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Original Article

Brucine suppresses ethanol intake and preference in alcohol-preferring Fawn-Hooded rats

Yu-ling LI[1], Qing LIU[1], Qi GONG[1], Jun-xu LI[2], Shou-peng WEI[1], Yan-ting WANG[1], Hui LIANG[1], Min ZHANG[1], Li JING[2], Zheng YONG[3], Andrew J LAWRENCE[4, *], Jian-hui LIANG[1, *]

[1]National Institute on Drug Dependence, Peking University, Beijing 100191, China; [2]Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY 14214, USA; [3]Institute of Pharmacology & Toxicology, Academy of Military Medical Sciences, Beijing 100850, China; [4]Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria 3010, Australia

Aim: Brucine (BRU) extracted from the seeds of *Strychnos nux-vomica* L is glycine receptor antagonist. We hypothesize that BRU may modify alcohol consumption by acting at glycine receptors, and evaluated the pharmacodynamic profiles and adverse effects of BRU in rat models of alcohol abuse.

Methods: Alcohol-preferring Fawn-Hooded (FH/Wjd) rats were administered BRU (10, 20 or 30 mg/kg, sc). The effects of BRU on alcohol consumption were examined in ethanol 2-bottle-choice drinking paradigm, ethanol/sucrose operant self-administration paradigm and 5-d ethanol deprivation test. In addition, open field test was used to assess the general locomotor activity of FH/Wjd rats, and conditioned place preference (CPP) was conducted to assess conditioned reinforcing effect.

Results: In ethanol 2-bottle-choice drinking paradigm, treatment with BRU for 10 consecutive days dose-dependently decreased the ethanol intake associated with a compensatory increase of water intake, but unchanged the daily total fluid intake and body weight. In ethanol/sucrose operant self-administration paradigms, BRU (30 mg/kg) administered before each testing session significantly decreased the number of lever presses for ethanol and the ethanol intake, without affecting the number of sucrose (10%) responses, total sucrose intake, and the number of lever presses for water. Acute treatment with BRU (30 mg/kg) completely suppressed the deprivation-induced elevation of ethanol consumption. Treatment with BRU (10, 20, and 30 mg/kg) did not alter locomotion of FH/Wjd rats, nor did it produce place preference or aversion.

Conclusion: BRU selectively decreases ethanol consumption with minimal adverse effects. Therefore, BRU may represent a new pharmacotherapy for alcoholism.

Keywords: alcoholism; ethanol; brucine; glycine receptor antagonist; Fawn-Hooded (FH/Wjd) rat

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Introduction

As a chronic psychiatric disorder, alcoholism imposes a significant medical and economic burden on both individuals and society[1, 2]. Alcohol consumption is the world's third largest risk factor for disease and disability and is considered to cause epilepsy, liver cirrhosis, pancreatitis, angiocardiopathy and several types of cancer[3–6]. Currently, there are only three FDA-approved medications available for the treatment of alcohol dependence, disulfiram, naltrexone and acamprosate. They each have only limited effectiveness in select alcoholic patients, and they also have significant adverse effects such as fatigue, abdominal pain, diarrhea, nausea, vomiting, blurred vision, depression and dizziness[7–10]. This lack of choice in medicine often impacts the effective treatment of alcohol abuse in clinical practice[1]. Thus, more effective pharmacotherapies for alcoholism are needed[11].

Semen strychni, the dried seed of the *Strychnos nux-vomica* L, has been effectively used in traditional Chinese medicine for hundreds of years[12]. Alkaloids are believed to be the major active components in *Semen strychni* and are responsible for its pharmacological and toxic effects[13]. There are 16 alkaloids isolated and identified from the seed, of which 70% is strychnine and brucine[13]. Strychnine is the alkaloid present at the highest concentration in the *Semen strychni*. It shows analgesic, anti-inflammatory and anti-tumor activities in preclinical studies[14]. However, it is a poison to humans and

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* To whom correspondence should be addressed.
E-mail liangjh@bjmu.edu.cn (Jian-hui LIANG);
andrew.lawrence@florey.edu.au (Andrew J LAWRENCE)
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most livestock. Brucine (BRU, Figure 1), an odorless white crystalline solid alkaloid (molecular weight, 394.45), is the second most abundant alkaloid in the *Semen strychnine*[15]. The LD_{50} values of strychnine and BRU in mice (ip) were reported to be 1.1 and 50.1 mg/kg, respectively, which means that the toxicity of BRU is much lower than strychnine[13]. The existing literature suggests that BRU exerts the following pharmacological effects: cough suppressant, microcirculation facilitation, cell protection, pain relief, anti-rheumatic and anti-tumor effects[12, 16, 17]. However, the effects of BRU on alcohol abuse have not been documented.

