Chlorzoxazone, an SK-Type Potassium Channel Activator Used in Humans, Reduces Excessive Alcohol Intake in Rats

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Background

Alcoholism imposes a tremendous social and economic burden. There are relatively few pharmacological treatments for alcoholism, with only moderate efficacy, and there is considerable interest in identifying additional therapeutic options. Alcohol exposure alters SK-type potassium channel (SK) function in limbic brain regions. Thus, positive SK modulators such as chlorzoxazone (CZX), a US Food and Drug Administration–approved centrally acting myorelaxant, might enhance SK function and decrease neuronal activity, resulting in reduced alcohol intake.

Methods

We examined whether CZX reduced alcohol consumption under two-bottle choice (20% alcohol and water) in rats with intermittent access to alcohol (IAA) or continuous access to alcohol (CAA). In addition, we used ex vivo electrophysiology to determine whether SK inhibition and activation can alter firing of nucleus accumbens (NAcb) core medium spiny neurons.

Results

Chlorzoxazone significantly and dose-dependently decreased alcohol but not water intake in IAA rats, with no effects in CAA rats. Chlorzoxazone also reduced alcohol preference in IAA but not CAA rats and reduced the tendency for rapid initial alcohol consumption in IAA rats. Chlorzoxazone reduction of IAA drinking was not explained by locomotor effects. Finally, NAcb core neurons ex vivo showed enhanced firing, reduced SK regulation of firing, and greater CZX inhibition of firing in IAA versus CAA rats.

Conclusions

The potent CZX-induced reduction of excessive IAA alcohol intake, with no effect on the more moderate intake in CAA rats, might reflect the greater CZX reduction in IAA NAcb core firing observed ex vivo. Thus, CZX could represent a novel and immediately accessible pharmacotherapeutic intervention for human alcoholism.

Key Words: Alcohol intake; intermittent; neuro-adaptation; nucleus accumbens; SK potassium channel

There is considerable interest in identifying new pharmacological treatments for alcoholism, in part because of the enormous and deleterious health, economic, and/or societal consequences of alcoholism ([1] , [2] , [3] and [4]). There are several existing pharmacotherapies for alcoholism, including naltrexone and acamprosate, but these can have only moderate benefits or act in a subset of alcoholics (3). Thus, we and other laboratories have used rodent models to identify alcohol-related cellular neuro-adaptations that might represent novel therapeutic interventions for alcoholism.

In this regard, long-term, voluntary alcohol self-administration and withdrawal is associated with reduced function of SK-type calcium-activated potassium channels (SK) and increased excitability in neurons from the nucleus accumbens (NAcb) core (5), a region critical for regulation of reward-related and motivated behaviors ([1] , [3] , [5] , [6] and [7]), with no changes in NAcb core SK function or firing ex vivo after sucrose self-administration. Importantly, intra-NAcb core infusion of an SK activator, which depresses action potential firing, reduces alcohol- but not sucrose-seeking (5). In addition, no changes in SK function are seen in the NAcb shell or dorsal stratum, and SK activators in these regions do not alter alcohol intake (5). Thus, we hypothesize that NAcb core SK neuro-adaptations enhance alcohol intake and allow SK activators to suppress alcohol intake. Also, passive alcohol exposure reduces SK function and increases excitability in the ventral tegmental area (8), another limbic region that regulates alcohol intake ([3] and [9]). However, these studies suffer from several limitations, including the low alcohol intake during operant self-administration and the need to operantly respond to procure alcohol, which is not present in human alcoholics, and the low face validity of passive exposure.

To better understand the relevance of SK neuro-adaptations for human alcoholism, we examined the impact of chlorzoxazone (CZX), a US Food and Drug Administration (FDA)-approved positive allosteric SK modulator (10) used for decades as a centrally acting muscle relaxant (11), on alcohol intake in an intermittent access to alcohol (IAA) paradigm ([12] and [13]). Alcohol intake levels in IAA rats are significantly greater than in rats with continuous alcohol access (CAA) ([12] and [13]) and are more similar to intake levels in alcohol-preferring rat strains (14). In addition, alcohol intake in IAA rats continues despite pairing alcohol with the aversive quinine, suggesting that IAA alcohol intake has become inflexible or maladaptive relative to the quinine-sensitive alcohol intake in CAA rats ([13] and [15]). Also, several compounds that can reduce alcohol intake in human alcoholics have a greater effect on IAA versus CAA alcohol intake (12). Thus, the IAA model has predictive validity for human alcoholism, whereas the more moderate alcohol intake in CAA rats might better model social drinking.

Here, we show that CZX significantly reduced alcohol intake in IAA but not CAA rats. Also, NAcb core neurons ex vivo from IAA but not CAA rats showed reduced SK function, enhanced excitability, and increased sensitivity to inhibitory effects of CZX on firing. Thus, CZX might represent a novel and readily available pharmacotherapy for the treatment of human alcoholism.

Methods and Materials

Alcohol Self-Administration

Adult male Wistar rats (250–275 g, Harlan, Livermore, California) drank 20% alcohol or water under a two-bottle choice, home-cage, IAA paradigm modified from Simms et al. (12). Rats had 24-hour access to alcohol 3 days/week (starting Monday, Wednesday, and Friday) for 5–6 weeks. Rats were then switched to only 3-hour access to alcohol on the 3 days/week to simplify detection of CZX-related intake changes. After 5–6 weeks of 3-hour/day, 3-day/week IAA drinking, the effects of CZX were examined.

Results from IAA rats were compared with rats with continuous access (24 hours/day, 7 days/week) to 20% alcohol and water under home-cage, two-bottle choice conditions (CAA), and CZX testing began after approximately 12 weeks CAA. The CAA intake levels were measured in the same 3-hour period in which IAA rats had access to alcohol and are only presented for the 3-hour consumption period, due to the lower level of CAA alcohol consumption.

Alcohol-drinking data were collected from 26 IAA rats and 19 CAA rats. Different doses of CZX were injected, once/week (Wednesday or Friday), in a counterbalanced manner across rats, with each rat receiving three of the four CZX doses (0, 10, 30, or 50 mg/kg). Chlorzoxazone was prepared in 25% cyclodextrin and 5% chremophor EL, pH approximately 7.6, and injected IP 20–30 min before onset of access to alcohol.

