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Effects of the benzodiazepine $GABA_A$ α1-preferring antagonist 3-isopropoxy-β-carboline hydrochloride (3-ISOPBC) on alcohol seeking and self-administration in baboons*

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Abstract

Background—The major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), modulates many of the behavioral effects of alcohol, including sedation, tolerance, and withdrawal. The α1 subunit of the benzodiazepine $GABA_A$ receptor is the most widely expressed alpha subunit in the brain, and has been implicated in the reinforcing- and abuse-related effects of alcohol. The aim of the present study was to examine whether treatment with a benzodiazepine $GABA_A$ α1-preferring ligand, 3-isopropoxy-β-carboline hydrochloride (3-ISOPBC), selectively decreases alcohol seeking and consumption.

Methods—Eight baboons self-administered alcohol (4% w/v; n=5; alcohol group) or a non-alcoholic beverage (n=3; control group) in Component 3 of a chained schedule of reinforcement. Responses in Component 2 provided indices of motivation to drink (seeking). Doses of 3-ISOPBC (5.0 – 30.0 mg/kg) and vehicle were administered before drinking sessions under both acute and chronic (5 day) conditions.

Results—Chronic, and not acute, administration of 3-ISOPBC significantly decreased self-administration responses, g/kg alcohol consumed, and the number of drinks in and duration of the first drinking bout in the alcohol group. In the control group, chronic administration of 3-ISOPBC did not significantly decrease any of these measures at any of the doses.

Conclusions—The $GABA_A$ α1-preferring ligand 3-ISOPBC may have therapeutic potential in the treatment of alcohol use disorder due to its ability to selectively reduce alcohol use.

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Contributions. All authors have contributed to the research and the article preparation. Holtyn performed the data analyses, wrote the first draft of the manuscript, and incorporated edits from the co-authors. Weerts contributed to the original conceptualization and design of the experiments, directed the present experiments, and edited the manuscript. Cook directed the development of the novel compound used in these experiments and contributed to the original conceptualization and design of the experiments. Tiruveedhula and Stephen synthesized the novel compound used in the experiments and edited the manuscript. All authors have approved the final manuscript.

Conflict of Interest. The authors have no conflicts of interest to declare.

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Keywords

Alcohol; 3-isopropoxy-β-carboline hydrochloride; 3-ISOPBC; Self-Administration; Baboon

1. Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and is an important target in the development of pharmacotherapies for alcohol use disorder. GABA binds to type A receptors, which have been implicated in the acute and chronic effects of alcohol, including sedation, tolerance, and withdrawal as well as the motivational effects of alcohol, including alcohol reinforcement and consumption (for reviews, see Enoch, 2008; Kumar et al., 2009; Lobo and Harris, 2008). $GABA_A$ receptors are composed of five subunits that form a central chloride channel and can belong to different subunit classes: α(1-6), β(1-3), γ(1-3), δ, ε, π, θ, and ρ(1-3). While many $GABA_A$ receptors are composed of one γ, two α, and two β subunits, the various subunit classes allow for extensive heterogeneity in receptor subunit composition. The subunit composition is a major determinant of the pharmacological profile of the receptor and the presence or absence of certain subunits may regulate specific behavioral effects of drugs such as alcohol (Olsen and Sieghart, 2009).

The α1 subunit of the $GABA_A$ receptor may play a role in the reinforcing- and abuse-related effects of alcohol. Knockout mice without the $GABA_A$ α1 receptor have been shown to consume less alcohol under a two-bottle alcohol versus water choice procedure and to respond less for alcohol under an operant self-administration procedure, although these were accompanied by reductions in saccharin and sucrose consumption (Blednov et al., 2003; June et al., 2007). In rodents bred for high alcohol intake, benzodiazepine $GABA_A$ α1-preferring antagonists – 3-propoxy-β-carboline hydrochloride (3-PBC) and β-carboline-3-carboxylate-tert-butyl ester (βCCT) – decreased alcohol intake when administered systemically or through microinfusions into the ventral palladium (Harvey et al., 2002; June et al., 2003). In primates, chronic administration of 3-PBC significantly decreased self-administration responses, volume consumed, and g/kg alcohol intake but also had some effects on self-administration of a non-alcoholic reinforcer in one study (Kaminski et al., 2013). In another study, chronic administration of 3-PBC and βCCT did not decrease alcohol intake or blood alcohol levels (Sawyer et al., 2014).

