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K_{Ca}2 channels: Novel therapeutic targets for treating alcohol withdrawal and escalation of alcohol consumption

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Small-conductance, calcium-activated potassium (K_{Ca}2, formerly SK (Wei et al., 2005)) channels link fluctuations in intracellular calcium levels with functional changes in central neuron membrane potentials. K_{Ca}2 channels are composed of 6 transmembrane domains, a channel pore located between S5 and S6, and intracellular N- and C-termini. Cloning studies have identified three *KCNN* genes that encode K_{Ca}2 channel α subunits (K_{Ca}2.1 (SK1), K_{Ca}2.2 (SK2), and K_{Ca}2.3 (SK3)) in brain (Kohler et al., 1996). K_{Ca}2 channel subunits are widely expressed throughout the brain, yet the expression pattern of the distinct subunits varies by brain region (Sailer et al., 2002; Sailer et al., 2004). K_{Ca}2.1 and K_{Ca}2.2 channels are highly expressed in the cerebral cortex, hippocampus, and lateral amygdala. K_{Ca}2.3 channels localize to the thalamus, brain stem nuclei, and basal ganglia, including the nucleus accumbens (NAc) and ventral tegmental area (VTA). In addition to distinct expression patterns in brain, K_{Ca}2 channel subunits are differentially blocked by the selective allosteric inhibitor apamin (Weatherall et al., 2011), a bee venom neurotoxin. Homomeric K_{Ca}2.2 channels are potently inhibited by apamin (EC₅₀ of 40 – 70 pM), whereas K_{Ca}2.3 and K_{Ca}2.1 channels are inhibited by apamin in the low nanomolar range (EC₅₀ of 0.6 – 6 and 1 – 10 nM, respectively)(Weatherall et al., 2010). While a number of non-selective K_{Ca}2 channel blockers (e.g., scyllatoxin, quaternary salts of bicuculline, D-turbocurarine) and positive modulators (e.g., 1-EBIO, NS309, chlorzoxazone) have been described, only a few toxins (i.e., tamapin, leiurotoxin) demonstrate some subunit selectivity for inhibition of K_{Ca}2 channels (Weatherall et al., 2010).

In mammalian cell lines, homomeric K_{Ca}2.2 or K_{Ca}2.3 produce functional cell surface channels and heteromeric K_{Ca}2.2 and K_{Ca}2.3 channel complexes give rise to functional channels (Monaghan et al., 2004). Expression of homomeric rat K_{Ca}2.1 channels does not produce detectable K_{Ca}2 currents (Bowden et al., 2001). Human K_{Ca}2.1 homomers can produce functional channels (Kohler et al., 1996), and rat K_{Ca}2.1 gives rise to functional channels when co-assembled with K_{Ca}2.2 or K_{Ca}2.3 in mammalian cells (Benton et al., 2003). The overlapping expression patterns of K_{Ca}2.1 and K_{Ca}2.2 channels, in particular, suggest that they may form functional heteromeric channels *in vivo*. Despite some overlapping expression patterns of K_{Ca}2.2 and K_{Ca}2.3 channels, K_{Ca}2.2 and K_{Ca}2.3 channels do not immunoprecipitate together (Sailer et al., 2002). However, a more recent study suggests that K_{Ca}2.2 and K_{Ca}2.3 heteromeric channels may depend on the splice variant (Strassmaier et al., 2005). These authors demonstrated that the long isoform of K_{Ca}2.2 (K_{Ca}2.2-L) channels co-assembles with K_{Ca}2.3 channels in mouse brain.