Previous studies found that Wistar IAA rats show approximately 50 mg% (approximately 10 mmol/L) blood alcohol concentrations after 30-min alcohol access (12). Here, with previously published methods (12), IAA blood alcohol concentrations were 32.3 ± 7.5 mg% after 1-hour alcohol access (n = 12).

Ex Vivo Electrophysiology

Electrophysiology methods were as previously described (5). Briefly, rats were sedated with 40 mg/kg pentobarbital (IP) before transcardial perfusion with Ringers' solution containing (in mmol/L): 225 sucrose, 119 NaCl, 2.5 KCl, 1.0 NaH_2PO_4, 4.9 $MgCl_2$, .1 $CaCl_2$, 26.2 $NaHCO_3$, 1.25 glucose, 1 ascorbic acid, and 3 kynurenic acid. The brain was removed, and coronal slices (300 µm) were cut in this same solution. Slices then recovered in 32°C carbogen-bubbled artificial cerebrospinal fluid containing (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 18 $NaHCO_3$, 11 glucose, pH 7.2–7.4, 301–305 mOsm. During electrophysiology experiments, slices were perfused with 31°–32°C carbogen-bubbled artificial cerebrospinal fluid and supplemented with 6-cyano-7-nitroquinoxaline-2,3-dione (10 µmol/L) and picrotoxin (50 µmol/L) to block α-amino-3-hydroxy-5-methylisoxazole propionate and γ-aminobutyric acid–A receptors.

Whole-cell current-clamp recordings were performed in medium spiny neurons, identified by small size, subthreshold depolarizing ramps with slow, repetitive firing, and resting potential < −85 mV (16), with a Multiclamp 700A patch amplifier and pClamp9.2 (Axon Instruments, Union City, California). The internal solution contained (in mmol/L): 130 KOH, 105 methanesulfonic acid, 17 hydrochloric acid, 20 HEPES, .2 EGTA, 2.8 NaCl, 2.5 mg/mL MgATP, and .25 mg/mL NaGTP. The resting membrane potential was brought to approximately –90 mV by passage of DC current before firing was generated with 300-msec depolarizing current pulses. For basal ex vivo electrophysiological differences between IAA and CAA rats, similar results were observed if all neurons across all rats were averaged together or if all neurons from a given rat were averaged together to yield a single value for each rat (not shown).

Brain slices from IAA animals were prepared 24–48 hours after alcohol intake, a time point equivalent to when the animal would have access for self administration. Functional properties were not different at 24 versus 48 hours after drinking (not shown).

Reagents

Chlorzoxazone and apamin were from Sigma-Aldrich (St. Louis, Missouri).

Statistical Analysis

Statistics were calculated with an unpaired t test or a one-way or two-way analysis of variance with Tukey post hoc, with SigmaStat 3.1 (SYSTAT Software, San Jose, California).

Results

As shown in Figure 1, IAA rats were trained to drink 20% alcohol versus water under a two-bottle choice, home-cage, intermittent access to alcohol paradigm, whereas CAA rats had continuous access to 20% alcohol concurrent with water. Chlorzoxazone significantly and dose-dependently reduced alcohol drinking in IAA rats, determined after 3 hours access to alcohol (Figure 2A) [$F(3,74) = 17.45$, $p < .001$] or 1 hour access to alcohol (Figure 2C) [$F(3,74) = 20.63$, $p < .001$]. Importantly, CZX did not significantly alter concurrent water intake in IAA rats after 3 hours (Figure 2B) [$F(3,74) = 1.15$, $p = .336$] or 1 hour access (Figure 2D) [$F(3,74) = 1.86$, $p = .144$]. CZX had no effect on alcohol intake in CAA rats, in strong contrast with IAA rats, (Figure 3A) [$F(3,53) = 1.00$, $p = .398$]—determined only after 3-hour access because of the lower amount of alcohol consumed—or on CAA water intake (Figure 3B) [$F(3,53) = 1.63$, $p = .195$]. Thus, CZX potently reduced alcohol intake in IAA rats, with no effect on alcohol intake in CAA rats or on water intake in IAA or CAA rats, suggesting that CZX effects on IAA alcohol intake occurred through a specific reduction of alcohol consumption rather than nonspecific motor effects.

Figure 1.

Intermittent (IAA) and continuous (CAA) access to alcohol intake paradigms. Adult male Wistar rats were trained to drink 20% alcohol versus water under a two-bottle choice paradigm with (A) IAA or (B) CAA. The IAA rats had 24-hour access to alcohol three days/week (starting Monday, Wednesday, and Friday) for 5–6 weeks. Rats were then switched so that they only had 3-hour access to alcohol on the three days/week to simplify detection of potential changes in intake after chlorzoxazone (CZX). After 5–6 weeks of 3-hour/day IAA drinking, the effects of CZX were examined. Different doses of CZX were injected, once/week, 20–25 min before access to alcohol, in a counterbalanced manner across rats. Results from IAA rats were compared with CAA rats that had continuous access to 20% alcohol versus water for an equivalent period of drinking, so that CZX testing began after approximately 12 weeks of CAA. The CAA rats were allowed access to alcohol 24 hours/day 7 days/week, but alcohol intake levels for this study were determined in the same 3-hour period in which IAA rats were allowed access to alcohol.

Figure 2.

Chlorzoxazone decreased IAA alcohol but not water intake. (A, B) Chlorzoxazone dose-dependently decreased IAA alcohol intake, determined after 3-hour access (A), with no effect on IAA water intake (B) (vehicle: n = 22; 10 mg/kg CZX: n = 21; 30 mg/kg CZX: n = 22; 50 mg/kg CZX: n = 13). Similarly, (C, D) CZX dose-dependently decreased IAA alcohol intake across 1-hour access (C) with no effect on water intake (D). (A, C) Post hoc $p < .05$, 10 mg/kg versus 30 or 50 mg/kg CZX. *$p < .05$, **$p < .01$ versus vehicle. Abbreviations as in Figure 1.

Figure 3.

