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Single-channel biophysical and pharmacological characterization of native human large conductance calcium-activated potassium channels in freshly isolated detrusor smooth muscle cells

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Abstract

Recent studies have demonstrated the importance of large-conductance Ca^{2+} -activated K^+ (BK) channels in detrusor smooth muscle (DSM) function *in vitro* and *in vivo*. However, in-depth characterization of human native DSM single BK channels has not yet been provided. Here, we conducted single-channel recordings from excised patches from native human DSM cells. Inside-out and outside-out recordings in high K^+ symmetrical solution (containing 140 mM KCl and ~300 nM free Ca^{2+}) showed single-channel conductance of 215–220 pS, half-maximum constant for activation of ~+75–+80 mV, low probability of opening (P_o) at +20 mV that increased ~10-fold at +40 mV and ~60-fold at +60 mV. Using the inside-out configuration at +30 mV, reduction of intracellular $[\text{Ca}^{2+}]$ from ~300 nM to Ca^{2+} -free decreased the P_o by ~85%, whereas elevation to ~800 nM increased P_o by ~50-fold. The BK activator NS1619 (10 μM) enhanced the P_o by ~10-fold at +30 mV; subsequent application of the selective BK inhibitor paxilline (500 nM) blocked the activity. Changes in intracellular $[\text{Ca}^{2+}]$ or the addition of NS1619 did not significantly alter the current amplitude or single-channel conductance. This is the first report to provide biophysical and pharmacological profile of native human DSM single BK channels highlighting their importance in regulating human DSM excitability.

Keywords

large-conductance Ca^{2+} -activated K^+ channel; conductance; calcium; open probability; urinary bladder; NS1619; paxilline

Introduction

One of the key functions of the large-conductance voltage- and Ca^{2+} -activated K^+ channels – also known as BK, maxi-K, *slol*, or $\text{K}_{\text{Ca}1.1}$ channels – is the regulation of excitability and contractility of detrusor smooth muscle (DSM) [38]. BK channels exist as a tetrameric assembly of the pore forming α subunits with or without associated regulatory β subunits. Unlike the α subunit, there are four isoforms of the β subunit: $\beta 1$ to $\beta 4$ [31]. The specific

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assembly of BK α - β complexes not only affects the biophysical properties of the channels but also shows preferential subunit expression in tissues [47,39].

The current understanding of the roles of BK channels in the control of bladder function is based on studies conducted primarily in rodents combined with a few reports in humans. RT-PCR and immunological methods have demonstrated the expression of BK α , β 1 and β 4 channel subunits in whole DSM strips and single DSM cells isolated from rodents and humans [21,19,5,6,24]. Functionally, the application of a pharmacological activator - NS1619, NS-004, NS-1608, or the recently discovered NS11021 - resulted in enhancement of whole cell BK current amplitude whereas the addition of iberiotoxin or paxilline, both selective BK channel blockers, had the opposite effect [28,41,22,39,21,19]. Tissue level studies involving intracellular microelectrodes and contractions further demonstrated the involvement of BK channels in shaping the repolarization phase of the DSM action potential, establishing the resting membrane potential, and influencing contractile activity of detrusor tissue strips [15,13,14,3]. In addition, BK channel activators, as reported for NS-8, may increase bladder capacity and induce alteration in measured patterns of *in vivo* activity [46]. Genetic knock-out mice lacking either the pore forming α - or the regulatory β 1-subunits exhibited enhanced myogenic and nerve-mediated contractility *in vitro*, and increased urination frequency, enhanced bladder pressure, abnormal pressure oscillations, and urine dripping *in vivo* mimicking detrusor overactivity [48,3,35,39]. Collectively, these studies point to the BK channels as critical regulators of DSM excitability and contractility.

Native and recombinant BK channels in the presence of submicromolar Ca^{2+} have very low open probability at voltages corresponding to the resting membrane potentials of DSM of ~ -40 mV. They show marked increase in activity at more positive potentials due to their voltage dependency [25,13]. Furthermore, micromolar Ca^{2+} concentrations obtained locally during normal physiological processes such as a Ca^{2+} spark or Ca^{2+} influx via L-type voltage-dependent Ca^{2+} channels strongly shift the voltage activation toward more negative voltages [27,25,26]. Thus during smooth muscle excitation when membrane potential depolarizes and intracellular Ca^{2+} levels increase, BK channel opening is thought to promote a reduction in DSM excitability and subsequent attenuation of contractility.

