Ethanol-mediated relaxation of guinea pig urinary bladder smooth muscle: involvement of BK and L-type Ca\(^{2+}\) channels

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Malysz J, Afeli SA, Provence A, Petkov GV. Ethanol-mediated relaxation of guinea pig urinary bladder smooth muscle: involvement of BK and L-type Ca\(^{2+}\) channels. Am J Physiol Cell Physiol 306: C45–C58, 2014. First published October 23, 2013; doi:10.1152/ajpcell.00047.2013.—Mechanisms underlying ethanol (EtOH)-induced detrusor smooth muscle (DSM) relaxation and increased urinary bladder capacity remain unknown. We investigated whether the large conductance Ca\(^{2+}\)-activated K\(^+\) (BK) channels or L-type voltage-dependent Ca\(^{2+}\) channels (VDCCs), major regulators of DSM excitability and contractility, are targets for EtOH by patch-clamp electrophysiology (conventional and perforated whole cell and excised patch single channel) and isometric tension recordings using guinea pig DSM cells and isolated tissue strips, respectively. EtOH at 0.3% vol/vol (~0.5 mM) enhanced whole cell BK currents at +30 mV and above, determined by the selective BK channel blocker paxilline. In excised patches recorded at +40 mV and ~300 nM intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), EtOH (0.1–0.3%) affected single BK channels (mean conductance ~210 pS and blocked by paxilline) by increasing the open channel probability, number of open channel events, and open dwell-time constants. The amplitude of single BK channel currents and unitary conductance were not altered by EtOH. Conversely, at ~10 μM but not ~2 μM intracellular [Ca\(^{2+}\)], EtOH (0.3%) decreased the single BK channel activity. EtOH (0.3%) affected transient BK currents (TBKCs) by either increasing frequency or decreasing amplitude, depending on the basal level of TBKC frequency. In isolated DSM strips, EtOH (0.1–1%) reduced the amplitude and muscle force of spontaneous phasic contractions. The EtOH-induced DSM relaxation, except at 1%, was attenuated by paxilline. EtOH (1%) inhibited L-type VDCC currents in DSM cells. In summary, we reveal the involvement of BK channels and L-type VDCCs in mediating EtOH-induced urinary bladder relaxation accommodating alcohol-induced diuresis.

smooth muscle; patch-clamp; contractility; detrusor; alcohol

**URINARY BLADDER FUNCTION DEPENDS on the contractile status of detrusor smooth muscle (DSM) cells. DSM relaxation allows for bladder expansion during urine storage, whereas its contraction, coupled with the opening of the bladder sphincter, permits urine voiding (3). Multiple ion channels and receptors are expressed in DSM cells regulating bladder function. Among them, the large-conductance voltage- and Ca\(^{2+}\)-activated K\(^+\) channels (also known as BK, Slo, or K\(_{Ca,1.1}\) channels) and L-type voltage-dependent Ca\(^{2+}\) channels (VDCCs) are recognized as key regulators of excitability and contractility of DSM (44). Supporting data, provided for DSM studies using rat, guinea pig, mouse, and importantly human tissues and cells (5, 17, 18, 22, 24, 25, 46), demonstrate the role of BK channels in the regulation of the resting membrane potential, modulation of the repolarization phase of DSM action potentials, and generation of spontaneous transient BK currents (TBKCs), also known as spontaneous transient outward currents. For example, the BK channel blockade with either iberiotoxin or charybdotoxin prolonged the action potentials correlating with enhanced contractility (16, 18), whereas the BK channel opener NS11021 reduced action potential generation and DSM contractility in guinea pigs (29). In human DSM, the selective BK channel inhibitor iberiotoxin and activator NS1619 increased and decreased, respectively, the contractility of tissue strips and excitability of freshly isolated DSM cells confirming the important regulatory role of this K\(^+\) channel subtype (22, 25). Key characteristics of the BK channels are 1) their large conductance whereby the opening or closure of few channels has pronounced effects on cellular excitability; 2) a dual sensitivity to metabolic factors (e.g., Ca\(^{2+}\) and cAMP-pathways) and membrane voltage; and 3) a close functional interrelationship with VDCCs, which are the primary regulators of contractility mediating the influx of Ca\(^{2+}\) to initiate DSM contractions (3, 24, 44, 46, 54).

Two types of VDCCs (L-type and T-type) have been identified in DSM cells similar to other smooth muscle cell types (1, 13, 48). The lower threshold of activation for the T-type VDCCs determines that they are active at the resting membrane potential of approximately ~40 mV and could regulate DSM excitability. The L-type VDCCs activate at more depolarized voltages, and their activity generates the upstroke phase of the DSM action potential allowing for net influx of Ca\(^{2+}\) to initiate contractility (12, 44).

Studies in non-DSM smooth muscle cells, neurons, and cells expressing recombinant BK channels demonstrated various direct effects of ethanol (EtOH) on BK channels and L-type VDCCs. The effects of EtOH on BK channel activity can be stimulatory, inhibitory, or neither. The type of response depends on multivariable factors including the species examined, the tissue or cell type investigated, the molecular composition of BK channel subunits expressed, the EtOH concentration used, and temporal exposure (4, 9, 35, 51). For example, in hypothalamic-neurohypophysial neurons, acute exposure to EtOH increased BK channel activity, whereas chronic 24-h treatment displayed much weaker effects (47). In cerebral artery smooth muscle cells, acute EtOH exposure in the presence of physiologically relevant high intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) has been shown to inhibit BK channel activity (6, 7, 31). In contrast, submicromolar concentrations of intracellular [Ca\(^{2+}\)] promoted enhancement of BK channel activity (6, 7). In the case of L-type VDCCs, studies on non-DSM smooth muscle cells, neurons, and recombinant,
cells demonstrated inhibitory effects of EtOH on the channel activity (10, 33).

In DSM, exposure to EtOH alters bladder function. In vitro studies with rabbit and rat DSM tissue strips revealed that EtOH evoked relaxation of pharmacologically induced contractions (38, 52, 56). In vivo cystometric measurements also demonstrated increased bladder capacity and residual urine volume in rats (56). In humans, clinical observations of acute intoxication-induced urinary retention, and experimental intravesicular or extravesicular EtOH application increased urinary bladder capacity (39, 42). These studies support the notion that EtOH directly modulates the responsiveness of DSM cells; however, the identity of molecular targets, in particular ion channels, remains unknown.

In this study, we investigated the effects of acute EtOH exposure on BK channels and L-type VDCC activity in freshly isolated DSM cells using the patch-clamp technique and isolectric tension recordings of isolated DSM strips. We tested the hypothesis that acute EtOH application evokes DSM relaxation in vitro by targeting both BK channels and L-type VDCCs.