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There are 32 splice variants that have been described for $K_{Ca2.1}$ channels in mouse brain, and $K_{Ca2.2}$ and $K_{Ca2.3}$ channels each have two isoforms (Shmukler et al., 2001; Wittekindt et al., 2004). The functional role and subcellular localization of these splice variants is just beginning to emerge. The short isoform of $K_{Ca2.3}$ that lacks an N-terminus appears to act as a negative dominant regulator of surface $K_{Ca2.3}$ channels (Tomita et al., 2003), and has been implicated in schizophrenia and cognition (Grube et al., 2011; Tomita et al., 2003). The two isoforms of $K_{Ca2.2}$ channels co-immunoprecipitate in brain (Strassmaier et al., 2005), and are both expressed in dendritic spines in the CA1 region of hippocampus (Allen et al., 2011); however, their subcellular expression patterns within dendritic spines and their function differ. $K_{Ca2.2-L}$, which has an extended N-terminal domain, is predominantly expressed in the postsynaptic density. The short isoform of $K_{Ca2.2}$ ($K_{Ca2.2-S}$) localizes to the plasma membrane of dendritic spines but is absent from the PSD. Interestingly, loss of $K_{Ca2.2-L}$ prevented the ability of apamin to influence long-term potentiation, enhanced non-spatial memory, and impaired spatial learning and memory (Allen et al., 2011). Similar to the short isoform of $K_{Ca2.3}$, $K_{Ca2.2-S}$ may also act as a dominant negative. Indeed, expression of $K_{Ca2.2-S}$ does not produce functional channels in HEK293 cells, and $K_{Ca2.2-S}$ mRNA expression levels were elevated in cortex from Alzheimer's disease patients (Murthy et al., 2008). These authors also demonstrated that cytokine exposure can increase $K_{Ca2.2-S}$ protein levels in cortical neurons. Together, these data emphasize the importance of understanding K_{Ca2} subunit pharmacology and complexes in disease processes.

K_{Ca2} Channels, Intrinsic Excitability, and Synaptic Plasticity

K_{Ca2} channels regulate membrane excitability by shaping excitatory postsynaptic potentials (EPSP) and controlling intrinsic activity, dendritic integration and pacemaker firing (Bond et al., 2005; Fakler and Adelman, 2008). K_{Ca2} channels are solely activated by transient elevations of intracellular Ca^{2+} and form functional heteromeric complexes with calmodulin that acts as a high-affinity Ca^{2+} sensor (Allen et al., 2007; Lee et al., 2003; Maylie et al., 2004). In hippocampus, amygdala and prefrontal cortex, $K_{Ca2.2}$ channels are highly expressed and are localized to the PSD of neuronal dendritic spines. Here they form a Ca^{2+} -mediated negative feedback loop with synaptic NMDA-type glutamate receptors (Bloodgood and Sabatini, 2007; Faber, 2010; Faber et al., 2005; Lin et al., 2008; Mulholland et al., 2011; Ngo-Anh et al., 2005). Activation of this feedback loop is thought to act as a postsynaptic shunt to decrease spine Ca^{2+} transients and synaptic depolarization. Consistent with this idea are studies showing that blocking K_{Ca2} channels in pyramidal neurons increases EPSP amplitude and facilitates NMDA receptor-dependent synaptic plasticity (Faber, 2010; Faber et al., 2008; Faber et al., 2005; Lin et al., 2008). K_{Ca2} channels also facilitate the acquisition of mPFC-, amygdala-, and hippocampal-dependent learning tasks (Brennan et al., 2008; Deschaux and Bizot, 1997; Deschaux et al., 1997; Hammond et al., 2006; Stackman et al., 2002), suggesting a prominent role for these channels in cellular mechanisms underlying memory encoding. In comparison, $K_{Ca2.3}$ channels are highly expressed in NAc core and shell subregions (Sailer et al., 2004; Stocker and Pedarzani, 2000) and contribute to action potential after-hyperpolarization (AHP) that is a critical regulator of basal firing rates (Hopf et al., 2010; Ishikawa et al., 2009). As will be described below, evidence suggests that K_{Ca2} channels may play an important role in alcohol addiction.

