Suppression of human detrusor smooth muscle excitability and contractility via pharmacological activation of large conductance Ca\(^{2+}\)-activated K\(^{+}\) channels

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Hristov KL, Parajuli SP, Soder RP, Cheng Q, Rovner ES, Petkov GV. Suppression of human detrusor smooth muscle excitability and contractility via pharmacological activation of large conductance Ca\(^{2+}\)-activated K\(^{+}\) channels. Am J Physiol Cell Physiol 302:C1632–C1641, 2012. First published March 14, 2012; doi:10.1152/ajpcell.00417.2011.—Overactive bladder syndrome is frequently associated with increased detrusor smooth muscle (DSM) contractility. We tested the hypothesis that pharmacological activation of the large-conductance voltage- and Ca\(^{2+}\)-activated K\(^{+}\) (BK) channel with NS-1619, a selective BK channel opener, reduces the excitability and contractility of human DSM. We used the amphotericin-perforated whole cell patch-clamp technique on freshly isolated human DSM cells, live-cell Ca\(^{2+}\) imaging, and isometric DSM tension recordings of human DSM strips obtained from open bladder surgeries. NS-1619 (30 μM) significantly increased the amplitude of the voltage step-induced whole cell BK currents, and this effect was abolished by pretreatment with 200 nM iberiotoxin (IBTX), a selective BK channel inhibitor. In current-clamp mode, NS-1619 (30 μM) significantly hyperpolarized the resting membrane potential, and the hyperpolarization was reversed by IBTX (200 nM). NS-1619 (30 μM) significantly decreased the intracellular Ca\(^{2+}\) level in isolated human DSM cells. BK channel activation with NS-1619 (30 μM) significantly inhibited the amplitude, muscle force, frequency, duration, and tone of the spontaneous phasic and pharmacologically induced DSM contractions from human DSM isolated strips. IBTX (200 nM) suppressed the inhibitory effects of NS-1619 on spontaneous contractions. The amplitude of electrical field stimulation (0.5–50 Hz)-induced contractions was significantly reduced by NS-1619 (30 μM). Our data suggest that pharmacological activation of BK channels could represent a novel treatment option to control bladder dysfunction in humans.

BK channels; iberiotoxin; NS-1619; patch clamp; urinary bladder

In DSM, the BK channel is composed of a pore-forming α-subunit and regulatory β1- or β4-subunits (6, 20, 34). Knockout mice lacking BK channel α-subunits or regulatory β1-subunits exhibit increased DSM contractility, elevated urination frequency, and increased intravesical pressure (28, 34, 41). These findings suggest that BK channel dysfunction may lead to the phenotype of overactive bladder (OAB). Recent studies in benign prostatic hyperplasia patients with detrusor overactivity (DO), as well as studies on a rabbit model of partial bladder outlet obstruction, suggest that downregulation of the BK channel contributes to DO under certain circumstances (5).

OAB is a major health issue. It is a highly prevalent condition, which increases with age and affects ~17% of the US population (39). Antimuscarinic drugs, which are currently the primary pharmacologic therapy for OAB, have adverse side effects and limited effectiveness (1). Since BK channels have been identified to play a prominent role in the regulation of DSM excitability and contractility, they may be a valid pharmacologic target for the treatment of bladder disorders such as OAB (5, 15, 20, 23, 31, 34, 35, 38). Development of drugs that can enhance BK channel activity have been proposed and investigated particularly as a treatment option for urinary bladder dysfunction (3, 4, 17, 37). Earlier studies have demonstrated that BK channel activation decreases DSM excitability and contractility in various experimental animal models (23, 37, 38). Studies involving genetic alterations in BK channel expression suggest that increasing BK channel activity may provide substantial therapeutic benefit in the treatment of DO (7, 26, 27). However, our knowledge about the functional role of the BK channel in human DSM is limited to a few publications (8, 11, 20, 31, 37). Recently, we showed that the BK channel is a main regulator of human DSM excitability and contractility (20). Inhibition of the BK channel with iberiotoxin (IBTX), a selective BK channel blocker, significantly decreases the whole cell outward current in freshly isolated human DSM cells, depolarizes the cell membrane potential, and increases human DSM contractility (20). However, it is unknown whether pharmacological activation of BK channels has the opposing effect in reducing human DSM excitability and contractility under physiological conditions.