MATERIALS AND METHODS

**DSM tissue harvesting.** Experiments were conducted in accordance with the Animal Use Protocol No. 1747 reviewed and approved by the University of South Carolina Institutional Animal Care and Use Committee. For this study, 60 adult male Hartley-Albino guinea pigs (300–570 g, average: 429.4 g) were euthanized with CO₂ followed by thoracotomy. Then, the whole bladder was cut above the urinary bladder capacity (39, 42). These studies support the notion that EtOH directly modulates the responsiveness of DSM cells; however, the identity of molecular targets, in particular ion channels, remains unknown.

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**Fresh DSM single cell isolation.** Guinea pig DSM cells were isolated as previously described (23, 41, 54). Briefly, small DSM strips were cut from the mucosa-free DSM specimens and placed in DS (2 ml) supplemented with 1 mg/ml BSA, 1 mg/ml papain, and 1 mg/ml dithiothreitol and incubated for 10–20 min at 37°C. Next, DSM strips were transferred to DS (2 ml) containing 1 mg/ml BSA, 0.5–1 mg/ml collagenase, and 100 μM CaCl₂ for 9–15 min at 37°C. After the incubation, DSM strips were washed with fresh DS solution containing BSA. Individual cells were released from the tissue by passing the enzyme-treated DSM strips through a fire-polished Pasteur pipette.

Conventional and perforated whole cell patch-clamp recordings. Electrophysiological experiments were performed based on methods and procedures previously described (23, 41, 54). Briefly, several drops of the DS containing freshly isolated DSM cells were placed into a recording chamber. After at least 20 min, the cells were washed several times with extracellular solution (see Solutions and compounds). We employed the conventional whole cell technique to record outward K⁺ currents and amphotericin-perforated whole cell recording method to measure TBKCs and L-type VDCC currents. Whole cell currents were recorded using an Axopatch 200B amplifier, Digitida 1440A, and pCLAMP version 10.2 software (Molecular Devices, Union City, CA). An eight-pole Bessel filter (Frequency Devices, Union City, CA) was used to filter the recorded currents. The patch-clamp pipettes were prepared from borosilicate glass (Sutter Instruments, Novato, CA) and pulled using a Narishige PCP-830 vertical puller (Narishige Group, Tokyo, Japan). The patch-clamp pipettes were fire-polished with a Microforge MF-830 to give a final tip resistance of approximately 3–6 MΩ. In conventional whole cell configuration, DSM cells were held at −60 mV, then depolarized from −80 mV up to +110 mV in 10-mV steps for 400 ms to elicit steady-state outward K⁺ channel currents, and then stepped back to −80 mV. TBKCs were recorded at −30 mV (corrected for the junction potential of approximately −10 mV). When the L-type VDCC currents were measured, DSM cells were held at −64 or −74 mV and stepped to voltages ranging from −54 to +46 in 10-mV steps for 400 ms and then returned to the holding voltage. Voltages in perforated patch-clamp experiments of Ca²⁺ currents were corrected for the liquid junction potential of approximately −14 mV. The stable control whole cell current was recorded for at least 6 min, and then EtOH (0.3 or 1%) or nifedipine (3 μM) was applied in the bath. All patch-clamp experiments were carried out at room temperature (22–23°C).

**Single-channel recordings.** Isolated DSM cells were allowed to adhere to a glass-plated chamber for at least 30 min and then rinsed with the 140 mM K⁺ solution containing free Ca²⁺ concentration of 300 mM (WEBMAXC Standard: http://www.stanford.edu/~cpatton/webmaxC.htm; Chris Patton; see Solutions and compounds). Single BK channel recordings were done on excised inside-out or outside-out patches using symmetrical 140 mM KCl solution for both the bath and pipette compartments based on previous reports (29, 34, 45) with 300 mM, ~2 μM, or 10 μM free [Ca²⁺], as indicated. In all excised patch single-channel recordings, the pipette solution contained ~300 mM free [Ca²⁺]. The recordings were made with the same equipment as used for whole cell patch-clamp experiments using the single-channel configuration mode. The final tip resistance of electrodes was 6–15 MΩ. All patch-clamp experiments were conducted at room temperature (22–23°C).

**Isometric DSM tension recordings.** The isometric DSM tension recording experiments were conducted as previously described (23, 41, 54). DSM strips were clipped between a stationary mount and a force-displacement transducer and then placed in tissue baths filled with Ca²⁺ containing physiological saline solution (PSS) thermostatically controlled at 37°C. DSM strips were stretched to an initial ~10 mN and washed with fresh PSS every 15 min for 45–60 min. The neuronal Na⁺ channel blocker tetrodotoxin at 1 μM was then added into the bath to attenuate neuronal activity. Increasing EtOH concentrations (0.01–1%) were applied at 10 to 12-min intervals into the baths in the absence or presence of paxilline (1 μM), a selective BK channel inhibitor. Paxilline was preapplied for at least 45 min, and the effect of EtOH was determined in the presence of the BK channel blocker only when a stable baseline was achieved.

**Solutions and compounds.** The DS solution with or without supplemented Ca²⁺ (100 μM) had the following composition (in mM): 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 Hepes, and 2 MgCl₂, pH 7.3, adjusted with NaOH. In patch-clamp experiments for single-channel recordings, the symmetrical K⁺ solution used contained (in mM): 140 KCl, 1.08 MgCl₂, 5 EGTA, and 3.16 CaCl₂, adjusted to pH 7.2 with NaOH (calculated [Ca²⁺]-free ~300 mM). In single-channel experiments requiring ~2 or 10 μM free [Ca²⁺], CaCl₂ was increased to 4.516 or 4.943 mM, respectively. In conventional whole cell experiments measuring outward K⁺ currents, the extracellular (bath) solution composition was as follows (in mM): 134 NaCl, 6 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose, and 10 Hepes, pH adjusted to 7.4 with NaOH; and the pipette solution was the same as used in the single-channel experiments. TBKCs were recorded with the perforated whole cell patch-clamp technique under conditions of the bath solution being the same as in the conventional whole cell experiments, and the following pipette solution components (in mM): 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl₂, 10 Hepes, and 0.05 EGTA with the pH adjusted to 7.2 with NaOH. For perforated whole cell patch-clamp experiments measuring L-type VDCC currents, the bath solution contained the following (in mM): 10 TEA-Cl, 6 CsCl, 124 NaCl, 1 MgCl₂, 2 CaCl₂, 10 Hepes, 10 glucose (pH 7.4 adjusted with CsOH), and pipette solution supplemented with freshly dissolved (every 1–2 h) 200–300 pg/ml amphotericin-B (in mM): 110 CsOH, 110 aspartate (free), 10 NaCl, 1 MgCl₂, 10 Hepes, 0.05 EGTA, and 30 CsCl (pH 7.2 adjusted with CsOH). The Ca²⁺-.
containing PSS for DSM contractility studies was prepared daily and had the following composition (in mM): 119 NaCl, 4.7 KCl, 24 NaHCO3, 1.2 KH2PO4, 2.5 CaCl2, 1.2 MgSO4, 11 glucose, and aerated with 95% O2-5% CO2 to obtain pH 7.4. Papain was obtained from Worthington (Lakewood, NJ), and dithiothreitol, collagenase type II, and paxilline were from Sigma-Aldrich (St. Louis, MO). Paxilline was dissolved as a stock solution in DMSO and diluted to the indicated concentrations. The final DMSO concentration in the bath did not exceed 0.05%.