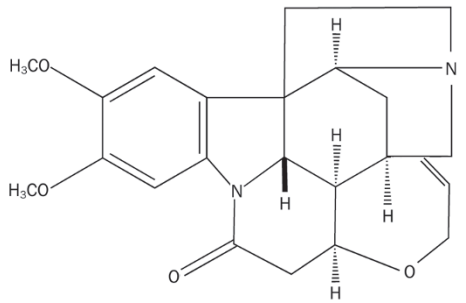

Figure 1. Chemical structure of brucine, a major alkaloid present in *Strychnos nux-vomica* seeds.

The central GABAergic and glycinergic systems are implicated in alcohol abuse and dependence[18]. In particular, the functions of α1 or α2 glycine receptors subtypes are enhanced by alcohol treatment[19]. Because BRU is an antagonist at the α1 and α1β glycine receptor subtypes[20], we hypothesize that BRU may be able to modify alcohol consumption by acting at glycine receptors in alcohol-preferring Fawn-Hooded (FH/Wjd) rats.

The goal of the current study was to examine the role of BRU in the modulation of ethanol consumption, ethanol seeking and deprivation-induced drinking in FH/Wjd rats. The specificity of BRU on ethanol effects was compared to its effect on sucrose drinking. The effect of BRU on locomotion and the Pavlovian conditioning of BRU were also studied to provide preliminary safety pharmacological information for further BRU drug development.

Materials and methods

Animals

The FH/Wjd rats were obtained from the Florey Institute of Neuroscience and Mental Health, University of Melbourne (Melbourne, Vic, Australia) and bred at the Department of Laboratory Animal Sciences, Peking University Health Science Center. Adult male FH/Wjd rats [Grade II, certificate number of the breeder: SCXK (Jing) 2011-0012], approximately 250–300 g at the start of the study, were individually housed and maintained with free access to food and water, except when stated otherwise (room temperature: 22±1 °C; relative humidity: 50%±10%). The animal facility was under a 12-h light/dark cycle (lights on at 8:00 AM). The rats were habituated to the housing room and experimenter handling for one week before the experimental procedures. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No 80–23, revised 1996) and approved by the Peking University Committee on Animal Care and Use.

Drugs

The BRU sulfate salt hydrate (Sigma-Aldrich, St Louis, MO, USA) was dissolved in 0.9% saline and injected subcutaneously at a volume of 1 mL/kg. The anhydrous ethanol and sucrose were purchased from Beijing Chemical Factory (Beijing, China) and prepared with tap water to various concentrations.

Ethanol 2-bottle-choice drinking paradigm

FH/Wjd rats were trained to drink ethanol using classical methods with minor modifications[21]. The alcohol-preferring FH/Wjd rats (ethanol preference >65%) were continuously (24 h per day) offered two bottles containing ethanol (5% *v/v*, in tap water) or tap water for 8 consecutive weeks. The ethanol and water were monitored once daily by bottle weighing immediately before the onset of the dark phase. The bottles were refilled daily with fresh solution and their left-right positions interchanged randomly to exclude the development of position preference. Standard rat chow was always available. At the end of the 8-week period, the rats were randomly divided into 4 groups (n=8) matched for similar daily ethanol consumption and preference over the last 7 d. During the test, all rats were subcutaneously administered with saline twice daily (at 8:00 AM and 7:30 PM) for the first 3 consecutive days, then they were injected with saline or various doses (10, 20, and 30 mg/kg) of BRU for 10 consecutive days and their consumption of ethanol, water and food were measured for 24 h. The ethanol concentration was selected on the basis of our previous studies, which showed that FH/Wjd rats exhibited higher preference for 5% ethanol[22]. The drug doses were chosen based on a pilot study.

Ethanol/sucrose self-administration procedure

To test ethanol or sucrose oral self-administration in adult FH/Wjd rats, a custom-made WS-1 operant self-administration apparatus was employed[23]. The device was composed of 6 operant chambers (29.5 cm×25.5 cm×25 cm) housed in sound-attenuating cubicles with an air-vent and house light. Levers (3 cm×2.3 cm) were installed on each side of the chamber, 5 cm above the floor. A stimulus light and buzzer were placed above the lever. Ethanol, sucrose or water (0.03 mL) was delivered from a 10 mL glass syringe connected to an automatic infusion pump. Pressing one of the two levers led to a 3-s combined tone and light cue. Fluid delivery and operant responses were recorded by a computer.

Before ethanol operant self-administration experiments, FH/Wjd rats were given a 10% ethanol solution as the only liquid source for 4 d. During the next 2 weeks, the rats had

free access to 5% ethanol and tap water. At the end of the 14th d, the rats were limited to 30 min of water per day for two successive days. On the evening of the second day of water limitation, the rats were put into the operant chambers for a 12-h response session under a fixed ratio 1 (FR1) schedule (1 reinforcer of 0.03 mL per lever press) with 10% sucrose as the reinforcer and both levers active. Once rats had learned to respond for sucrose, they were water restricted and exposed to 45 min FR1 sessions for the next 5 d with a 10% sucrose solution as the reinforcer. The animals had free access to water in their home cages and received an additional 2 d training in accordance with the above protocol. After this initial training phase, the session time was shortened to 30 min and the response ratio was increased to 3 (FR3). At this point, a modified sucrose fade protocol was introduced with minor changes[24–26]. Various sucrose solutions (10%, 8%, 6%, 4%, and 2%) were blended with 5% ethanol and the rats received 3 training sessions for each mixture until they responded reliably under the FR3 schedule for 5% ethanol alone and a second lever that delivered water was introduced. In the sucrose operant self-administration experiment, the sucrose concentration remained constant (10%) and another lever delivered tap water. In all studies, the positions of the ethanol/sucrose solution and water were switched to prevent side bias.

