Ethanol Effect on BK Channels is Modulated by Magnesium

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Background: Alcoholics have been reported to have reduced levels of magnesium in both their extracellular and intracellular compartments. Calcium-dependent potassium channels (BK) are known to be one of ethanol (EtOH)'s better known molecular targets.

Methods: Using outside-out patches from hippocampal neuronal cultures, we examined the consequences of altered intracellular Mg^{2+} on the effects that EtOH has on BK channels.

Results: We find that the effect of EtOH is bimodally influenced by the Mg^{2+} concentration on the cytoplasmic side. More specifically, when internal Mg^{2+} concentrations are ≤200 μM, EtOH decreases BK activity, whereas it increases activity when Mg^{2+} is at 1 mM. Similar results are obtained when using patches from HEK cells expressing only the α-subunit of BK. When patches are made with the actin destabilizer cytochalasin D present on the cytoplasmic side, the potentiation caused by EtOH becomes independent of the Mg^{2+} concentration. Furthermore, in the presence of the actin stabilizer phalloidin, EtOH causes inhibition even at Mg^{2+} concentrations of 1 mM.

Conclusions: Internal Mg^{2+} can modulate the EtOH effects on BK channels only when there is an intact, internal actin interaction with the channel, as is found at synapses. We propose that the EtOH-induced decrease in cytoplasmic Mg^{2+} observed in frequent/chronic drinkers would decrease EtOH's actions on synaptic (e.g., actin-bound) BK channels, producing a form of molecular tolerance.

Key Words: Ethanol, Magnesium, BK Channel.

IN THIS STUDY, we examined the consequences of altered Mg^{2+} concentrations on one of ethanol (EtOH)'s better known molecular targets, the calcium-dependent potassium channel (BK). BK activity is potentiated by EtOH, and this modulation is dependent on the intracellular Ca^{2+} concentration (Liu et al., 2008, 2013; Yuan et al., 2011). More specifically, EtOH increases the activity of the channel when internal Ca^{2+} concentrations (1 to 30 μM) are at physiological levels (Feinberg-Zadek and Treistman, 2007; Feinberg-Zadek et al., 2008; Liu et al., 2008). EtOH's potentiation of BK channel activity would lead to a stronger action potential after hyperpolarization, thus reducing firing frequency. The final effect would be a less responsive neuronal system (Gruss et al., 2001).

The Ca^{2+} dependence of EtOH's effects on BK channels has been well documented (see Mulholland et al., 2009). Cytoplasmic Ca^{2+} is thought to be the only cation necessary for the EtOH potentiation of these channels (Liu et al., 2008). This is concluded despite the fact that Mg^{2+} affects BK activity (Cui et al., 2009; Horrigan and Ma, 2008; Hu et al., 2003, 2007; Yang et al., 2008; Zhang et al., 2001) in a similar manner as does Ca^{2+}. Mg^{2+}, at physiological levels, increases BK channel open probability and is not only competitive with Ca^{2+} at some binding sites but also has independent Mg^{2+}-specific binding sites at the c-terminus of BK (Chen et al., 2011; Cui, 2010; Cui et al., 2009; Latorre and Brauchi, 2006; Yang et al., 2008; see Fig. 6). Thus, it would be reasonable to expect that Mg^{2+} could also modulate EtOH's effects on BK channels.

The cytoskeleton influences the activity of BK channels. It is well known that the presence of polymerized actin (as is found at synapses; Frotscher et al., 2014; Gordon-Weeks and Fournier, 2014; Loebrich, 2014; Mori et al., 2014) causes a decrease in the activity of some types of BK channels (Brainard et al., 2005; O'Malley et al., 2005) perhaps through interaction with sites at the c-terminus (Tian et al., 2006). Moreover, actin polymerization is dependent on the Mg^{2+} concentration (Galińska-Rakoczy et al., 2009; Hild et al., 2010). Interestingly, EtOH affects cytoskeletal actin filament integrity (Allansson et al., 2001; Loureiro et al., 2011; Offenhäuser et al., 2006; Popp and Dertien, 2008; Romero et al., 2010; Szabo et al., 2007). Importantly, alcohol consumption and/or exposure results in the depletion of Mg^{2+} levels in serum (Altura and Altura, 1999; Brown et al., 2002; Poikolainen and Alho, 2008; Romani, 2008) as well as cellular internal Mg^{2+} (Babu et al., 1999; Li et al., 2001; Romani, 2008). These are compelling reasons to explore whether cytoplasmic Mg^{2+} and actin can modulate the effects of EtOH on ionic channels.

We report here that EtOH's effects on BK channel activity are influenced by internal Mg^{2+} and that these effects are dependent on the integrity of the internal actin structure. Furthermore, this influence is observed at Ca^{2+} concentrations reported to be high enough (with respect to magnesium

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concentrations) to avoid Ca^{2+}-binding site competition (Shi and Cui, 2001; see Zhang et al., 2001). Thus, the influence of Mg^{2+} should be considered to be independent of other modulators (e.g., Ca^{2+}) of EtOH's effects on BK channels. We also discuss the possible regulation of BK channels by the well-known "Mg^{2+} deficit" syndrome resulting from chronic EtOH consumption, where the decrease in potentiation of channel activity by acute EtOH could be a form of tolerance resulting from these changes in internal Mg^{2+} levels.

