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Modulation of $\alpha 5$ subunit-containing GABA_A receptors alters alcohol drinking by rhesus monkeys

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Abstract

Background—Alcohol's ability to potentiate the activity of γ -aminobutyric acid (GABA) at GABA_A receptors has been implicated as a key mechanism underlying the behavioral effects of alcohol. The complex molecular biology of these receptors raises the possibility that particular receptor subtypes may play unique roles in alcohol's abuse-related effects and that subtype-selective ligands with therapeutic specificity against alcohol might be developed. This study evaluated the capacity of $\alpha 5$ GABA_A receptor ligands to alter selectively the reinforcing effects of alcohol.

Methods—Two groups of rhesus monkeys were trained to orally self-administer alcohol or sucrose under fixed-ratio schedules and limited daily access conditions. Additionally, following daily self-administration sessions, the behavior of each monkey was scored for both species-typical and drug-induced behaviors.

Results—Concentrations of 1–6% alcohol maintained self-administration above water levels, engendered pharmacologically-relevant blood alcohol levels ranging from 90–160 mg/dl, and produced changes in behavior typical of alcohol intoxication. Concentrations of 0.3–3% sucrose also reliably maintained self-administration. The $\alpha 5$ GABA_A receptor agonist QH-ii-066 enhanced and the $\alpha 5$ GABA_A receptor inverse agonist L-655,708 inhibited alcohol but not sucrose drinking. The changes in alcohol drinking could be reversed with the $\alpha 5$ GABA_A receptor antagonist XLI-093. However, L-655,708 increased yawning in both alcohol- and sucrose-drinkers, possibly indicative of an anxiogenic effect.

Conclusions—These findings suggest a prominent and specific role for $\alpha 5$ GABA_A receptor mechanisms in the reinforcing effects of alcohol. Moreover, these results suggest that $\alpha 5$ GABA_A

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receptors may represent a novel pharmacological target for the development of medications to reduce drinking. Of ligands modulating this receptor, $\alpha 5$ GABA_A receptor inverse agonists may hold the most promise as alcohol pharmacotherapies.

Keywords

GABA_A receptors; alcohol self-administration; monkey; pharmacotherapy; QH-ii-066; L-655,708; XLi-093; naltrexone

Introduction

Alcohol's ability to potentiate the activity of γ -aminobutyric acid (GABA) at GABA_A receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both human and non-human subjects (e.g., Hyytiä and Koob, 1995; June et al., 1995; 1998; Korpi, 1994; Söderpalm and Hansen, 1998). Molecular biological studies have shown that the mammalian GABA_A receptor is a pentamer consisting of subunits from at least five different families, including the α , β , and γ subunits (McKernan and Whiting, 1996; Pritchett et al., 1989; Rudolph et al., 2001). In addition, most of these subunits have a number of different isoforms (e.g., $\alpha 1$ - $\alpha 6$, $\beta 1$ - $\beta 3$, $\gamma 1$ - $\gamma 3$; Olsen and Sieghart, 2008). The complex molecular biology of GABA_A receptors raises the possibility that subtype-selective agents with therapeutic specificity against alcohol might be developed.

GABA_A receptors expressing the $\alpha 5$ subunit ($\alpha 5$ GABA_A receptors) comprise only a small proportion of native GABA_A receptors and are found primarily in the hippocampus (Sur et al., 1999; Uusi-Oukari and Korpi, 2010). Projections from the CA1 and CA3 hippocampal fields have been shown to innervate brain regions implicated in the reinforcing effects of alcohol (e.g., Sesack and Grace, 2010). Several lines of evidence support a potential role for $\alpha 5$ GABA_A receptor mechanisms in the abuse-related effects of alcohol. In this regard, using data from the Collaborative Study on the Genetics of Alcoholism, Song and colleagues (2003) reported a significant association between *GABRA5* (the gene encoding the $\alpha 5$ subunit of the GABA_A receptor) and alcohol dependence. In alcohol discrimination studies in squirrel monkeys, we have shown that the $\alpha 5$ GABA_A receptor-selective agonists QH-ii-066 and panadiplon mimicked the discriminative stimulus effects of alcohol (Platt et al., 2005). Moreover, the discriminative stimulus effects of alcohol and the alcohol-like discriminative stimulus effects of QH-ii-066 were dose-dependently blocked by L-655,708, an $\alpha 5$ GABA_A receptor-selective inverse agonist (Platt et al., 2005). With respect to the reinforcing effects of alcohol, intrahippocampal or i.p. administration of other $\alpha 5$ GABA_A receptor-selective inverse agonists decreased alcohol self-administration in rats at doses that did not disrupt self-administration of saccharin or water (June et al., 2001; McKay et al., 2004). Finally, mice lacking the $\alpha 5$ subunit of the GABA_A receptor show both reduced preference for and reduced consumption of alcohol (Boehm et al., 2004).