Chronic Alcohol and Glutamate

Accumulating evidence suggests that chronic and repeated episodes of alcohol exposure and withdrawal modify the function of glutamatergic processes in key brain regions in the addiction circuitry. Glutamatergic-based neuroadaptations are thought to contribute to the severity of the withdrawal syndrome, consumption of high amounts of alcohol, and relapse

to alcohol-seeking behaviors (Fadda and Rossetti, 1998; Krystal et al., 2003; Vengeliene et al., 2008). Chronic ethanol exposure and withdrawal produce morphological adaptations in the size and density of dendritic spines (Carpenter-Hyland and Chandler, 2006; Moonat et al., 2011; Pandey et al., 2008; Zhou et al., 2007) and elevate the synaptic expression of GluN1 and GluN2B subunits of the NMDA receptor in a number of regions in the addiction circuitry (Carpenter-Hyland et al., 2004; Clapp et al., 2010; Hendricson et al., 2007; Kash et al., 2009; Mulholland et al., 2011; Qiang et al., 2007; Roberto et al., 2004). Enhanced activation and number of NAc NMDA receptors and elevated extracellular glutamate levels actively regulate expression of ethanol-seeking behaviors (Gremel and Cunningham, 2009, 2010; Obara et al., 2009; Szumlinski et al., 2008). These neuroadaptations in glutamatergic processes in the NAc shell are associated with a potentiation of NMDA receptor-dependent excitatory postsynaptic currents and a metaplastic shift from long-term depression to long-term potentiation (Jeanes et al., 2011). Additional evidence suggests that up-regulation of GluN2B in the dorsomedial striatum may be responsible for driving excessive alcohol intake and relapse (Wang et al., 2007; Wang et al., 2010). It is thought that cycling between periods of heavy alcohol drinking and abstinence promotes enhanced relapse vulnerability. Indeed, results from Becker and colleagues have shown that repeated cycles of chronic intermittent ethanol exposure and withdrawal produce escalations in voluntary ethanol consumption in mice (Becker and Lopez, 2004; Griffin et al., 2009; Lopez and Becker, 2005). As relapse is the most challenging clinical problem encountered during treatment of addiction (O'Brien, 2005), there is a substantial need to identify novel pharmacological targets that have higher therapeutic efficacy with fewer side effects. Even though our understanding of the neurobiological basis of alcohol addiction has grown considerably over the past two decades, it is essential that we identify novel therapeutic targets to reduce the uncontrolled drinking that is the hallmark of alcohol use disorders. Mounting evidence suggests that K_{Ca2} channels play an important role in alcohol addiction (Table 1), and that K_{Ca2} channels are a novel therapeutic target for treating alcohol dependence (Mulholland et al., 2009). Although not discussed here, additional studies have also identified an important role for calcium- and voltage-activated BK (K_{Ca1}) channels in alcohol tolerance and consumption (Brodie et al., 2007; Martin, 2010; Mulholland et al., 2009; Treistman and Martin, 2009).

Chronic Alcohol and Hippocampal K_{Ca2} Channels

A recent study from our laboratory has examined the role of $K_{Ca2.2}$ channels in regulating neuroadaptations in hippocampal glutamatergic synapses using well-characterized in vitro and in vivo models of chronic alcohol exposure and withdrawal. We demonstrated that continuous ethanol exposure reduced K_{Ca2} -mediated tail currents recorded in CA1 pyramidal neurons from organotypic hippocampal slices (Mulholland et al., 2011). These data are consistent with previous work showing reduced hippocampal AHP currents in rats treated with a chronic ethanol liquid diet (Durand and Carlen, 1984). Continuous ethanol exposure of organotypic slices also reduced $K_{Ca2.2}$ channel surface expression. This reduction in $K_{Ca2.2}$ channels was accompanied by elevations in synaptic expression of GluN1 and GluN2B subunits supporting the suggestion that the bidirectional changes in $K_{Ca2.2}$ channels and synaptic NMDA receptors mediate ethanol-associated plasticity of glutamatergic synapses. We next tested whether this leads to a functional uncoupling of the feedback loop between $K_{Ca2.2}$ channels and NMDA receptors in dendritic spines after chronic ethanol. As expected, the K_{Ca2} channel inhibitor apamin increased the amplitude of subthreshold EPSPs recorded from CA1 pyramidal neurons in untreated slices. In contrast, the ability of apamin to potentiate postsynaptic potentials was greatly reduced in CA1 neurons exposed to chronic ethanol. Thus, chronic ethanol bi-directionally regulates K_{Ca2} channels and NMDA receptors in hippocampal pyramidal neurons that lead to disruption of the K_{Ca2} channel-NMDA receptor feedback loop and hyperexcitability during acute ethanol withdrawal.

