Pharmacology, Biochemistry and Behavior 167 (2018) 36–41

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Inhibition of *Glyoxalase 1* reduces alcohol self-administration in dependent and nondependent rats

Giordano de Guglielmo[a,*], Dana E. Conlisk[a], Amanda M. Barkley-Levenson[b], Abraham A. Palmer[b,c], Olivier George[a,*]

[a] Department of Neuroscience, The Scripps Research Institute, La Jolla, CA 92037, USA
[b] Department of Psychiatry, University of California San Diego, La Jolla, CA 92037, USA
[c] Institute for Genomic Medicine, University of California San Diego, La Jolla, CA 92037, USA

ARTICLE INFO

Keywords:
Alcohol
AUD
Glo1
Addiction
Drinking
GABA

ABSTRACT

Previous studies showed that the glyoxalase 1 (*Glo1*) gene modulates anxiety-like behavior, seizure susceptibility, depression-like behavior, and alcohol drinking in the drinking-in-the-dark paradigm in nondependent mice. Administration of the small-molecule GLO1 inhibitor *S*-bromobenzylglutathione cyclopentyl diester (pBBG) decreased alcohol drinking in nondependent mice, suggesting a possible therapeutic strategy. However, the preclinical therapeutic efficacy of pBBG in animal models of alcohol dependence remains to be demonstrated. We tested the effect of pBBG (7.5 and 25 mg/kg) on operant alcohol self-administration in alcohol-dependent and nondependent rats. Wistar rats were trained to self-administer 10% alcohol (v/v) and made dependent by chronic intermittent passive exposure to alcohol vapor for 5 weeks. Pretreatment with pBBG dose-dependently reduced alcohol self-administration in both nondependent and dependent animals, without affecting water self-administration. pBBG treatment was more effective in dependent rats than in nondependent rats. These data extend previous findings that implicated *Glo1* in alcohol drinking in nondependent mice by showing even more profound effects in alcohol-dependent rats. These results suggest that the pharmacological inhibition of GLO1 is a relevant therapeutic target for the treatment of alcohol use disorders.

1. Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder that is characterized by compulsive alcohol use (Koob and Le Moal, 1997), which places an enormous burden on society. In addition to its psychological and societal toll, AUD was estimated to cost the United States' economy USD$249 billion in 2010 alone (Sacks et al., 2015). Current behavioral and pharmacological treatments have limited efficacy, and there is an urgent need for new and more effective treatments (Koob, 2010). Alcohol use disorder has high comorbidity with several psychiatric disorders, including generalized anxiety disorder and depression. Thus, the development of treatments for AUD that may also address psychiatric comorbidities is critically important.

The enzyme glyoxalase 1 (GLO1) is the rate-limiting enzyme in the glyoxalase system, a metabolic pathway that detoxifies α-oxoaldehydes, particularly methylglyoxal (Mannervik, 2008; Thornalley, 1990, 1993, 2003b). Methylglyoxal (MG) is naturally produced by several mechanisms, including by the degradation of glycolytic intermediates, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate (Thornalley, 1993). In conjunction with GLO2, GLO1 enzymatically converts MG into D-lactate (Thornalley, 2003a). Methylglyoxal is a competitive partial agonist at γ-aminobutyric acid A ($GABA_A$) receptors, and physiological levels of MG have been implicated in several processes that involved $GABA_A$ signaling. For example, voluntary binge-like alcohol drinking was recently shown to be modulated by *Glo1* gene expression (McMurray et al., 2017b). The overexpression of *Glo1* increased drinking, and the genetic knockdown of *Glo1* and pharmacological inhibition of the enzyme (GLO1) that is encoded by *Glo1* decreased alcohol drinking in nondependent mice in the drinking-in-the-dark (DID) paradigm (McMurray et al., 2017b). Interestingly, a role for *Glo1* in two other behavioral domains that are often comorbid with alcoholism has also been demonstrated. The genetic and pharmacological manipulation of *Glo1* affects anxiety-like behavior (Distler et al., 2012; McMurray et al., 2016; Williams et al., 2009) and depression-related behavior (McMurray et al., 2017b). This raises the possibility that GLO1 inhibitors may be useful for the treatment of AUD and other common comorbid conditions. However, the effect of small-molecule inhibitors of GLO1 on alcohol self-administration in animal

* Corresponding authors at: Department of Neuroscience, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2400, La Jolla, CA 92037, USA.
E-mail addresses: giordano@scripps.edu (G. de Guglielmo), ogeorge@scripps.edu (O. George).

https://doi.org/10.1016/j.pbb.2018.03.001
Received 6 December 2017; Received in revised form 1 February 2018; Accepted 1 March 2018
Available online 02 March 2018
0091-3057/

models of alcohol dependence remains to be demonstrated.