As soon as stable lever pressing for ethanol/sucrose was established for at least 20 sessions, FH/Wjd rats in both groups were divided into 4 subgroups (n=5–6) that then received either a saline or BRU (10, 20, and 30 mg/kg, sc) treatment 2 h before each testing session.

Ethanol deprivation test

The ethanol intake of FH/Wjd rats increases following an ethanol deprivation process[27, 28]. A previous test found that the daily ethanol consumption of FH/Wjd rats in a 10% ethanol group is higher than in a 5% ethanol group[22]. Thus, we studied the effects of BRU on deprivation-induced drinking in a group of FH/Wjd rats that had been drinking 10% (*v/v*) ethanol voluntarily for at least 2 months. One group was tested for the baseline and the ethanol bottle of other 3 groups was removed from the cage for 5 d. On the test day (at 8:00 AM and 7:30 PM), 4 groups (n=6–9) of rats received a subcutaneous injection of 30 mg/kg BRU, saline or no treatment (2 groups). The ethanol bottle was replaced approximately 30 min prior to the beginning of the dark cycle (20:00 PM). Fluid consumption was recorded at 2, 4, 12, 24, 36, and 48 h after the ethanol bottle was replaced.

Locomotor activity test

Locomotor activity was measured in 4 chambers (49 cm×49 cm×59 cm) situated in sound-attenuating cabinets (96 cm×96 cm×96 cm) using Digbehv spontaneous activity monitors (DigBehv-LG, Shanghai Jiliang Software Technology Co Ltd, Shanghai, China)[29]. The total distance of horizontal locomotor activity was recorded with a video camera located above the chamber and analyzed with the Digbehv software (Version 2.0, Shanghai Jiliang Software Technology Co Ltd, Shanghai, China). The rats were divided into 4 groups (n=6) matched for body weight. The rats received saline or BRU (10, 20, and 30 mg/kg, sc) and were then immediately put into the test chambers individually to measure their locomotion for 240 min[30]. Rats from the different experimental groups were tested in random order.

Conditioned Place Preference (CPP) test

The present study used an unbiased paradigm as described in previous studies[29, 31, 32]. FH/Wjd rats were conditioned in the CPP apparatus, which consisted of three distinct chambers (two end-chambers of 28 cm×23 cm×20 cm, L×W×H, and one middle chamber of 14 cm×23 cm×20 cm, L×W×H) separated by a retractable guillotine door. The two end-chambers, defined as the conditioning chambers, were distinguishable by two somatosensory cues (tactile: stainless steel rod floor or stainless steel mesh floor; visual: radial or square shaping of five low-power red light bulbs). The movements of the animals through the apparatus were monitored by three infrared photocells (3 cm above floor) in each chamber, and the data were recorded using a computer. The CPP procedures included a 15-min pretest session (D 0; drug free), eight 45-min conditioning sessions (D 1 to D 8; drug or saline training), and a 15-min test session (D 9; drug free). On D 0, each rat was placed in the central compartment without the guillotine doors to allow free access to all three compartments. The amount of time spent in each compartment was recorded and used to assess the natural place preference tendency (exclusion criteria: time difference >120 s between the two end-compartments; attrition rate of 15%). During the conditioning days, rats received saline or BRU (10, 20, and 30 mg/kg, n=7–10) and were immediately confined to the saline-paired or drug-paired compartment for 45 min. On test days, the rats were placed in the central compartment without an injection, and allowed to explore all three chambers freely for 15 min. The CPP scores were calculated using the time (seconds) spent in the drug-paired compartment minus the time spent in the saline-paired side on the test days.

Statistical analysis

Data from the 2-bottle-choice test and locomotor activity test were analyzed using a two-factor repeated measures analysis of variance (RM-ANOVA). When three or more groups were compared, a one-way ANOVA was performed and, if significant, followed by post hoc analysis using the LSD tests. Statistical significance was considered to be P<0.05. The data shown are the mean±SEM.