We next wanted to confirm ethanol-induced down-regulation of $K_{Ca2.2}$ channels and also determine the subcellular localization of the neuroadaptation using an in vivo model of ethanol dependence. Adult male C3H/He mice were exposed to chronic ethanol in vapor inhalation chambers, and PSD-enriched and extrasynaptic fractions were prepared from hippocampus immediately following removal of mice from the chambers. As expected, $K_{Ca2.2}$ channels localize to synaptic and extrasynaptic dendritic sites in hippocampal neurons, an observation we confirmed using immunohistochemistry and confocal imaging (Mulholland et al., 2011). Ethanol vapor inhalation significantly reduced $K_{Ca2.2}$ channel expression in the PSD-enriched fraction, but not at extrasynaptic sites in hippocampus. Together, these data show that in vivo and in vitro continuous ethanol exposure reduces surface expression of $K_{Ca2.2}$ channels specifically at synaptic sites. Prolonged ethanol consumption is well-documented to produce deleterious effects on hippocampal-dependent learning and memory. More recent evidence has detailed the importance of neuroadaptations in the hippocampus that contribute to convulsive behaviors during ethanol withdrawal (Gililand-Kaufman et al., 2008; Mark and Finn, 2002; Martin-Garcia and Pallares, 2005). Our data raise the exciting possibility that chronic ethanol-induced changes in hippocampal K_{Ca2} channels play a role in withdrawal severity and impaired performance on hippocampal-dependent cognitive tasks.

A potential target to reduce NMDA receptor hyperactivity during acute ethanol withdrawal is to restore K_{Ca2} channel activity. To elevate K_{Ca2} channel function, we used 1-EBIO, a K_{Ca2} channel positive modulator, that increases the Ca^{2+} sensitivity of K_{Ca2} channels by stabilizing the Ca^{2+} -calmodulin interface on the α -subunit thus slowing channel deactivation and prolonging channel open time (Pedarzani et al., 2001; Syme et al., 2000; Wolfart et al., 2001). We demonstrated that 1-EBIO attenuated acute withdrawal-related epileptiform burst firing and neurotoxicity in CA1 neurons from organotypic hippocampal slices (Mulholland et al., 2011). Chlorzoxazone (CZX), a centrally-acting FDA approved medication used to treat muscle spasms, also functions as a K_{Ca2} channel positive modulator by increasing the open probability of the K_{Ca2} channel (Cao et al., 2001; Syme et al., 2000). CZX has a similar structure to 1-EBIO (Cao et al., 2001), appears to have a longer half-life than 1-EBIO (Wan et al., 2006; Yasuhara and Levy, 1988), readily crosses the blood-brain barrier (Yasuhara and Levy, 1988), and is orally available (Alvina and Khodakhah, 2010a, b). Like 1-EBIO, treatment of organotypic hippocampal slices with CZX (100 μ M) during 24 hr withdrawal from 8 day ethanol exposure significantly prevented NMDA receptor-mediated withdrawal neurotoxicity in the CA1 region (Fig 1). In vivo, systemic administration of 1-EBIO reduced the severity of handling-induced convulsions in C3H/He mice undergoing withdrawal from ethanol vapor inhalation (Mulholland et al., 2011). In addition, 1-EBIO treatment completely prevented the most severe form of convulsive activity observed in ethanol-dependent mice (Fig 2). Thus, these data demonstrate that down-regulation of $K_{Ca2.2}$ channels contributes to alcohol-associated plasticity of hippocampal glutamatergic synapses, and that reduced expression of $K_{Ca2.2}$ channels in dendritic spines plays a role in the alcohol withdrawal syndrome. The ability of a positive modulator of K_{Ca2} channel function to attenuate hyperexcitability identifies the K_{Ca2} channel as a novel therapeutic target for treating alcohol withdrawal and dependence.