The purpose of the present study was to evaluate the contribution of $\alpha 5$ GABA_A receptor mechanisms to the reinforcing effects of alcohol in rhesus monkeys. We determined the ability of $\alpha 5$ GABA_A receptor-selective ligands to augment and/or attenuate alcohol self-administration. The selective ligands included the agonist QH-ii-066, the antagonist XLi-093 and the inverse agonist L-655,708. Results with the $\alpha 5$ GABA_A receptor ligands were compared with results obtained with naltrexone, an FDA approved pharmacotherapy to treat alcohol dependence. Drugs that reduce alcohol self-administration may have potential as candidate anti-alcohol medications. An important consideration in the development of an alcohol pharmacotherapy is the pharmacological specificity of the candidate medication's effects. One technique used to evaluate the specificity of a medication's effect is to compare the doses of a drug to reduce behaviour maintained by alcohol relative to behaviour

maintained by a non-drug reinforce. To that end, we investigated the extent to which L-655,708 modulated the reinforcing effects of alcohol at doses that did not alter sucrose self-administration. Finally, compliance with any medication regimen is tied closely with the extent to which the medication produces untoward side effects. We evaluated the observable behavioral effects of $\alpha 5\text{GABA}_A$ receptor ligands in both alcohol- and sucrose-drinking monkeys to assess the degree to which the drugs elicited behavioral effects that might limit their clinical utility (e.g., sedation, anxiety, convulsions).

Materials and Methods

Subjects

Nine adult male rhesus monkeys (*Macaca mulatta*), weighing 9.5 to 12.1 kg, were studied in daily experimental sessions. Three of the monkeys (two assigned to the alcohol group; one assigned to the sucrose group) had prior experience in operant i.v. self-administration (either cocaine or midazolam) procedures. The remaining monkeys were experimentally naive. Monkeys were housed in a colony room with a 12-h light/dark cycle. Monkeys had unrestricted access to water via the standard cage-associated automatic sipper line and were fed a diet of monkey chow (Harlan Teklad Monkey Diet, Harlan Teklad, Madison, WI), supplemented with fresh fruit. All animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the “Guide for Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Apparatus

Monkeys were housed individually in stainless steel primate cages that also served as the experimental chambers. One side of the cage was customized to form an operant drinking panel. The panel consisted of two retractable sippers (model #: ENV-652AM; Med Associates, Inc., Georgia, VT) equipped with solenoids to minimize dripping. Each sipper was connected with Tygon tubing to a stainless steel liquid reservoir mounted on the outside of the drinking panel. A response lever (Med Associates, Inc., Georgia, VT) was positioned below each sipper. Each press of a lever produced an audible click and was recorded as a response. Colored lights mounted above the sippers could be illuminated to serve as visual stimuli. In these experiments, only one side of the drinking panel was active.

Alcohol self-administration

Five monkeys were trained to respond under a fixed-ratio (FR) schedule of oral alcohol reinforcement. In the presence of a white light, completion of every 10th response (FR 10) resulted in a change in illumination from white to red light and extension of the drinking spout. Displacement of the drinking spout up, down, left or right during extension resulted in alcohol delivery. Fluid flow continued for as long as the monkey displaced the spout or for 30-s, whichever was shorter. Within the 30-s sipper extension time, the monkey could stop drinking (i.e., release the spout) and resume drinking (i.e., displace the spout) as many times as it wanted. The actual duration and volume of intake, within the constraints of the schedule, were under the control of the subject. Between extensions of the drinking spout, all lights were off briefly, and responses had no scheduled consequences. Daily self-administration sessions lasted 3-h. The reinforcing effects of a range of concentrations of alcohol (0.5 – 16% w/v), as well as water, were evaluated initially in each monkey. Each alcohol concentration (or water) was made available for a minimum of five consecutive self-administration sessions and until stable intake was observed (i.e., no upward or downward trend in mls and/or g/kg consumed over a period of at least three consecutive days).

The effects of pretreatment with varying doses of the $\alpha 5\text{GABA}_A$ agonist QH-ii-066 (0.1 – 5.6 mg/kg), the $\alpha 5\text{GABA}_A$ antagonist XLi-093 (0.03 – 0.3 mg/kg) or the $\alpha 5\text{GABA}_A$ inverse agonist L-655,708 (0.1 – 1.8 mg/kg) and their vehicles were determined on self-administration of a 2% alcohol solution. This alcohol concentration was chosen because it maintained intakes significantly higher than that of water. In addition, it was on the ascending limb of the alcohol concentration-response function, allowing for detection of both increases and decreases in intake as a consequence of drug pretreatment. Each dose of QH-ii-066, XLi-093 and L-655,708 was studied for a minimum of five consecutive sessions and until intake was stable. Between evaluation of different doses of the $\alpha 5\text{GABA}_A$ ligands, monkeys were returned to baseline self-administration (i.e., a minimum of five days of self-administration of a 2% alcohol solution in the absence of any pretreatment). This step allowed assessment of baseline levels of intake over the course of the study. The $\alpha 5\text{GABA}_A$ ligands were studied in random order in individual animals, and all doses of a given ligand were studied before moving on to another drug. For comparison, the effects of the opioid antagonist naltrexone (0.01 – 0.3 mg/kg) also were determined.