Data analysis and statistics. Clampfit software version 10.2 and GraphPad Prism version 4.03 (La Jolla, CA) were used for analysis of patch-clamp data. Whole cell BK currents were analyzed before and after the addition of EtOH as means of steady-state currents over the last 200 ms for each voltage. The amplitude of inward L-type VDCC currents was obtained by measuring peak negative inward current for each voltage step. The constants for half-maximum activation (V0.5) of L-type VDCC currents were calculated using the Boltzmann sigmoidal fit (GraphPad Prism) to the G/Gmax vs. voltage relationship. In the case of single BK channel recordings, the values for open probability of each patch (NPo) were obtained using the build-in algorithm in Clampfit, which calculates NPo as T(o)/(T(o) + T(c)), where T(o) and T(c) correspond, respectively, to total open time and total closed time during the recording interval. Single BK channel currents were either filtered off-line with the Gaussian build-in option (11 coefficients) in Clampfit or not filtered before analysis. In excised patch-clamp experiments, the unitary BK channel conductance (determined from the current-voltage relationship) and the effects on P_o at +40 mV were typically determined in the same patches. First, the unitary current amplitudes were measured at various voltages in the absence of EtOH. Second, the patches were stepped to +40 mV and the effects of EtOH were determined over 5- to 10-min intervals before and after its application. Third, in the continued presence of EtOH the unitary current amplitudes were recorded again at various voltages. When examining the single BK channel activity at other voltages, the analysis interval was ~1–3 min for voltages of +80 mV and below and 15–60 s for +90 mV and higher. The graphs for mean single BK channel amplitude-voltage and P_o-voltage were plotted with GraphPad Prism by respectively fitting linear regression (slope providing the conductance). The total number of BK channels present (N#) in each patch was determined at +80 to +100 mV. The determination of channel P_o was done by dividing the open single-channel probability of each patch (NPo) by the number of ion channels present in the patch (N#). The single-channel amplitudes were calculated from all-point histograms using the Gaussian distribution function to qualify the values for closed and open states. The open dwell time constants were obtained in Clampfit using the two-term exponential equation from curve fitting of open-dwell time histograms. This function also provided the relative fraction of events for each component. To estimate the actual number of events for each component, the relative fraction was multiplied by the total number of events detected during the analysis of pre- and EtOH-treated intervals of 5–10 min. For normalization, the τ constant and the number of events values were expressed as fold increases over the pre-EtOH control taken as 1. TBKCs were analyzed using the threshold of 7.5 pA, which is three times the BK channel unitary current of 2.5 pA at −30 mV (21, 43), and the event detection function in Clampfit.

Isometric DSM tension recordings were measured with MyoMed software (Med Associates, St. Albans, VT). MiniAnalysis software (Synaptosoft, Decatur, GA) was used for data analysis of DSM phasic contraction amplitude and muscle force integral (determined by integrating the area under the curve of the phasic contractions) over the last 5-min interval before the addition of a higher concentration of EtOH. Increasing concentrations of EtOH were added at 10 to 12-min intervals. GraphPad Prism 4.03 software (GraphPad Software, La Jolla, CA) was used for statistical analysis, and CorelDraw Graphic Suite software (Corel, Ottawa, ON, Canada) and Microsoft PowerPoint (Seattle, WA) for data illustration. The data are summarized as means ± SE (n = number of different DSM patches, cells, or tissue strips; N = number of guinea pigs) and compared using paired or unpaired Student’s t-test. A P value of <0.05 was considered statistically significant.

RESULTS

EtOH increases whole cell BK currents in freshly isolated native DSM cells. To examine whether EtOH modulates BK channel function, we first employed the conventional whole cell patch-clamp method with [Ca2+] of ~300 nM and 2 mM for the pipette (intracellular) and the bath (extracellular), respectively. We utilized this approach rather than the perforated patch-clamp technique since we were interested in addressing...
the direct effects of EtOH on BK currents under the conditions of experimentally controlled intracellular Ca\(^{2+}\) levels. As shown in Fig. 1, whole cell currents were evoked by changing the voltage from \(-80\) to \(+110\) mV in steps of 10 mV in 11 cells (\(N = 10\)). The DSM cells had a mean capacitance of 43.1 \(\pm\) 2.9 pF (\(n = 11, N = 10\)). This protocol allowed us to record the whole cell BK currents, especially at voltages above \(+30\) mV. They were characterized by the noisy current profile indicative of channel openings with large conductance. The addition of EtOH at 0.3% significantly enhanced the responses measured at \(+30\) mV and above (Fig. 1). For example, at \(+40\) and \(+110\) mV, the mean outward current density significantly increased by 1.43-fold (from \(3.49 \pm 0.47\) to \(4.96 \pm 0.86\) pA/pF) and 1.36-fold (from \(33.81 \pm 6.2\) to \(46.58 \pm 9.30\) pA/pF), respectively (\(n = 11, N = 10, P < 0.05\)). These experiments suggest that in DSM cells, EtOH directly enhances the activity of the BK channels. This was further supported by the experiments in which, in the continued presence of EtOH, the selective BK channel blocker paxilline (500 nM) was applied. Paxilline inhibited currents at 0 mV and above (\(P < 0.05\), Fig. 1B) consistent with the previous observations in DSM cells showing contribution of BK channels at voltages of \(-0\) to \(+10\) mV and above (19, 22). For instance, at \(+40\) and \(+110\) mV the mean current density value significantly decreased to \(2.18 \pm 1.28\) pA/pF and to \(4.18 \pm 5.69\) pA/pF (\(n = 6, N = 6, P < 0.05\)), respectively. These results indicate that the majority of the outward K\(^+\) current recorded under the experimental conditions was carried by the BK channels and was also sensitive to the modulation by EtOH.