Results

Effects of BRU on ethanol intake in FH/Wjd rats

We first examined the effects of BRU on ethanol consumption in a 2-bottle free-choice drinking paradigm in FH/Wjd rats, a strain that naturally drinks a large amount of ethanol[33]. During the first 3 d of saline administration, no significant difference was observed between the 4 groups [EtOH intake: F(3,28)=0.215, NS; water intake: F(3,28)=1.731, NS; total fluid

intake: $F(3,28)=0.046$, NS; preference: $F(3,28)=1.426$, NS; food intake: $F(3,28)=0.668$, NS; body weight: $F(3,28)=0.056$, NS]. During the period of drug delivery, BRU (20 and 30 mg/kg) resulted in a significant reduction in daily ethanol intake, which persisted throughout the 10-d treatment period [$F(3,28)=8.043$, $P<0.01$] (Figure 2A). The reduction in daily ethanol intake of the BRU treatment groups was associated with a compensatory increase in water intake [$F(3,28)=6.305$, $P<0.01$] (Figure 2B), so that the daily total fluid intake [$F(3,28)=2.706$, NS] remained unchanged (Figure 2C), and the

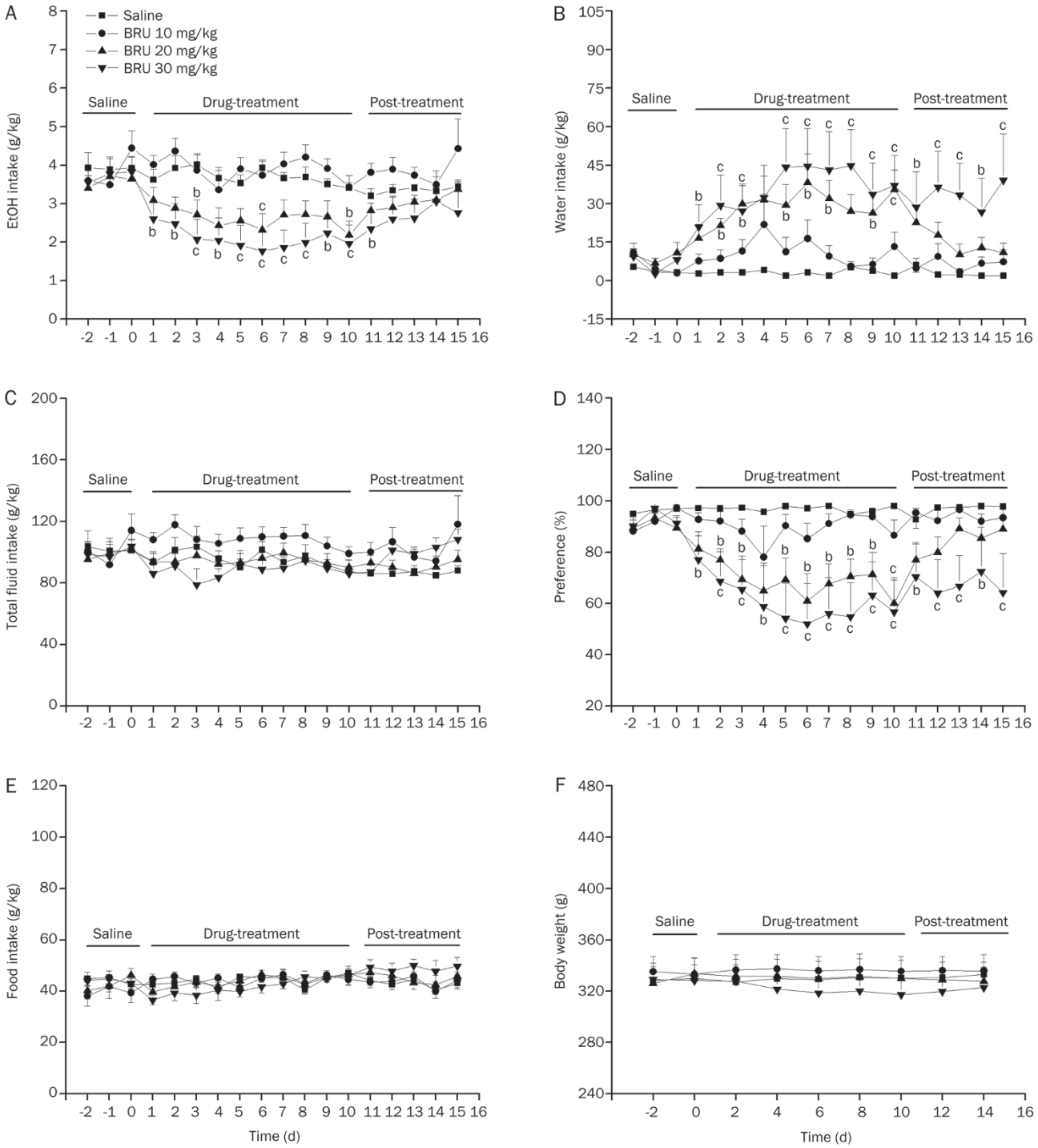

Figure 2. Effects of BRU (10, 20, and 30 mg/kg, *sc*, bid) on EtOH (5%, *v/v*) intake (A), water intake (B), total fluid intake (C), preference (D), food intake (E) and body weight (F) in the ethanol 2-bottle-choice paradigm in FH/Wjd rats. The experimental phases shown (from left to right) were the saline phase (d 2–d 0), drug-treatment phase (d 1–d 10) and post-treatment phase (d 11–d 15). The data are expressed as the mean±SEM (n=8). $^{b}P<0.05$, $^{c}P<0.01$ versus the saline group.