Subsequently, maximally effective doses of QH-ii-066 (0.3 mg/kg) and L-655,708 (1.8 mg/kg) and their vehicles were given as pretreatments before sessions in which a wider range of alcohol concentrations were made available for self-administration. These studies determined the degree to which the ligands altered the shape and/or position of the alcohol concentration-response function. Finally, the capacity of XLi-093 to reverse the effects of QH-ii-066 and L-655,708 was determined. In all instances, the effects of the $\alpha 5\text{GABA}_A$ ligands at each alcohol concentration were studied for a minimum of five consecutive sessions and until intake was stable.

Sucrose self-administration

A separate group of four monkeys was trained to respond under a FR 10 schedule of oral sucrose reinforcement. This schedule and solution delivery was identical to that for alcohol self-administration, except that a sucrose solution instead of alcohol was available for self-administration. The reinforcing effects of a range of concentrations of sucrose (0.1 – 3% w/v), as well as water, were evaluated initially in each monkey. Each sucrose concentration (or water) was made available for a minimum of five consecutive self-administration sessions and until stable intake was observed.

The effects of pretreatment with varying doses of the QH-ii-066 or L-655,708 and their vehicles were determined on self-administration of a 0.3% sucrose solution. This sucrose concentration was chosen because it maintained intakes that were significantly higher than that of water and roughly equivalent to intakes maintained by 2% alcohol in the alcohol self-administration group. Each dose of QH-ii-066 and L-655,708 was studied for a minimum of five consecutive sessions and until intake was stable. Between evaluation of different doses of the $\alpha 5\text{GABA}_A$ ligands, monkeys were returned to baseline self-administration. The $\alpha 5\text{GABA}_A$ ligands were studied in irregular order in individual animals, and all doses of a given ligand were studied before moving on to another test drug.

Observable behavior

Following daily self-administration sessions, the behavior of each monkey (regardless of whether the animal self-administered alcohol or sucrose) was scored during a five-min sampling period using a focal animal approach as described in Platt et al. (2000; 2002) and as modified for the rhesus monkey. That is, after the self-administration session, an observer who was not informed about the drugs under investigation watched a specific animal for five min. During this sampling period, the observer recorded each instance a particular behavior (Table 1) occurred in 15-s intervals during the period. Scores were calculated from these

data as the number of 15-s intervals in which a particular behavior occurred (maximum possible score for each behavior was 20). A randomization schedule was employed so that order effects between animals were minimized. Eight observers performed the scoring across the duration of the study. Typically, two observers were used daily – one observing the alcohol drinkers, one observing the sucrose drinkers. To ensure reliability across observers, each individual underwent at least 20 h of training until they reached an inter-observer reliability criterion of 90% based on percent agreement scores calculated between all possible pairs.

Blood alcohol levels (BAL)

At select time points across the study (e.g., once stable intake was obtained at a particular alcohol concentration or after a particular drug treatment), monkeys self-administering alcohol were anesthetized with ketamine immediately following the session and three – five mls of blood were collected from the femoral vein. Samples were immediately centrifuged at 3200 rpm for 8–12 minutes. The plasma was drawn off, transferred to polypropylene tubes, and then frozen until analysis. Triple determinations of BALs were conducted using a rapid high performance plasma alcohol analysis using alcohol oxidase with an AM1 series analyzer and Analox Kit GMRD-113 (Analox Instruments USA, Lunenburg, MA). This process detects BAL ranges from 0 to 350 mg/dl using an internal standard of 100 mg/dl.

Data analysis

After each alcohol self-administration session, intake for individual subjects was determined in two ways: volume (mls) and alcohol dose (g/kg) consumed. Dose was calculated as: [volume consumed (mls) $\times$ alcohol concentration (g/ml)]/weight (kg). After each sucrose self-administration session, intake for individual subjects was the volume consumed (mls). Data are expressed as mean intake over the last three sessions of water or alcohol/sucrose availability. Because the intake measurements for alcohol self-administration are correlated, with the exception of Fig. 1, we present only the data representing alcohol dose. A one-way repeated measures ANOVA was used to evaluate the effect of alcohol/sucrose concentration on total volume consumed.

To compare the effects of the $\alpha 5\text{GABA}_A$ ligands and naltrexone on alcohol self-administration, intakes following pretreatment with QH-ii-066, XLi-093; L-655,708 or naltrexone were converted to % of baseline intake. Baseline intake was considered to be the mean dose of alcohol consumed across the three days of no pretreatment immediately prior to tests with a given dose of a compound. Separate one-way repeated measures ANOVAs followed by Bonferroni t-tests compared the effects of the pretreatment drugs to the effect of vehicle on intake. In curve-shifting experiments, separate two-way repeated measures ANOVAs compared the effects of either QH-ii-066 or L-655,708, alone or combined with XLi-093, to the effect of vehicle on intake.

To compare the effects of the $\alpha 5\text{GABA}_A$ ligands on sucrose self-administration, intakes following pretreatment with QH-ii-066 or L-655,708 were converted to % of baseline intake. Baseline intake was considered to be the mean mls of sucrose consumed across the three days of no pretreatment immediately prior to tests with a given dose of a compound. Separate one-way repeated measures ANOVAs compared the effects of the pretreatment drugs to the effect of vehicle on intake.