MATERIALS AND METHODS

Preparations

Hippocampal CA1 neurons from embryonic rats were cultured for 7 to 14 days before use. Neurons from these cultures are known to only express α and α–β4 subunits of BK channels (Misonou et al., 2006; Piwonska et al., 2008). The culture media were then slowly changed to external solutions, usually requiring 20 to 30 volume equivalents. The procedure would last about 5 minutes and did not show any visible sign of damage or trauma to the cells, which could be patched within approximately 4 hours after the solution exchange.

Solutions

In all cases, the external (bath) solution used was (in mM): 130.5 K-gluconate, 14.5 KCl, 10 HEPES, 3 NaCl, 2 HEGTA, pH 7.35 (using KOH) with $MgCl_2$ and $CaCl_2$ added to make free concentrations of 1 mM and 30 μM, respectively. The amounts used for the free ion concentrations were calculated using the program WEBMAXC STANDARD (online program, 2009 version, Stanford University), with ionic strength at 0.16, and at 20°C. Internal (pipette) solutions were similar to external, except the amounts of $MgCl_2$ and $CaCl_2$ were prepared to achieve different free ion concentrations, as indicated. All tests for voltage shifts ($V_{1/2}$, see below) were made with internal 30 μM free Ca^{2+}, to minimize possible competition with free Mg^{2+} on the high-affinity Ca^{2+} sites (Chen et al., 2011; Cui, 2010; Lee and Cui, 2010) of BK channels. One millimolar free external Mg^{2+} was used because, first, the approach of studying internal Mg^{2+} effects would be simplified by changing only its internal concentration. Second, it was thought that one important question to answer would be what would happen if conditions mimic the internal Mg^{2+} depletion caused by EtOH (see Discussion). Finally, it is much easier to obtain stable outside-out configurations when patches are made with high external/bath Mg^{2+}.

The agents used were dimethyl sulfoxide (DMSO), cytochalasin D (both from Sigma-Aldrich, St. Louis, MO), and phalloidin (Invitrogen, Eugene, OR). All were diluted to their final concentrations in the internal solutions. Dilutions of cytochalasin D and phalloidin would yield a final DMSO concentration of 0.1% (v/v).

The "moderate" EtOH concentration range of 20 to 25 mM is the lower-limit of "intoxicating" in terms of human and animal consumption (see Eckardt et al., 1998; Harris and Mihic, 2004) and has been previously used for studies in EtOH effects on decreasing internal cellular Mg^{2+} (see, e.g., Babu et al., 1999; Li et al., 2001). Thus, in all cases here, EtOH was applied directly to the bath to make a final concentration of 20 to 25 mM.

Patching Procedure

Borosilicate glass pipettes were made with a resistance of 7 to 10 Mega-Ohms (MΩ). Pipettes were first tip-filled with the particular internal solution to be tested without any agents, and then backfilled with the same solutions plus agents, whenever such were tested. High-resistance on-cell patches were created (>2 Giga-Ohms: GΩ) followed by the whole-cell, and finally outside-out configurations. To improve the stability of the seals, in most cases, the patches were left unperturbed for at least 5 minutes after obtaining the outside-out configuration.

Data Gathering

Pulse protocols and data recordings were made using a HEKA EPC10 amplifier controlled with PATCHMASTER program (all by HEKA Elektronik, Dr. Schulze GmbH, Lambrecht/Pfalz, Germany). A series of criteria were followed to include recordings in our analysis. First, in accordance with what is known from hippocampal BK channels (Gong et al., 2001), patches with channel openings with conductances below 220 pS were not considered. Second, voltage-dependent activity was monitored through the use of voltage ramps, from which a range of voltage-dependent activity was determined (minimum to maximum activity), and an initial visual estimate of the voltage for NPo = 0.5 could be made. One of 2 protocols was followed once the patch was determined as stable.

Tests Using Changes in Specific Value of NPo. Patches were held at a holding potential where NPo was estimated to be about 0.5 (from the voltage ramps). A series of square pulses were then given, in increments of 1 mV and of 5 seconds duration, from −5 mV to +5 mV from that holding potential, until a recording yielded a control NPo = 0.5 to 0.6.

NPo as a Function of Voltage. Recordings were made using square pulses, each of 3 to 5 seconds duration, in increments of 5 to 10 mV from the lowest to slightly above the highest used in the voltage ramps (see Data Gathering). The higher limit was obtained by setting the voltage to where no closed states could be distinguished. The criteria for the duration of each test took into account that a minimum of 3 consecutive stable sets should be taken just prior to EtOH application and that the total time lapsed for all 3 should not be more than 2 to 5 minutes. This is of particular importance when considering that acute EtOH effects on the BK channel are usually reported to occur within the first 2 to 5 minutes after drug application (e.g., see Martin et al., 2008). Thus, recordings in the presence of EtOH would be limited to the next 2 to 5 minutes after EtOH application.

Analysis. All-points amplitude histograms were obtained from recordings where discrete channel openings, as well as closed states, could be distinguished. From there, the NPo was calculated using

$$\mathrm{NPo} = \frac{\sum_i iA_i}{\sum_i A_i},$$

where N is the number of open levels, i the level number (i = 0 is the closed state), and A_i is the area of a Gaussian fit to the ith level in the all-points histogram. The NPo for each voltage, from at least 3 consecutive sets (see above), was averaged and either included in a set of n's (0.5 to 0.6 NPo bar graphs) or plotted versus applied voltage.