Currently, there is a gap in our understanding of single-channel properties of native human detrusor BK channels. The sporadic data, available at present, provide very limited information about their biophysical and pharmacological properties [42,19]. As interspecies differences may influence bladder function and receptor pharmacology [2,1], it is important to determine the biophysical and pharmacological characteristics of native human DSM single BK channels. The comparison of DSM single BK channel profiles from different species [39,42,28] with those of other cell types [39,42,28], will provide an insight into the translational value of various animal models. This is crucially important as DSM BK channel modulation through genetic manipulation or direct pharmacological means is considered to be a valid therapeutic approach to control bladder dysfunction [38,11]. In this study we addressed the lack of knowledge of native human DSM BK channel properties by conducting a thorough characterization of single BK channel currents in excised patch recordings, both inside-out and outside-out configurations, from patient-donors determining the voltage sensitivity of the open channel probability and the single channel conductance. We further evaluated the sensitivity of the single-channel currents to intracellular Ca^{2+} and two BK channel modulators, the activator NS1619 and the selective blocker paxilline identifying the effects on the open channel probability and single channel conductance under the conditions of this study. This report defines the properties of native human DSM BK channels at the level of single channels and complements the initial identification of their properties provided by molecular biology approaches, perforated whole cell patch-clamp recordings, and DSM contractility studies [19].

Materials and methods

Human DSM tissue collection

Human DSM tissue specimens were obtained from routine open bladder surgeries for bladder cancer in accordance with the reviewed and approved protocol HR 16918 by the Medical University of South Carolina (MUSC) Institutional Review Board. All specimens, harvested from bladder regions not affected by cancer, were collected from patients with no preoperative history of overactive bladder. We studied DSM cells obtained from 14 patients (9 males, 5 females, 10 Caucasians, 3 African-American, and 1 unclassified race), 57 to 78 years of age (mean=67.8 ±1.9 years).

Fresh DSM single cell isolation

Human DSM cells were isolated as previously described [21,19]. Briefly, small DSM strips were cut from the mucosa-free DSM specimens and placed in dissection solution (DS, 2 ml) supplemented with 1 mg/ml BSA, 1 mg/ml papain, and 1 mg/ml dithiothreitol and incubated for 30–40 min at 37°C. Next, DSM strips were transferred to DS (2 ml) containing 1 mg/ml BSA, 0.5–1 mg/ml collagenase (type II), and 100 µM CaCl₂ for 15–20 min at 37°C. After the incubation, DSM strips were washed with fresh BSA containing DS solution. Individual cells were released from the tissue by passing the enzyme-treated DSM strips through a Pasteur pipette.

Single-channel recording

Isolated human 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. The free Ca²⁺ concentration was calculated to be ~300 nM (WEBMAXC Standard, <http://www.stanford.edu/~cpatton/webmaxcS.htm>, Chris Patton). Single-channel recordings of BK currents were obtained from excised inside-out or outside-out patches using symmetrical 140 mM KCl solution for both the bath and pipette compartments based on previous reports [39,28]. The recordings were made with an Axopatch 200B amplifier, Digidata 1440A, pCLAMP software ver. 10.2 (Molecular Devices LLC, Sunnyvale, CA) and an eight-pole Bessel filter 900CT/9L8L (Frequency Devices) to filter the recorded signals. Patch-clamp pipettes were made from borosilicate glass (Sutter Instruments, Novato, CA) and polished with a Micro Forge MF-830 fire polisher (Narishige Group, Tokyo, Japan) to give a final tip resistance of 6–15 MΩ. All patch-clamp experiments were conducted at room temperature (22–23°C).