Observational data were treated in two ways. To evaluate the effect of increasing intake of alcohol on observable behavior, frequency scores for each behavior were averaged across the alcohol self-administration subjects to obtain group means. Mean scores ($\pm$ SEM) for each behavior then were plotted as a function of intake. For these analyses, intake (g/kg) was

converted to “number of drinks”. We use an operational definition of a “drink” as 0.25 g/kg alcohol or 17 g alcohol in a 70 kg human, representing the upper range of worldwide definitions of a drink (cf. Grant and Bennett, 2003; Kalant and Poikolainen, 1999). To determine statistical reliability of treatment effects on each behavior, the effect of drink number was determined by separate one-way ANOVAs. Only the behavioral data generated during determination of the alcohol-concentration response function were included in these analyses. To evaluate the effect of $\alpha 5\text{GABA}_A$ ligands on observable behavior, frequency scores for each behavior were averaged separately across the alcohol self-administration subjects and the sucrose self-administration subjects to obtain means. For each group, mean scores ($\pm$ SEM) for each behavior were plotted as a function of dose. For normally distributed data (as assessed using the Shapiro-Wilk test), separate two-way mixed factor ANOVAs determined the effects of the between-group factor (alcohol vs. sucrose) and within-group factor (dose) and their interaction on each behavior. In all cases, significant main effects were examined post-hoc with Bonferroni t-tests. The alpha level for all tests was $P = 0.05$.

Drugs

Ethanol (95%; Pharmco Products, Brookfield, CT) and diluted to the appropriate concentrations with tap water. QH-ii-066 and XLi-093 were synthesized as described in Huang et al. (2000) and Li et al. (2003), respectively. These compounds were dissolved in 50–80% propylene glycol/20–50% 0.9% saline solution. L-655,708 (Tocris Bioscience, Ellsville, MO) was dissolved in 50% propylene glycol/50% 0.9% saline solution. Naltrexone HCl (Sigma/RBI, St. Louis, MO) was dissolved in 0.9% saline solution. Intramuscular injection volumes were 0.02 – 0.05 ml/kg body weight. Pretreatment times were 10 min for the $\alpha 5\text{GABA}_A$ ligands and 30 min for naltrexone.

Results

Alcohol self-administration

Oral presentation of alcohol maintained self-administration in all monkeys under the FR schedule of alcohol delivery and limited access conditions (Fig. 1). As the concentration of alcohol was increased, intake first increased and then decreased, resulting in an inverted u-shaped concentration-response function. For the group, alcohol concentrations between 1 and 6% (w/v) maintained intakes significantly higher than that during water availability (Fig. 1A; $F(7,28)=17.6$, $P<0.001$, Bonferroni t-tests, $P<0.05$). At these concentrations, monkeys drank approximately 0.8 – 1.9 g/kg of alcohol (Fig. 1B). Analysis of blood samples verified that the volumes consumed when concentrations $\geq 2\%$ (w/v) were available produced BALs in excess of 80 mg/dl in all monkeys (Fig. 1C).

In addition to producing orderly effects on drinking behavior, alcohol altered several observable behaviors (Fig. 2). All five self-administration monkeys had self-administration episodes resulting in intake of 1 – 8 drinks; while three of the five monkeys had self-administration episodes resulting in intake of nine or more drinks. These higher intakes were observed after 13% of the self-administration sessions. While intakes below the equivalent of 7 drinks failed to alter any observable behavior, intakes of at least 7 drinks produced reliable changes in measures of passive visual, locomotion, tactile/oral exploration, stereotypy, scratching, and ataxia compared to levels of these behaviors observed during water availability. Locomotion, across a range of 7 – 9 drinks, was significantly increased compared to levels observed during water availability ($F(10,716)=6.2$, $P<0.001$, Bonferroni t-tests, $P<0.05$). At the highest intake (≥ 10 drinks), though, locomotion returned to water levels, likely coinciding with a significant increase in passive visual behavior ($F(10,716)=2.0$, $P<0.05$, Bonferroni t-tests, $P<0.05$). During water availability, ataxia was

not observed. However, a significant drink-dependent increase in ataxia occurred at intakes of 8 drinks ($F(10,716)=4.0$, $P<0.001$, Bonferroni t-tests, $P<0.05$). Significant drink-dependent decreases in behaviors also were observed. At intakes of 7 drinks, scratching was significantly reduced compared to water levels ($F(10,716)=8.0$, $P<0.001$, Bonferroni t-tests, $P<0.05$). Alcohol also produced an overall drink-dependent decrease in levels of tactile/oral exploratory behavior ($F(10,716)=2.2$, $P<0.05$, Bonferroni t-tests, n.s.) and stereotypic behavior ($F(10,716)=2.4$, $P<0.01$, Bonferroni t-tests, n.s.). Alcohol had no other significant effects on observable behavior over the range of intakes observed during determination of the alcohol concentration-response function.