Chlorzoxazone did not decrease CAA alcohol intake. Chlorzoxazone had no effect on alcohol (A) or water (B) intake in CAA rats (vehicle: n = 17; 10 mg/kg CZX: n = 15; 30 mg/kg CZX: n = 16; 50 mg/kg CZX: n = 9). CAA rats were allowed access to alcohol 24 hours/day 7 days/week, but alcohol intake levels were determined in the same 3-hour period in which IAA rats were allowed access to alcohol. Abbreviations as in Figure 1.

The preference ratio, the amount of alcohol consumed relative to the total liquid (alcohol plus water) consumed, has been considered to reflect the motivational relevance of alcohol (1). Preference for alcohol was very high in IAA rats and dose-dependently decreased by CZX (Figure 4A) [$F(3,74) = 7.24$, $p < .001$]. In contrast, the preference ratio in CAA rats was lower than in IAA and was

not altered by CZX (Figure 4B) [$F(3,53) = 1.64$, $p = .191$]. Thus, CZX only reduced alcohol preference in IAA rats.

Figure 4.

Alcohol preference across the 3 hours of intake was decreased by CZX in IAA but not CAA rats. (A, B) Chlorzoxazone decreased the alcohol preference (the level of alcohol intake divided by the level of alcohol plus water intake) in IAA (A) but not CAA (B) rats. *$p < .05$ versus vehicle. Abbreviations as in Figure 1.

We also examined the impact of CZX on alcohol or water consumption in the first hour of access relative to the entire period of access. The IAA rats consumed most alcohol within the first hour of the 3-hour access session, and CZX significantly reduced the proportion of total alcohol consumed within the first hour of access (Figure 5A) [$F(3,74) = 10.48$, $p < .001$]. In addition, in CAA rats, CZX did not significantly alter the amount of alcohol consumed within the first hour relative to the 3-hour drinking period (Figure 5C) [$F(3,53) = 1.08$, $p = .364$] or the amount of water consumed in the first hour relative to total water consumption (Figure 5D) [$F(3,53) = 1.59$, $p = .202$]. Thus, CZX not only reduced the total alcohol consumed in IAA rats but also altered the pattern of alcohol intake only in IAA rats.

Figure 5.

The IAA rats consumed most alcohol within the first hour of access. (A) The IAA rats consumed most alcohol within the first hour of access, relative to the amount consumed across all 3 hours of access. Chlorzoxazone significantly reduced this higher initial consumption in IAA rats. (B) Chlorzoxazone did not alter water intake in IAA rats. Chlorzoxazone did have an overall significant effect on the pattern of IAA water consumption [$F(3,74) = 4.25$, $p = .008$], but this reflected a significant difference post hoc between 30 and 50 mg/kg rather than differences between any CZX dose and vehicle. (C, D) Chlorzoxazone did not alter the percentage of total alcohol (C) or water (D) consumed in the first hour of access, relative to all 3 hours of access, in CAA rats. *$p < .05$ versus vehicle. Abbreviations as in Figure 1.

Because IAA rats undergo periods of withdrawal, we examined whether CZX could reduce CAA alcohol intake after a 24-hour withdrawal from alcohol. Thirty mg/kg CZX had no significant effect on CAA alcohol intake after withdrawal [vehicle: .74 ± .08 g/kg in 3 hours, $n = 7$; CZX: .60 ± .09 g/kg in 3

hours, n = 7; t(12) = 1.21, p = .248], although CAA alcohol intake was increased after withdrawal relative to no withdrawal [t(22) = 2.69, p = .013], consistent with the widely observed alcohol deprivation effect (1). Furthermore, CZX did not alter alcohol preference [vehicle: 38.0 ± 3.7%; CZX: 33.2 ± 5.7%; t(12) = .685, p = .506] or amount of alcohol consumed in the first hour relative to total [vehicle: 48.7 ± 6.0%; CZX: 53.6 ± 8.9%; t(12) = .457, p = .656] in CAA rats after 24-hour withdrawal. Also, 24-hour withdrawal in CAA rats significantly enhanced preference [t(22) = 6.408, p < .001] but not the timing of consumption [t(22) = .159 p < .875] relative to no withdrawal. Thus, although withdrawal increased alcohol intake and preference in CAA rats, these data suggest that the differential effect of CZX on IAA and CAA alcohol intake might not reflect the experience of withdrawal per se.

To further examine whether CZX reduction of IAA alcohol intake might reflect nonspecific motor effects, we examined the impact of CZX on open-field locomotion. As shown in Figure 6, CZX produced a dose-dependent decrease in locomotion in IAA rats and CAA, with a similar effect on locomotion in both groups [CZX: F(1,35) = 12.957, p < .001; group: F(1,35) = .301, p = .587; interaction: F(1,35) = .001, p = .999; post hoc p < .01 for 30 mg/kg CZX and p > .4 for 10 mg/kg CZX in both groups]. Taken together with a lack of effect of 30 mg/kg CZX on IAA water intake and CAA water and alcohol intake, these results indicate that the CZX reduction in IAA alcohol intake reflected a specific effect on IAA alcohol consumption.

Figure 6.

Chlorzoxazone similarly depressed open-field locomotion in IAA rats (vehicle: n = 10; 10 mg/kg CZX: n = 9; 30 mg/kg CZX: n = 6) and CAA rats (vehicle: n = 9; 10 mg/kg CZX: n = 9; 30 mg/kg CZX: n = 6). Similar results were observed for stereotypies (not shown). *p < .05 versus vehicle. Abbreviations as in Figure 1.

Previous studies suggest that the ability of SK activators to reduce alcohol intake is enabled by the presence of neuro-adaptations in SK channels within the NAcb core, where NAcb core SK currents are reduced after operant self-administration of alcohol but not sucrose and SK activation within the NAcb core reduces intake of alcohol but not sucrose (5). Thus, we examined whether the differential behavioral effects of CZX in IAA and CAA rats might be reflected in ex vivo excitability of NAcb core neurons. Basal firing ex vivo was significantly elevated in NAcb core neurons from IAA relative to CAA and alcohol-naive rats (Figure 7C) [F(2,54) = 4.809, p = .012 at 200 pA; F(2,53) = 7.729, p = .001 at 220 pA] or as input-output slope (see figure legend; Figure 7D) [F(2,54) = 20.53, p < .001]. In addition, the enhancement of NAcb core firing by the SK antagonist apamin (100 nmol/L) was significantly smaller in IAA versus CAA and naive rats (Figures 7A and 7E) [F(2,14) = 16.93, p < .001].