NS-1619 (1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one) is a selective activator of the BK channel and has been shown to modulate cell excitability in various tissues including smooth muscle (9, 18, 24). NS-1619 (EC\(_{50}\) = 10–30 μM) decreases contractility in guinea pig DSM by activation of the BK channel (36). Our

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recent study revealed that BK channel activation with NS-1619 significantly inhibits spontaneous, pharmacologically induced and nerve-evoked contractions in rat DSM (38). However, functional studies performed on human DSM specimens have not addressed the role of BK channel pharmacological activation under normal physiological conditions of spontaneous activity but rather after DSM depolarization with KCl (31).

The present study evaluates the effect of pharmacological activation of BK channels with NS-1619 in human DSM with respect to excitability, intracellular Ca$^{2+}$, and contractility. Because human is the target species of interest for therapeutic intervention, the present study of human DSM is critical to validate animal models reflecting human bladder function. Utilizing a multidisciplinary experimental approach including a perforated patch-clamp technique, live-cell Ca$^{2+}$ imaging, and isometric DSM tension recordings, we evaluated the effect of pharmacological activation of BK channels on the excitability, intracellular Ca$^{2+}$ levels, and contractility of human DSM.

**MATERIALS AND METHODS**

*Human DSM tissue collection.* All human studies have been reviewed and approved by the institutional review board of the Medical University of South Carolina (MUSC) (protocol HR 16918). Human DSM tissues were obtained from routine bladder surgeries including radical cystectomy for bladder cancer and other open bladder surgeries for malignant or nonmalignant conditions of the lower urinary tract. Tissue utilized in this study was obtained only from donor patients without a preoperative history of OAB. DSM tissues were obtained from a total of 28 patients with an average age of 64.4 ± 1.7 yr (range 46–84). Demographics included 26 Caucasians and two African Americans, of which there were 23 males and 5 females. After surgery, the bladder specimens were immediately transported from the operating room to the laboratory in ice-cold Ca$^{2+}$-free HEPES-buffered dissection solution (DS) (see section Solutions and drugs). The mucosal layer including the urothelium and lamina propria were removed from the specimen. DSM strips were further processed for fresh single DSM cell isolation and isometric DSM tension recordings.

*Fresh human DSM single cell isolation.* Human DSM single cells were isolated as previously described (20). Briefly, small DSM strips (5–10 mm long, 2–4 mm wide) were cut from the human DSM specimens. Next, two or three DSM strips were incubated in 2 ml of DS supplemented with 1 mg/ml bovine serum albumin (BSA), 1 mg/ml papain (Worthington, Lakewood, NJ), and 1 mg/ml dithiothreitol for 30 min at 37°C. Then, the DSM strips were transferred to prewarmed 2 ml of DS containing 1 mg/ml BSA, 1 mg/ml collagenase type II (Sigma Aldrich), 0.5 mg/ml trypsin inhibitor (Sigma Aldrich), and 100 μM CaCl$_2$ for 7–14 min at 37°C. Enzyme-treated DSM strips were then washed two or three times with fresh DS containing BSA. Individual cells were released from the DSM tissue by passing the strips through the tip of a Pasteur pipette. The freshly isolated single DSM cells were used for patch-clamp recordings and live-cell intracellular Ca$^{2+}$ measurements within 12 h after isolation.