It is clear from the work of Licata et al (2009) in primates, June et al. (2003) and Harvey et al (2002) in rodents, and Platt et al. (2002) in rhesus macaques, that both βCCT and 3-PBC have been shown to be α1-preferring antagonists in vivo. Moreover, both βCCT and 3-PBC have been shown to be potent antagonists in vitro (Harvey et al., 2002; Yin et al., 2010). Based on these and the above data, the synthesis of an analog of 3-PBC, 3-isopropoxy-β-carboline hydrochloride (3-ISOPBC), was undertaken (Tiruveedhula et al., 2015). This choice was guided by molecular modeling (He et al., 2000; Huang et al., 2000) wherein it is well established that a major change in the structure of a benzodiazepine $GABA_A$ (α1-6β2/3γ2) receptor subunit selective ligand can dramatically alter the subunit selectivity (Clayton et al., 2007, 2015). 3-ISOPBC displays a 7-fold selectivity for the α1 subunit over

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the α2 and α3 subunits as well as a 30-fold selectivity over the α5 subunit (supplemental Table 1s[1]). 3-PBC binding affinities have been published previously (Harvey et al., 2002). 3-ISOPBC did not bind to any other receptors in the 43 receptor panel tested (supplemental Table 2s[2]) in the psychoactive drug screening program (UNC, B. Roth; Besnard et al., 2012; Huang et al., 2010), analogous to the ligand 3-PBC. In addition, 3-ISOPBC did not exhibit sedative nor ataxic activity, as illustrated by results from rotarod testing (supplemental Figure 1s[3]). Even though 3-ISOPBC binds more potently to α1 receptors (supplemental Table 1s[4]), it is clear from the rotarod data that it does not affect positive allosteric modulation at benzodiazepine α1 $GABA_A$ receptors. The ligand 3-PBC also had no agonist activity at α1 subunits, even though it binds more potently to the α1 subunit than other DS sites (Yin et al., 2010). The choice of 3-ISOPBC was also based on the structure of the branched isopropyl group, which would hinder metabolism by beta (omega-1) oxidation. Since 3-ISOPBC may undergo phase 1 metabolism by cytochrome P450 enzymes – potentially by beta (omega-1) oxidation of the linear n-propyl group in 3-PBC (Foye et al., 2013) – at a much slower rate than 3-PBC, it was hypothesized that this would increase the duration of action and provide a ligand more active in vivo than 3-PBC.

The present study investigated whether acute and chronic administration of 3-ISOPBC could selectively reduce alcohol seeking and self-administration in baboons. The baboons consumed alcohol daily under a chained schedule of reinforcement at levels that produce blood alcohol levels exceeding 0.08%. The use of the chain schedule allows for examination of drug effects on responding in the presence of alcohol-related cues that is maintained by conditioned reinforcement (i.e., responding that produces access to alcohol or “seeking”), as well as alcohol self-administration within the same session. To determine the specificity of effects on alcohol-related behaviors, chronic administration of 3-ISOPBC was also conducted with baboons that self-administered a preferred, non-alcoholic beverage under the chained schedule.

2. Material and Methods

2.1. Subjects

Eight singly-housed adult male baboons (*Papio anubis*; Southwest Foundation for Biomedical Research, San Antonio, TX) weighing on average 28.1 kg (+ 4.2 SD) served as subjects. For the alcohol group (N=5), the reinforcer delivered was 4% w/v alcohol. For the control group (N=3), the reinforcer delivered was a preferred non-alcohol beverage (orange-flavored, sugar-free Tang®), diluted to a concentration that functioned as a comparable reinforcer (Duke et al., 2014). All baboons had extensive histories of self-administration of either alcohol or the non-alcoholic beverage under the chained schedule of reinforcement as reported previously (Duke et al., 2014; Holtyn et al., 2014; Kaminski and Weerts, 2014; Kaminski et al., 2008, 2012, 2013). Each day, the baboons were fed standard primate chow that was adjusted to maintain sufficient caloric intake for normal baboons of their size, age,

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and activity level (about 50-73 kcals/kg); fresh fruit or vegetables; and a children's chewable multivitamin. Water was available ad libitum except during sessions. The housing room was maintained under a 12–hour light/dark cycle (lights on at 6:00 AM). Facilities were maintained in accordance with USDA and AAALAC standards. The protocol was approved by the JHU Animal Care and Use Committee and followed the Guide for the Care and Use of Laboratory Animals (2011).