These results strongly suggest that action potential firing in NAcb core neurons was only enhanced in IAA rats and that increased excitability of IAA neurons was a consequence of reduced SK function.

Figure 7.

Ex vivo whole-cell patch-clamp electrophysiology experiments in nucleus accumbens (NAcb) core neurons from IAA and CAA rats. (A) Examples showing that SK-type potassium channel (SK) inhibition with apamin (100 nmol/L) produced a smaller enhancement of firing in IAA versus CAA neurons and that the CZX reduction in firing was prevented by apamin exposure in both IAA and CAA neurons. (B) Examples showing greater CZX inhibition of firing in IAA versus CAA neurons. (C) Greater basal excitability, measured with applied current versus action potential generation, was observed in IAA compared with CAA or naive NAcb core neurons ex vivo (CAA: n = 19; naive: n = 21; IAA: n = 17). To generate firing, neurons were depolarized with a series of 7 or 8 300-msec current pulses, with 20 pA between each current pulse, where the initial current pulse for each neuron was just subthreshold for firing. For one naive cell, firing was not determined at 220 pA. (D) Greater basal excitability measured with the input-output slope, calculated by fitting a line relating the number of action potentials generated in the first three suprathreshold current pulses and the last subthreshold current pulse in a given neuron ([5] and [62]). (E) Decreased apamin enhancement of firing in IAA compared with CAA or naive NAcb core neurons ex vivo (CAA: n = 8; naive: n = 5; IAA: n = 7). (F) Chlorzoxazone produced a greater inhibition of firing in IAA neurons (n = 6 for 100 µmol/L CZX and 4 for 30 µm CZX for both groups), and CZX inhibited firing in IAA and CAA NAcb core neurons through SK channels, because the CZX reduction in firing was blocked by apamin (n = 5 for both groups). Example traces were from a current step to 140 pA for IAA and 140 pA for CAA in (A) and 120 pA for IAA and 180 pA for CAA in (B). *p < .05, **p < .01, IAA versus CAA and naive. Abbreviations as in Figure 1.

We next examined CZX inhibition of NAcb core firing ex vivo. One hundred µmol/L but not 30 µmol/L CZX significantly reduced firing in both IAA and CAA neurons, but 100 µmol/L CZX produced a significantly greater inhibition of firing in IAA versus CAA NAcb core neurons, and pre-exposure to apamin greatly reduced the CZX inhibition of firing in both groups (Figures 7B and 7F) [group: $F(1,18) = 5.884$, $p = .026$; with or without apamin pre-exposure: $F(1,18) = 30.247$, $p < .001$; group × apamin: $F(1,18) = 5.751$, $p = .028$; post hoc $p < .01$ IAA vs. CAA, $p < .05$ apamin block of CZX]. Thus, CZX inhibited NAcb core firing through activation of SK channels, with a significantly greater reduction in firing in IAA versus CAA NAcb core neurons.

Discussion

The present study demonstrates that CZX, an FDA-approved SK activator (10) used for decades in humans as a centrally acting myorelaxant (11), reduced excessive alcohol intake in IAA rats but not moderate alcohol intake in CAA rats. CZX significantly and dose-dependently decreased alcohol

intake in IAA rats, with no effect on concurrent water intake. In contrast, CZX did not reduce alcohol or water intake in CAA rats; the lack of effect of CZX in CAA rats was not due to a floor effect, because adulteration of alcohol with quinine reduces CAA alcohol intake (13). CZX also reduced alcohol preference in IAA but not CAA rats. Furthermore, IAA rats drank most alcohol within the first hour of the 3-hour access period, and CZX reduced this tendency for strong initial alcohol consumption. Chlorzoxazone reduction of IAA drinking did not reflect effects on motor activity. In addition, ex vivo electrophysiology experiments found enhanced basal firing in the NAcb core neurons from IAA versus CAA and age-matched, alcohol-naive rats. Increased IAA basal firing was associated with a smaller enhancement of firing by the SK blocker apamin, suggesting that increased excitability reflected reduced SK function. Furthermore, CZX inhibited firing in both IAA and CAA NAcb core neurons, but the CZX inhibition of firing was significantly greater in IAA neurons. Thus, CZX selectively reduced the excessive alcohol intake in IAA rats but not the moderate alcohol intake in CAA rats, and differential sensitivity to CZX might result from a neuro-adaptation in NAcb core SK function only in IAA rats. Chlorzoxazone could therefore represent a novel and potent pharmacotherapeutic intervention for human alcoholism.

Although CZX reduced alcohol intake in IAA but not CAA rats, some caution might be warranted for differential CZX effects on preference and intake pattern. For example, higher preference in IAA rats might simply reflect the restricted access to alcohol, whereas CAA animals have no time constraints and thus might spread their drinking out across time, which could reduce apparent alcohol preference. Also, although weekly IAA and CAA alcohol intake is similar (with CAA rats drinking less per day but with access to alcohol more days/week), we describe IAA alcohol intake as excessive (14), because the amount consumed in a given session of access is greater in IAA versus CAA rats, and heavy intake within a session is considered a better model of human alcoholism ([14] and [15]) (e.g., where intermittent but not continuous access produces aversion-resistant alcohol intake) ([13] and [15]). Finally, IAA rats experience repeated withdrawal from alcohol, whereas CAA rats do not. In this regard, CAA rats after 24-hour withdrawal increased alcohol intake and preference, although with no changes in intake pattern, but CZX did not reduce CAA alcohol intake after withdrawal from alcohol. These results suggest that the differential effects of CZX on IAA and CAA alcohol intake might not reflect withdrawal per se.

CZX is used in humans as a centrally acting muscle relaxant, and reduced IAA alcohol intake could reflect more general motor or motivational impairments ([17] and [18]). Thirty milligrams/kilogram CZX produced a similar, strong locomotor inhibition in both groups, but this dose of CZX significantly decreased IAA alcohol intake with no significant effect on IAA water intake or CAA water or alcohol intake. Thus, open-field locomotion might represent an inadequate control for possible nonspecific motor effects of CZX on home-cage alcohol intake. Instead, the lack of effect of CZX on CAA alcohol or water intake or IAA water intake might represent better internal controls, which suggests that the CZX reduction in IAA alcohol intake did not reflect motor impairment.