$V_{1/2}$ *Measurements.* When $V_{1/2}$ was measured, the criterion for using each plot was to have at least 2 points in the voltage ranges where saturation of NPo was observed. Fits of the NPo-versus-voltage plots were made using

$$N_m PO = \frac{N_m}{1 + e^{k(V_{1/2} - V)}}$$

(IgorPro, v. 6.1; WaveMetrics, Inc., Portland, OR), where N_m is the maximum number of levels larger than zero. The fits corresponds to a Boltzmann function multiplied by N_m, with k having the usual value zF/KT. From this, the EtOH-induced shifts in $V_{1/2}$ (from controls) were determined.

Statistics. In order to avoid tolerance to EtOH, only 1 cell per dish was used. Thus, n represents the number of patches = number of cells = number of culture dishes used. For the $V_{1/2}$-versus-voltage tests, a minimum of $n = 5$ per case was used to comply with the basic binomial distribution requirements (Shapiro–Wilk test). Two-way analyses of variance (ANOVAs) were performed for the tests with specific values of NPo (i.e., = 0.5 to 0.6, fixed variables were X_1: with/without EtOH and X_2: [Mg^{2+}]) as well as for the results from NPo versus voltage (fixed variables were X_1: [Mg^{2+}] = 200 μM/[Mg^{2+}] = 1 mM and X_2: treatments). Tukey tests were made post hoc the ANOVA tests, to determine specific pair differences. Significant differences in all tests were considered when $p < 0.05$ and are reported following the indication of the type of test.

RESULTS

EtOH-Induced Changes in BK Activity are Regulated by Cytoplasmic Magnesium

It is well known that EtOH changes the open probability (NPo) of BK channels. We worked at NPo values near 0.5 to 0.6 as controls (see Methods) to detect either positive or negative EtOH-induced changes. Measurements were conducted with the cytoplasmic side of the patch exposed to buffered free Ca^{2+} at 30 μM but different concentrations of buffered free Mg^{2+} (Fig. 1). Surprisingly, significant EtOH-induced decreases in NPo were observed with cytoplasmic-side Mg^{2+} concentrations $\leq$200 μM (Fig. 1). In contrast, significant EtOH-induced increases were observed in the presence of 1 mM Mg^{2+}. There was no change in NPo at 400 μM. Similar results (data not shown) are observed with 5 μM cytoplasmic Ca^{2+}. These results demonstrate that (i) there is a Mg^{2+}-dependent EtOH effect on the activity of BK channels in outside-out patches and (ii) the effect is not affected by Ca^{2+} at the physiological concentrations used here for both ions (Shi and Cui, 2001).

Magnesium-Dependent EtOH Effects versus Voltage

BK channels are regulated by both Ca^{2+} and voltage. Therefore, BK channel NPo was recorded as a function of voltages that included observed minima and maxima of NPo. Unless associated with a change in conductance or channel density, changes in specific values of NPo (at particular voltages) are the result of shifts in the $V_{1/2}$ of such NPo-versus-voltage curves. Thus, to obtain a more clear and complete description of the NPo-versus-voltage characteristics, the $V_{1/2}$ shift was used to determine EtOH-induced potentiation (negative shift) or depression (positive shift) of BK activity. A total of 200 μM Mg^{2+} was used as a comparison with 1 mM Mg^{2+}, both at 30 μM internal Ca^{2+}. Figure 2 shows an example of EtOH-induced increases in BK activity when internal Mg^{2+} is at 1 mM. The EtOH-induced increase in channel activity is an effect that is within the voltage ranges that include the observed minima and maxima of NPo (Fig. 2*D*). In contrast, EtOH induces a decrease in BK activity when internal Mg^{2+} is at 200 μM (Fig. 3*D*). A similar behavior was observed when patches from HEK cells, expressing only the BK-α subunit, were used, indicating that this phenomenon could be a property of α-BK channels without other subunits (Fig. 4). Thus, the EtOH-induced effects on the activity of the BK channels can be observed throughout the physiological voltage range.

The Magnesium-Dependent EtOH Effects on BK Channels are Dependent on Associated Actin Filaments

In contrast to our results, potentiation by EtOH was previously reported to be independent of internal Mg^{2+} (e.g., see Liu et al., 2008). As actin filaments interact with BK channels (Tian et al., 2006), an explanation for this difference could be the preservation of actin filaments in the outside-out patch configuration (Ruknudin et al., 1991), as opposed to other configurations (where internal components are expected to be scarce or absent), such as inside-out (e.g., Liu et al., 2008, 2013; Yuan et al., 2011) or artificial bilayers