2.2. Apparatus

Sessions were conducted in modified primate cages as described in detail previously (Kaminski et al., 2008; Weerts et al., 2006) and contained (1) a panel with three colored "cue" lights, (2) an intelligence panel with two vertically operated levers and two different colored "jewel" lights each located above one of the levers, (3) a "drinkometer" connected to a calibrated 1000-ml bottle, and (4) a speaker mounted above the cages for presentation of auditory stimuli (tones). A computer interfaced with Med Associates hardware and software remotely controlled the experimental conditions and data collection.

2.3. Chained Schedule of Reinforcement Procedure

Sessions were conducted seven days per week and began at the same time (8:30 AM) each day. The start of a session and the onset of Component 1 was signaled by a 3-s tone. During Component 1, a red cue light was illuminated and all instrumental responses were recorded but had no programmed consequence. After 20 min, Component 1 ended and Component 2 was initiated.

Component 2 was signaled by the illumination of a yellow cue light and consisted of two links. During the first link, the jewel light over the left lever was turned on, and a concurrent fixed interval 10 min, fixed time 20 min (FI 10-min FT 20-min) schedule was in effect. The first link ended either a) with the first response on the left lever after 10 min elapsed or b) automatically after 20 min, whichever occurred first. During the second link, the jewel light over the left lever flashed and a fixed-ratio (FR) 10 schedule was in effect on the left lever. Completion of the FR response requirement ended Component 2; the yellow cue light and the jewel light were turned off and Component 3 was initiated. If the FR 10 requirement was not completed within 90 min, the session terminated without transitioning to Component 3 (i.e., no access to alcohol or the non-alcoholic beverage for the day).

Component 3 was signaled by the illumination of the blue cue light. A blue jewel light over the right lever was also illuminated, and the opportunity to self-administer alcohol or the non-alcoholic beverage (depending on group assignment) was available under an FR 10 schedule on the right lever. Completion of each FR and subsequent contact with the drinkometer spout delivered fluid for the duration of spout contact or for a programmed duration (5 sec), whichever came first. This defined a single drink. Component 3 and the session ended after 120 min.

2.4. Drugs

All solutions for oral consumption were mixed using reverse osmosis (RO) purified drinking water. Ethyl alcohol (190 Proof, Pharmco-AAPER, Brookville CT) was diluted with RO

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water to 4% w/v alcohol. Orange-flavored, sugar-free, Tang® powder (Kraft Foods) was dissolved in RO water as described previously (Duke et al., 2014). The 3-ISOPBC was synthesized in the laboratory of Dr. James Cook (University of Wisconsin-Milwaukee; Tiruveedhula et al., 2015). Doses of 3-ISOPBC (5.0 – 30.0 mg/kg) were dissolved in a vehicle of 50% saline, 37.5% propylene glycol, and 12.5% ethanol and administered via the intramuscular route (2-3 mls/injection). Vehicle tests were completed using the same volume and procedures as detailed below.

2.5. 3-ISOPBC Test Procedures

The baseline stability criterion was defined as stable self-administration of alcohol or non-alcoholic beverage (i.e., ± 20%) for three consecutive sessions. To evaluate acute effects of 3-ISOPBC on alcohol-related behaviors and to verify the safety of the dose range, in Experiment 1 doses of 3-ISOPBC (10.0 – 30.0 mg/kg) or its vehicle were administered acutely in the alcohol group only. The baseline stability criterion was met before each test dose of 3-ISOPBC. Doses were tested in mixed order, with active doses tested no more than once per week. In Experiment 2 doses of 3-ISOPBC (5.0 – 20.0 mg/kg) or vehicle were administered daily for 5 consecutive days to baboons in both groups. For both experiments, doses of 3-ISOPBC were administered 30 min before sessions.