K_{Ca2} Channels and the Alcohol Reward Circuitry

In addition to ethanol-induced neuroadaptations in hippocampal K_{Ca2} channels, recent studies have addressed the possibility that acute and chronic ethanol exposure and withdrawal alters firing properties of VTA dopamine (DA) and NAc core medium spiny neurons through adaptations in K_{Ca2} channel function and expression (Brodie et al., 1999; Hopf et al., 2010; Hopf et al., 2007). The studies focused on the VTA and NAc core because of their roles in motivated and goal-directed behaviors, including alcohol addiction. Acute

bath application of ethanol has been shown to excite VTA neurons and reduce the AHP that follows spontaneous action potentials (Brodie and Appel, 1998; Brodie et al., 1990). Brodie and colleagues (1999) further demonstrated that blockade of K_{Ca2} channels with apamin in VTA DA neurons potentiated the ethanol-associated increase in firing rate and the attenuation of the AHP. These authors argued that the magnitude of K_{Ca2} currents can influence the response of VTA DA neuron to acute ethanol (Brodie et al., 1999; Brodie et al., 2007). Hopf and colleagues (2007) showed that the amplitude of the AHP and K_{Ca2} -mediated tail currents were significantly reduced in rats 7 days following a 5 day ethanol (2 g/kg, ip, twice a day) treatment paradigm (Hopf et al., 2007). Interestingly, K_{Ca2} channels can modulate the ability of NMDA receptors to induce burst firing in midbrain DA neurons (Johnson and Seutin, 1997; Seutin et al., 1993). In agreement with these studies, bath application of NMDA induced burst firing in control neurons when apamin was co-applied with NMDA (Hopf et al., 2007). However, NMDA bath application alone readily produced burst firing in VTA DA neurons in slices prepared from ethanol-treated rats. These authors concluded that a consequence of reduced K_{Ca2} function by ethanol exposure and withdrawal is to facilitate NMDA-induced burst firing in VTA DA neurons. In a separate study, Hopf and colleagues showed that 5 weeks of operant self-administration of 10% ethanol by rats increased intrinsic excitability of neurons in the NAc core (Hopf et al., 2010). Alcohol self-administration also reduced the amplitude of the AHP and K_{Ca2} -mediated tail currents in these neurons. These functional adaptations in K_{Ca2} channels by ethanol self-administration were associated with a significant reduction in $K_{Ca2.3}$ channel expression in NAc core. Thus, these data suggest that withdrawal from ethanol treatment and ethanol self-administration can reduce function of K_{Ca} channels in the VTA and NAc core, and that these adaptations are important for regulating intrinsic excitability and NMDA receptor-dependent burst firing.

Emerging evidence suggests that chronic ethanol-associated changes in K_{Ca2} channels in the mesolimbic circuitry can influence alcohol consumption and alcohol-seeking behaviors in dependence and non-dependence rodent models of ethanol addiction. Indeed, microinjection of 1-EBIO into the NAc core, but not NAc shell or dorsolateral striatum reduced the motivation of rats to consume ethanol following abstinence from 5 weeks of operant ethanol self-administration (Hopf et al., 2010). In a separate study using an intermittent access paradigm, systemic administration of CZX to rats decreased voluntary alcohol consumption and alcohol preference without affecting water intake (Hopf et al., 2011). In a mouse dependence model characterized by escalation of voluntary drinking (Becker and Lopez, 2004; Griffin et al., 2009; Griffin et al., 2007; Lopez and Becker, 2005), our preliminary data demonstrate a significant reduction in $K_{Ca2.3}$ channels in the NAc core from ethanol-dependent mice (Mulholland et al., 2010). These data are consistent with a previous report showing a down-regulation of $K_{Ca2.3}$ channel in the NAc core of ethanol-treated rats (Hopf et al., 2010). Because K_{Ca2} channel positive modulators have off-target actions (Cuthbert, 2001; Dutta et al., 2009; Pedarzani et al., 2001; Syme et al., 2000), we next examined the ability of apamin, a potent and selective K_{Ca2} channel inhibitor, to influence voluntary alcohol consumption using a limited-access, two-bottle choice paradigm. Consistent with previous reports, repeated cycles of CIE vapor inhalation significantly increased voluntary ethanol consumption. Preliminary data demonstrate that bilateral microinjection of apamin into the NAc (verified by histological analysis) significantly increased voluntary drinking in non-dependent mice. In strong contrast, microinjection of apamin did not increase drinking in ethanol-dependent mice. Together, these preliminary data together with reported findings from Hopf and colleagues support the suggestion that CIE-induced down-regulation of $K_{Ca2.3}$ channels in NAc contributes to altered intrinsic excitability of NAc core neurons and the escalation of voluntary consumption in ethanol-dependent mice.