Solutions and compounds

The DS solution with and without supplemented Ca²⁺ (100 µM) had the following composition (in mM): 80 monosodium glutamate, 55 NaCl, 6 mM KCl, 10 glucose, 10 HEPES, and 2 MgCl₂, pH 7.3, adjusted with NaOH. The symmetrical K⁺ solution contained (in mM): 140 KCl, 1.08 MgCl₂, 5 EGTA, and 3.16 CaCl₂, adjusted to pH 7.2 with NaOH. To obtain the Ca²⁺-free solution, 3.16 mM CaCl₂ was omitted from the symmetrical K⁺ solution whereas for the elevated Ca²⁺ solution 4.16 mM CaCl₂ was used instead of 3.16 mM yielding the calculated free Ca²⁺ concentration of ~800 nM. Papain was obtained from Worthington (Lakewood, NJ); dithiothreitol, collagenase, NS1619, and paxilline from Sigma-Aldrich (St. Louis, MO). NS1619 and paxilline were dissolved as stock solutions in DMSO and diluted to the indicated concentrations.

Data analysis and statistics

Clampfit software version 10.2 and GraphPad Prism version 4.03 (La Jolla, CA), were used for analysis of data. The values for single-channel NP_o were obtained using the build-in algorithm in Clampfit, which calculates NP_o 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-channel currents were analyzed over ~2–5 min intervals prior to and after the experimental testing of the effects of altering intracellular $[Ca^{2+}]$ (both Ca^{2+} -free and elevated) and NS1619 (with and without paxilline) on P_o at +30 mV. When examining the single-channel activities at other voltages, the analysis interval was ~0.5–3 min for voltages of +60 mV and below, and 0.25–1 min for +70 – +100 mV. The graphs for mean single-channel amplitude-voltage and P_o -voltage were made in GraphPad Prism by respectively fitting linear regression (slope providing the conductance) and non-linear Boltzmann sigmoidal fit function (calculating $V_{0.5}$ value assuming maximum P_o value of 1). The total number of BK channels present ($N\#$) in each patch was determined at +80 to +100 mV. The determination of channel open probability (P_o) was done by dividing the open single-channel probability of each patch (NP_o) by the number of ion channels present in the patch ($N\#$). The single-channel amplitudes were calculated from all-point histograms using Gaussian distribution function to qualify the values for closed and open states. The data are summarized as mean $\pm$ SE (n =number of different patches; N =number of patients) and compared using paired or unpaired Student's t-test. A P value of < 0.05 was considered statistically significant.

Results

Voltage-dependence of human DSM single BK channel currents

Single-channel recordings were measured from excised membrane patches obtained from freshly isolated native human DSM cells using symmetrical 140 mM K^+ solution containing ~300 nM free Ca^{2+} . In total, 45 separate single-channel recordings were made from DSM patches derived from 14 different patients, 30 with the inside-out configuration (13 patients) and 15 with outside-out configuration (8 patients) detecting single BK channel currents at different membrane potentials. Representative traces for the single-channel currents obtained with the inside-out and outside-out configurations are shown in Fig. 1 and 2, respectively. In both cases, increasing the membrane potential toward more positive voltages caused enhancement of single-channel activity. At voltages below ~+30 mV, the probability of single-channel events was very low as exemplified in Fig. 1D and 2D. When the currents were recorded below 0 mV, their amplitude was comparable to that recorded at the corresponding potential in the positive range except for much lower probability of opening as illustrated in Fig. 1A and 2A. Above the threshold of ~+40 mV, the single-channel activity increased robustly. At voltages above +60 mV, the single-channel current amplitude deviated noticeably from the straight line (Fig. 1C and 2C), which reflects rectification properties of BK channels under the experimental conditions utilized. We, hence, used data-points +60 mV and below in the determination of the mean single BK channel conductance (based on the slope of the I-V graph). Fig.3 summarizes the individual data points for all inside-out and outside-out patches depicting single-channel conductance, half-maximum voltage constant for activation ($V_{0.5}$), and P_o values measured for +20, +40, and +60 mV. There were no statistical differences in any of the biophysical parameters examined for the two configurations used (Fig. 3A–D). The mean values were ~215–220 pS for single BK channel conductance, +75–80 mV for $V_{0.5}$, and when normalized to the +20 mV for each patch, the P_o increased by ~10-fold and 60-fold at +40 and +60 mV, respectively. These experiments collectively indicate that the single-channel currents recorded in DSM excised patches are highly voltage-dependent and exhibit the expected mean conductance values of BK channels.