2.6. Data Analysis

The primary variables of interest included measures of seeking (Component 2: FI responses and latency to complete the FI requirement) and measures of consumption (Component 3: FR self-administration responses, drink contacts, and volume consumed). Total g/kg and ml/kg consumed were calculated based on individual body weights and the total volume of alcohol or non-alcoholic beverage consumed, respectively. The patterning of drinking was analyzed as a function of drinking “bouts” as in our previous study (Kaminski and Weerts, 2014). A drinking bout was defined as 2 or more drinks with less than 5 minutes between each drink, beginning with the first drink.

For each baboon, the mean of the 3 sessions that preceded each test condition was used as the baseline for comparison with doses of 3-ISOPBC and vehicle. To determine whether there were any differences in baseline responding in the alcohol and control groups, baseline responding in Experiment 2 was compared using independent-samples *t*-tests (baseline responding of the alcohol group in Experiment 1 is not included because corresponding control group sessions were not conducted). In Experiment 2, data analyzed were the last 3 of the 5 days of 3-ISOPBC or vehicle administration. Data were analyzed using separate statistical analysis of variance (ANOVA) for each group (Alcohol or Control) with 3-ISOPBC dose (BL, 0.0 – 30.0 mg/kg) as a repeated measure. Bonferroni post-hoc tests were used for pair-wise comparisons of vehicle with 3-ISOPBC doses.

3. Results

During baseline sessions, stable drinking was observed in all baboons, in both groups and few or no responses were recorded on the inactive operanda (all operanda in Component 1, right lever and drinkometer in Component 2, and left lever in Component 3). Systematic

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differences between the groups during baseline sessions were not observed for measures of seeking (Component 2: FI responses and latency to complete the FI requirement). During the baseline sessions preceding drug test sessions, the grand mean (+ SEM) latency to complete the FI schedule was 639.5 (20.2) seconds for the alcohol group and 609.7 (5.3) seconds for the control group [$t(6) = 1.09$, $p = .317$]. The grand mean (+ SEM) number of FI responses was 141.2 (68.4) for the alcohol group and 144.9 (96.2) for the control group [$t(6) = 0.03$, $p = .975$].

During baseline sessions, the grand mean (+ SEM) alcohol intake was 748.1 (78.6) ml and 1.07 (0.07) g/kg, comparable to intake which has previously been reported to produce blood-alcohol levels (BAL) of >0.08% in baboons (Holtyn et al., 2014; Kaminski et al., 2008). The grand mean non-alcoholic beverage intake during baseline sessions was 1000.0 (0.0) ml. While volume of intake of the non-alcoholic beverage was higher than the volume of intake of alcohol [$t(6) = 2.40$, $p = .053$], we have previously demonstrated that 4% w/v alcohol and the nonalcoholic beverage function as equivalent reinforcers (i.e., maintain similar breaking points under a progressive ratio procedure) despite the fact that they maintain different intake volumes (Duke et al., 2014).

3.1. Experiment 1: Effects of Acute Administration of 3-ISOPBC

Table 1 shows effects of acute administration of 3-ISOPBC on alcohol seeking and consumption under the chained schedule of reinforcement. Acute administration of 3-ISOPBC did not significantly change any of the measures of seeking (Component 2: FI responses and latency to complete the FI requirement) or consumption (Component 3: FR self-administration responses and g/kg alcohol consumed). Behavioral observations conducted by laboratory personnel that included the recording of any signs or symptoms of drug side effects (e.g., sedation, muscle relaxation, motor incoordination, gastro-intestinal symptoms, etc.) confirmed that administration of doses up to and including 30.0 mg/kg were safe and did not produce adverse effects. However, there was some difficulty with solubility at the 30.0 mg/kg dose. Because of this, in combination with the difficulty in synthesizing the large quantities needed for testing in baboons, the 30.0 mg/kg dose was not tested under chronic conditions. Acute administration of 3-ISOPBC was not conducted in the control group because it did not significantly reduce seeking or consumption in the alcohol group.