The primary metabolic pathway for CZX is the CYP2E1 cytochrome P450, and the effective concentration of CZX could be altered if alcohol influences CYP2E1 activity. Prolonged alcohol exposure can upregulate CYP2E1 activity ([19] , [20] and [21]), including in the brain (22), which could decrease CZX concentrations in alcohol-drinking rats. However, alcohol can act as a moderate competitive inhibitor of CYP2E1 (23) and increase CZX peak concentrations, although this effect would be more pronounced across time and less evident in the first several hours ([24] , [25] and [26]). We have not examined CZX metabolism here, in part because repeated blood collection would likely disrupt ongoing alcohol intake. However, 10 mg/kg CZX significantly decreased IAA alcohol intake, whereas 30 mg/kg CZX did not alter CAA alcohol intake. Thus, alcohol-related CYP2E1 changes are unlikely to explain the differential effect of CZX on IAA versus CAA alcohol intake.

In addition to the CZX reduction of alcohol intake only in IAA rats, we also observed decreased SK regulation of NAcb core firing ex vivo only in IAA rats. Mesolimbic regions such as the NAcb core can promote alcohol seeking and intake ([3] , [5] , [9] , [27] , [28] and [29]), and the human NAcb can be activated by alcohol-related cues that induce cravings ([30] and [31]). Action potential firing is a predominant mechanism through which neurons transmit information, and NAcb firing (e.g., in response to reinforcer-predictive cues) ([32] , [33] and [34]) is thought to contribute to initiation of motivated and addictive behaviors ([6] , [7] , [32] and [33]). Interestingly, we found that basal NAcb core firing ex vivo was enhanced in neurons from IAA rats relative to CAA and alcohol-naive rats. In addition, the SK antagonist apamin produced a smaller increase in IAA versus CAA firing, suggesting that increased basal IAA excitability reflected reduced SK regulation of firing. Also, CZX activation of SK produced a greater inhibition of firing in IAA versus CAA NAcb core neurons. We previously showed (5) that operant alcohol self-administration is associated with reduced NAcb core SK function and greater inhibition of firing by SK activators, with no changes in SK function in other striatal regions or in the NAcb core of sucrose self-administering animals. Importantly, local infusion of an SK activator only decreased reward-seeking behavior under conditions where reduced SK function was observed ex vivo. Thus, we hypothesize that reduced SK function in the NAcb core is required for SK activators to depress alcohol intake. Also, reduced SK function might be expected to decrease the ability of CZX to modulate firing. However, the smaller CZX inhibition of CAA firing might instead reflect a floor effect, where CZX inhibition of firing is more moderate under conditions of stronger basal SK function. Finally, alcohol-related SK changes are also seen in the ventral tegmental area (8) and hippocampus (35), and thus we cannot rule out the possibility that CZX could reduce alcohol intake by acting in these or other brain regions.

Interestingly, NAcb SK function is also altered after cocaine exposure but with increased SK function in the NAcb shell (36), in contrast to decreased SK function with alcohol in the NAcb core. The SK function is also reduced after several forms of learning ([37] and [38]), suggesting that SK might undergo plastic changes in relation to many types of salient events. One interesting possibility is that SK changes represent a homeostatic response (36). For example, increased SK after hyperactivation associated with cocaine might serve to reduce excitability, whereas, conversely, decreased SK function might occur in response to the depressive effects of alcohol.

Although we hypothesize that CZX reduces drinking by SK activation (10), CZX can also act on other ion channels, including the large- (BK) and intermediate-conductance calcium–activated potassium channels ([10] and [39]). However, the SK antagonist apamin prevented the CZX inhibition of NAcb core firing ex vivo, suggesting a preeminent CZX action through SK. Other CZX effects are also more consistent with action on SK. Chlorzoxazone produces a similar reduction in burst firing in midbrain dopamine neurons as 1-ethyl-2-benzimidazolinone (1-EBIO) ([40] and [41]), another SK channel activator which does not activate BK ([42] and [43]). Furthermore, CZX prevents contraction of the isolated aorta in a manner consistent with SK suppression of excitability (44). Thus, we consider it likely that CZX reduces IAA alcohol intake through activation of SK.

There are three SK subunits (SK1–SK3), and SK3 subunits are particularly abundant in the NAcb, whereas SK1 is relatively uncommon in the striatum but highly enriched in areas like the hippocampus, and SK2 is distributed throughout the brain ([45] and [46]). If alcohol alters just one subunit, a compound selective for that subunit could be more efficacious than CZX with fewer nonspecific effects. In this regard, operant alcohol intake reduces NAcb core SK function and decreases proteins levels of the SK3 but not SK2 subunit (5), and there are relatively selective activators for SK3/SK2 versus SK1 (47) and for SK1 versus SK3/SK2 (48). The fact that CZX is already approved for use in humans makes it of immediate potential clinical utility, although SK subunit-selective compounds could represent better pharmacological therapies in the future.

The SK activators dampen neuronal excitability and thus have been considered possible therapeutic agents to treat episodic ataxia Type-2 ([49] and [50]), epilepsy (51), Parkinson's disease (52), anxiety (53), and alcohol addiction (5). CZX might represent just such an agent. However, the SK activator 1-EBIO only counteracted seizure activity at relatively high doses that also produced motor impairments (51), and CZX has been considered only moderately effective as a centrally acting muscle relaxant (11). In contrast, even quite low doses of 1-EBIO reduce burst firing in midbrain dopamine neurons (40), and CZX reduced IAA alcohol intake even at 10 mg/kg, which had little effect on locomotor activity. Thus, we speculate that CZX could be more potent as an antidrinking compound relative to the presently approved use as a centrally acting myorelaxant.