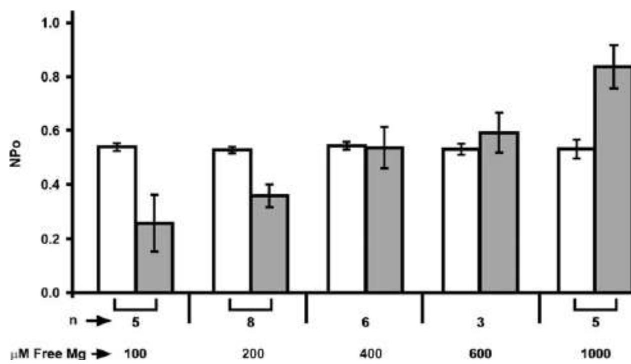

Fig. 1. Magnesium-dependent ethanol (EtOH) influence on calcium-dependent potassium channels (BK) from hippocampal neurons. Shown are EtOH-induced changes from initial control NPo values near 0.5 to 0.6. Significant EtOH-induced NPo reduction is observed at $\leq$200 μM free Mg^{2+} suggesting that the transition from potentiation to depression might be mostly dependent on the Mg^{2+} concentration. These results show that the 200 μM magnesium cases are a reasonable and consistent choice (see Methods) for comparison with the 1 mM Mg^{2+} cases in the more rigorous tests that followed. Data shown are for internal 30 μM free Ca^{2+}. Similar effects seen using 5 μM free Ca^{2+} (data not shown). White-filled bars are control and gray-filled are +EtOH. Error bars are SE. The brackets under the horizontal axis indicate significant difference (t-test's $p < 0.016$) between a paired-case with the same free Mg^{2+} (bottom row), with n indicated below each control/+EtOH pair (row above magnesium concentrations). The control data were targeted to NPo values between 0.5 and 0.6. As voltages for controls were different throughout the experiments, the +EtOH NPo is reported as that obtained at the same voltage as that of the corresponding control. Following ANOVA of all data ($\pm$ EtOH vs. [Mg^{2+}], $p < 0.0001$), Tukey tests indicated a significant difference limit at 200 μM Mg^{2+} when +EtOH is compared to its control (at specific [Mg^{2+}] for Control vs. +EtOH; 100 μM: $p < 0.0145$, 200 μM: $p < 0.0485$, 400 μM: $p = 1$, 600 μM: $p > 0.9997$, 1 mM: $p < 0.003$).

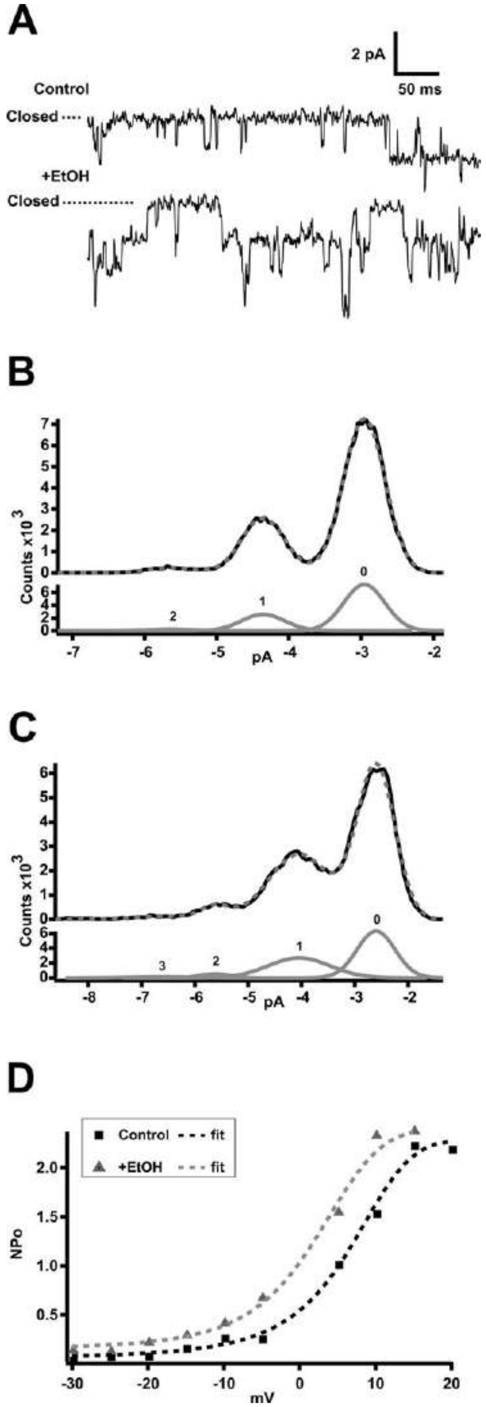

Fig. 2. Example of ethanol (EtOH)-induced potentiation of calcium-dependent potassium channels (BK) obtained from hippocampal neurons. Internal solution contained 30 μM free Ca^{2+} and 1 mM free Mg^{2+}. (**A**) Addition of 20 to 25 mM EtOH causes an increase in activity. All-points histograms (sample of **B** = control and **C** = +EtOH) were used to obtain plots of NPo versus applied voltage (**D**). In **D**, fits made of these plots (dashed black = control, dashed gray = +EtOH) indicate that EtOH causes a leftward voltage shift. The examples given in **A** (and for **B** and **C**) were from records taken at a voltage of −5 mV. In **B** and **C**, raw histograms are shown as black traces, Gaussian fits as gray traces, and composite of Gaussian fits as dashed gray traces. Similar effects seen using 5 μM free Ca^{2+} (data not shown).