Calcium sensitivity of human DSM single BK channel currents

In addition to voltage dependency, BK channels exhibit sensitivity to intracellular Ca^{2+} [25,38,26,44]. To confirm that DSM single BK channel activity is regulated by intracellular $[Ca^{2+}]$, inside-out patch-clamp recordings were made whereby the bath solution

(intracellular compartment) was changed from control $[Ca^{2+}]$ of ~ 300 nM to ~ 0 nM (using 5 mM EGTA and 0 $CaCl_2$, see Materials and Methods). Continuous single-channel recordings were made at the V_m of +30 mV as exemplified in Fig. 4A. In this example, the P_o value was reduced by 88.9% (Fig. 4B) without affecting the single-channel current amplitude at +30 mV or mean single-channel conductance, obtained from I-V plots of control and Ca^{2+} -free conditions (Fig. 4C). The effect was readily reversible. In 12 patches ($N=7$), the control P_o at +30 mV of 0.0364 ± 0.0130 was reduced by $\sim 85\%$ ($P < 0.0001$, Table 1 and Fig. 4D). There were no significant effects ($P > 0.05$) by the Ca^{2+} -free condition on single BK channel current amplitude at +30 mV or single-channel conductance (Table 1).

In separate experiments, also conducted with the inside-out configuration, the Ca^{2+} concentration in the intracellular (bath) compartment was increased from the control of ~ 300 nM to ~ 800 nM (see Materials and Methods). A representative experiment from this series of recordings is shown in Fig. 5A–C depicting current traces at +30 mV, and single-channel open probability-voltage and current-voltage plots prior and after the increase in the Ca^{2+} concentration. As illustrated, the channel activity robustly increased. There were numerous channel openings in the ~ 800 nM Ca^{2+} solution. In 10 patches ($N=5$) recorded at +30 mV as represented in Fig. 5D, the mean P_o increased by ~ 50 -fold ($P < 0.00001$) from the control (~ 300 nM) of 0.0492 ± 0.0253 while the current amplitude did not significantly change. The values for the channel conductance were also similar ($P > 0.05$) for the control and elevated Ca^{2+} conditions (Table 1). These experiments demonstrate that intracellular or cytosolic Ca^{2+} is an important regulator of the activity of native human DSM single BK channels influencing the P_o without significant effects on current amplitude and the single BK channel conductance.

Sensitivity of human DSM single BK channel currents to pharmacological modulators

Both native and recombinant BK channel activities are enhanced by the BK channel activator NS1619 and inhibited by the selective blocker paxilline [12,10,17,21]. We tested the effects of these two compounds on native human DSM single BK channel currents. The sensitivity to $10 \mu M$ NS1619 was examined at the V_m of +30 mV during its continuous presence and at different voltages before and after its application, typically in the same patches. A representative experiment is depicted in Fig. 6A–C showing enhancement of single-channel activity by NS1619. As illustrated for this example, the single-channel P_o increased by ~ 10 -fold or 1000% of the control. We examined the effect of $10 \mu M$ NS1619 in 11 different patches (8 inside-out and 3 outside-out, $N=6$) at +30 mV. The control P_o of 0.0615 ± 0.0337 increased by ~ 9.8 -fold ($P < 0.03$) following application of NS1619 at $10 \mu M$ (Fig. 6B). This activator did not significantly change single BK channel current amplitude at +30 mV of ~ 7 pA or the single BK channel conductance of ~ 200 pS (Table 1). Thus, the primary effect of the BK channel activator NS1619 was to increase the P_o or channel gating.

In 8 patches (5 inside-out and 3 outside-out, $N=6$) in the continuous presence of NS1619 exhibiting the mean P_o of 0.247 ± 0.083 , we applied 500 nM paxilline. The application of this selective BK channel blocker in 6 out of 8 patches completely inhibited the channel activity as exemplified by Fig. 6A. In the remaining two recordings, the P_o value decreased by 95% and 99%, respectively.

These experiments showed that the single-channels recorded are indeed mediated by the voltage- and Ca^{2+} -sensitive BK channels known to be potentiated by NS1619 and inhibited by paxilline.