EtOH positively modulates the activity of single BK channels in freshly isolated native DSM cells in the presence of low intracellular \([Ca^{2+}]\). To determine whether EtOH directly modulates the activity of BK channels, the single-channel recordings were conducted by the cell-free excised patch-clamp method, both inside-out and outside-out configurations. In all experiments, a symmetrical high KCl solution containing 140 mM KCl and \(-300\) nM free \([Ca^{2+}]\) was used for pipette and bath compartments. Under these experimental conditions, single BK channel currents were recorded at potentials above \(+20\) mV as exemplified in Fig. 2, A and B. In total, single BK channel currents were measured in 14 outside-out and 16 inside-out patches (Table 1). There were no differences in the single-channel conductance and \(P_o\) values measured for the two excised configurations. As indicated in Table 1, the mean single-channel conductance values were \(-210\) pS. At \(+20\) mV, the mean \(P_o\) of \(-0.002\) increased by approximately

![Table 1. Single BK channel parameters in excised membrane patches derived from freshly isolated DSM cells measured at \(-300\) nM \([Ca^{2+}]\)](attachment)

<table>
<thead>
<tr>
<th>Inside-Out ((n = 16, N = 12))</th>
<th>Outside-Out ((n = 14, N = 12))</th>
<th>Combined Excised Patches ((n = 30, N = 18))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductance, pS</td>
<td>212.6 (\pm) 4.8</td>
<td>206.8 (\pm) 5.4</td>
</tr>
<tr>
<td>(P_o) at (+20) mV</td>
<td>0.00206 (\pm) 0.00109</td>
<td>0.00097 (\pm) 0.00034</td>
</tr>
<tr>
<td>(P_o) at (+40) mV</td>
<td>0.00584 (\pm) 0.00187</td>
<td>0.00891 (\pm) 0.00368</td>
</tr>
<tr>
<td>(P_o) at (+60) mV</td>
<td>0.0428 (\pm) 0.0150</td>
<td>0.0601 (\pm) 0.0228</td>
</tr>
<tr>
<td>Normalized (P_o) at (+20) mV</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normalized (P_o) at (+40) mV</td>
<td>7.7 (\pm) 1.7</td>
<td>8.2 (\pm) 1.0</td>
</tr>
<tr>
<td>Normalized (P_o) at (+60) mV</td>
<td>76.9 (\pm) 20.4</td>
<td>70.2 (\pm) 13.1</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE; \(n\) = number of different detrusor smooth muscle (DSM) patches; \(N\) = number of guinea pigs. \(P_o\), open channel probability.
eightfold at +40 mV and ~75-fold at +60 mV (normalized to $P_c$ at +20 mV) indicating strong voltage dependence. To further confirm that the measured single-channel currents were indeed mediated by BK channels, the effect of the selective BK channel inhibitor paxilline (500 nM) was examined in seven patches (5 inside-out and 2 outside-out, $N = 3$) at the $V_h$ of +40 mV. A representative experiment is shown in Fig. 2C. Before the paxilline application, the control $P_o$ was 0.0045 ± 0.0015 ($n = 7, N = 3$) and the single-channel current amplitude 8.8 ± 0.9 pA ($n = 7, N = 3$). Paxilline (500 nM) inhibited all of the single-channel activity (Fig. 2C). In the 3-min recording following the application of paxilline, there were no single-channel openings.

In subsequent experiments, the effects of EtOH at 0.1 and 0.3% were examined on single BK channel currents measured at +40 mV. A representative recording from this series of experiments is shown in Fig. 3A, whereas Fig. 4 provides the summary data for the mean single BK channel unitary conduc-
tance, $P_o$, current amplitude, and total number of events in the absence or presence of EtOH. As summarized, EtOH at 0.1 and 0.3% caused significant enhancement of $P_o$ and increased the number of events but did not significantly change the single BK channel current amplitude. The single-channel conductance was determined from the slope of the single BK channel current-voltage relationship as exemplified in Fig. 4A and summarized in Fig. 4B. Consistent with the lack of an effect on the single BK channel amplitude, the mean single-channel conductance values were not significantly different in the absence or presence of EtOH. Figure 3 (histograms at right) further illustrates the effect of EtOH on the open dwell time constants. Similar to a previous study (8), we fitted the open dwell time histograms with the two-component function. This revealed two types of channel openings: one with a shorter time constant of $\sim 1–2$ ms and the other of $\sim 10$ ms. For each of the two time constants, we obtained the relative fraction of activities and the total number of events. For normalization purposes, we expressed the values as fold increase over the pre-EtOH control levels. In the example shown in Fig. 3, the two open dwell time constants at 0.3% EtOH increased by 1.1- and 1.5-fold and the relative events by 4.6- and 2.4-fold, respectively, for components 1 and 2. Table 2 provides the summary of the effects by EtOH on single BK channel parameters. These data show that EtOH changed the proportion of the BK channel openings favoring the channel activity with the shorter dwell time constant (i.e., component 1). This was accomplished by an increase in the number of events for this component. As summarized in Table 2, at 0.1 and 0.3% EtOH, the number of events for component 1 increased on average by $\sim 2$- and 5-fold, respectively. In comparison, the incidence of component 2 increased by 1.6- and 1.8-fold for 0.1 and 0.3% EtOH, respectively. At 0.3% EtOH there were $\sim 1.5$- and $\sim 1.8$-fold increases in the dwell time constants for components 1 and 2, respectively. The increases in the dwell time constants and overall number of events underlie the observed enhancement of the $P_o$ (Table 2 and Fig. 4). Collectively, these single-channel recordings reveal that EtOH in the presence of low intracellular $[\text{Ca}^{2+}]$ of $\sim 300$ nM positively modulates the activity of BK channels by increasing $P_o$, the incidence of single-channel events, and open dwell time constants.

**EtOH induces differential effects on single BK channel activity in the presence of elevated intracellular $[\text{Ca}^{2+}]$: no significant change at $\sim 2$ $\mu$M and inhibition at $\sim 10$ $\mu$M.** Since previous reports in non-DSM cells indicated that the effect of EtOH may depend on intracellular $[\text{Ca}^{2+}]$ (4, 8, 30), additional single BK channel recordings using the inside-out configuration under moderately elevated intracellular free $[\text{Ca}^{2+}]$ of $\sim 2$ $\mu$M were performed. In the control condition (without EtOH), the single BK channel activity was very robust as illustrated in Fig. 5. Single BK channel openings were observed at negative voltages and increased with depolarization. At $+40$ mV, the mean single BK channel open probability was $0.84 \pm 0.03$ ($n = 14, N = 9, P < 0.001$ vs. $P_o$ at $\sim 300$ nM $[\text{Ca}^{2+}]$, see Table 1). In 12 patches ($N = 8$), current-voltage relationships were determined, as exemplified in Fig. 5, A and B, revealing the mean single BK channel unitary conductance of $228.3 \pm 7.0$ pS under these experimental conditions used, which was not significantly different ($P > 0.05$) from the value obtained for $\sim 300$ nM free $[\text{Ca}^{2+}]$ (see Table 1). Subsequently, the effect of EtOH at 0.3% was determined at $+40$ mV and revealed no statistically significant changes in the channel open probability nor in the single BK channel unitary current ($n = 11, N = 8, P > 0.05$ for both parameters; Fig. 6, A–C).

