D. Levin

Department of Psychiatry and Behavioral Sciences and Department of Psychology and Neuroscience, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form
30 June 2014
Accepted 1 July 2014
Available online 10 July 2014

Keywords:
Alcohol drinking
Alcoholism
Treatment
Neuroactive steroids
GABAergic system

ABSTRACT

Neuroactive steroids can modulate a variety of neurobehavioral functions via the GABAergic system. This study was conducted to determine the importance of the neurosteroid pregnenolone on the regulation of alcohol intake. The effects of acute and chronic administration of pregnenolone on alcohol intake were assessed in alcohol preferring (P) rats. The rats were injected i.p. with the vehicle or pregnenolone (25, 50 or 75 mg/kg) and their alcohol and water intake were recorded at 2, 4, 6 and 24 h. Also, the chronic effects of 50 mg/kg (i.p.) pregnenolone on alcohol intake were determined. Our results show that although the main effect of i.p. injection of pregnenolone in reducing alcohol intake was not quite significant compared with the vehicle, pregnenolone at 75 mg/kg significantly ($P<0.025$) reduced alcohol intake. Regarding alcohol preference, acute administration of pregnenolone both at 50 mg/kg ($P<0.05$) and at 75 mg/kg ($P<0.025$) significantly reduced alcohol preference. In chronic experiments pregnenolone given for 10 consecutive days did not show a significant effect on alcohol intake and alcohol preference. Overall, although pregnenolone given i.p. acutely and significantly reduced alcohol intake and preference, the fact that chronic treatment did not show an effect diminishes its promise to be considered for the treatment of alcoholism. However, its profile of effects might be different in human alcoholics.

1. Introduction

Neuroactive steroids have been shown to exert a variety of behavioral effects including anxiolytic (Bitran et al., 1991; Wieland et al., 1991), sedative-hypnotic (Mendelson et al., 1987; Wiebe and Kavaliers 1988), anti-convulsant (Finn and Gee, 1994), cognitive impairing effects or cognitive improving effects (Flood et al., 1992; Marx et al., 2009; 2011; Ritsner et al., 2010; Savitz, 2010). Neurosteroids such as pregnenolone also contribute to the behavioral effects of alcohol both in rodents (O'Dell et al., 2004; Barbaccia et al., 1999) and humans, as it has been shown that alcohol ingestion increases the level of neurosteroids in both brain and plasma (Barbaccia et al., 1999; Pierucci-Lagha et al., 2006).

The GABAergic systems play an important role in the regulation of alcohol intake in rodents and humans (June et al., 2003; Quintanilla et al., 2008; Agabio et al., 2012; Stopponi et al., 2012). The GABAergic neuroactive steroids have been shown to have alcohol-like discriminative stimulus properties in rodents (Shannon et al., 2005) and primates (Grant et al., 1997). These steroids act as allosteric modulators of $GABA_A$, NMDA glutamate and sigma receptors (Wu et al., 1991; Baulieu, 1997; Belelli and Lambert, 2005; Morrow et al., 2006). The neurosteroid pregnenolone has an opposing effect on NMDA glutamate and $GABA_A$ receptors; increasing NMDA glutamate receptor activity but decreasing $GABA_A$ receptor activity (Majewska et al., 1990,1992). Consistent with their specific actions on different receptors, neurosteroids have been implicated in several behavioral actions of alcohol including excessive drinking in rats (Besheer et al., 2010). In a clinical study, it has been shown that endogenous neurosteroids mediate some of the subjective effects of alcohol, as alcohol ingestion modifies neurosteroid hormones bidirectionally; i.e. increasing plasma pregnenolone and dehydroepiandrosterone (DHEA) concentrations and decreasing progesterone and allopregnanolone concentrations (Pierucci-Lagha et al., 2006).

Although these findings suggest a role for these neurosteroids in some of the subjective effects of alcohol, they do not address the effects of these hormones on rewarding effects of alcohol. Recently, a group of researchers investigated the effect of pregnenolone on alcohol intake and found that pregnenolone when given systemically (i.p.), dose-dependently reduced 30-min operant alcohol self-administration in alcohol preferring P rats without having an impairing effect on their locomotion (Besheer et al., 2010). In this study the effect of pregnenolone on alcohol intake

* Correspondence to: Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Box 104790 Durham, NC, 27710, USA.
Tel.: +1 919 668 1880; fax: +1 919 681 3416.
E-mail address: azadi@duke.edu (A.H. Rezvani).

http://dx.doi.org/10.1016/j.ejphar.2014.07.003
0014-2999/

was measured only for 30 min following the treatment using an operant behavior. Although this finding may be suggestive, it does not portray the effect of this compound post-30 min. Furthermore, the chronic effect of this compound on alcohol intake has not been studied and currently is unknown.

Considering the important role of the GABAergic system in the brain in the regulation of alcohol intake, and the interaction of neurosteroids with the GABAergic system, we decided to study the effects of both acute and chronic administration of pregnenolone on alcohol intake in selectively-bred alcohol preferring (P) rats. It was hypothesized that pregnenolone, based on the existing literature, will reduce alcohol intake and preference in alcohol preferring rats.