One important concern for use of CZX in humans is a CZX-related, rare, idiopathic liver toxicity ([54] and [55]). Although the mechanism of CZX toxicity is unclear, CYP2E1 in the liver metabolizes CZX to OH-CZX ([23] , [56] and [57]). Interestingly, alcoholics who do not have CYP2E1 induction after alcohol drinking show less clinical and biochemical signs of liver disease relative to CYP2E1-induced alcoholics (58). Thus, CYP2E1 metabolism of CZX could contribute to CZX toxicity, and compounds that inhibit CYP2E1 activity ([23] and [56]) and prevent alcohol-related liver damage in rats (57) might reduce the risk of liver toxicity and also the CZX dose required for effective plasma levels (59). In addition, hepatotoxic effects of CZX in humans are considered quite rare ([54] and [55]), and agents such as statins present liver-related side effects, but the benefits to human health as well as the presence of relatively inexpensive liver screens have not precluded their widespread use in humans ([60] and [61]).

In conclusion, CZX, an FDA-approved positive allosteric SK modulator, significantly and selectively reduced the excessive alcohol intake in IAA rats, with no effect on the more moderate alcohol intake in CAA rats. Ex vivo electrophysiology experiments showed that IAA NAcb core neurons exhibit reduced SK regulation of firing and increased excitability. Also, CZX produced a greater inhibition of firing ex vivo in IAA NAcb core neurons, and we hypothesize that reduced NAcb core SK function in IAA rats might allow SK activation by CZX to reduce alcohol intake. These results support the importance of SK channels in the regulation of alcohol intake and the use of CZX as a novel, potent, and immediately accessible therapeutic intervention for alcoholism.

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Figures

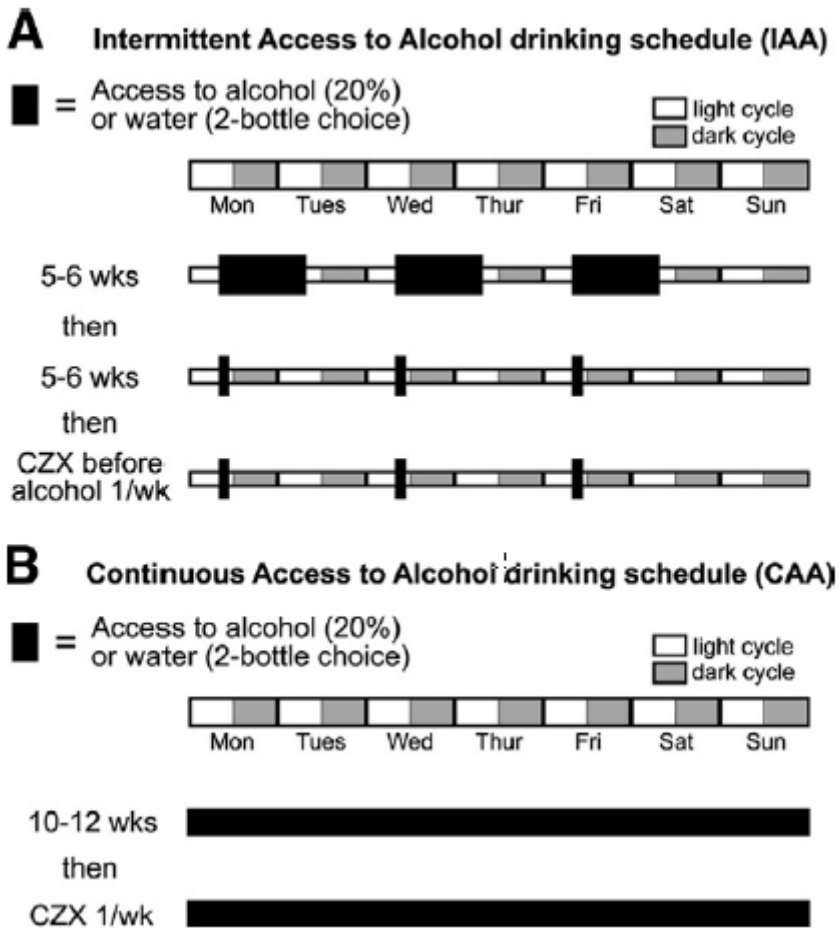


Figure 1.

Intermittent (IAA) and continuous (CAA) access to alcohol intake paradigms. Adult male Wistar rats were trained to drink 20% alcohol versus water under a two-bottle choice paradigm with (**A**) IAA or (**B**) CAA. The IAA rats had 24-hour access to alcohol three days/week (starting Monday, Wednesday, and Friday) for 5–6 weeks. Rats were then switched so that they only had 3-hour access to alcohol on the three days/week to simplify detection of potential changes in intake after chlorzoxazone (CZX). After 5–6 weeks of 3-hour/day IAA drinking, the effects of CZX were examined. Different doses of CZX were injected, once/week, 20–25 min before access to alcohol, in a counterbalanced manner across rats. Results from IAA rats were compared with CAA rats that had continuous access to 20% alcohol versus water for an equivalent period of drinking, so that CZX testing began after approximately 12 weeks of CAA. The CAA rats were allowed access to alcohol 24 hours/day 7 days/week, but alcohol intake levels for this study were determined in the same 3-hour period in which IAA rats were allowed access to alcohol.

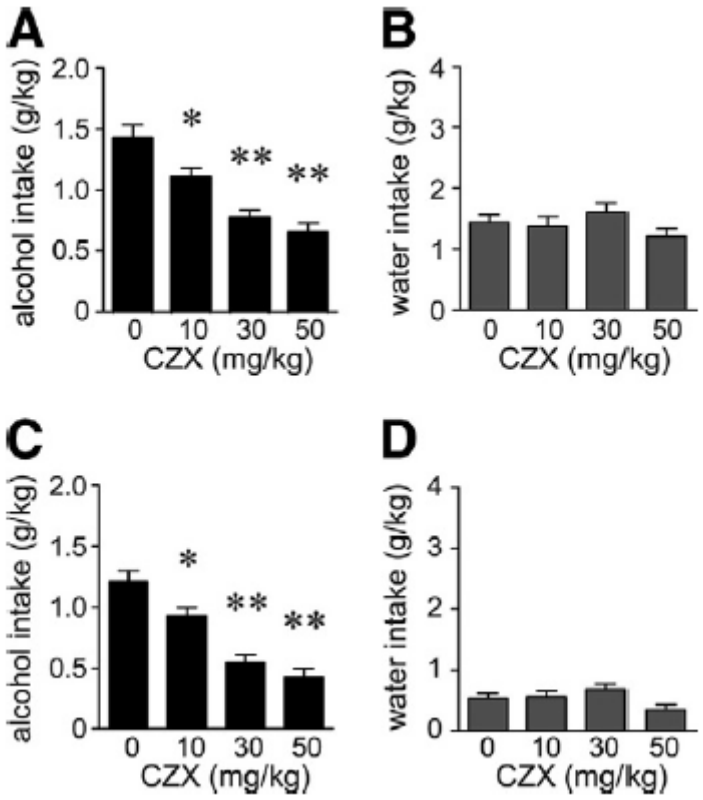


Figure 2.