Next, the effect of EtOH (0.3%) was assessed in the presence of $\sim 10$ $\mu$M intracellular free $[\text{Ca}^{2+}]$, which corresponds to a relatively high level of cytosolic $[\text{Ca}^{2+}]$. As shown in Fig. 7, A–C, under this condition at $+40$ mV, EtOH caused a significant reduction of $P_o$ ($n = 8, N = 4, P < 0.05$) and had no effect on the unitary current amplitude ($P > 0.05$). The $P_o$ values were $0.817 \pm 0.038$ and $0.783 \pm 0.037$ in the absence and in the presence of EtOH, respectively ($P < 0.05$). Thus EtOH reduced the single BK channel activity on average by $P_o$ of $\sim 0.04$, which corresponds approximately to the activity of untreated BK channels measured at $+60$ mV under the condition of $\sim 300$ nM free $[\text{Ca}^{2+}]$ (Table 1). In addition, the unitary single BK channel conductance was not altered by EtOH at $\sim 10$ $\mu$M intracellular free $[\text{Ca}^{2+}]$ (Fig. 8, A–C). The values were $222.3 \pm 5.3$ pS for the control and $220.3 \pm 6.2$ pS with EtOH ($n = 8, N = 4, P > 0.05$). The pre-EtOH control conductance values in the presence of $\sim 10$ $\mu$M were not significantly different ($P > 0.05$) from those obtained at $\sim 300$ nM (Table 1) and $\sim 2$ $\mu$M $[\text{Ca}^{2+}]$. These experiments reveal dependence on intracellular $[\text{Ca}^{2+}]$ for the effects of EtOH on DSM BK channels, specifically enhancement of channel activity at $\sim 300$ nM, no significant modulation at $\sim 2$ $\mu$M, and a decrease in $P_o$ at $\sim 10$ $\mu$M.

**EtOH modulates TBKCJs by either increasing the frequency or decreasing the amplitude depending on basal level of activity.** DSM cells exhibit robust TBKC activity, which regulates excitability of these cells (44). Whole cell perforated

### Table 2. Summary of the effects by EtOH on single BK channel parameters

<table>
<thead>
<tr>
<th>Component</th>
<th>Pre-EtOH ($n = 13, N = 10$)</th>
<th>0.1% EtOH ($n = 11, N = 8$)</th>
<th>0.3% EtOH ($n = 10, N = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of events</td>
<td>0.37 ± 0.05</td>
<td>0.42 ± 0.06$^*$</td>
<td>0.63 ± 0.06$^*$</td>
</tr>
<tr>
<td>Relative fold increase in number of events</td>
<td>1</td>
<td>2.34 ± 0.36$^*$</td>
<td>5.01 ± 0.78$^*$</td>
</tr>
<tr>
<td>$\tau$, ms</td>
<td>1.43 ± 0.15</td>
<td>1.58 ± 0.18</td>
<td>2.15 ± 0.36$^*$</td>
</tr>
<tr>
<td>Relative fold increase in $\tau$</td>
<td>1</td>
<td>1.21 ± 0.15</td>
<td>1.46 ± 0.13$^*$</td>
</tr>
</tbody>
</table>

Component 2

| Fraction of events | 0.63 ± 0.06 | 0.58 ± 0.06$^*$ | 0.37 ± 0.06$^*$ |
| Relative fold increase in number of events | 1 | 1.56 ± 0.21$^*$ | 1.78 ± 0.32$^*$ |
| $\tau$, ms | 9.96 ± 0.90 | 10.26 ± 0.78 | 18.27 ± 3.50$^*$ |
| Relative fold increase in $\tau$ | 1 | 1.02 ± 0.09 | 1.77 ± 0.28$^*$ |

Values are means ± SE; $n =$ number of different DSM patches; $N =$ number of guinea pigs. BK channel, large conductance $\text{Ca}^{2+}\text{-activated K}^+$ channel; EtOH, ethanol. Comparison versus control (pre-EtOH) values: $^*P < 0.05$, $^{†}P < 0.01$, $^{‡}P < 0.001$. 

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patch-clamp recordings were obtained in 17 cells \((N = 13)\) at \(V_h\) of \(-30\) mV with a mean cell capacitance of \(45.5 \pm 2.3\) pF and a frequency and amplitude of TBKCs of \(1.2 \pm 0.4\) Hz and \(26.0 \pm 6.0\) pA, respectively. This high number of experiments was needed since DSM cells showed differential responses to EtOH. In certain DSM cells, as exemplified in Fig. 9A, EtOH induced enhancement of TBKCs by increasing their frequency. In other cells, shown in Fig. 9B, the primary effect of EtOH appeared on the TBKC amplitude. A key characteristic that determined how DSM cells responded was the TBKC frequency. As summarized in Fig. 9, C and D, DSM cells exhibiting the basal level of TBKC frequency of \(<0.5\) Hz responded to EtOH with increases in frequency, whereas those above this threshold did not. The analysis of the basal pre-EtOH control characteristics of these two types of cells, which we refer to as low- and high-frequency TBKC DSM cells, revealed a 10-fold separation for the mean frequency, \(0.17 \pm 0.05\) \((n = 8, N = 8)\) vs. \(2.1 \pm 0.5\) Hz \((n = 9, N = 7)\), and no statistically significant differences in the amplitude of TBKCs and cell capacitance. The values for low \((n = 8, N = 8)\) and...
high (n = 9, N = 7) frequency TBKC DSM cells were, respectively, 15.9 ± 1.5 and 34.9 ± 10.8 pA (P > 0.05) for the amplitude, and 41.2 ± 2.7 and 49.3 ± 3.1 pF (P > 0.05) for the cell capacitance. Low-frequency TBKC DSM cells responded to 0.3% EtOH by an average of approximately fourfold increase in the frequency and no effect on the amplitude (Fig. 9D). In contrast, 0.3% EtOH in high-frequency TBKC DSM cells caused inhibition of the amplitude of TBKCs to ~0.8-fold of the control and no effect on the frequency (Fig. 9D). The specific effects of EtOH depending on the basal level of activity would not have been obvious if all data for DSM cells were analyzed together. Both amplitude and frequency of TBKCs showed overall significant effects (n = 17, N = 13, P < 0.05; Fig. 9D).