2. Materials and methods

2.1. Animals

Adult male selectively-bred alcohol preferring (P) rats were used. The rats were 535 ± 6.27 g at the initiation of the first treatment. This line of rats was originally derived from Wistar rats at Indiana University. We obtained these rats directly from a breeding colony maintained at Dr. Lawrence Lumeng's Laboratory at Indiana University, Indianapolis. Upon arrival at Duke University, they were kept in a standard laboratory with controlled temperature of 21 ± 2 °C and humidity of 50 ± 10 percent and reversed light cycle (lights off: 0700–2200). Rats were housed individually in specialized cages that were fitted with two 100-ml graduated Richter drinking tubes for the recording of water and alcohol consumption. The rats were initially given free access to a solution of 10% (v/v) alcohol for three consecutive days, and then had a free choice between water and alcohol solution (15%) in two different graduated Richter tubes for the remainder of the study. Three days of forced alcohol exposure made the rats accustomed to the taste and pharmacological effects of alcohol (Rezvani et al., 2009; 2010). Animals were fed 5001 Rodent Chow (Lab Diet, Brentwood, MO, USA) and water ad libitum. Rats were on alcohol and water for at least 6 weeks before the drug treatment began. All treatments and care of the animals were conducted according to a protocol approved by the Animal care and Use committee of Duke University in an AAALAC- approved facility.

2.2. Drug preparation

Pregnenolone (5-Pregnen-3β-OL-20-ONE) was purchased from Steraloids, Inc., Newport RI, USA and was dissolved in a solution of 100% alcohol, then diluted with saline. The amount of alcohol in the drug solution and in the vehicle was equal to 0.1 g/kg. Solutions of alcohol were prepared from a solution of 100% ethanol mixed with tap water. Fresh alcohol and water bottles were presented to rats 15 min after each injection.

2.3. Experimental protocol

The following experiments were carried out to study the effects of acute and chronic administration of pregnenolone on alcohol intake and preference in alcohol preferring rats.

2.4. Acute effects

After establishment of a stable level of alcohol drinking (4.88 ± 0.19 g/kg/day), rats ($n=13$) were injected i.p. with 25, 50 or 75 mg/kg pregnenolone or the same volume of the control vehicle following a counterbalanced order with random assignment. Thus, all animals received all treatments. The volume of drug or vehicle injected i.p. was 2 ml/kg. Treatments were given 15 min before alcohol exposure and alcohol intake and preference as well as water intake were measured at 2, 4, 6 and 24 h after the treatment. To avoid a possible carryover effect a 3-day interval was allowed between injections. Alcohol intake returned to the pre-treatment baseline levels after this interval.

2.5. Chronic effects

To determine the chronic effect of pregnenolone on alcohol intake, the same P rats were injected i.p. with either 50 mg of pregnenolone ($n=7$) or the control vehicle ($n=6$) for 10 consecutive days. There was a 2-weeks interval between the last injection in the acute study and the initiation of the chronic study. The baseline for alcohol intake before the initiation of the chronic treatment was 4.72 ± 0.4 g/kg/day. Alcohol intake and alcohol preference as well as water intake were measured at 6 and 24 h after each treatment.

2.6. Statistical analysis

The results were evaluated for statistical significance by the analysis of variance with repeated measures of drug treatment dose and hour after administration. The drug doses were given in a counterbalanced order with the vehicle as the control. Planned comparisons between the vehicle administered control condition and each drug dose were made. As a follow-up to significant interactions, tests of the simple main effects were performed. Fisher Least Significant Difference (Fisher LSD) was used as post-hoc test. The threshold of $P<0.05$ (two-tailed) was used as the threshold for significance. Alcohol preference (i.e. % alcohol intake) was calculated as the percent of alcohol consumed over total fluid intake (total alcohol intake/total fluid intake) × 100 (Rezvani et al., 2010). Data values are expressed as means ± S.E.M.

3. Results

3.1. Acute effects

To study the acute effects of pregnenolone, rats ($n=13$) were treated i.p. with 25, 50 or 75 mg/kg pregnenolone or the control vehicle and their alcohol and water intake were measured. In the dose-effect study, the main effects of i.p. administration of pregnenolone in reducing alcohol intake and alcohol preference were nearly significant ($F(3,36)=2.76$, $P=0.056$ and $F(3,36)=2.83$, $P=0.052$ for alcohol intake and alcohol preference, respectively). Compared with control vehicle, only the 75 mg/kg dose significantly ($F(1,36)=6.25$, $P<0.025$) reduced alcohol intake (Fig. 1). Alcohol intake was significantly ($P<0.005$) reduced by acute administration of 75 mg/kg of pregnenolone at 4-h and 6-h time points but not at 2 or 24 h. The 25 and 50 mg/kg doses did not show a significant effect on alcohol intake (Fig. 1). Regarding alcohol preference, both 50 and 75 mg/kg doses significantly ($F(1,36)=5.38$, $P<0.05$ and $F(1,36)=7.17$, $P<0.025$ for 50 and 75 mg/kg, respectively) reduced alcohol preference while the lower dose of 25 mg/kg dose did not show a significant effect (Fig. 2). Compared with control vehicle, no dose of pregnenolone had a significant effect on water or total fluid intake (Table 1).

3.2. Chronic effects

To study the chronic effects of pregnenolone on alcohol intake, rats were injected with 50 mg/kg of compound or vehicle for 10 consecutive days and their alcohol and water intake was recorded. No significant effects of pregnenolone were seen on alcohol