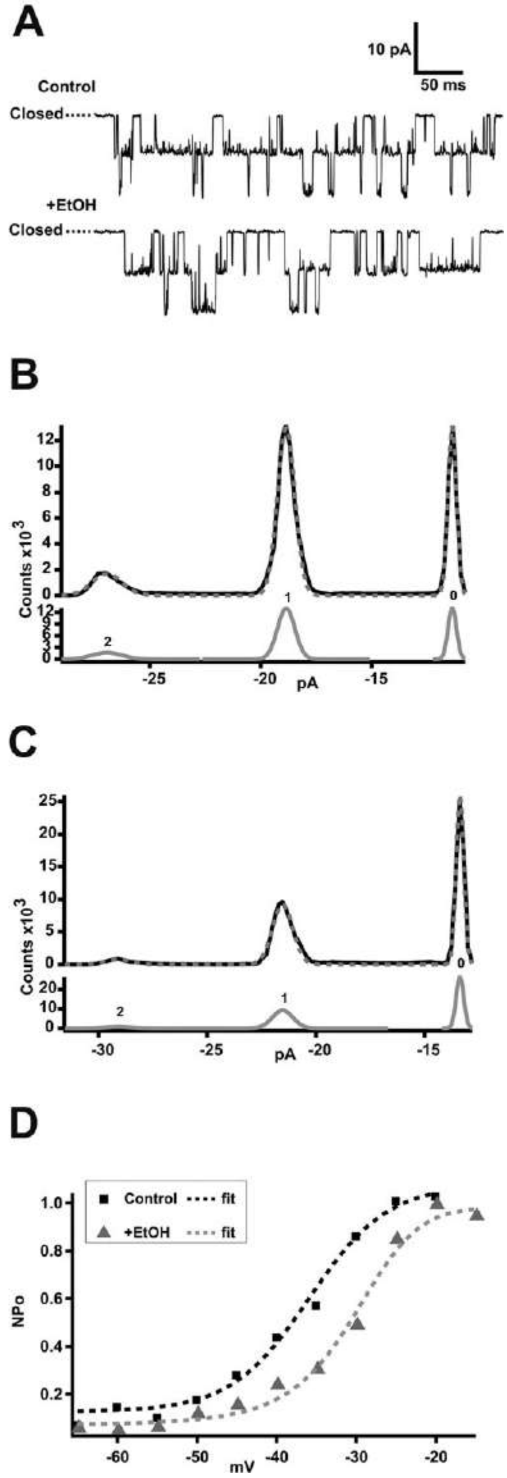

Fig. 3. Example of ethanol (EtOH)-induced depression of calcium-dependent potassium channels (BK) obtained from hippocampal neurons. Internal solution contained 30 μM free Ca^{2+} and 200 μM free Mg^{2+}. (**A**) Addition of EtOH causes a decrease in activity. All-points histograms (sample of **B** = control and **C** = +EtOH) were used to obtain plots of NPo-versus-applied voltage (**D**). In **D**, fits made for these plots (dashed black = control, dashed gray = +EtOH) indicate that EtOH causes a rightward voltage shift. The examples given in **A** (and for **B** and **C**) were from records taken at a voltage of −30 mV. All other plot details are as explained in Fig. 2.

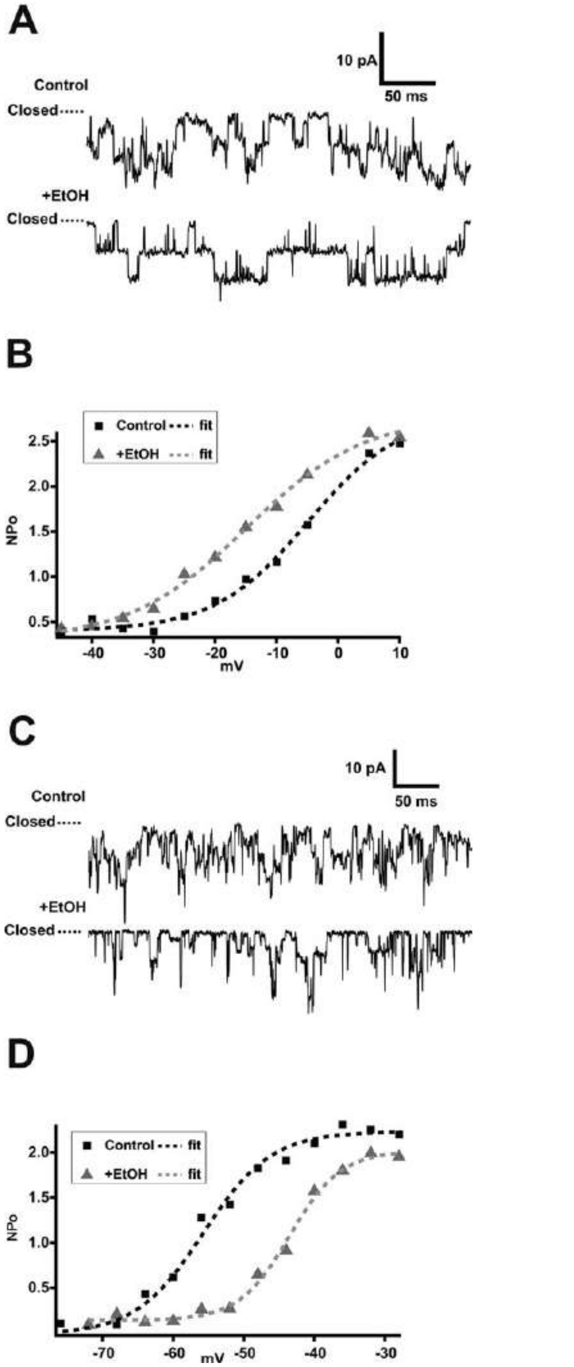

Fig. 4. Examples of ethanol (EtOH)-induced changes on calcium-dependent potassium channels (BK) activity from HEK cells expressing only the BK-α subunit. With 1 mM internal (cytoplasmic) free Mg^{2+}, addition of EtOH causes an increase in activity (**A**). This activity increase is seen in the plots of NPo-versus-applied voltage (**B**) as a leftward shift in $V_{1/2}$ (dashed black = control, dashed gray = +EtOH). There were 4 trials ($n = 4$) made for this case, giving an average $V_{1/2}$ shift of (mV $\pm$ SE) -12.14 ± 2.37. The example given in **A** was from records taken at -20 mV (contained in **B**). With 200 μM internal free Mg^{2+}, addition of EtOH causes a decrease in activity (**C**), also seen in the plots of NPo-versus-applied voltage (**D**) as a right-ward shift in $V_{1/2}$ (dashed black = control, dashed gray = +EtOH). There were 4 trials ($n = 4$) made for this case, giving an average $V_{1/2}$ shifts of (mV $\pm$ SE) 11.27 ± 0.77, significantly different from the $V_{1/2}$ shift obtained with 1 mM free Mg^{2+} (t-test $p < 0.0002$). The example given in **C** was from records taken at -60 mV (contained in **D**). Internal solution for all tests had 30 μM free Ca^{2+}.