EtOH attenuates DSM spontaneous phasic contractions by BK channel-dependent and BK channel-independent mechanisms. To examine the effects on DSM contractility, increasing concentrations of EtOH (0.01–1%) were applied to strips exhibiting spontaneous phasic contractions. EtOH inhibited DSM spontaneous phasic contraction amplitude and muscle force in a...
concentration-dependent manner (Fig. 10). At the maximal concentration of 1%, EtOH decreased DSM spontaneous phasic contraction amplitude and muscle force by 70.2%/H1100610.3 and 72.3%/H1100611.4%, respectively (n = 8, N = 4, P < 0.001 for both; Fig. 10B).

Because BK channels are known to critically regulate DSM contractility (44), we further tested the effects of EtOH under the condition of BK channel blockade with paxilline. We found that paxilline (1 µM) attenuated the EtOH inhibitory effects on DSM phasic contraction amplitude and force (Fig. 10B). These results provide evidence that BK channels are involved in mediating the EtOH inhibitory effects on the spontaneous phasic contractions of DSM isolated strips. However, in the presence of paxilline EtOH was still able to cause DSM relaxation. In particular, at 1% EtOH, the effects were comparable in the absence or presence of paxilline. This points to the involvement of an additional non-BK channel-dependent mechanism, which we address below.

EtOH inhibits L-type VDCC currents in freshly isolated native DSM cells. Reports indicate that in various non-DSM cells EtOH inhibits L-type VDCC channel activity (10, 33). Here, we examined whether EtOH could attenuate DSM...
VDCC activity and thus contribute to DSM relaxation. We recorded L-type VDCC currents using the whole cell perforated patch-clamp technique and solutions appropriate to isolate VDCC currents (see *Solutions and compounds*). In response to the voltage protocol in which cells were stepped from -74 to +36 mV in steps of 10 mV, the currents peaked at ~+5 mV. At +6 mV, the average inward current density was -1.02 ± 0.2 pA/pF (n = 13, N = 9). The cell capacitance and the half-maximum constant for activation (V0.5) averaged 44.8 ± 3.4 pF and -7.5 ± 1.7 mV (n = 13, N = 9), respectively. This profile suggests that these inward currents were due to opening of L-type VDCC channels. To confirm this, we examined the effect of the selective L-type VDCC inhibitor nifedipine. Nifedipine (3 μM) completely inhibited the inward current. At +6 mV, the values before and after nifedipine were -0.99 ± 0.16 pA/pF and 0.07 ± 0.08 pA/pF (n = 5, N = 5, P < 0.001; Fig. 11). This demonstrates that L-type VDCCs were primarily responsible for the inward current recorded.

The effect of EtOH was further examined on the L-type VDCC current. As shown in Fig. 11, EtOH at 0.3% had minimal effects on the L-type VDCC current (P > 0.05 for all voltages except for +6 mV). The subsequent increase to 1% EtOH significantly inhibited the L-type VDCC peak current for measured voltages at -14 mV and above. When measured at +6 mV, the current density decreased from the pre-EtOH control level of -1.28 ± 0.26 pA/pF to -1.15 ± 0.13 pA/pF (n = 6, N = 5, P < 0.05) at 0.3% EtOH and further to -0.83 ± 0.19 pA/pF (n = 6, N = 5, P < 0.001) at 1% EtOH. The constant for half-maximum activation was not significantly altered: -9.5 ± 2.7 mV and -6.5 ± 2.1 mV in the presence or absence of 1% EtOH, respectively (n = 6, N = 5, P > 0.05). This series of experiments indicates that in DSM cells EtOH inhibited L-type VDCCs in the concentration range producing non-BK channel-dependent relaxation. This provides support that EtOH mediates relaxation of DSM also by inhibiting L-type VDCCs when applied at higher concentrations (>0.3% EtOH).

**DISCUSSION**

The data presented here reveal that BK channels and L-type VDCCs are molecular targets for EtOH, thus supporting their role in mediating alcohol-induced relaxation of DSM. EtOH at concentrations as low as 0.1%, corresponding to blood alcohol concentration (BAC) achieved in moderate-heavy drinking, caused attenuation of DSM contractility. EtOH at 0.3% in the presence of ~300 nM intracellular free [Ca2+] enhanced the whole cell outward BK current and at 0.1 and 0.3% increased the P0 of single BK channel currents. The single BK channel recordings further revealed open-state-dependent effects of EtOH causing an increase in the number of events and open dwell time constants. In contrast, at 2 μM intracellular free [Ca2+] no statistically significant effects were observed for the open channel probability and single BK channel currents; whereas in the presence of 10 μM intracellular free [Ca2+], the open channel probability was significantly decreased demonstrating a strong dependence of the EtOH effects on [Ca2+]. The effect of EtOH (0.3%) on TBKCs depended on the basal level of activity in the frequency. Low-frequency TBKC DSM cells, those with the frequency <0.5 Hz, responded to EtOH by an increase in the frequency and no effect on the amplitude, whereas high-frequency TBKC cells showed no change in the frequency and a decrease in the amplitude of TBKCs. In the presence of the BK channel blocker paxilline, the inhibitory effects of EtOH on DSM spontaneous phasic contraction amplitude and muscle force, and on whole cell BK currents were attenuated supporting the involvement of these channels in...
mediating the DSM relaxation. However, EtOH at 1% evoked similar DSM relaxation in the presence or absence of the selective BK channel inhibitor paxilline. L-type VDCC blockade by EtOH likely plays a role in relaxation, as in isolated DSM cells these Ca\(^{2+}\) currents were directly inhibited by EtOH. Our experiments collectively support that EtOH-induced relaxation of DSM involves at least enhancement of BK channel activity and blockade of L-type VDCCs.

In acute EtOH intoxication, BAC may reach 0.5% and the loss of bladder control often occurs at \(-0.3\%\) (37). At EtOH concentrations of 0.5–1%, various types of ion channels are affected, among them are BK channels and L-type VDCCs (9). The evidence for their involvement has been obtained in studies investigating neuronal or cardiovascular cells (4, 9). Additional insights have been provided in recombinant expression systems (10, 30). In contrast, relatively little work has been carried out in the urinary bladder. To our knowledge, no previous study addressed whether BK channels and L-type VDCCs are molecular targets for EtOH in DSM. Prior studies with DSM tissue strips demonstrated the EtOH-induced relaxation of carbachol-, purinergic agonist-, and electrical field stimulation-evoked DSM contractions (38, 52, 56). In this study, EtOH induced relaxation of DSM spontaneous phasic contractions by attenuating phasic contraction amplitude and muscle force at concentrations of 0.1% and higher. The observed DSM relaxation, hence, occurred at physiologically relevant BAC. Furthermore, the EtOH-mediated effects were attenuated by paxilline, a selective BK channel inhibitor, providing strong evidence for a role of BK channels.

