Behavioural Brain Research 272 (2014) 238–247

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Intra-nucleus accumbens shell injections of R(+)- and S(−)-baclofen bidirectionally alter binge-like ethanol, but not saccharin, intake in C57Bl/6J mice

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HIGHLIGHTS

- R(+)- and S(−)-baclofen were microinjected into the nucleus accumbens shell.
- Binge-like ethanol and saccharin intake were monitored.
- Time-course of ethanol and saccharin intake was monitored.
- We report a bidirectional, enantioselective effect on binge-like ethanol intake.

ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 3 July 2014
Accepted 7 July 2014
Available online 12 July 2014

Keywords:
Nucleus accumbens shell
Baclofen
Drinking-in-the-Dark
Ethanol
Binge-like
$GABA_B$ receptor

ABSTRACT

The $GABA_B$ agonist baclofen has been widely researched clinically and preclinically as a treatment of alcohol use disorders (AUDs). However, the efficacy of baclofen remains uncertain. The clinically used racemic compound can be separated into separate enantiomers. These enantiomers have produced different profiles in behavioral assays, with the S- compound often being ineffective compared to the R- compound, or the S- compound antagonizing the effects of the R- compound. We have previously demonstrated that the R(+)-baclofen enantiomer decreases binge-like ethanol intake in the Drinking-in-the-Dark (DID) paradigm, whereas the S(-)-baclofen enantiomer increases ethanol intake. One area implicated in drug abuse is the nucleus accumbens shell (NACsh).The current study sought to define the role of the NACsh in the enantioselective effects of baclofen on binge-like ethanol consumption by directly microinjecting each enantiomer into the structure. Following bilateral cannulation of the NACsh, C57Bl/6J mice were given 5 days of access to ethanol or saccharin for 2 h, 3 h into the dark cycle. On Day 5 mice were given an injection of aCSF, 0.02 R(+)-, 0.04R(+)-, 0.08 S(-)-, or 0.16 S(-)-baclofen (μg/side dissolved in 200 nl of aCSF). It was found that the R(+)-baclofen dose-dependently decreased ethanol consumption, whereas the high S(−)-baclofen dose increased ethanol consumption, compared to the aCSF group. Saccharin consumption was not affected. These results further confirm that $GABA_B$ receptors and the NACsh shell are integral in mediating ethanol intake. They also demonstrate that baclofen displays bidirectional, enantioselective effects which are important when considering therapeutic uses of the drug.

1. Introduction

Dopaminergic and GABAergic projections to and from the nucleus accumbens shell (NACsh[1]) to areas including the ventral tegmental area (VTA) and ventral pallidus [1–4] have been implicated in addiction. Specifically, the NACsh has been implicated in stimulant reinforcement [5]. The "incentive arousal" view of dopamine response suggests that accumbal dopamine is not responsible for the behavioral reaction to the stimuli, but rather works as an amplifier signal for the stimuli–moderating whether or not the stimuli result in a behavioral response [6]. Bassareo et al. [7] showed that ethanol and a chocolate + sucrose tastant both elicit a dopaminergic response from the NACsh upon an animal's first experience with the reinforcers. However, upon second presentation of the reinforcers, although the chocolate + sucrose tastant no longer elicited a dopaminergic response, the ethanol

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[1] Artificial cerebrospinal fluid (aCSF), Alcohol use disorders (AUDs), Blood ethanol concentration (BEC), C57Bl/6J (B6), Drinking-in-the-Dark (DID), High Alcohol Preferring (HAP), nucleus accumbens core (NACcore), nucleus accumbens shell (NACsh), prefrontal cortex (PFC), ventral tegmental area (VTA).

http://dx.doi.org/10.1016/j.bbr.2014.07.011
0166-4328/

reinforcer elicited an even greater dopaminergic response in the NACsh. Dopaminergic habituation to a tastant and maladaptive dopaminergic responses to drugs of abuse have not been associated with the nucleus accumbens core (NACcore) or prefrontal cortex (PFC) [8–10], making the NACsh a particularly important region for eliciting a response to drugs of abuse [6].

Ethanol interacts with GABAergic neurons of the NACsh. 80% of ethanol reactive cells in the NACsh are GABAergic; a higher percentage than the NACcore and PFC [11]. However, to date, few studies have attempted to alter ethanol intake in preclinical models of consumption by manipulation of NACsh GABAergic function. Rewal et al. [12] and Nie et al. [13] demonstrated that viral knock-down of extrasynaptic $GABA_A$ receptor subunits in the NACsh, but not NACcore, reduced ethanol intake without affecting saccharin intake. While these results suggest a role for $GABA_A$ receptors, $GABA_B$ receptors have not been investigated, even though they comprise and entire third of the GABA receptors of the NAC [14].

Baclofen, a $GABA_B$ receptor agonist used to treat severe forms of epilepsy, has been repeatedly shown to alter ethanol consumption. Clinically, baclofen has been used to effectively treat symptoms of alcohol use disorders (AUDs), such as craving and anxiety, in European populations [15]. However, the results in U.S. populations have not been as conclusive [16]. These conflicting results may be due to different alcoholic populations, with European studies often focusing on a population of severe AUD sufferers. A U.S. study examining hospital inpatients at a high risk for alcohol withdrawal syndrome did find baclofen to be effective at reducing these symptoms [17].