3.2. Experiment 2: Effects of Chronic Administration of 3-ISOPBC

Table 2 shows effects of chronic administration of 3-ISOPBC on seeking for alcohol and the non-alcoholic beverage under the chained schedule of reinforcement. Chronic administration of 3-ISOPBC did not significantly change any of the measures of seeking (Component 2: FI responses and latency to complete the FI requirement) in both the alcohol and control groups.

Figure 1 shows effects of chronic administration of 3-ISOPBC on consumption. In the alcohol group, chronic administration of 3-ISOPBC decreased the number of self-administration responses (Component 3: FR responses), with a significant decrease relative to vehicle at the 10.0 mg/kg dose. Chronic administration of 3-ISOPBC decreased g/kg alcohol consumed, with significant decreases relative to vehicle at the 10.0 and 20.0 mg/kg

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doses. In the control group, 3-ISOPBC did not reduce the number of self-administration responses or ml/kg consumed at any of the doses.

Figure 2 shows effects of chronic administration of 3-ISOPBC on the pattern of drinking in the first drinking bout. In the alcohol group, chronic administration of 3-ISOPBC decreased the number of drinks in the first drinking bout, with a significant decrease relative to vehicle at the 10.0 and 20.0 mg/kg doses. Chronic administration of 3-ISOPBC decreased the duration of the first drinking bout, with significant decreases relative to vehicle at the 20.0 mg/kg dose. In the control group, 3-ISOPBC did not reduce the number of drinks in or the duration of the first drinking bout at any of the doses.

4. Discussion

The α1 subunit of the $GABA_A$ receptor has been implicated in the reinforcing- and abuse-related effects of alcohol in some studies (Blednov et al., 2003; Harvey et al., 2002; June et al., 2007; Kaminski et al., 2013). The present study investigated whether acute and chronic administration of the benzodiazepine $GABA_A$ α1-preferring ligand 3-ISOPBC could selectively reduce alcohol seeking and self-administration in baboons. Pretreatment with 3-ISOPBC did not reduce alcohol seeking in a group that self-administered alcohol or in a control group that self-administered a non-alcoholic beverage. This is consistent with a prior similar study in which acute and chronic administration of 3-PBC did not reduce alcohol seeking in baboons responding under a chained schedule of alcohol reinforcement (Kaminski et al., 2013). Chronic, and not acute, administration of 3-ISOPBC reduced alcohol consumption in the alcohol group without reducing consumption of the non-alcoholic reinforcer in the control group. Thus, 3-ISOPBC may have therapeutic potential in the treatment of alcohol use disorder due to its ability to selectively reduce alcohol use.

Identification of the precise role of the $GABA_A$ α1 receptor in alcohol reinforcement and consumption is ongoing. Knockout mice without the $GABA_A$ α1 receptor have been shown to consume less alcohol under a two-bottle alcohol versus water choice procedure (Blednov et al., 2003) and an operant self-administration procedure (June et al., 2007). The $GABA_A$ α1-preferring antagonist 3-PBC has been shown to decrease alcohol self-administration in rodents bred for high alcohol intake (Harvey et al., 2002), as well as binge-like drinking in a maternally deprived rodent model (Gondre-Lewis et al., 2016). A similar reduction in alcohol maintained responding was observed following administration of 3-ISOPBC in the maternal deprivation model (Tiruveedhula et al., 2015). In baboons, 3-PBC reduced self-administration of alcohol but also had some effects on self-administration of a non-alcoholic reinforcer (Kaminski et al., 2013). In contrast to the findings in rodents and baboons, both 3-PBC and βCCT failed to attenuate alcohol drinking in rhesus macaques (Sawyer et al., 2014). The reason for this contradictory result is unclear; however, the highest dose of 3-PBC tested in Sawyer et al.'s study (10.0 mg/kg) was lower than that which reduced total g/kg alcohol intake in the baboon model (18.0 mg/kg). The choice of 3-ISOPBC for the present study was based on the structure of the branched isopropyl group, which would hinder metabolism by beta (omega-1) oxidation. It was hypothesized that this would increase the duration of action and provide a ligand more active in vivo than 3-PBC; this appears to be the case. In the present study, 3-ISOPBC selectively reduced alcohol drinking.