ethanol preference [$F(3,28)=6.824$, $P<0.01$] (Figure 2D) was significantly decreased. Daily food intake [$F(3,28)=0.870$, NS] (Figure 2E) and body weight [$F(3,28)=0.332$, NS] (Figure 2F) were not significantly altered by drug treatment, suggesting that changes in ethanol intake were independent of food intake. During the post-treatment sessions, the ethanol intake [$F(3,28)=2.447$, NS] (Figure 2A) and preference [$F(3,28)=3.723$, $P<0.05$] (Figure 2D) of the BRU treated groups remained lower without a rebound increase.

Effects of BRU on ethanol/sucrose self-administration in FH/Wjd rats

In the ethanol operant self-administration paradigm, BRU reduced the number of lever presses for 5% ethanol in a dose-related manner compared to saline. Statistical significance was observed at the high dose (30 mg/kg) for the number of lever presses for ethanol [$F(3,18)=4.379$, $P<0.05$)] (Figure 3A) and ethanol intake [$F(3,18)=3.794$, $P<0.05$] (Figure 3B). The ethanol intake (in mg/kg, mean±SEM) for the saline, 10, 20,

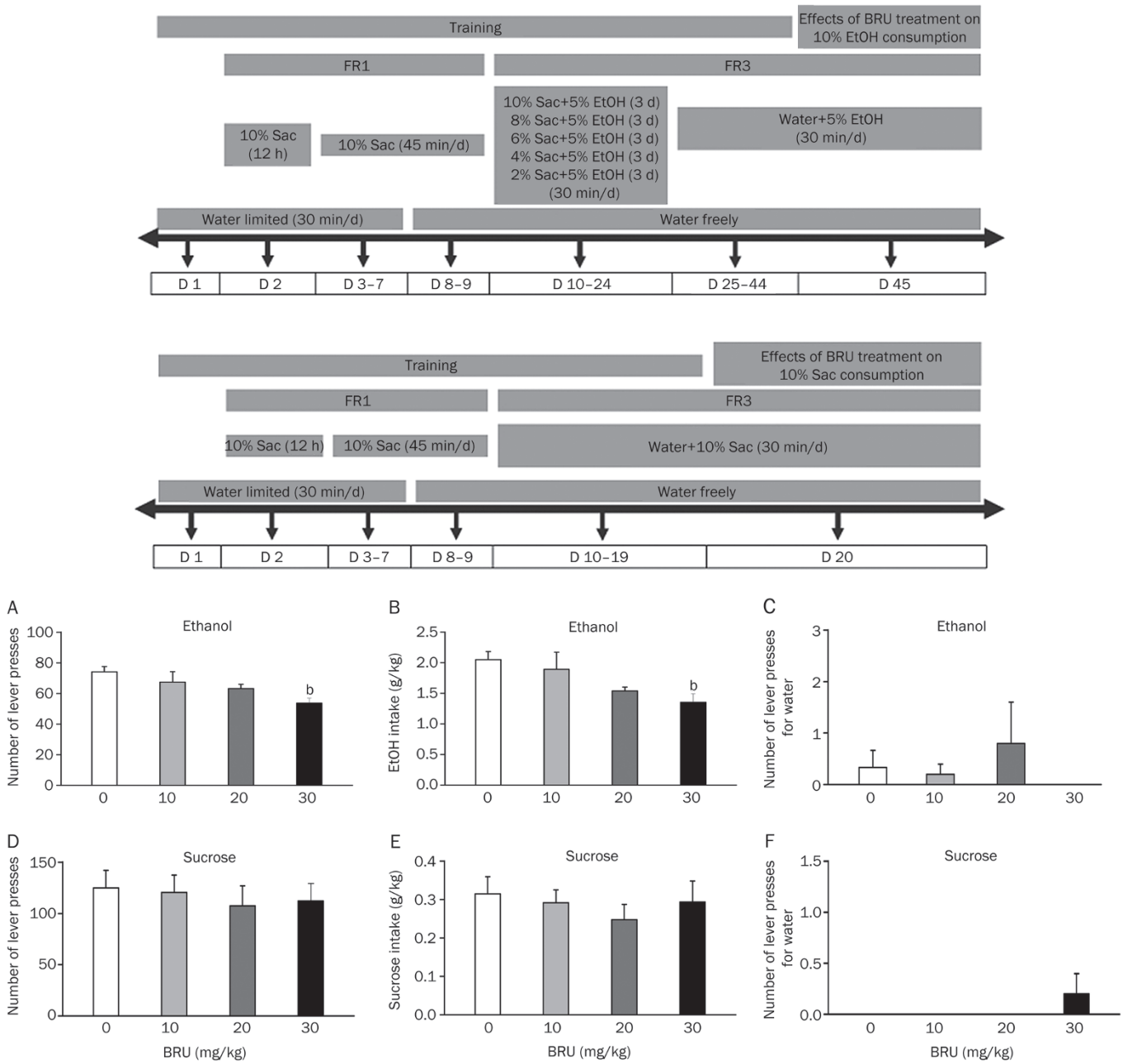

Figure 3. Effects of BRU (10, 20, and 30 mg/kg, sc) on ethanol or sucrose self-administration during a 30 min FR3 session in FH/Wjd rats. The drug was administered (sc) 2 h before the beginning of the sessions. BRU (30 mg/kg) decreased the number of lever presses for ethanol (A) and the ethanol intake (B), but not the water responses (C). BRU did not decrease the number of lever presses for sucrose (D), sucrose intake (E) or the water responses (F) when sucrose was given. The number of lever presses for water in the 30 mg/kg BRU-treated group is zero (C). This is also the case for the saline-, 10- and 20- mg/kg treated groups (F). The data represent the mean±SEM (n=5–6). $^bP<0.05$ versus the saline group.