Chlorzoxazone decreased IAA alcohol but not water intake. (**A**, **B**) Chlorzoxazone dose-dependently decreased IAA alcohol intake, determined after 3-hour access (**A**), with no effect on IAA water intake (**B**) (vehicle: $n = 22$; 10 mg/kg CZX: $n = 21$; 30 mg/kg CZX: $n = 22$; 50 mg/kg CZX: $n = 13$). Similarly, (**C**, **D**) CZX dose-dependently decreased IAA alcohol intake across 1-hour access (**C**) with no effect on water intake (**D**). (**A**, **C**) Post hoc $p < .05$, 10 mg/kg versus 30 or 50 mg/kg CZX. $^{*}p < .05$, $^{**}p < .01$ versus vehicle. Abbreviations as in Figure 1.

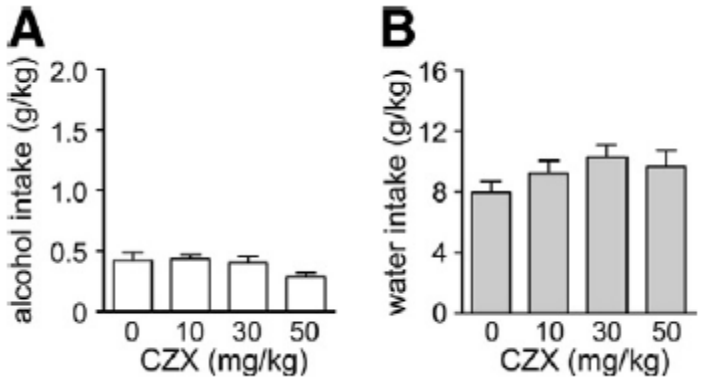


Figure 3.

Chlorzoxazone did not decrease CAA alcohol intake. Chlorzoxazone had no effect on alcohol (**A**) or water (**B**) intake in CAA rats (vehicle: $n = 17$; 10 mg/kg CZX: $n = 15$; 30 mg/kg CZX: $n = 16$; 50 mg/kg CZX: $n = 9$). CAA rats were allowed access to alcohol 24 hours/day 7 days/week, but alcohol intake levels were determined in the same 3-hour period in which IAA rats were allowed access to alcohol. Abbreviations as in Figure 1.

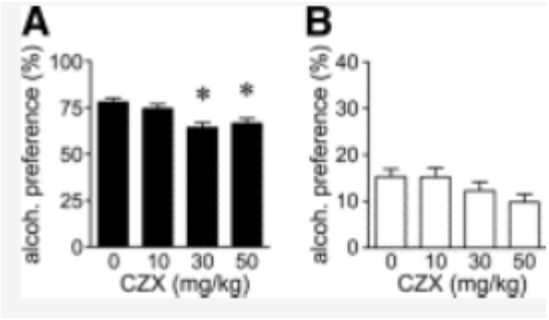
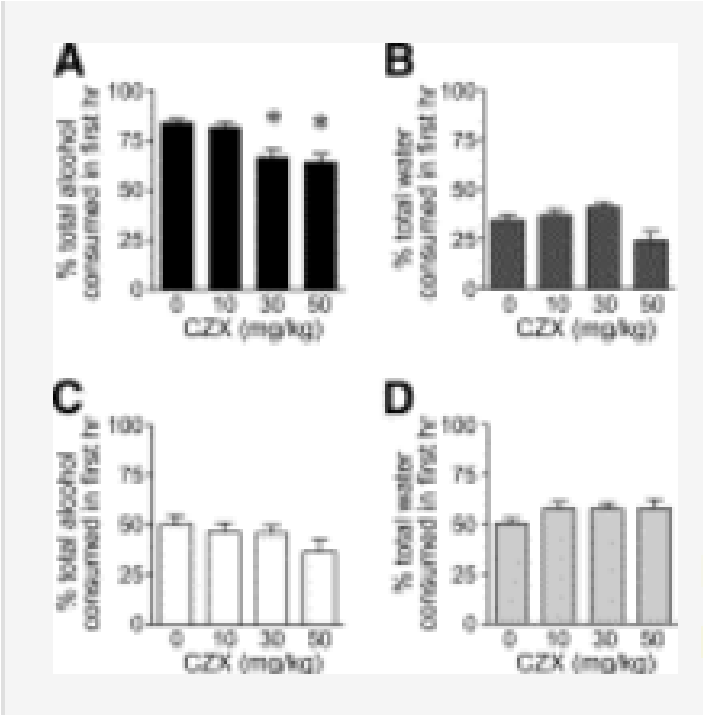


Figure 4.

Alcohol preference across the 3 hours of intake was decreased by CZX in IAA but not CAA rats. (**A**, **B**) Chlorzoxazone decreased the alcohol preference (the level of alcohol intake divided by the level of alcohol plus water intake) in IAA (**A**) but not CAA (**B**) rats. *$p < .05$ versus vehicle. Abbreviations as in Figure 1.



The IAA rats consumed most alcohol within the first hour of access. (**A**) The IAA rats consumed most alcohol within the first hour of access, relative to the amount consumed across all 3 hours of access. Chlorzoxazone significantly reduced this higher initial consumption in IAA rats. (**B**) Chlorzoxazone did not alter water intake in IAA rats. Chlorzoxazone did have an overall significant effect on the pattern of IAA water consumption [$F(3,74) = 4.25$, $p = .008$], but this reflected a significant difference post hoc between 30 and 50 mg/kg rather than differences between any CZX dose and vehicle. (**C**, **D**) Chlorzoxazone did not alter the percentage of total alcohol (**C**) or water (**D**) consumed in the first hour of access, relative to all 3 hours of access, in CAA rats. *$p < .05$ versus vehicle. Abbreviations as in Figure 1.