*Electrophysiological (patch-clamp) recordings.* All electrophysiological experiments were performed as previously described (20). Briefly, several drops of the DS containing freshly isolated human DSM cells were placed into a recording chamber. After 20 min the cells were washed several times with extracellular solution (see section Solutions and drugs). We employed the amphoterin-perforated whole cell configuration of the patch-clamp technique (10, 19), which preserves the native environment and the signal transduction pathways of the cells. Whole cell currents and resting membrane potential were recorded using an Axopatch 200B amplifier, Digidata 1440A, and pCLAMP version 10.2 software (Molecular Devices, Union City, CA). An eight-pole Bessel filter 900CT/9LFL (Frequency Devices) 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 PP-830 vertical puller (Narishige Group, Tokyo, Japan). The patch-clamp pipettes were coated with dental wax to reduce capacitance and polished with a Micro Forge MF-830 fire polisher to give a final tip resistance of ~4–6 MΩ. Voltage-step protocols were applied by holding DSM cells at −70 mV and then depolarized from 0 mV up to +80 mV in 20 mV steps for 200 ms to elicit steady-state K$^{+}$ outward currents and then stepped back to −70 mV. The control whole cell current was recorded for at least 10 min and then NS-1619 (30 μM) was applied in the bath. The resting membrane potential of freshly isolated human DSM cells was recorded in the current-clamp mode of the perforated patch-clamp technique. All patch-clamp experiments were carried out at room temperature (22–23°C).

**Measurement of live DSM intracellular Ca$^{2+}$ levels.** To measure intracellular Ca$^{2+}$ levels, 250 μl poly-l-lysine D (0.1 mg/ml) was added to 35 mm glass-bottom dishes, incubated in a 37°C water bath for 1 h, then the dishes were aspirated and washed three times with 2 ml sterile double-distilled water. Suspension of fresh single DSM cells (500 μl) was added into the dishes and kept at room temperature for 30 min to allow the cells to adhere to the glass bottom. Fura 2-AM solution was added to the cell suspension to make the final concentration of 2 μM and incubated for 30 min in the dark at room temperature. DSM cells were washed three times with a fresh bath solution. Ca$^{2+}$ imaging was acquired with a ×40/1.35 oil Iris objective and an OLYMPUS IX81 motorized inverted research microscope. The light was generated by TILL LB-LS/30 300 Watt Xenon Arc Lamp and then filtered into monochromatic light (340 nm and 380 nm) with an emission wavelength of 510 nm. A region without any cells was selected as background, which was subtracted from the measured values. The elicited light was detected with a digital charge-coupled device camera (Hamamatsu Photonics C10600-10B-H), and the camera exposure time was 200 ms. Image acquisition and hardware control were performed with MetaFluo 7.7.2.0. software.

**Isometric DSM tension recordings.** Isometric DSM tension recordings were conducted as previously described (20). Briefly, small strips (~2 × 5 mm) were cut from the DSM specimen and transferred to a sterile double-distilled water. The DSM strip was then suspended vertically in water-jacketed tissue baths filled with physiological saline solution (PSS) and equilibrated with 95% O$_2$/5% CO$_2$ at 37°C. The DSM strips were preloaded with an initial resting tension of ~1 g, and the changes in DSM tension were recorded isometrically. The bath solution was changed every 15 min with fresh PSS while spontaneous phasic contractions were stabilized. The spontaneous phasic contractions were generally observed within 20–30 min of the equilibration period. Three groups of treatments were set up for DSM strips after the equilibration period. For the first group of DSM strips exhibiting spontaneous contractions, 30 μM NS-1619 was added directly to the bath and DSM contractions were recorded for at least 30–40 min. To confirm the selectivity of NS-1619, DSM strips were preincubated for at least 10 min with IBTX and after stabilization of contractions, NS-1619 was added and DSM contractions were recorded for at least 35 min. In the second group, DSM phasic and tonic contractions were pharmacologically induced by addition of either a cholinergic agonist (carbachol, 0.1 μM) or by a depolarizing agent (20 mM or 60 mM KCl), and after stabilization of phasic and tonic contractions, NS-1619 (30 μM) was introduced into the bath solution. In the third set of experiments, nerve-evoked contractions were induced by electrical field stimulation (EFS) using a pair of platinum electrodes mounted in the tissue bath in parallel to the DSM strip. EFS pulses were generated using a PHM-1521 stimulator (MED Associates, Georgia, VT), and the pulse parameters were as follows: 0.75 ms pulse width, 20 V pulse amplitude, 3 s stimulus duration, and the polarity was reversed for alternating pulses. For EFS studies, after the equilibration period, DSM...
strips were subjected to either continuous repetitive stimulation of a frequency of 20 Hz at 1-min intervals or to increasing frequencies from 0.5–50 Hz at 3-min intervals. The contractions were recorded using a Myomed myograph system (MED Associates).