Conclusions

The data presented here suggest that chronic ethanol-associated plasticity critically involves K_{Ca2} channels in hippocampus, VTA, and NAc core. These neuroadaptations contribute to alterations in neuronal firing properties, enhanced intrinsic excitability, and NMDA receptor-dependent burst firing and withdrawal hyperexcitability. We recently showed that chronic ethanol disrupts the calcium-dependent K_{Ca2} channel-NMDA receptor negative feedback loop in hippocampal dendritic spines and leads to aberrant excitability during withdrawal. Restoring K_{Ca2} channel activity can prevent the deleterious consequences of alcohol withdrawal in vivo and in vitro. This is particularly important in light of evidence that withdrawal-associated plasticity promotes increased consumption and that the severity of withdrawal and relapse rates increase with conventional benzodiazepines treatment (Becker and Lopez, 2004; Griffin et al., 2009; Malcolm et al., 2002; O'Dell et al., 2004). Direct pharmacological modulation of K_{Ca2} channels using positive modulator or channel inhibitors can bi-directionally influence drinking behavior in non-dependent models of ethanol seeking and voluntary consumption. Perhaps most interesting, chronic ethanol regulates expression of $K_{Ca2.3}$ channel in the NAc core, and this down-regulation appears to be a critical factor for influencing consumption of high amounts of ethanol. Collectively, these studies using various rodent models have clearly identified K_{Ca2} channels as a novel therapeutic target to alleviate the symptoms of alcohol withdrawal and reduce high amounts of voluntary alcohol consumption.

Future Studies

There are a number of outstanding questions that need to be considered before embarking on large scale clinical studies for the treatment of alcohol use disorders. We do not yet know the time course of chronic ethanol-associated down-regulation of K_{Ca2} channels or if repeated withdrawal contributes to this phenomenon. What are the mechanisms for the reduction in K_{Ca2} channels? Does protein kinase A play a role in the reduction of surface K_{Ca2} channel by chronic ethanol as has been suggested for K_{Ca2} internalization following induction of synaptic plasticity? Does chronic ethanol shift expression patterns of heteromeric K_{Ca2} channel complexes or enhance the dominant negative subunits of $K_{Ca2.2}$ or $K_{Ca2.3}$? Pharmacological targeting of heteromeric complexes or specific isoforms could lead to more effective treatments with less severe side effects. Chlorzoxazone, a proposed compound to treat alcoholism, is metabolized by cytochrome P450 2E1 (Carriere et al., 1993), and chronic ethanol exposure is known to increase CYP2E1 synthesis and decrease CYP2E1 degradation in the liver (Lu and Cederbaum, 2008; Tsukamoto and Lu, 2001; Zerilli et al., 1995). Does this mean that higher doses of CZX are needed to reduce voluntary drinking and the severity of withdrawal in ethanol dependent versus non-dependent rodents? If so, will the side effects of higher doses be tolerable? Although these pre-clinical findings are promising, future work is necessary to determine if these results on K_{Ca2} channels will translate into efficacious pharmacotherapies for treating alcohol use disorders.