and 30 mg/kg groups was 2.05±0.13, 1.89±0.28, 1.53±0.06, and 1.35±0.14, respectively. In contrast, all doses tested with BRU had no effect on the number of sucrose (10%) responses [$F(3,17)=0.196$, NS] (Figure 3D) or total sucrose intake [$F(3,17)=0.406$, NS] (Figure 3E). Similarly, the number of lever presses for water were not significantly changed at any dose of BRU in both operant self-administration experiments (Figure 3C and 3F).

BRU inhibited deprivation-induced drinking in FH/Wjd rats

Ethanol consumption of rats markedly increases after a period of abstinence, known as the ethanol deprivation effect[34]. The ethanol intake and preference were significantly increased between the baseline and untreated groups after a period of forced deprivation in heavy drinking FH/Wjd rats (ethanol intake: at 2, 4, 12, 24, 36, and 48 h, $P<0.01$, $P<0.01$, $P<0.05$, $P<0.01$, $P<0.01$, and $P<0.01$, respectively; preference: NS, $P<0.05$, $P<0.01$, $P<0.01$, $P<0.01$, and $P<0.01$ at every time point; Figure 4). BRU abolished the increase in ethanol consumption observed in the saline group after the 5-d deprivation. A one-way ANOVA followed by a *post hoc* analysis revealed a significant difference between the 30 mg/kg BRU and saline groups on ethanol intake after 2, 4, 12, 24, 36, and 48 h ($P<0.01$). Importantly, BRU continued to suppress deprivation-induced drinking and the preference to below baseline levels for the entire 48-h experiment (Figure 4). Total fluid intake was not affected by the BRU treatment over 24 or 48 h compared to the untreated and saline groups (data not shown).

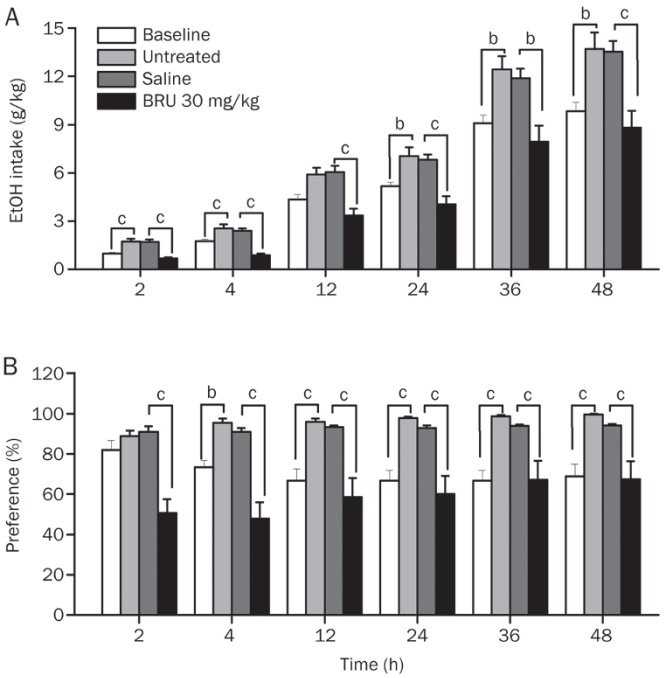

Figure 4. BRU (30 mg/kg, sc) attenuated ethanol consumption after being deprived of ethanol for 5 d in FH/Wjd rats. Once the level of ethanol intake remained stable over 2 months, FH/Wjd rats were tested for baseline or deprived of ethanol for 5 d, and then the rats received a subcutaneous injection of BRU 30 mg/kg, saline or no treatment. The ethanol intake and preference were determined over the following 48 h. All data are expressed as the mean±SEM (n=6–9). $^bP<0.05$, $^cP<0.01$ compared to the control group.