Discussion

We provide the first thorough characterization of human single BK channel activities in excised patches of freshly isolated DSM cells from donor-patients. The human DSM single BK channel currents exhibited properties consistent with those reported for these channels in other systems: the mean single-channel conductance of more than 200 pS, P_o highly dependent on the voltage and intracellular Ca^{2+} concentration, enhancement of single-channel activity by the BK channel activator NS1619 and blockade by the selective BK channel inhibitor paxilline. The single-channel conductance and amplitude were not affected by modification of intracellular $[Ca^{2+}]$ or NS1619.

The biophysical properties of native DSM single BK channels have not been investigated to the same detail as those of neuronal, kidney or other smooth muscle cell types [27]. Only a few reports in the literature characterize the single BK channel currents in DSM cells. Nearly all used rodent derived cells conducted with cell-attached, inside-out, or outside-out patch-clamp configurations, and the whole cell perforated recordings [15,39,42,28]. There are only two very limited and preliminary reports of human DSM single-channel activity assessed by either the cell-attached configuration or the perforated whole cell patch-clamp recording [42,19]. Rodent and human studies demonstrated robust DSM single BK channel currents with mean conductance values in the range of ~200–250 pS using symmetrical K^+ solutions or 100–150 pS with asymmetrical solutions [4,17,22,42,50,39,28,20]. In this study, using native human DSM BK channels, we observed the mean single BK channel conductance of 215–220 pS for inside-out and outside-out configurations. Our data are comparable with the literature conducted using symmetrical high K^+ solutions that maximize the current amplitude and minimize the complications associated with current rectification especially at positive voltages [27,45]. As observed in myometrial smooth muscle cells [23], presynaptic nerve terminals [45], and skeletal myocytes [8], the presence of cations such as Na^+ or Mg^{2+} reduces the single BK channel conductance over certain voltages attributed to channel inhibition. To our knowledge, our study is the first report to provide detailed characterization of single BK channels for native human DSM with excised patches evaluating voltage dependence, sensitivity to intracellular Ca^{2+} and pharmacological effects to the activator NS1619 and inhibitor paxilline.

We identified a marked increase in the channel open probability following membrane depolarization. At membrane potentials of +20 mV under the experimental conditions of relatively low cytosolic Ca^{2+} of ~300 nM, which is close to the resting level of ~100 nM [16,9], very low probability of channel openings was noted. On average at +20 mV, we obtained the P_o of ~0.010 (inside-out) or ~0.017 (outside-out) – the values not statistically different from each other. When the holding potential was changed to +40 and +60 mV, the mean P_o increased respectively by ~10-fold and 60-fold when normalized to the P_o of +20 mV for each patch (Fig. 3D). When the Ca^{2+} concentration was reduced to apparent Ca^{2+} -free, the P_o decreased by ~85%. In contrast, the elevation in the intracellular Ca^{2+} concentration to ~800 nM from the control of ~300 nM enhanced the P_o by ~50-fold. The single-channel conductance and the single-channel current amplitude at +30 mV were not altered ($P > 0.05$) by either $[Ca^{2+}]$ modification. The observations of an increase in P_o with depolarization and sensitivity to cytosolic Ca^{2+} further confirm the properties of native human single BK channels being similar to those reported for other cell types [15,39,42,28,27,45].

It is likely that native human DSM BK channels are primarily composed of the heteromeric assembly α - β 1 or α - β 4 subunits based on the co-expression of these subunits in human, rodent, and rabbit DSM tissues or cells [20,19,6,24]. Our single-channel recordings also support the existence of heteromeric channels. We identified the apparent threshold for