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In both the alcohol and control groups, the majority of drinks occurred within the first drinking bout. Prior studies also have shown rats (Samson et al., 2000), baboons (Weerts et al., 2006), and other primates (Boyle et al., 1998) to engage in "loading," wherein the highest rate of alcohol drinking occurs early in the alcohol self-administration period followed by lower rates of drinking for the remainder of the period. Chronic administration of 3-ISOPBC reduced the number of drinks in and the duration of the first drinking bout in the alcohol group, but not in the control group. This suggests that 3-ISOPBC may reduce alcohol intake once consumption is initiated, which could be important in preventing drinking episodes from becoming a full relapse to heavy drinking. Chronic administration of 3-PBC also has been shown to decrease the number of drinks during the first 20 minutes of alcohol availability in Component 3 of the chained schedule (Kaminski et al., 2013). In Kaminski et al.'s (2013) study, the highest doses of 3-PBC tested (10.0 and 18.0 mg/kg) also decreased the number of drinks in the first 20 minutes in a control group that self-administered a non-alcoholic beverage. Harvey et al. (2002) observed a decrease in saccharin-maintained responding when the highest dose of 3-PBC (20.0 mg/kg) was administered to rodents. The authors suggested that the non-selective effects at higher doses of 3-PBC may be due to a saturation of all α receptors as 3-PBC binds to other α receptors to some degree. It is worth emphasizing, however, that in both studies, responding maintained by the non-alcoholic reinforcer was reduced at higher doses than required to suppress alcohol self-administration. In the present study, effects of 3-ISOPBC were selective for alcohol. The mechanism by which 3-ISOPBC selectively attenuated alcohol response in the present study is not known. Possible differences between 3-PBC and 3-ISOPBC include differences in metabolism or ligand transport.

Although the mechanisms of action of the α1 -preferring antagonists are not well understood, one possibility rests on the tonic control in the central nervous system by opposing systems, including GABA. It is possible that these α1 -preferring antagonists simply stabilize the benzodiazepine α1 GABA receptor system in the antagonist conformation, the result of which would be to slightly decrease the normal flow of chloride ions through the $\alpha 1\beta_{2/3}\gamma 2$ ion channel. The effect via the projections from the ventral tegmental area (Harvey et al., 2002) to the nucleus accumbens would then effect the levels of dopamine release; this slight decrease may be why Warnock, June et al. (personal communication) did observe a decrease in alcohol self-administration in a binge drinking model (rodents) in the complete absence of anhedonia or depression. Tiruveedhula, et al. (2015) reported that 3-ISOPBC decreased alcohol consumption in a maternally deprived rodent model. Gondre-Lewis et al. (2016) reported a similar effect with 3-PBC and proposed some involvement of α2-receptor subunits; however, this effect could not be reversed by administration of flumazenil. Consequently, this cannot be due to an effect at $\alpha 2\beta_{2/3}\gamma 2$ benzodiazepine $GABA_A$ receptors. It is possible that the observed effect was mediated by a different set of α2-related receptors (Gondre-Lewis et al., 2016) or to the α1-preferring antagonist effect at benzodiazepine α1 $GABA_A$ receptors. Much work remains to understand this observation. Nevertheless, the real strength of the use of α1-preferring antagonists in the treatment of alcohol use disorder stems from the fact that this type of ligand lacks sedating, amnesic, and ataxic properties (Ator et al., 2010; Licata et al., 2009) because it is an antagonist at this α1 benzodiazepine $GABA_A$ site.

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The present study examined whether the $GABA_A$ α1-preferring ligand 3-ISOPBC possesses therapeutic potential in regard to its ability to selectively reduce alcohol seeking and consumption. Alcohol use disorders are heterogeneous and development of more efficacious and safe pharmacotherapies is needed to expand the number of individuals who may benefit from treatment. In the present study, 3-ISOPBC did not decrease alcohol seeking, but did selectively reduce alcohol self-administration and consumption by primarily altering the pattern of drinking. 3-ISOPBC selectively reduced the number of drinks and the duration of drinks in the first alcohol drinking bout. No changes in drinking patterns were found for the non-alcoholic reinforcer. These data suggest that 3-ISOPBC may be clinically useful for reducing alcohol use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Acute doses of 3-ISOPBC did not decrease alcohol seeking or consumption in baboons.
- Chronic doses of 3-ISOPBC reduced alcohol consumption.
- Chronic doses of 3-ISOPBC did not affect consumption of a non-alcoholic beverage.
- Effects of chronic doses of 3-ISOPBC on consumption were specific to alcohol.