(e.g., Crowley et al., 2003; Pau et al., 2011; Yuan et al., 2011).

To test this, agents that would either disrupt (10 μM cytochalasin D) or stabilize (10 μM phalloidin) actin were used when testing for EtOH effects with both high and low internal Mg^{2+}. The possible effect of DMSO was also considered, as it is the vehicle solvent for phalloidin and cytochalasin, but it yielded no significant effects on the EtOH-induced $V_{1/2}$ shifts in NPo. The average control $V_{1/2}$ values with 200 μM free Mg^{2+} (=mV $\pm$ SE): normal = -7.12 ± 4.22 ($n = 15$), +DMSO = -26.98 ± 5.55 ($n = 10$), +Phalloidin = -14.30 ± 5.67 ($n = 11$), and +Cytochalasin D = -7.98 ± 3.40 ($n = 15$); for 1 mM free Mg^{2+}: normal = 1.02 ± 3.90 ($n = 8$), +DMSO = -17.98 ± 6.68 ($n = 7$), +Phalloidin = -8.28 ± 7.44 ($n = 9$), and +Cytochalasin D = 10.18 ± 7.19 ($n = 7$). The effects found for EtOH on $V_{1/2}$ values did not depend on their initial (or control) $V_{1/2}$ values. A summary of the EtOH-induced effect $V_{1/2}$ shifts in NPo is given in Fig. 5.

Interestingly, when Mg^{2+} is 200 μM, then EtOH decreases BK activity unless actin is disrupted by cytochalasin D. In contrast, EtOH potentiates the activity with 1 mM Mg^{2+} unless actin is stabilized by phalloidin.

Thus, when actin filaments are destabilized, then EtOH induces a potentiation of BK channel activity, with either high or low internal Mg^{2+}. In contrast, when actin filaments are stabilized by phalloidin, then EtOH depresses BK channel activity, regardless of the Mg^{2+} concentration. It is clear that the EtOH-induced changes of BK channel activity are determined by internal Mg^{2+}, but that this reliance is contingent on the integrity of the actin filaments associated with the channel.

DISCUSSION

Here, we report for the first time that, in outside-out patches, Mg^{2+} alters, in a concentration-dependent manner, the effects of EtOH on BK channel activity. Previously, experimental conditions used to test for EtOH effects on BK channels involved the use of cell-attached techniques, inside-out patches, or reconstituted channels in artificial bilayers. In these, internal Mg^{2+} is not controlled (e.g., cell attached), or the participation of internal components has been minimized (e.g., inside-out) or eliminated (e.g., artificial bilayers). In our case, internal cellular components should be retained with the outside-out patch configuration (Ruknudin et al., 1991). Therefore, the conditions obtained for single channel recordings better approximate those at the surface of intact cells. Furthermore, we used $[Mg^{2+}]/[Ca^{2+}]$ ratios that are lower than that reported for Mg^{2+}-Ca^{2+} competition for the high-affinity Ca^{2+} binding site (see Shi and Cui, 2001). Thus, it appears that any influence of actin on the high-affinity binding site is minimal, and therefore, we only considered influences on low-affinity binding sites for Mg^{2+} (see Fig. 6).