Effects of BRU on locomotor activity and CPP in FH/Wjd rats

A two-factor repeated measures ANOVA indicated that there were no differences between the groups in locomotor activity when the data were analyzed as 10-min bins [$F_{drug}(3,20)=1.100$, NS; $F_{drug\times time}(69,460)=0.743$, NS] (Figure 5). The cumulative distances in 4 h were also statistically analyzed using a one-way ANOVA, and no statistical significance was found for BRU on the general locomotor activity [$F(3,20)=1.148$, NS] (Figure 5 inset).

Neither group of treated rats exhibited a significant change in the time spent in the drug-paired compartment side [$F(3,28)=0.010$, NS] (Figure 6). There was no evidence of conditioned place preference or aversion in FH/Wjd rats when treated with BRU (10, 20, and 30 mg/kg) in the CPP paradigm. The average time (in s, mean±SEM) in the drug-paired compartment for Sal-treated and BRU-paired (10, 20, and 30 mg/kg) was 286.85±29.88, 266.07±26.70, 209.70±28.25, and 297.04±25.81, respectively. These data suggest that BRU does not appear to possess any intrinsic rewarding or aversive effects.

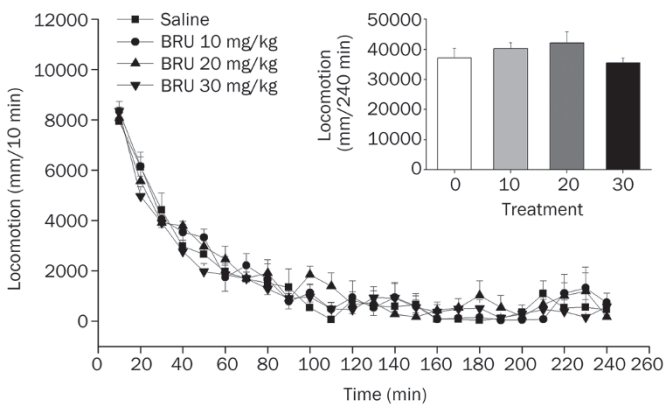

Figure 5. BRU (10, 20, and 30 mg/kg, sc) did not affect the locomotor activity in the open-field test in FH/Wjd Rats. Animals were injected with BRU or saline immediately before the test. Locomotion was recorded every 10 min for 4 h. The data are expressed as the mean±SEM (n=6).

Discussion

Here, we show that BRU significantly suppressed voluntary ethanol intake and reduced ethanol preference as measured using the ethanol 2-bottle-choice drinking paradigm in alcohol-preferring FH/Wjd rats. This effect was dose-related and apparent across the 10 d of drug treatment. The effect of BRU was selective for ethanol intake, as there was a compensatory increase in water intake and no consistent effects on total fluid intake. We also found that systemic administration of BRU (30 mg/kg) reduced ethanol operant self-administration but did not decrease sucrose or water self-administration.

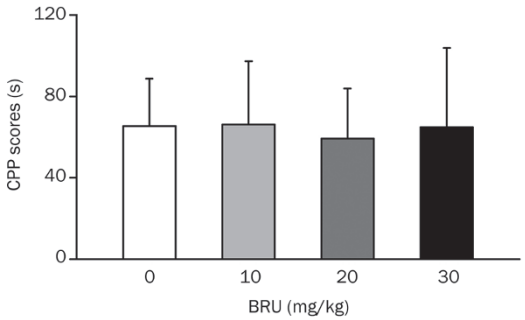

Figure 6. BRU (10, 20, and 30 mg/kg, sc) did not induce conditioned place preference or aversion in FH/Wjd rats in an unbiased CPP test. Each point shows the mean±SEM (n=7–10).

Naltrexone can cause an initial decrease in free-choice ethanol consumption by FH/Wjd rats, but this effect diminished after a period of 7 d[35]. Tolerance also develops to acamprosate for voluntary ethanol intake and ethanol-seeking behaviors with repeated treatments[36]. The development of tolerance to the effects of these drugs in pre-clinical models has raised questions about their clinical utility[37]. In the course of a 10-d BRU treatment, no tolerance was observed. BRU has an elimination half-life ($T_{1/2}$) of 2.9 h after transdermal administration (40 mg/kg) to ICR mice[13]. The inhibitory effect lasted for up to 5 d after subcutaneous injection in our study, suggesting that the duration of drug action is not tightly linked to the plasma concentration. The mechanisms underlying such a potent and long-lasting effect require further investigation. Nonetheless, this finding suggests that BRU may significantly decrease the frequency and volume of drinking in alcoholics[38]. In addition, taste preference may influence ethanol consumption. In the sucrose self-administration test, the development of preference for sucrose solution was not changed, suggesting that BRU does not decrease the taste of natural rewards.