Baclofen has also produced inconsistent results preclinically. For example, baclofen decreased operant responding for ethanol [18,19], lowered the breakpoint for ethanol in alcohol preferring rats [20], and decreased intake of ethanol [21,22] when administered systemically. Baclofen also decreased binge-like ethanol consumption when microinjected directly into the anterior, but not posterior, VTA [23]. Conversely, baclofen has also been demonstrated to increase ethanol consumption following acute and chronic systemic injections [24–26].

The baclofen that is used clinically is a racemic compound that breaks down into absolute configurations of R- and S- and positive (+) and negative (-) molecular rotations. We have observed enantioselective effects of baclofen on the binge-like ethanol consumption produced by Drinking-in-the-Dark (DID); R(+)-baclofen reduced drinking whereas S(-)-baclofen increased drinking [27]. Enantioselective effects of baclofen have also been demonstrated in other behavioral paradigms, with R-baclofen often being more behaviorally active. Olpe et al. [28] quantified the effects of R-, S-, and racemic baclofen on reflexes and electro-shock seizures. S- was completely ineffective, even at the highest dose, whereas R- was equally effective as the racemic compound. Paredes and Agmo [29] demonstrated that S(-)-baclofen was ineffective at inhibiting sexual behavior, whereas R-baclofen was twice as effective as the racemic compound. S-baclofen has been demonstrated to depress the trigeminal nucleus oralis response, but at a dose 20 times larger than the least effective R-dose [30]. In the periphery, S-baclofen reduced the response to R-baclofen [30]. While Olpe et al. [28] suggest that S-baclofen may play a role in potentiating the action of R-baclofen without being active itself, Fromm et al. [30] show that S-baclofen is not only active at higher doses, but that it also may inhibit the actions of R- in certain cases. Collectively, these studies show that the behavioral effects of baclofen are enantiomer dependent, and highlight the importance of considering enantioselective aspects of drug effects when accounting for the therapeutic use of the drug [31].

A second aspect of considering therapeutic potential is choosing an appropriate model of behavior to test the drug [30]. One of many such models is DID, which has been used extensively in our lab to test drug treatments via systemic and microinjection approaches [23,32–34]. Developed by Rhodes et al. [35,36], DID achieves high levels of binge-like drinking by introducing ethanol 3 h into the dark cycle with a 2 h access period. In a relatively short period of time, B6 mice reliably ingest enough ethanol to reach pharmacologically relevant BECs of over 1 mg/ml without introducing food or water restrictions. As such, DID presents a simple model that can be used to screen potential AUD treatments that target various receptor systems, including the GABA, endocannabinoid, glutamate, and dopamine receptor systems [23,33,37].

We hypothesize that microinjection of R(+)-baclofen into the NACsh will reduce ethanol intake, but that microinjection of S(-)-baclofen will increase ethanol intake, in the binge-like DID paradigm. Further, we hypothesize that microinjection of baclofen into the NACsh will not affect saccharin intake, as the NACsh no longer releases dopamine in response to a second exposure to a saccharin reinforcer [7].

2. Method

2.1. General design

In brief, male B6 mice underwent surgery for bilateral cannulation of the NACsh. Following at least 48 h of recovery, mice began a 5-day DID procedure where 20% ethanol or 0.2% saccharin was available for 2 h, 3 h into the dark period each day. On Day 5, animals received one of five possible microinjection doses; 0.02 or 0.04 μg R(+)-baclofen, 0.08 or 0.16 μg S(-)-baclofen, or artificial cerebrospinal fluid (aCSF) (Fig. 1).

2.2. Subjects

Male B6 mice were bred in-house. Breeders for our colony were obtained from Jackson Laboratory (Bar Harbor, ME) and replaced every few months by new breeder pairs purchased from Jackson Laboratory. A total of 118 animals were used in this study. One aCSF control group (dose = 0.0 μg) was used for each reinforcer (ethanol or saccharin) to reduce the number of animals associated with this project (see Fig. 1).

Animals received food at all times and water ad lib apart from during implementation of DID. Lights were kept on a reverse light-dark schedule with lights off at 8 am. Animals were group housed until the time of surgery, after which time they were individually housed. Animals were at least 58 days of age at the time of surgery, and at least 60 days of age at the time DID was

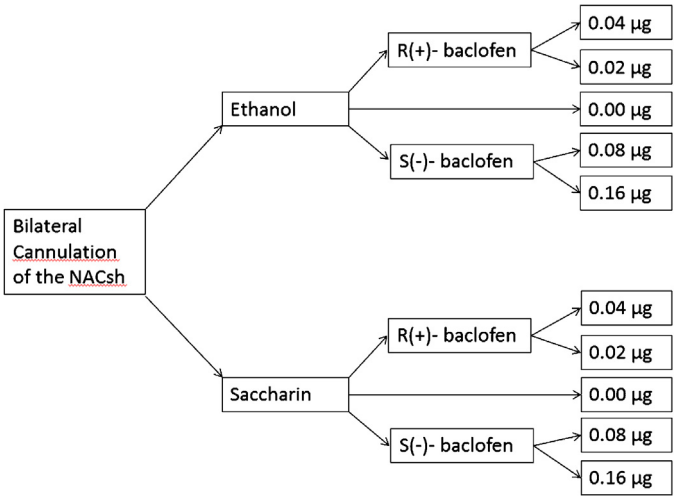

Fig. 1. Study design.