The present study tested the effect of the small-molecule GLO1 inhibitor *S*-bromobenzylglutathione cyclopentyl diester (pBBG) on alcohol self-administration in alcohol-dependent and nondependent rats. We used the chronic intermittent ethanol (CIE) model combined with operant self-administration, a model that has been shown to have robust predictive validity for alcoholism and construct validity for the neurobiological mechanisms of alcohol dependence (Heilig and Koob, 2007; Koob, 2009). Rats that are made dependent by CIE exhibit clinically relevant blood alcohol levels (BALs; 150–250 mg/100 ml), an increase in alcohol drinking when tested during early and protracted abstinence, and compulsive-like alcohol drinking (e.g., responding despite adverse consequences; Kimbrough et al., 2017b; Leao et al., 2015; O'Dell et al., 2004; Roberts et al., 1996; Schulteis et al., 1995; Vendruscolo et al., 2012). Alcohol dependence that is induced by alcohol vapor results in withdrawal symptoms during both acute withdrawal and protracted abstinence (de Guglielmo et al., 2017; Kallupi et al., 2014; Macey et al., 1996; Vendruscolo and Roberts, 2014), anxiety-like behavior (Valdez et al., 2002), irritability-like behavior (Kimbrough et al., 2017a), and the development of mechanical hyperalgesia (Edwards et al., 2012). We hypothesized that if GLO1 is a relevant target for the treatment of AUD, then pBBG treatment will reduce alcohol self-administration in both dependent and nondependent rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 12$; Charles River, Raleigh, NC, USA), weighing 225–275 g at the beginning of the experiments, were housed in groups of two per cage in a temperature-controlled (22 °C) vivarium on a 12 h/12 h light/dark cycle (lights on at 8:00 PM) with *ad libitum* access to food and water. All of the behavioral tests were conducted during the dark phase of the light/dark cycle. All of the procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2. Operant self-administration

Self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT, USA). The animals were first trained to self-administer 10% alcohol (v/v) and water solutions until stable responding was maintained. First, to facilitate the acquisition of operant self-administration, the rats were initially provided free-choice access to 10% alcohol (v/v) and water for 1 day in their home cages to habituate them to the taste of alcohol. Second, the rats were subjected to an overnight session in the operant chambers with access to one lever (right lever) that delivered water on a fixed-ratio 1 (FR1) schedule of reinforcement. Food was available *ad libitum* during this training. Third, after 1 day off, the rats were subjected to a 2 h session on an FR1 schedule for 1 day and a 1 h session on an FR1 schedule the next day, with one lever delivering alcohol (right lever). All of the subsequent sessions lasted 30 min, and two levers were available (left lever: water; right lever: alcohol) until stable levels of intake were reached.

2.3. Alcohol vapor chambers

The rats were made dependent by chronic intermittent exposure to alcohol vapors as previously described (Gilpin et al., 2008; O'Dell et al., 2004). They underwent cycles of 14 h ON (BALs during vapor exposure ranged between 150 and 250 mg%) and 10 h OFF, during which behavioral testing for acute withdrawal occurred (i.e., 6–8 h after the vapor was turned OFF when brain and blood alcohol levels are negligible; Gilpin et al., 2009). In this model, rats exhibit somatic withdrawal signs and negative emotional symptoms, reflected by anxiety-like responses and elevated brain reward thresholds (de Guglielmo et al., 2016; Edwards et al., 2012; O'Dell et al., 2004; Rimondini et al., 2002; Schulteis et al., 1995).

2.4. Operant self-administration during alcohol vapor exposure

Behavioral testing during alcohol vapor exposure occurred three times per week. The rats were tested for alcohol (and water) self-administration on an FR1 schedule in 30-min sessions. Operant self-administration on an FR1 schedule requires minimal effort by the animal to obtain the reinforcement and herein was considered a measure of intake.

2.5. Drugs

The 10% alcohol (v/v) drinking solution was prepared by diluting 95% alcohol (v/v) in water. pBBG was synthesized in the laboratory of Prof. Alexander Arnold (University of Wisconsin, Milwaukee; McMurray et al., 2017a; McMurray et al., 2017b). pBBG was dissolved in vehicle (8% dimethylsulfoxide, 18% Tween-80, and 74% distilled water) and administered intraperitoneally 30 min before the test session.

2.6. Effect of systemic pBBG on alcohol self-administration in nondependent and dependent rats

Wistar rats ($n = 12$) were trained to self-administer 10% alcohol (v/v) and water until stable self-administration was established (± 10% over the last three sessions). The rats were then intraperitoneally injected with pBBG (0, 7.5, and 25 mg/kg/ml) according to a Latin-square design with a baseline alcohol self-administration session between tests. At the end of the treatment, the rats were re-baselined for alcohol self-administration to exclude possible long-lasting effects of the compound. After three self-administration tests, the animals were moved to the vapor chambers for 3 weeks. Blood samples were collected once weekly to determine BALs. The rats were then tested for alcohol (and water) self-administration on an FR1 schedule of reinforcement in 30-min sessions until the escalation of self-administration was observed. At this point, the treatment began, and the animals were intraperitoneally injected with pBBG (0, 7.5, and 25 mg/kg/ml) according to a Latin-square design with a baseline alcohol self-administration session between tests.

The experimental timeline is illustrated in Fig. 1.

2.7. Statistical analysis

The data were analyzed using one-way repeated-measures analysis of variance (ANOVA), followed by the Newman Keuls *post hoc* test. The escalation data and changes vs. baseline were analyzed using Student's *t*-test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Systemic pBBG reduces alcohol self-administration in nondependent and dependent rats

pBBG significantly reduced alcohol self-administration in nondependent rats. This effect was confirmed by the one-way ANOVA, which revealed a significant effect of treatment ($F_{2,11} = 9.53$, $p < 0.01$). The Newman Keuls *post hoc* test showed that pBBG significantly reduced alcohol self-administration at the dose of 25 mg/kg ($p < 0.01$; Fig. 2A, a). The effect was dose-dependent, in which a significant difference was detected between the 7.5 and 25 mg/kg doses ($p < 0.05$; Fig. 2A, a). Water self-administration was unaffected by the treatment ($F_{2,11} = 0.36$, $p > 0.05$). Further analysis of the percent