To confirm that BK channels directly mediate EtOH-induced effects in DSM, whole cell patch-clamp and excised patch (both inside-out and outside-out configurations) single-channel recordings were carried out. In the case of single BK channel recordings in the presence of \(-300\) nM free [Ca\(^{2+}\)] for both pipette and bath, we have combined all of the data regardless of the configuration used since EtOH crosses the plasma membrane through simple diffusion (50). At the steady state, EtOH in the vicinity of the recorded patch should distribute at comparable levels intracellularly and extracellularly. There were no statistical differences, under the experimental conditions of this study, between the two inside- and outside-out configurations used (Table 1). Both whole cell (Fig. 1) and single-channel (Figs. 2 and 3) recordings showed that EtOH potentiated the activity of the BK channels. The enhancement by EtOH appeared to be dependent on the basal level of BK channel (current) activity. Only when the activity was not very low, an increase in the BK channel current occurred in the presence of \(-300\) nM intracellular free [Ca\(^{2+}\)] (Fig. 1). Since in the whole cell configuration direct and indirect effects of EtOH are difficult to distinguish, the data obtained with the excised patches clearly demonstrate the direct modulation of BK channels by EtOH. To our knowledge, no previous study investigated the effects of EtOH on DSM electrical properties with any configuration of the patch-clamp technique. In nondSM cells, similar to our findings, acute EtOH exposure increased the whole cell BK currents in a subset of dorsal root ganglion neurons (15) and recombinant BK channels (mSlo) expressed in Xenopus laevis oocytes (30). The latter study also demonstrated the dependency of EtOH-mediated responses on intracellular Ca\(^{2+}\) levels. In the absence of Ca\(^{2+}\) or in the presence of high concentrations (\(\geq 10\) \(\mu\)M), no change or inhibition, respectively, was observed. In our study, we noted similar effects of EtOH (0.3%) for \(P_o\), observing no significant change for \(\approx 2\) \(\mu\)M (Fig. 6) and an inhibition for \(\approx 10\) \(\mu\)M intracellular [Ca\(^{2+}\)] (Fig. 7) when measured with the inside-out configuration. The unitary single BK channel amplitude or the slope conductance was insensitive to EtOH at \(-2\) or \(10\) \(\mu\)M intracellular free [Ca\(^{2+}\)] (Figs. 6–8). Thus, in DSM cells, the effect of EtOH on BK channels is also dependent on [Ca\(^{2+}\)]. In the presence of \(-2\) or \(10\) \(\mu\)M intracellular free [Ca\(^{2+}\)], it was not possible to reliably calculate open dwell time constants due to frequent simultaneous BK channel openings and high \(P_o\). Other investigators using excised patches derived from neuronal, recombinant, and GH3 cells with the [Ca\(^{2+}\)] ranging from 100 nM to \(10\) \(\mu\)M reported EtOH-induced enhancements in the \(P_o\), increases in the open dwell time constant, and decreases in the closed dwell time constant without changing the single-channel conductance (8, 15, 26, 30). In our study, in excised patches derived from DSM cells at \(-300\) nM intracellular free [Ca\(^{2+}\)] where the \(P_o\) was low, we also identified an enhancement of the \(P_o\), an increase in the open dwell time constants and no change in single BK channel conductance. The effects of EtOH on the open dwell time in DSM patches were different from those described previously. Unlike in the recombinant cells expressing mSlo in oocytes, where either decreases in mean open dwell time constant or increases in the longest time constant were seen at \(-0.3\%\) (\(-50\) nM) (8, 30), in DSM patches at \(-300\) nM free [Ca\(^{2+}\)] we report increases for both shorter and longer time constants (Table 2). We also noted an increase in the proportion of dwell time events favoring the shorter time constant, in contrast to recombinant mSlo channels (8, 30), as well as an overall increase in the incidence of BK channel openings. The differences in the single BK channel properties may be due to species differences, mouse vs. guinea pig, or our experimental approach using native DSM cells vs. recombinant mSlo (8, 30).

TBKCs constitute a fundamental mechanism, which regulates excitability of DSM cells and other cell types (44). They are evoked by Ca\(^{2+}\) sparks, localized releases of subplasmalemmal Ca\(^{2+}\) via sarcoplasmic reticulum (SR) ryanodine receptors (20). In the current-clamp mode measuring membrane potential, Ca\(^{2+}\) spark-BK channel events manifest as spontaneous transient hyperpolarizations (STHs). STHs provide a hyperpolarizing influence regulating smooth muscle excitability. Indeed, DSM cells show a high degree of correlation for activities of TBKCs and STHs (55). The mechanisms regulating activity of TBKCs are of considerable interest. Previous studies have demonstrated that in DSM cells enhancement of the PKA signaling pathway via inhibition of phosphodiesterases (54, 55) or activation of muscarinic subtype 3 receptors with carbachol (40) enhanced or attenuated TBKCs, respectively. In addition, the \(\beta_3\)-adrenergic agonist BRL37344 (24) and the nonselective adrenergic receptor agonist isoproterenol (46) increased TBKC activity in DSM cells. In this study, we identified that another mechanism for TBKC regulation involves EtOH. Interestingly, the manner by which EtOH altered DSM TBKCs depended the basal level of TBKC activity, in particular the frequency. DSM cells with the basal frequency of \(< 0.5\) Hz, referred to as low-frequency TBKC DSM cells, responded to EtOH (0.3%) with an increase in the frequency and no effect on the amplitude of TBKCs. In contrast, high-frequency TBKC DSM cells (\(> 0.5\) Hz) showed
differential effects, no effect on the frequency, and a decrease in the amplitude of TBKCs (Fig. 9). A potential explanation might be related to the basal level of [Ca\(^{2+}\)] present in SR of DSM cells. In cerebral artery smooth muscle cells, EtOH increased the [Ca\(^{2+}\)] load of the SR (31). Thus, it may be that low-frequency TBKC DSM cells have relatively low SR [Ca\(^{2+}\)] load and EtOH by increasing the SR [Ca\(^{2+}\)] load allows for faster Ca\(^{2+}\) sparks and an increase in the TBKC frequency. In the case of high-frequency TBKC DSM cells, the high basal SR [Ca\(^{2+}\)] load probably cannot be further increased. The fact that the amplitude of TBKCs was decreased in high-frequency TBKC DSM cells suggests that [Ca\(^{2+}\)] within the Ca\(^{2+}\) sparks reached high concentrations of ~10 µM. Such concentrations have been reported for Ca\(^{2+}\) sparks in smooth muscle cells (20, 57). In excised patches in the presence of ~10–20 µM intracellular [Ca\(^{2+}\)], EtOH caused inhibition of single BK channel open probability, as observed by us (Fig. 7) and others (30, 31). Since low-frequency TBKC DSM cells responded to EtOH without a significant effect on the amplitude, the [Ca\(^{2+}\)] of Ca\(^{2+}\) spark perhaps reaches low micromolar levels in these cells. This would be consistent with the lack of an effect of EtOH on DSM single BK channel activity at 2 µM [Ca\(^{2+}\)] (Fig. 6). The effects of EtOH in high-frequency TBKC DSM cells resemble those previously reported for cerebral arteries (31). In both cell types the amplitudes were decreased; however, the frequency was only attenuated in the vascular myocytes. This difference, the presence of low-frequency TBKC DSM cells, and enhancement of BK channel activity by EtOH at the low intracellular [Ca\(^{2+}\)] could contribute to alcohol-mediated relaxation of the urinary bladder.