$\alpha 5\text{GABA}_A$ ligands and alcohol self-administration

Daily pretreatment with the $\alpha 5\text{GABA}_A$ ligands or naltrexone produced qualitatively different effects on alcohol (2% w/v) intake depending on the compound (Fig. 3). For example, the two lowest doses (0.1 and 0.3 mg/kg) of QH-ii-066 induced small but significant increases in alcohol consumption (125 – 135% of the baseline intake of 1.4 ± 0.2 g/kg) compared to vehicle ($F(5,20)=18.2$, $P<0.001$, Bonferroni t-tests, $P<0.05$). At higher doses of QH-ii-066, intake returned to baseline levels and did not differ from that following vehicle administration. Problems with solubility prohibited testing higher doses of QH-ii-066. In the case of XLi-093, none of the doses evaluated altered self-administration of alcohol compared to vehicle, and intake was maintained at baseline levels (1.8 ± 0.02 g/kg). In contrast, daily pretreatment with L-655,708 resulted in dose-dependent reductions in alcohol self-administration compared to vehicle ($F(4,16)=37.6$, $P<0.001$), with the two highest doses (1 and 1.8 mg/kg) producing significant decreases in intake (Bonferroni t-tests, $P<0.05$). At the highest dose of L-655,708, intake was suppressed maximally to approximately 40% of the baseline intake of 1.6 ± 0.2 g/kg. The suppression of alcohol self-administration by L-655,708 was similar to that induced by naltrexone. As expected, naltrexone engendered dose-dependent decreases in alcohol self-administration that reliably differed from vehicle at doses of 0.1 and 0.3 mg/kg ($F(4,16)=7.7$, $P<0.01$, Bonferroni t-tests, $P<0.05$). The highest dose of naltrexone maximally suppressed alcohol self-administration to 38% of the baseline intake of 1.7 ± 0.2 g/kg. In general, for all drugs, the effect observed on the first day of a given pretreatment was similar to that observed on the final day of administration (5 – 7 days), providing no evidence of the development of tolerance to the effects of QH-ii-066, L-655,708 or naltrexone (data not shown).

Based on the results of the study described above, doses of the $\alpha 5\text{GABA}_A$ ligands that maximally altered alcohol self-administration were studied in combination with a wider range of alcohol concentrations (Fig. 4). In general, pretreatment with QH-ii-066 (0.3 mg/kg) enhanced self-administration of 0.5 – 4% (w/v) alcohol solutions and resulted in a leftward shift in the ascending limb of the alcohol concentration-response function (Fig. 4, left). By and large, this leftward shift could be reversed if the animals also received a pretreatment of the highest dose of XLi-093 (0.3 mg/kg). These findings are confirmed by a significant alcohol concentration $\times$ treatment interaction ($F(6,24)=5.2$, $P<0.01$, Bonferroni t-tests, $P<0.05$). In contrast, pretreatment with L-655,708 (1.8 mg/kg) attenuated self-administration of 1 – 6% (w/v) alcohol solutions and resulted in an overall rightward shift in the ascending limb of the alcohol concentration-response function (Fig. 4, right). As with QH-ii-066, pretreatment with the highest dose of XLi-093 completely reversed the L-655,708-induced rightward shift in the alcohol concentration-response function. These findings are confirmed by a significant alcohol concentration $\times$ treatment interaction ($F(6,24)=3.9$, $P<0.01$, Bonferroni t-tests, $P<0.05$).

α 5GABA_A ligands and sucrose self-administration

Oral presentation of sucrose maintained self-administration in all monkeys under the FR schedule of sucrose delivery and limited access conditions (Fig. 5A). As the concentration of sucrose was increased, intake also increased. Patterns of responding were comparable to those observed during alcohol self-administration (data not shown). For the group, sucrose concentrations between 0.3 and 3% (w/v) maintained intakes significantly higher than that during water availability ($F(4,12)=9.5$, $P<0.01$, Bonferroni t-tests, $P<0.05$). At 0.3% (w/v), individual subjects displayed intakes ranging from ~450 to 1100 mls. The average intake ($\pm$ SEM) for the group at this concentration was 839 ± 157 mls and did not differ significantly from the intake of the alcohol self-administration group at 2% (w/v) (906 ± 92 mls; $t(7)=0.4$, $P=n.s.$). Daily pretreatment with either QH-ii-066 or L-655,708 did not markedly alter sucrose self-administration at any dose compared to their respective vehicles (Fig. 5B).