exponential BK current increase of $\sim +40$ mV and the $V_{0.5}$ of $\sim +75$ – 80 mV, both of which are consistent with the co-assembly of α and β subunits [36,18,31,25]. Recombinant HEK-293 cells, recorded in the presence of intracellular Ca^{2+} in ~ 200 – 300 nM, expressing only the α subunit exhibited the threshold of $\sim +50$ mV whereas for complexes co-expressing the α subunit with either $\beta 1$ or $\beta 4$, the activation threshold occurred at much lower voltages [30]. In addition, the $V_{0.5}$ constant for recombinant α subunits expressed alone was reported to be above $+100$ mV for intracellular Ca^{2+} concentrations of 300 nM or less [33,7,37] whereas in our study the constant was $\sim +75$ – 80 mV. The inter-relations among intracellular Ca^{2+} concentration, the $V_{0.5}$ constant, and the presence and molecular identity of β subunit are complex. At physiological resting Ca^{2+} concentrations of 100 nM or less, the $V_{0.5}$ constant remains unchanged and comparable for homomeric (α) and heteromeric (α - β) BK channels. Only for intracellular Ca^{2+} concentrations of 300 nM or higher, is the effect on $V_{0.5}$ more pronounced for β -subunit containing BK channels [33,25,7,37,49]. Since our data revealed variation in the $V_{0.5}$ and P_o values among different patches recorded (see fig. 3B), the native human native BK channels likely consist of both homomeric and heteromeric subunit complexes. Future studies are needed to fully elucidate the roles and molecular subunit identity of BK channels in the control of human DSM function especially under pathological conditions.

We observed robust potentiation of the human DSM BK channel activity by the channel activator NS1619 (Fig. 6). When normalized to the pre-addition control, NS1619 enhanced the P_o by ~ 10 -fold or 1000% of the control. The primary effect occurred on gating since the values for slope conductance or the current amplitude at $+30$ mV were not significantly affected (Table 1). Our observations for the effects of NS1619 on human DSM BK channels are consistent with the reported data of this compound on single BK channel activity in rat cerebral artery smooth muscle [17] and guinea pig DSM [41]. To our knowledge, our study is the first to determine the effect of NS1619 on human native DSM single BK channel activity. NS1619 was reported to increase whole-cell outward BK currents in isolated human [21] and guinea pig [41] DSM cells and to inhibit spontaneous and evoked detrusor contractility in human, rat, guinea pig, rabbit, and Landrace pig tissue strips [43,21,32,5]. Other BK channel activators – specifically NS1608, NS004 and NS11021- have been studied and their effects determined in single-channel patch-clamp experiments conducted on rodent DSM BK channels. Similar to our observation, the addition of these compounds increased the P_o without effects on current amplitude or single-channel conductance [22,42,28]. The modulatory sites for the action of the BK activators remain to be fully elucidated. At least for NS1619 the interaction likely involves an intracellular site on the α subunit [10]. In our study, NS1619 was effective in both inside-out or outside-out patch-clamp configurations. Since this compound is highly hydrophobic and easily crosses the plasma membrane, we could not identify whether NS1619 binds intracellularly or extracellularly. In the case of paxilline, a negative allosteric mechanism has been proposed involving interaction with the S5–P loop–S6 module of the α subunit with a crucial role of Glycine311 in BK S6 helix [52]. In recombinant systems or native vascular and neuronal cells, paxilline effectively inhibited the BK channel activity [40,29]. In DSM cells, the blocking effects of paxilline have been previously described for BK currents potentiated by the non-selective phosphodiesterase inhibitor IBMX [51] and for hyperpolarization-evoked by the BK activator NS1608 [42]. In our study, we observed that the addition of paxilline (at 500 nM in the presence of NS1619) inhibited the single BK channel activity (Fig. 6A). This indicates that paxilline is an effective inhibitor of human DSM BK channels and can be used in studies aimed at identifying the physiological and regulatory roles of these channels. Since iberiotoxin or related toxins do not effectively inhibit all BK α - β channel complexes [34], the use of paxilline provides an advantage when the blocking all of BK channel subtypes is desired.

In summary, this report is the first to provide detailed characterization of native human single BK channels in excised patches derived from freshly isolated DSM cells. The human DSM single BK channel currents exhibit strong dependence on voltage and intracellular Ca^{2+} concentrations, as well as potentiation of activity by the opener NS1619 and attenuation by the selective inhibitor paxilline. This study advances the understanding of biophysical and pharmacological properties of native human DSM BK channels that until now remained elusive.

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Fig. 1.