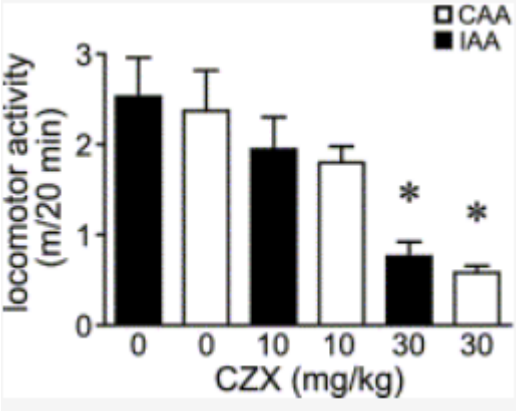


Figure 6.

Chlorzoxazone similarly depressed open-field locomotion in IAA rats (vehicle: $n = 10$; 10 mg/kg CZX: $n = 9$; 30 mg/kg CZX: $n = 6$) and CAA rats (vehicle: $n = 9$; 10 mg/kg CZX: $n = 9$; 30 mg/kg CZX: $n = 6$). Similar results were observed for stereotypies (not shown). $^{*}p < .05$ versus vehicle. Abbreviations as in Figure 1.

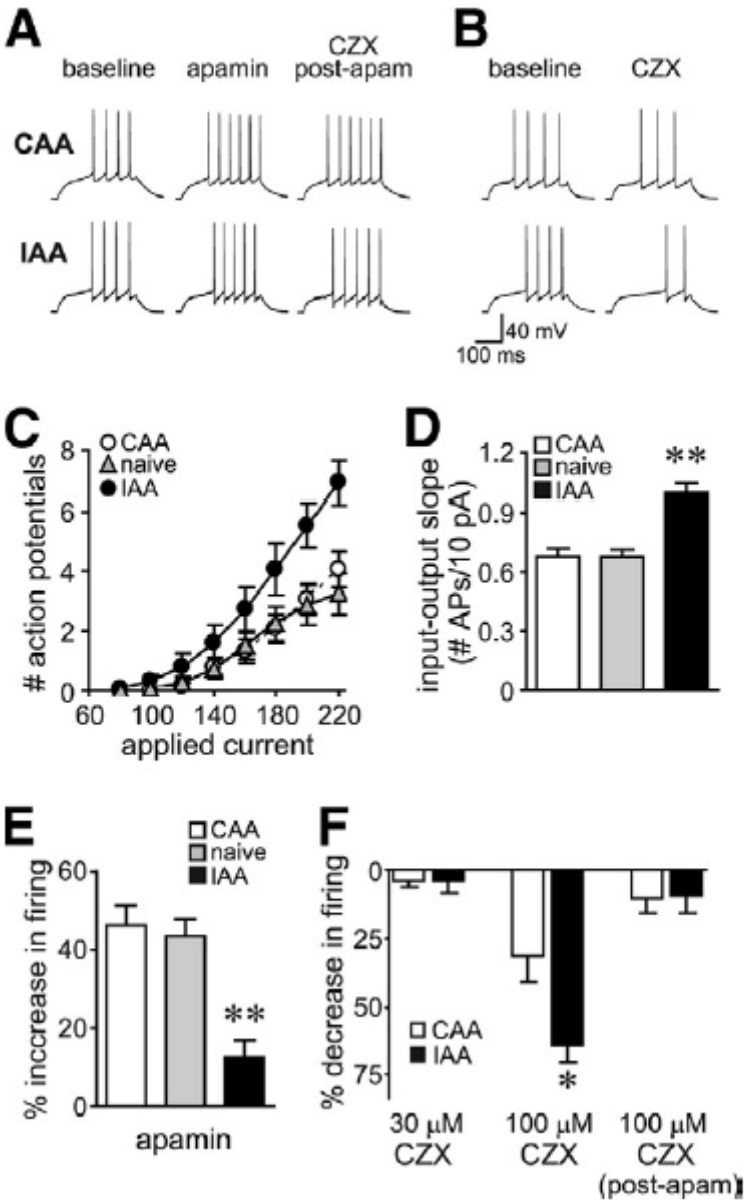


Figure 7.

Ex vivo whole-cell patch-clamp electrophysiology experiments in nucleus accumbens (NAcb) core neurons from IAA and CAA rats. (**A**) Examples showing that SK-type potassium channel (SK) inhibition with apamin (100 nmol/L) produced a smaller enhancement of firing in IAA versus CAA neurons and that the CZX reduction in firing was prevented by apamin exposure in both IAA and CAA neurons. (**B**) Examples showing greater CZX inhibition of firing in IAA versus CAA neurons. (**C**) Greater basal excitability, measured with applied current versus action potential generation, was observed in IAA compared with CAA or naive NAcb core neurons ex vivo (CAA: $n = 19$; naive: $n = 21$; IAA: $n = 17$). To generate firing, neurons were depolarized with a series of 7 or 8 300-msec current pulses, with 20 pA between each current pulse, where the initial current pulse for each neuron was just subthreshold for firing. For one naive cell, firing was not determined at 220 pA. (**D**) Greater basal excitability measured with the input-output slope, calculated by fitting a line relating the number of action potentials generated in the first three suprathreshold current pulses and the last subthreshold current pulse in a given neuron ([5] and [62]). (**E**) Decreased apamin enhancement of firing in IAA compared with CAA or naive NAcb core neurons ex vivo (CAA: $n = 8$; naive: $n = 5$; IAA: $n = 7$). (**F**) Chlorzoxazone produced a greater inhibition of firing in IAA neurons ($n = 6$ for 100 µmol/L CZX and 4 for 30 µm CZX for both groups), and CZX inhibited firing in IAA and CAA NAcb core neurons through SK channels, because the CZX reduction in firing was blocked by apamin ($n = 5$ for both groups). Example traces were from a current step to 140 pA for IAA and 140 pA for CAA in (**A**) and 120 pA for IAA and 180 pA for CAA in (**B**). $^{*}p < .05$, $^{**}p < .01$, IAA versus CAA and naive. Abbreviations as in Figure 1.