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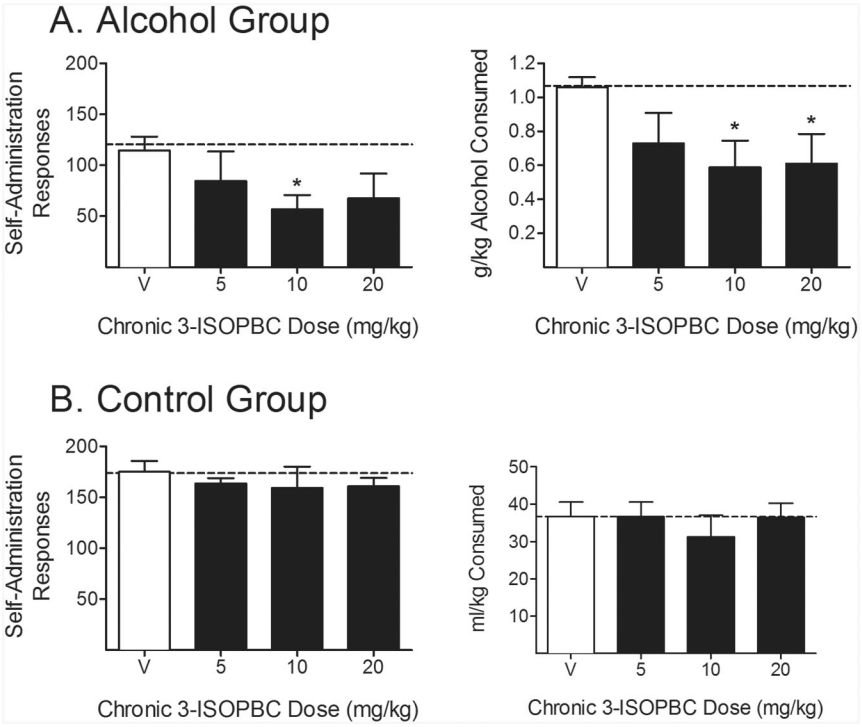

Figure 1. Experiment 2

Effects of chronic (5 day) administration of 3-ISOPBC (5.0 – 20.0 mg/kg) on consumption in Component 3 of the chained schedule of reinforcement in the (A) Alcohol Group and (B) Control Group. Data shown are the group means (+ SEM) of self-administration responses (left panels), and g/kg alcohol consumed for the alcohol group and ml/kg consumed for the control group (right panel). Baseline responding is indicated by the horizontal, dashed lines. *indicates $p < .05$ for pair-wise comparison for each 3-ISOPBC dose vs. vehicle.

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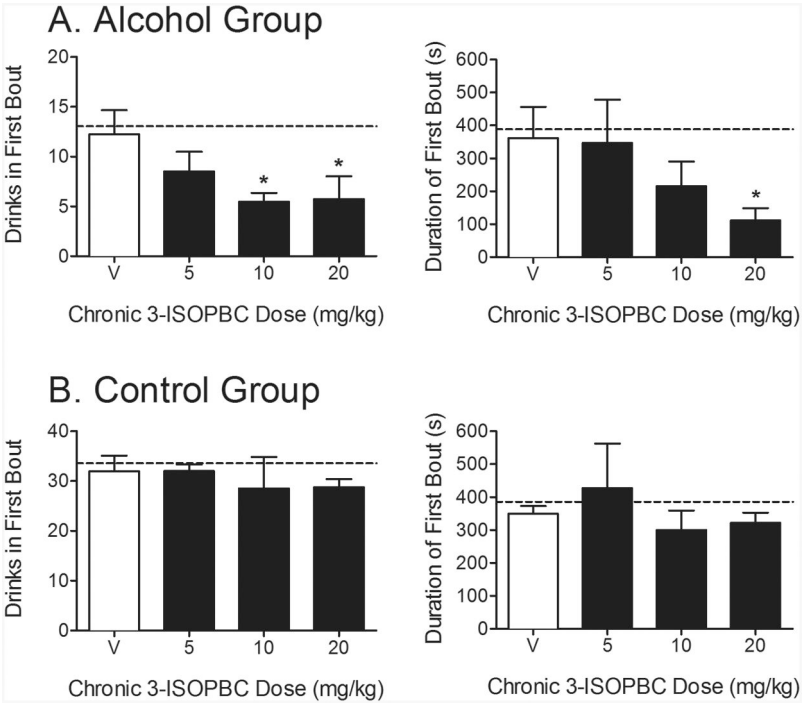