Voltage-dependence of native human DSM single BK channel currents recorded from excised patches in inside-out configuration. (A) Representative recordings obtained using symmetrical 140 mM KCl solution (~ 300 nM free Ca^{2+}) illustrating increase in BK channel activity with depolarization in a single patch. C > indicates the closed channel state with inward channel openings for -20 mV and outward for $+20$, $+40$, and $+60$ mV. (B) Histograms for traces in (A). Plotted intervals were 0.5 s for each potential using the bins of 0.6 pA. The dotted bell shaped curves represent fitted data with the Gaussian distribution function for each state. (C) Single-channel current-voltage relationship for the single representative patch shown in (A). The mean single-channel conductance for this patch was 226 pS. (D) Single BK channel open probability (P_o) – voltage relationship for the single patch represented by the traces in (A). The $V_{0.5}$ was estimated to be $+65$ mV. The lower panel depicts the P_o values on the logarithmic scale illustrating very low probability of single-channel opening at -30 to $+40$ mV.

Fig. 2.

Voltage-dependence of native human DSM single BK channel currents recorded from excised patches with outside-out configuration. (A) Representative recordings using the symmetrical 140 mM KCl solution (~ 300 nM free Ca^{2+}) depicting increase in channel activity with depolarization in a single patch. C > indicates the closed state with inward channel openings occurring for -20 mV and their direction is outward for $+20$, $+40$, and $+60$ mV. (B) Histograms for traces in (A). Plotted intervals were of 0.5 sec for each potential with the bins of 0.6 pA. The dotted bell shaped curves represent fitted data with the Gaussian distribution function for each state. (C) Single-channel amplitude-voltage graph for the single patch represented in (A). The mean single-channel conductance for this patch was 231 pS. (D) Single-channel open probability (P_o)-voltage relationship for the single patch represented by traces in (A). The $V_{0.5}$ constant was estimated to be $+68.9$ mV. The lower panel in (D) depicts the P_o values on the logarithmic scale for the voltages in the range of -30 to $+40$ mV illustrating very low P_o values.

Fig. 3.

Comparison of biophysical properties of native human DSM single BK channel activity measured in inside-out and outside-out configurations. Shown are the summary graphs for (A) mean single-channel conductance, (B) constant for half-maximum activation ($V_{0.5}$), and (C) single-channel open probability measured at +20, +40, and +60 mV, and (D) the normalized value for open channel probability relative to the P_o at +20 mV for each patch recorded. Each data-point represents a value obtained from a separate patch recording; the horizontal line for each condition depicts the mean. The values for (A) single BK channel conductance were: 221.2 ± 3.6 pS (inside-out) and 213.8 ± 5.0 pS (outside-out); (B) $V_{0.5}$: $+81.5 \pm 2.6$ mV (inside-out) and $+76.7 \pm 5.6$ mV (outside-out); (C) P_o in inside-out configuration at +20, +40, and +60 mV respectively 0.010 ± 0.003 , 0.065 ± 0.014 , and 0.199 ± 0.030 ; and for P_o in outside-out configuration at +20, +40, and +60 mV respectively 0.013 ± 0.006 , 0.084 ± 0.036 , and 0.276 ± 0.071 ; (D) the normalized P_o values (+20 mV taken as 1) in inside-out configuration at +40 and +60 mV respectively 11.1 ± 1.2 and 58.8 ± 10.9 ; and for P_o in outside-out configuration at +40 and +60 mV respectively 9.6 ± 1.2 , and 64.2 ± 12.0 . The data are based on 30 inside-out single-channel patch-clamp recordings (N=13) and 15 outside-out patch-clamp recordings (N=8).

Fig. 4.

Effect of removal of intracellular Ca^{2+} on native human DSM single BK channel activity. (A) A representative experiment illustrating the effect of removing intracellular Ca^{2+} (5 mM EGTA and 0 CaCl_2) using the inside-out configuration at V_m of +30 mV showing attenuation of single BK channel activity measured in a single patch. C > indicates the closed channel state and the outward currents the channel openings. (B) The plot of the P_o for the patch illustrated in (A). In this example the P_o values were 0.0089 for the control and 0.0010 for the $\text{Ca}^{2+}\text{-free}$ condition. (C) The graph of the single-channel BK current-voltage relationship for the single patch represented in (A). The mean single-channel conductance values in this example were 209.9 pS for the control and 205.3 pS for the $\text{Ca}^{2+}\text{-free}$ solution. (D) Summary of the effect of the $\text{Ca}^{2+}\text{-free}$ solution on the P_o measured at V_m of +30 mV in 12 inside-out patches (N=7). The connected filled circles depict individual experiments, and the open circles with error bars and connected line the mean values, * indicates $P < 0.05$ (paired Student's t-test).