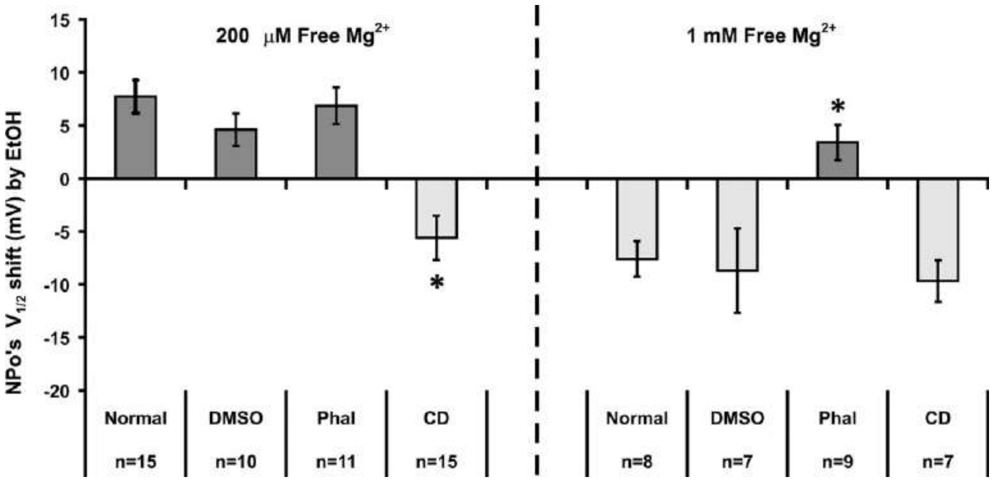

Fig. 5. Summary of ethanol (EtOH)-induced $V_{1/2}$ shifts from hippocampal neurons. Potentiation corresponds to negative $V_{1/2}$ shifts (bars below horizontal axis), while depression corresponds to positive $V_{1/2}$ shifts (bars above horizontal axis). Dimethyl sulfoxide (DMSO) was at 0.1%, also present at this concentration when phalloidin (**Phal**; 10 μM) or cytochalasin D (**CD**: 10 μM) were used. The **n**s are given in the lowest row. EtOH-induced potentiation is present with 1 mM free Mg^{2+}, while depression is induced with 200 μM free Mg^{2+}. The presence of CD causes a persistent EtOH-induced potentiation, while Phal causes a persistent depression. Following ANOVA of all data (treatment versus [Mg^{2+}], $p < 0.0001$), Tukey tests indicated that within the 200 μM Mg^{2+}, the results with CD were significantly different from the other 3 (largest $p < 0.0110$), whereas at 1 mM Mg^{2+}, it was Phal which was significantly different from the other 3 (largest $p < 0.030$). The "*" indicates the case which was significantly different from the others with the same Mg^{2+} concentration. Black capped lines are standard errors (SE).

The EtOH-Induced Shift in $V_{1/2}$ *and Cytoskeletal Components*

EtOH-induced activity changes are contingent on the cytoplasmic Mg^{2+} concentrations. The Mg^{2+}-dependent EtOH effect on BK channels is only evident if they are still associated with actin filaments. Furthermore, these effects demonstrate, for the first time, that Mg^{2+} is a possible modulator of EtOH's effects. However, there is not yet enough information to determine whether the effects are a direct influence on the BK channel's Mg^{2+} binding sites, or on the ion's effect on actin polymerization, or some mixture of both (see Fig. 6). In accordance with this model, actin filaments are known to be stabilized by increasing Mg^{2+} (Galińska-Rakoczy et al., 2009; Hild et al., 2010). Thus, EtOH-induced depression would have been expected for high Mg^{2+} concentrations. That this is not the case indicates that the causes for the Mg^{2+}-dependent EtOH effect cannot be explained by the direct effect of Mg^{2+} on the actin filaments. Still, the Mg^{2+}–EtOH interaction appears to be a result of the presence of actin filaments or actin components, such as found at synapses (Frotscher et al., 2014; Gordon-Weeks and Fournier, 2014; Loebrich, 2014; Mori et al., 2014).

Hypothesis on the Dependency of Mg^{2+} *Action on Actin*

Figure 6 is a model that includes the participation of the c-terminus of the BK channel in the EtOH-Mg^{2+}-actin effect. In this model, actin modulates the voltage-dependent openings of the BK channel, such modulation being in turn dependent on the Mg^{2+} concentration. The modulation of open probability is simplified as directly associated to movement of the BK c-terminus (Fig. 6). The model considers only binding sites for Mg^{2+} (which are at the c-terminus), and actin, as well as possible EtOH interaction sites at the channel protein and bilayer sides.

The main premise of the model is that the channel's activity would depend on the stabilization of the c-terminus with respect to the membrane, with the highest open probability occurring when the terminus is closest to the main transmembrane segments of the α subunit. There is already precedence for this feature of the model, as others have suggested that a similar re-conformation of the c-terminus is associated with the open state of K-channels (Cui et al., 2009; Jiang et al., 2002). Thus, any condition that increases the *possibility* of having the c-terminus closer to the membrane would also increase the channel's open probability. The model includes certain assumptions that are focused on effects stabilizing the position of the c-terminus: (i) Mg^{2+} binding to its site in the channel would favor stabilization of the c-terminus near the membrane, (ii) actin filament binding would hinder movement of the c-terminus, and (iii) EtOH binding would favor c-terminus movement toward or away from the membrane, depending on the c-terminus position previous to EtOH application. Taken together, the presence of bound, polymerized actin would stabilize the conditions present previous to EtOH application. Notice that although the model does not include the effect of Mg^{2+} on the actin filaments' stability, it is still consistent with what is known about Mg^{2+}-dependent actin polymerization. Namely, under low Mg^{2+}, it is expected that the effect of "less polymerized" actin would result in a more negative control's $V_{1/2}$, but having an opposite effect with higher Mg^{2+}.