α 5GABA_A ligands and observable behavior

In the absence of drug pretreatment and under water availability, the alcohol- and sucrose-drinking monkeys did not differ from each other for the majority of behaviors (Supplemental Figure 1). However, the sucrose drinkers did exhibit significantly higher levels of tactile/oral exploration ($t(7)=-4.3$, $P<0.005$) and locomotion ($t(7)=-2.4$, $P<0.05$) and tended to present more ($t(7)=-2.5$, $P<0.05$). Neither QH-ii-066 nor L-655,708, at any dose evaluated, markedly altered the majority of observable behaviors. Likewise, naltrexone failed to alter any of the behaviors. There were, however, two exceptions (Fig. 6A and 6B). QH-ii-066 dose-dependently decreased tactile/oral exploration in both the alcohol-drinking and sucrose-drinking monkeys, with a dose of 3 mg/kg significantly reducing this behavior compared to vehicle levels. This effect was confirmed by a significant main effect of QH-ii-066 dose on this behavior (Shapiro-Wilk Normality test – passed; $F(4,28)=4.6$, $P<0.01$, Bonferroni t-tests, $P<0.05$). On the other hand, L-655,708 dose-dependently increased yawning in both self-administration groups, with the highest dose (1.8 mg/kg) engendering significantly more yawning compared to vehicle (Shapiro-Wilk Normality test – passed; $F(4,28)=2.8$, $P<0.05$, Bonferroni t-tests, $P<0.05$).

Discussion

In the present study, alcohol served as a reinforcer in all monkeys, maintaining intake levels above those of water. At concentrations of 2% (w/v), monkeys self-administered pharmacologically relevant doses of alcohol and produced BALs ranging from 90 – 160 mg/dl. The overall intakes (i.e., g/kg) observed in this study are generally typical of intakes observed for macaques self-administering alcohol under limited access conditions (Grant and Johanson, 1988; Henningfield and Meisch, 1978; 1979; 1981; Katner et al., 2004; Vivian et al., 1999). Differences in intake between the present study and those cited could be due to a number of methodological differences including length of self-administration session, addition of flavorants, induction techniques, concentrations of alcohol made available for self-administration, and the self-administration apparatus itself. In fact, the latter difference could be especially important in the cases where the intakes observed in the present study are higher than those of the cited studies. In our procedure, the monkey must press a lever to gain access to a drinking spout connected to an alcohol reservoir. Depression of the drinking spout during extension results in alcohol delivery and fluid flow continues for as long as the monkey displaces the spout or for 30-s, whichever is shorter. The actual duration and volume of intake, within the constraints of the schedule, is under the control of the subject. Depending upon the available alcohol concentration, we observed average volumes of 12.5 mls of alcohol delivered per spout extension (range: ~3.5 mls/extension at 16% w/v; ~21 mls/extension at 2% w/v; data not shown). This average volume/delivery is much larger than the volumes/delivery in the cited studies in which the drinking spouts are

designed to deliver a set amount of alcohol (typically 0.5 – 1 ml) each delivery. Evidence from rats would suggest that alcohol intake should increase as the volume per reinforcement increases (Henningfield and Meisch, 1975).

When given the opportunity to consume a wide range of alcohol concentrations, monkeys drank sufficient quantities of alcohol to alter observable behavior. In many instances, these behavioral changes mirrored the effects of alcohol in intoxicated humans or those exposed to an acute alcohol challenge. For example, it is well-established that alcohol has a biphasic effect on activity (e.g., Martin et al., 1993; Morean and Corbin, 2010) such that, in human subjects, alcohol-induced stimulant effects (e.g., feelings of energized, stimulated, vigorous) are generally observed at lower BALs on the ascending limb of the blood alcohol curve, and alcohol-induced sedative effects (e.g., feelings of heavy head, inactive, sluggish) are reported at higher BALs on the descending limb of the blood alcohol curve. In monkeys, we observed a reliable increase in locomotor activity across a lower range of drinks (and presumably lower BALs) compared to the range where we observed increases in passive visual behavior and decreases in tactile/oral exploration, possible indices of sedative-like effects. Alcohol also is known to reduce the response to stressors and can produce anxiolytic-like effects in humans (e.g., Eckardt et al., 1998; Thomas et al., 2003). Similarly, alcohol produced a drink-dependent decrease in stereotypy and scratching, two behaviors that have been associated with anxiety in nonhuman primates and which are amenable to reduction by typical anxiolytics (e.g., Birkett et al., 2005; Castles et al., 1999; Schino et al., 1996; Tiefenbacher et al., 2005a; 2005b; Troisi, 2002). Finally, alcohol has been shown to dose-dependently increase the amount of body sway or static ataxia in humans (e.g., Schuckit, 1985); likewise, in monkeys, observable ataxia increased in a drink-dependent manner. Together, these findings illustrate and reinforce the translational nature of our non-human primate drinking model.

Using this model and GABA_A receptor subtype-selective ligands, we assessed the contribution of $\alpha 5$ GABA_A receptor mechanisms to the reinforcing effects of alcohol. We found that the agonist QH-ii-066 modestly increased, and the inverse agonist L-655,708 decreased, intake of alcohol (2% w/v). Subsequently, the maximally effective dose of QH-ii-066 was found to enhance intake of a wider range of alcohol concentrations; whereas, the maximally effective dose of L-655,708 was found to inhibit intake of a wider range of alcohol concentrations. Both leftward (QH-ii-066) and rightward (L-655,708) shifts in the alcohol concentration-response function were abolished by XLi-093, despite it not altering drinking on its own. These observations are in agreement with results from rodent studies showing that other $\alpha 5$ GABA_A receptor inverse agonists can reduce drinking (June et al., 2001; McKay et al., 2004). In conjunction with observations that $\alpha 5$ GABA_A receptor-subunit knockout mice exhibit reduced preference for and consumption of alcohol (Boehm et al., 2004), these findings suggest a prominent role for $\alpha 5$ GABA_A receptor mechanisms in the reinforcing effects of alcohol. Moreover, the lack of effect on drinking behavior with XLi-093 alone raises the possibility that neutral efficacy (i.e., antagonism) at this specific GABA_A receptor subtype is not sufficient to inhibit drinking, rather inverse efficacy is required.