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Figure 1.

The K_{Ca2} channel positive modulator chlorzoxazone (CZX) reduces NMDA receptor-mediated excitotoxicity during ethanol withdrawal. Organotypic hippocampal slice cultures were treated with chronic ethanol, and withdrawal toxicity was measured as previously described (Mulholland et al., 2011). Cultures were exposed to CZX (100 μ M) during the 24 hr withdrawal period, and propidium iodide staining of dead and dying neurons in the CA1 region was assessed 24 hr after the removal of ethanol. (A) Representative images of propidium iodide (PI) staining in organotypic hippocampal slice cultures. (B) As expected, withdrawal from 8 day ethanol produced significant uptake of PI in the CA1 region. Treatment of cultures with CZX alone did not alter cell viability; however, CZX significantly prevented withdrawal neurotoxicity (One-way ANOVA, $F(3,14) = 5.32$, $P < .05$, Newman-Keuls post-hoc, * $P < .05$ versus CTRL, ** $P < .05$ versus EWD; $n = 8-10$ slices/group/replicate, 4 replicates).

Figure 2.

Positive modulation of K_{Ca2} channels significantly attenuates the occurrence of severe handling-induced convulsions (HIC) during withdrawal from continuous ethanol vapor inhalation. 1-EBIO (20 mg/kg, ip.) was administered 30 min prior to assessing HICs at 4 h (left panel) and 8 h (right panel). HIC scoring scale: 0 = no convulsion activity on lift or spin; 1 = no activity on lift, but facial grimace on spin; 2 = tonic convulsion after tail lift and spin; 4 = tonic convulsion on tail lift. Adapted from Mulholland et al., 2011.

Table 1

Summary of published findings on the effects of acute and chronic ethanol on K_{Ca}2 channel function and expression and the effects of K_{Ca}2 channel positive modulators on EtOH-seeking, consumption, and withdrawal severity

Brain Region	EtOH Exposure Model	EtOH Concentration or Consumption	K _{Ca} 2 Function	K _{Ca} 2 Expression	K _{Ca} 2 Modulation of Behavior
CA1 & DG ¹	20 wk EtOH liquid diet plus 3 wk WD	—	↓ AHP amplitude and duration	—	—
VTA ²	Acute bath application of VTA slices with EtOH	20–120 mM	Apamin ↑ acute EtOH-induced ↓ in the AHP	—	—
VTA ³	2 g/kg EtOH (ip) 2× d/5 d plus 7 d WD	—	↓ AHP & K _{Ca} 2 tail currents; ↓ K _{Ca} 2 control of NMDA burst firing	—	—
NAC ⁴	Operant self-administration of 10% EtOH for 42–50 d	0.42 g/kg/30 min	↓ AHP & K _{Ca} 2 currents; ↓ K _{Ca} 2 control of intrinsic excitability	↓ K _{Ca} 2.3	↓ EtOH-seeking behavior
NAC ⁵	Consumption of Intermittent 20% EtOH for 10–12 wks	~32 mg/dL	↓ K _{Ca} 2 control of intrinsic excitability	—	↓ EtOH consumption
CA1 ⁶	64 h EtOH vapor inhalation in-vivo; ~8 d in-vitro	~160 mg/dL in-vivo; 25–100 mM in-vitro	↓ K _{Ca} 2 currents & K _{Ca} 2 control of EPSPs	↓ K _{Ca} 2.2	↓ HICs

EtOH = ethanol; CA = Cornu Ammonis; DG = dentate gyrus; WD = withdrawal; AHP = after-hyperpolarization; VTA = ventral tegmental area; NAC = nucleus accumbens; EPSPs = excitatory postsynaptic potentials; - = not reported or tested; HIC = handling-induced convulsions; 1. Durand & Carlen, 1984, 2. Brodie et al., 1999, 3. Hopf et al., 2007, 4. Hopf et al., 2010, 5. Hopf et al., 2011, 6. Mulholland et al., 2011