Figure 2. Experiment 2

Effects of chronic (5 day) administration of 3-ISOPBC (5.0 – 20.0 mg/kg) on the pattern of drinking in the first drinking bout in Component 3 of the chained schedule of reinforcement in the (A) Alcohol Group and (B) Control Group. Data shown are the group means (+ SEM) of the number of drinks in the first drinking bout (left panels), and the duration (seconds) of the first drinking bout (right panels). Baseline responding is indicated by the horizontal, dashed lines. *indicates $p < .05$ for pair-wise comparison for each 3-ISOPBC dose vs. vehicle.

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Table 1

Experiment 1

Effects of acute administration of vehicle (V) and doses of 3-ISOPBC on seeking in Component 2 (C2) and consumption in Component 3 (C3) for alcohol under the chained schedule of reinforcement. Baseline (BL) is the grand mean of the 3 days preceding each acute administration.

					3-ISOPBC dose (mg/kg)			
			BL	**V**	**10.0**	**20.0**	**30.0**	**F(4,16)**
C2	Left Lever FI Resp	Mean	125.5	574.8	582.8	272.0	417.0	1.91
		SEM	48.0	270.1	307.3	166.6	236.6	
	Left Lever FI Resp Latency (s)	Mean	632.3	625.4	615.1	629.9	613.6	0.46
		SEM	24.8	19.2	13.7	13.2	10.7	
C3	Right Lever FR Resp	Mean	117.9	126.2	108.0	109.4	106.0	0.82
		SEM	14.6	12.6	13.0	16.1	19.5	
	g/kg Alc Consumed	Mean	1.1	1.2	1.1	1.0	1.0	0.60
		SEM	0.1	0.1	0.1	0.1	0.1	

Note: FI, Fixed Interval; FR, Fixed Ratio; Resp, Response; Alc, Alcohol.

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Table 2

Experiment 2

Effects of chronic (5 day) administration of vehicle (V) and doses of 3-ISOPBC on seeking in Component 2 (C2) for alcohol (Alcohol Group) and a non-alcoholic beverage (Control Group) under the chained schedule of reinforcement. Baseline (BL) is the grand mean of the 3 days preceding each chronic condition.

					3-ISOPBC dose (mg/kg)			
Alcohol Group			**BL**	**V**	**5.0**	**10.0**	**20.0**	**F(4,16)**
C2	Left Lever FI Resp	Mean	141. 2	394.5	212.3	161.5	198.2	1.93
		SEM	68.4	217.4	118.1	107.4	113.1	
	Left Lever FI Resp Latency (s)	Mean	639. 5	620.4	706.0	676.5	755.3	1.05
		SEM	20.2	10.8	58.1	52.0	93.2	
Control Group			BL	V	5.0	10.0	20.0	F(4,8)
C2	Left Lever FI Resp	Mean	144. 9	128.2	200.4	154.8	75.0	1.03
		SEM	96.2	55.3	131.1	85.1	37.7	
	Left Lever FI Resp Latency (s)	Mean	609. 7	674.6	612.8	624.7	735.0	1.33
		SEM	5.3	20.7	11.3	18.3	95.1	

Note: FI, Fixed Interval; FR, Fixed Ratio; Resp, Response; Alc, Alcohol.

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