Fig. 5. Effect of elevating intracellular Ca^{2+} on native human DSM single BK channel activity. (A) A representative recording demonstrating the enhancement of single BK channel activity by increase in intracellular $[\text{Ca}^{2+}]$ from the control of $\sim 300 \text{ nM}$ (5 mM EGTA and 3.18 mM CaCl_2) to $\sim 800 \text{ nM}$ (5 mM EGTA and 4.18 mM CaCl_2) recorded in a single patch using the inside-out configuration at V_m of +30 mV. C > indicates the closed channel state. Visible are instances of multiple simultaneous channel openings especially for the solution containing $\sim 800 \text{ nM}$ free $[\text{Ca}^{2+}]$. (B) Single-channel P_o -voltage relationship for the single patch illustrated in (A). In this example, the P_o values were 0.0046 for the control ($\sim 300 \text{ nM Ca}^{2+}$) and 0.4694 for $\sim 800 \text{ nM Ca}^{2+}$ solution. (C) Single BK channel current-voltage relationship for the patch represented in (A). The mean single-channel conductance values in this example were 254.9 pS for the control and 255.5 pS for the elevated Ca^{2+} solution. (D) Summary of the effect of the elevating free intracellular $[\text{Ca}^{2+}]$ to $\sim 800 \text{ nM}$ on single-channel P_o measured at +30 mV in 10 inside-out patches (N=5). The connected filled circles depict individual experiments and the open circles with error bars and connected line the mean values, # indicates $P < 0.0001$ (paired Student's t-test).

Fig. 6. Pharmacological modulation of human single BK channels by the opener NS1619 and inhibitor paxilline in excised patches. (A) A representative experiment demonstrating potentiation of the single BK channel activity by the addition of NS1619 (10 μ M) and blockade by the subsequent application of paxilline (500 nM in the continuous presence of 10 μ M NS1619) in a single patch. The recording was obtained using the inside-out configuration at V_m of +30 mV; C > indicates the closed channel state. (B) The graph of the single-channel P_o for the single patch illustrated in (A). In this recording, the P_o values were 0.023 for the control prior to the addition and 0.165 in the presence of NS1619. (C) Single BK channel current-voltage relationship for the patch represented in (A). The mean single-channel conductance values in this example were 253.1 pS for the control and 247.1 pS in the presence of NS1619. (D) Summary of the effect of NS1619 on single BK channel P_o measured at +30 mV in 11 excised patches (N=6). The connected filled circles depict individual experiments, and the open circles with error bars and connected line the mean values, ** indicates $P < 0.01$ (paired Student's t-test).

Table 1

Summary of the effects of altering intracellular $[Ca^{2+}]$ and the addition of NS1619 on single BK channel activity in human excised DSM patches.

	P_o, Relative to Control (%)	Current @ +30 mV (pA)	Channel Conductance (pS)
Control	100	7.0 ± 0.3	199.2 ± 8.0
Ca ²⁺ -free	12.4 ± 2.6 ***	7.3 ± 0.2	203.2 ± 7.0
Control	100	7.3 ± 0.2	230.6 ± 5.6
~800 nM [Ca ²⁺]	5142.3 ± 1620.8 *	7.0 ± 0.2	231.1 ± 6.7
Control	100	7.3 ± 0.4	219.3 ± 6.4
10 μM NS1619	982.6 ± 327.1 *	7.3 ± 0.5	218.8 ± 7.4

Control condition contains 300 nM free Ca²⁺. Each data-point is n=8–12, N=5–7;

* indicates $P < 0.05$,

*** $P < 0.001$ versus respective controls (paired Student's t-test comparison).