Relapse to heavy ethanol drinking after a period of abstinence is a serious problem in the treatment of human alcoholism[27]. The ethanol deprivation effect, raised first in 1967 by Sinclair and Senter, appears to be an index of craving for ethanol[39, 40]. The time course of FH/Wjd rats on 10% ethanol consumption for 2, 4, 6, 12, and 24 h has been reported[27, 28]. In the present study, we found that the ethanol intake of FH/Wjd rats was similar to the previous studies at every time point and had the tendency to increase after 5 d of ethanol deprivation. BRU (30 mg/kg, sc, bid) reduced ethanol intake and preference after a 5-d deprivation in rats over a 48 h period. Thus, if these finding can be translated clinically, BRU may reduce the relapse rate and prolong abstinence in alcoholic patients.

The place-conditioning paradigm is widely used to assess the rewarding or aversive properties of drugs[41]. BRU at behaviorally effective doses (20 and 30 mg/kg) displayed no rewarding or aversive effects in the CPP test. In addition, BRU did not alter the general locomotor activity of the rats suggesting that BRU is not a general CNS depressant. Moreover, BRU did not alter sucrose drinking behavior. Together, these data strongly suggest that BRU decreases ethanol drinking and ethanol preference via specific neurobiological mechanisms but not via non-specific behavioral suppression or drug-induced taste aversion. Pharmacokinetic studies following dermal administration show that liposomal BRU distributes widely and could be detected in the liver, heart, spleen, lung, kidney, brain and muscle tissues[15]. BRU can also pass the blood brain barrier and reach stable drug levels in the mouse brain[13]. BRU is also believed to produce anti-nociceptive effects in a hot-plate test through central mechanisms[12]. In view of the above, it is likely that BRU may exert its inhibitory actions on ethanol drinking in the central nervous system.

Electrophysiological studies show that ethanol can enhance glycine receptor function in mouse and chick embryonic spinal neurons in a concentration-dependent manner[42]. Meanwhile, Mascia *et al* found that ethanol could potentiate homomeric α1 or α2 glycine receptors expressed in *Xenopus oocytes*[19]. Ethanol has its enhancing effects on glycine receptor function mainly by increasing burst durations[43]. The basic mechanism of alcohol appears to be its antagonism of glycine unbinding from the glycine receptors[43]. Ethanol can increase glycine-mediated chloride uptake into rat brain synaptoneurosomes[44]. Work by Jonsson *et al* found that α1 subunit expression in the nucleus accumbens is related to ethanol intake in ALKO Alcohol (AA) rats[45]. Microinjection of glycine into the ventral tegmental area selectively decreases ethanol consumption in rats[46]. However, there is currently no report showing a synthetic glycine receptor ligand can specifically decrease ethanol consumption. BRU acts as an antagonist at the α1 and α1β glycine receptors in human embryonic kidney 293 cells with K_i values (μmol/L) of 1.7 and 1.4, respectively[20]. Therefore, it is possible that ethanol and BRU modulate behavior via opposing actions on the glycine receptors of rats. If so, then glycine receptors could be an interesting drug target for the treatment of alcoholism[46]. Bilateral accumbal microinjection of glycine enhances the DA-activating effects of ethanol, thus decreasing ethanol self-administration in Wistar rats[47]. Given the complexity of the neural circuitry that participates in the control of alcohol reinforcement, it may not be surprising that systemic versus targeted injection of glycine receptor ligands produce inconsistent behavioral effects, and thus further highlight the complicated role of glycine receptors in alcohol use. BRU also binds to serotonin 5-HT_3 receptors with a K_i value of 6.2 μmol[20]. It is well known that 5-HT_3 receptor antagonists such as ondansetron, block alcohol stimulated DA release in the mesolimbic system and attenuate ethanol consumption during acquisition, maintenance and deprivation in male P rats[48]. Studies demonstrate that antimuscarinic agents induce a comparable decrease in alcohol intake in FH/Wjd and P rats[49]. BRU is also an allosteric modulator of muscarinic acetylcholine receptors[50]. Thus, 5-HT_3 and muscarinic receptor antagonism could be two other potential mechanisms that may mediate the pharmacological effects of BRU observed in the current study.

In conclusion, this study found that BRU exerts marked and specific effects, reducing ethanol drinking and preference

behaviors in a strain of alcohol-preferring rats, with little evidence for the development of tolerance. BRU has a good safety profile in preliminary toxicology studies. Because alcohol abuse and alcoholism remain significant psychiatric disorders that can be resistant to current treatments, these preclinical findings encourage further examination of BRU for treating alcohol use disorders.

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Author contribution

Yu-ling LI, Jian-hui LIANG, and Andrew J LAWRENCE contributed to the research design; Yu-ling LI, Qing LIU, Qi GONG, Shou-peng WEI, Yan-ting WANG, Hui LIANG, Min ZHANG, and Li JING performed the experiments; Yu-ling LI and Jian-hui LIANG performed the data analysis; Yu-ling LI, Jun-xu LI, Zheng YONG, Andrew J LAWRENCE, and Jian-hui LIANG wrote or contributed to the writing of the manuscript.

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