In our hands, L-655,708 was as effective as the prototypical alcohol pharmacotherapy naltrexone in reducing drinking in the monkeys. Coupled with the observation that L-655,708 also can attenuate the subjective/interoceptive effects of alcohol (Platt et al., 2005), a growing body of research suggests that $\alpha 5$ GABA_A receptor inverse agonists may have potential as candidate anti-alcohol medications. An important consideration in the development of an alcohol pharmacotherapy is the pharmacological specificity of the candidate medication's effect. One technique used to evaluate the specificity of a medication's effect is to compare the doses of a drug needed to reduce behavior maintained

by alcohol relative to behavior maintained by a non-drug reinforcer. For example, if a drug reduces alcohol-maintained behavior to a greater extent than it reduces behavior maintained by the non-drug reinforcer, then the drug may have selective effects on alcohol self-administration (c.f. Mello and Negus, 1996). In the present study, L-655,708, across the same dose range studied in the alcohol drinkers, did not alter drinking in the sucrose drinkers. This result suggests that the inhibitory effects of L-655,708 are selective for alcohol. Moreover, that sucrose drinking also was not modified by QH-ii-066 provides additional support for a key role for $\alpha 5$ GABA_A receptor mechanisms in modulating the abuse-related effects of alcohol in particular, rather than drinking behavior in general.

Because adverse side effects can limit the utility of a pharmacotherapy, and because non-selective inverse agonists at the GABA_A/benzodiazepine site often can have convulsant/proconvulsant effects (e.g., Braestrup et al., 1983), it was of interest to determine the behavioral profile of L-655,708. In both alcohol- and sucrose-drinkers, L-655,708 was relatively benign and there was no indication of any interaction between L-655,708 and alcohol. Notably, even with repeated administration, L-655,708 was devoid of convulsant activity. This agrees with studies in rats showing that L-655,708 does not induce seizures on its own, nor does it alter the dose of pentylenetetrazole required to induce seizures (Atack et al., 2006). The sole observable effect of L-655,708 was to increase yawning in both groups of monkeys. In nonhuman primates, increases in yawning often have been interpreted as indicating increased stress and anxiety (Castles et al., 1999; Lutz et al., 2004), and yawning can be induced in monkeys with administration of an anxiogenic drug (Major et al., 2009). Likewise, L-655,708 exhibited anxiogenic-like activity in mice in an elevated plus-maze procedure (Navarro et al., 2002). While the effects of L-655,708 have not been determined in alcoholics, there is evidence for transient anxiogenic effects of flumazenil, a benzodiazepine receptor antagonist, in alcoholics in early withdrawal (Nutt et al., 1993). Taken together, these findings certainly would suggest that an $\alpha 5$ GABA_A receptor inverse agonist is likely to have anxiogenic effects in humans. While a relatively mild side effect, anxiogenesis would likely limit how an L-655,708-type drug might be used effectively in a treatment setting. For example, given that stress-induced negative affective states and withdrawal-induced anxiety are important facilitators of relapse to excessive alcohol use (e.g., Breese et al., 2005; Heilig et al., 2010), a drug that induces anxiety would not be a practical anti-relapse medication. Rather, our results would suggest that an $\alpha 5$ GABA_A receptor inverse agonist may be most useful as a pharmacotherapy to assist individuals with achieving abstinence.

In summary, our findings suggest a prominent and selective role for $\alpha 5$ GABA_A receptor mechanisms in the reinforcing effects of alcohol. The results implicate $\alpha 5$ GABA_A receptors as potential pharmacological targets for medications to reduce drinking and assist individuals in achieving abstinence. In particular, $\alpha 5$ GABA_A receptor inverse agonists may hold the most promise as alcohol pharmacotherapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Oral self-administration of varying alcohol concentrations and water under a fixed-ratio schedule and limited access conditions in monkeys. For each monkey, mean intakes for the last three sessions at each concentration were calculated. Data points represent the mean intake $\pm$ SEM (A. mls; B. g/kg) for the group (N = 5). * $P < 0.05$ vs. water (gray bar = SEM for water availability). C. Mean $\pm$ SEM blood alcohol levels (mg/dl) determined once stable intake was obtained at a particular alcohol concentration for the group (N = 5).

Figure 2.

Effects of increasing number of drinks on observable behavior (one drink = 0.25 g/kg alcohol). Data points represent the mean $\pm$ SEM at each drink (N = 34 – 115). * P<0.05 vs. water (gray bar = SEM for water availability).

Figure 3.