Effects of EtOH on visceral smooth muscle contractility and the underlying mechanisms involved appear to be tissue-dependent. In DSM, similar to the intestine and corpus cavernosum, EtOH induced relaxation of isolated smooth muscle tissue strips (11, 28, 32). In the intestine, the excitation-contraction coupling mechanism for EtOH-mediated inhibition of contractility has been shown to involve smooth muscle hyperpolarization consistent with the activation of K\(^+\) channels (32). However, it still remains to be established if these K\(^+\) channels are indeed the BK channels. In contrast, preliminary evidence in the gastrointestinal tract has been provided for intermediate conductance Ca\(^{2+}\) activated (IK) channels playing a role in mediating responses to EtOH on contractility (11). In DSM, although IK channels are expressed, they do not contribute to excitability and contractility based on the lack of an effect by the selective IK channel inhibitor TRAM-34 in guinea pig and human DSM (2, 41). Our study points to DSM BK channels being directly modulated by EtOH. In corpus cavernosum smooth muscle, a role for BK channels has also been identified in EtOH-mediated relaxation, but the provided evidence suggested a potential indirect modulation via increase of intracellular Ca\(^{2+}\) levels (28). In cerebral arteries, comparable concentrations of EtOH caused vasoconstriction relating to increases in cerebral blood pressure after binge drinking (6, 7). In this tissue, inhibition of BK channels by EtOH mediates this contractile effect, dependent on the presence of the BK channel regulatory β\(_2\)-subunit (6, 7) and also on inhibition of TBKCs due to reduction of Ca\(^{2+}\) sparks and observed direct inhibition of BK channels at high [Ca\(^{2+}\)] reached during high amplitude TBKCs, estimated to be ~10 µM (20, 57). As a result, attenuation of the negative feedback loop mediated by BK channels on cerebral artery smooth muscle excitability leads to depolarization, increase in [Ca\(^{2+}\)] influx, and vasoconstriction (31). In contrast, DSM responded differently to the same concentrations of EtOH by relaxation. Our study provides evidence that EtOH-induced attenuation of DSM contractility involves BK channel modulation via two distinct mechanisms. First, under submicromolar [Ca\(^{2+}\)], present for most of the time in DSM cells, EtOH enhanced the activity of BK channels assessed by conventional whole cell (Fig. 1) and excised patch single-channel recordings (Figs. 3 and 4). Second, EtOH increased the TBKC frequency in DSM cells exhibiting a low level of basal TBKC activity (Fig. 9). Although a differential effect of EtOH was observed in the high-frequency TBKC DSM cells, in whole DSM tissue low-frequency DSM cells may predominate and drive overall responsiveness to EtOH. Importantly, our report identifies novel mechanisms involving BK channels in mediating the inhibitory effects of EtOH on DSM contractility and adds to the consideration of tissue or cell type specificity in elucidating the pathways underlying responses to EtOH.

When BK channels were blocked with the selective BK channel inhibitor paxilline, EtOH at the highest concentration tested of 1% induced similar DSM relaxation in the presence or absence of paxilline. This suggests that at such relatively high concentrations of EtOH, BK channels are unlikely to be the primary mediators of the EtOH inhibitory effects on DSM contractility. Since L-type VDCCs are key regulators of DSM excitability and contractility (12, 48), and in non-DSM cells, EtOH has been shown to inhibit these channels (10, 31, 33), we investigated the effect of EtOH on L-type VDCC currents in DSM cells. We used the perforated whole cell patch-clamp technique since this method preserves the intracellular signaling pathways and molecules with minimal current run-down. To our knowledge, our study is the first to record DSM L-type VDCC currents with the perforated patch-clamp technique using conditions ideally suited to optimally record Ca\(^{2+}\) currents. Unlike previous reports (21, 41, 46) that used K\(^+\)-based solutions and thus contained contaminating K\(^+\) channel currents, the recordings of our study were carried out in the presence of external TEA and Cs\(^+\)-containing solutions to minimize the influence of K\(^+\) conductance and maximize the ability to record stable L-type VDCC currents. We further analyzed the VDCC voltage dependency identifying the V\(_{0.5}\) constant of approximately ~7 mV and the peak current occurring at approximately ~5 mV, expected for the L-type VDCCs, and demonstrated inhibition of the currents by the selective L-type VDCC blocker nifedipine. Our report further extends the characterization of VDCC currents provided by the conventional whole cell patch-clamp method (14, 27, 48). Although DSM cells also express T-type VDCC currents (49) under our recording conditions, their contribution was negligible. DSM L-type VDCC currents were inhibited ~33% by 1% EtOH without significantly changing the constant for half maximum activation. The attenuation of L-type VDCC activity was likely due to EtOH decreasing the channel Po, shortening the open dwell time without altering the single-channel conductance as well as promoting steady-state inactivation as observed in neurohypophysial terminals and PC-12 cells (36, 53). Thus inhibition of L-type VDCC currents and potentially another mechanism or multiple, yet to be identified, could
explain the BK channel-resistant DSM relaxation in response to EtOH.

In summary, our study reveals that both BK channels and L-type VDCCs are molecular targets for EtOH in DSM. We provide evidence that the enhancement of BK channel activity and inhibition of L-type VDCC currents by EtOH are involved in mediating the alcohol-induced relaxation of the urinary bladder. The effects on BK channels occurred at physiologically relevant BAC levels during moderate-to-heavy acute alcohol intake. The EtOH-mediated DSM relaxation allows for expansion of the urinary bladder resulting in higher urine storage capacity accommodating alcohol-induced diuresis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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