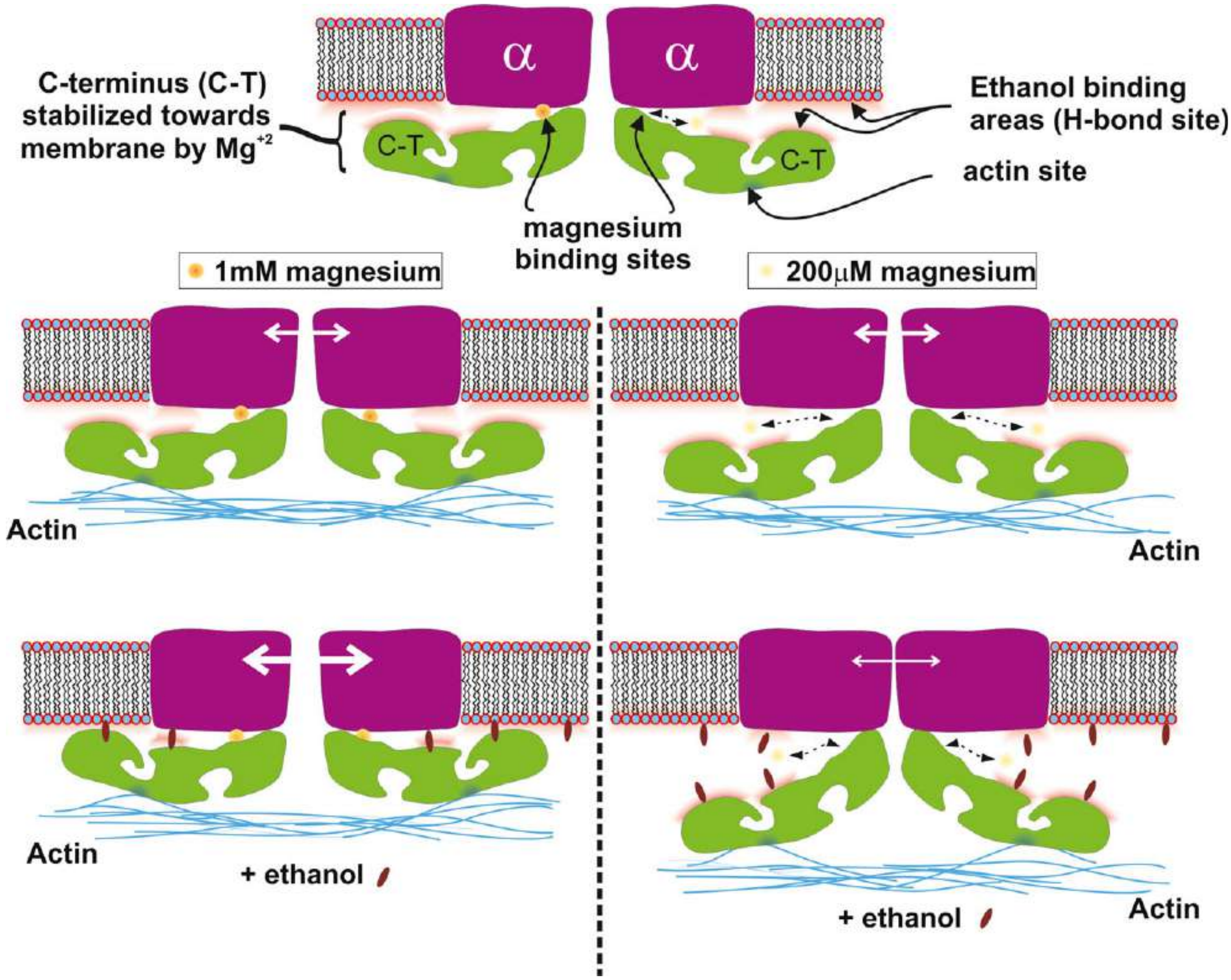

Fig. 6. Model of the mechanism of action of ethanol (EtOH)-induced changes in calcium-dependent potassium channels (BK) activity. An inherent mobility of the c-terminus (C-T) part of the channel is assumed and associated with BK activity. The basis for modulation of activity would be the C-T's interaction with the α trans membrane sections (and bilayer). Thus, one main feature of the model is the proposition that anything that alters the probability of the C-T being closer to the bilayer would also increase the probability of channel openings (depicted by thickness of the white arrows inside the trans membrane section "α", at top). The high/low Mg^{2+} (left/right of vertical dashed line) would correspondingly increase/decrease the open probability. Actin filaments would stabilize either state, thus determining the modulation of the system by EtOH. Thus, if the C-T was stabilized close to the bilayer (by actin, bottom drawing at left of vertical dashed line), addition of EtOH will further stabilize (or bring closer) the C-T. On the other hand, if the C-T were actin-stabilized away from the bilayer, addition of EtOH would further stabilize it away from the bilayer (bottom drawing at right of vertical dashed line). See Discussion for more details.

Another View of EtOH Tolerance

One major consequence of alcohol consumption and/or exposure is the depletion (down to 200 to 400 μM from 1 mM) of cellular internal Mg^{2+} (see Babu et al., 1999; Li et al., 2001; Romani, 2008). This depletion can occur even with small amounts of EtOH and as early as the first few minutes after ingestion or exposure (Babu et al., 1999). The long-term consequences are usually highly symptomatic, and therapeutic strategies for Mg^{2+} replenishment have been used as remedies for alcoholism (Poikolainen and Alho, 2008). We now hypothesize that the BK channel is affected by the Mg^{2+} depletion caused by EtOH. Namely, that the EtOH-induced reduction of intracellular Mg^{2+} would differentially alter BK channel activity in cellular areas where BK channels are highly associated with actin filaments. More explicitly, synapses, where there is high actin polymerization, would show a decrease in BK activity when internal Mg^{2+} is reduced by EtOH. Although at the cellular level, this would have various consequences; at the systemic level, the overall effect can be interpreted as another mechanism for tolerance in habitual (or chronic) EtOH drinkers. Given that while in occasional drinkers, the Mg^{2+} levels would recover shortly after cessation of EtOH consumption, in habitual drinkers, the Mg^{2+} levels would remain low for extended periods (Eckardt et al., 1998; Poikolainen and Alho, 2008; Torres et al., 2009).

Thus, we hypothesize that in occasional drinkers, EtOH induces potentiation of BK activity, but that there would be no potentiation (i.e., tolerance) at synaptic (i.e., actin-bound) BK channels, because of the decrease in cytoplasmic Mg^{2+} observed in frequent/chronic drinkers.

In summary, EtOH effects on BK channel are modulated by the concentration of internal (cytoplasmic) Mg^{2+}. This effect is dependent upon the integrity of actin elements associated with BK channels such as those at synaptic membranes. Thus, for most alcoholics, the low levels of internal Mg^{2+} would contribute to tolerance of the EtOH-induced potentiation of BK channel activity.

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