Effects of daily pretreatment with QH-ii-066, XLi-093, L-655,708 or naltrexone on intake maintained by a 2% (w/v) alcohol solution. For each monkey, mean intakes for the last three sessions at each dose were calculated. Data points represent the mean intake $\pm$ SEM presented as a % of baseline g/kg for the group (N = 5). * $P < 0.05$ vs. vehicle. Baseline intake for QH-ii-066 = 1.4 ± 0.2 g/kg; XLi-093 = 1.8 ± 0.02 g/kg; L-655,708 = 1.6 ± 0.2 g/kg; naltrexone = 1.7 ± 0.2 g/kg (one-way ANOVA; $F(3,16) = 0.97$, n.s.).

Figure 4.

Effects of daily pretreatment with QH-ii-066, XLi-093, L-655,708 or naltrexone on intake maintained by a 2% (w/v) alcohol solution. For each monkey, mean intakes for the last three sessions at each dose were calculated. Data points represent the mean intake $\pm$ SEM presented as a % of baseline g/kg for the group ($N = 5$). * $P < 0.05$ vs. vehicle. Baseline intake for QH-ii-066 = 1.4 ± 0.2 g/kg; XLi-093 = 1.8 ± 0.02 g/kg; L-655,708 = 1.6 ± 0.2 g/kg; naltrexone = 1.7 ± 0.2 g/kg (one-way ANOVA; $F(3,16) = 0.97$, n.s.).

Figure 5.

A. Oral self-administration of varying sucrose concentrations and water under a fixed-ratio schedule and limited access conditions in monkeys. For each monkey, mean intakes for the last three sessions at each concentration were calculated. Data points represent the mean intake $\pm$ SEM (mls) for the group ($N = 4$). * $P < 0.05$ vs. water (gray bar = SEM for water availability). B. Effects of daily pretreatment with QH-ii-066 or L-655,708 on intake maintained by a 0.3% (w/v) sucrose solution. For each monkey, mean intakes for the last three sessions at each dose were calculated. Data points represent the mean intake $\pm$ SEM presented as a % of baseline mls for the group ($N = 4$). Baseline intake for QH-ii-066 = 739 ± 178 mls; L-655,708 = 953 ± 142 mls (t -test; $t(6) = -0.94$, n.s.).

Figure 6. Observable effects of QH-ii-066 on tactile/oral exploration (A) and L-655,708 on yawning (B). Bars/points are group means $\pm$ SEM in the sucrose self-administration group (N = 4) and the alcohol self-administration group (N = 5). * $P < 0.05$ vs. vehicle.

Table 1Behavioral categories, abbreviations, and definitions^a

SPECIES-TYPICAL:		
Passive visual	(VIS)	Animal is standing or sitting motionless with eyes open
Rest/Sleep posture	(RSP)	Idiosyncratic posture adopted by monkeys during rest or sleep; eyes closed, easily roused by external stimulation (e.g., tapping on cage)
Locomotion	(LOC)	At least two directed steps in the horizontal and/or vertical plane
Tactile/oral exploration	(TAC)	Any tactile or oral manipulation of the cage or environment
Forage	(FOR)	Sweeping and/or picking through wood chip substrate
Self-groom	(GRM)	Picking, scraping, spreading or licking of an animal's own hair
Scratch	(SCR)	Vigorous strokes of the hair with the finger or toenails
Vocalization	(VOC)	Species-typical sounds emitted by monkey (not differentiated into different types)
Yawn	(YWN)	To open mouth wide and expose teeth
Present	(PRE)	Posture involving presentation of rump, belly, flank, and/or neck) to observer or other monkey
Threat/aggress	(THR)	Multifaceted display involving one or more of the following: open mouth stare with teeth partially exposed, eyebrows lifted, ears flattened or flapping, rigid body posture, piloerection, attack (e.g., biting, slapping) of inanimate object or other monkey
Fear grimace	(FGR)	Grin-like facial expression involving the retraction of the lips exposing clenched teeth; may be accompanied by flattened ears, stiff, huddled body posture, screech/chattering vocalizations
Body spasm	(BSP)	An involuntary twitch or shudder of the entire body; also "wet dog" shake
Lip smack	(LIP)	Pursing the lips and moving them together to produce a smacking sound; often accompanied by moaning
Cage shake	(CSH)	Any vigorous shaking of the cage that may or may not make noise
Stereotypy	(STY)	Any repetitive, ritualized pattern of behavior that serves no obvious function
Other	(OTH)	Any notable behavior not indicated above (e.g., masturbation, nose rub, lip droop, vomit/retch)
PROCEDURE-RELATED:		
Lever press	(LVR)	Depression of lever manipulanda on the drinking panel
Drink	(DRI)	Mouth contact to fluid delivery sippers
DRUG-INDUCED:		
Ataxia	(ATX)	Any slip, trip, fall or loss of balance
Procumbent posture	(PRO)	Loose-limbed posture (sitting or lying on cage bottom) accompanied by eye closure; not easily aroused by external stimulation (e.g., tapping on cage)

^a Adapted from Novak et al., 1992; Weerts et al., 1998; Platt et al., 2000, 2002.