Solutions and Drugs. The Ca^{2+}-free DS had the following composition (in mM): 80 monosodium glutamate; 55 NaCl; 6 KCl; 10 glucose; 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES); and 2 MgCl2. pH 7.3, adjusted with NaOH. The Ca^{2+}-containing PSS was prepared daily and contained (in mM): 119 NaCl, 4.7 KCl, 24 NaHCO3, 1.2 KH2PO4, 2.5 CaCl2, 1.2 MgSO4, and 11 glucose and aerated with 95% O2/5% CO2 to obtain pH 7.4. The extracellular (bath) solution used in the patch-clamp experiments contained (in mM): 134 NaCl; 6 KCl; 1 MgCl2; 2 CaCl2; 10 glucose; and 10 HEPES, pH adjusted to 7.4 with NaOH. The patch-clamp pipette solution contained (in mM): 110 potassium aspartate; 30 KCl; 10 NaCl; 1 MgCl2; 10 HEPES; and 0.05 EGTA, pH adjusted to 7.2 with NaOH and supplemented with freshly dissolved (every 1–2 h) 200 μg/ml amphotericin-B. IBTX was purchased from Alomone Labs (Jerusalem, Israel). BSA and amphotericin-B were obtained from Thermo Fisher Scientific (Pittsburgh, PA). All other drugs were obtained from Sigma-Aldrich (St. Louis, MO). NS-1619 was dissolved freshly in DMSO at 10 mM stock solution and a final concentration of 30 μM was used for all experiments. Carbachol, IBTX, and tetrodotoxin (TTX) were dissolved in double-distilled water. The DMSO concentration in the bath solution did not exceed 0.1%.

Data analysis and statistics. The parameters of DSM phasic and tonic contractions were analyzed using MiniAnalysis software version 6.0.7 (Synaptosoft, Decatur, GA). GraphPad Prism 4.03 software (GraphPad Software, San Diego, CA) was used for further statistical analysis, and CorelDraw Graphic Suite X3 software (Corel, Ottawa, Canada) was used for data illustration. Data for DSM contractions were normalized to the control (taken to be 100%) and were expressed as percentages. For the EFS-induced contractions, the contraction amplitude at an EFS frequency of 50 Hz under control conditions was taken to be 100%. Net muscle force (muscle force integral) was determined by integrating the area under the curve of the phasic contractions. Muscle tone was determined by measuring the phasic contraction baseline curve. Clampfit version 10.2 was used to analyze the patch-clamp data. Results were summarized as means ± SE for the n (the number of DSM strips or cells) isolated from N (the number of patients). Data were compared by two-way ANOVA followed by Bonferroni posttest or two-tailed paired Student’s t-test, and a P value <0.05 was considered statistically significant.

RESULTS

Pharmacological activation of BK channels with NS-1619 increased the voltage-step induced whole cell outward K+ current in freshly isolated human DSM cells. Our previous study showed that IBTX suppressed the majority of the whole cell outward K+ current in human DSM cells (20), indicating that the BK channels are the major determinant of human DSM cell excitability under physiological conditions. Here, we tested whether pharmacological activation of the BK channel with NS-1619 affects the whole cell outward K+ current in human DSM cells. The average cell capacitance was 26.2 ± 1.5 pF (n = 72, N = 28). Brief square voltage step protocol (MATERIALS AND METHODS) was applied, and the cells responded with gradual increases in the outward currents with each depolarizing voltage-step (Fig. 1A). NS-1619 (30 μM) significantly increased the whole cell outward K+ current (n = 10, N = 8; P < 0.05; Fig. 1A). To determine if NS-1619-induced activation of the BK currents was due to BK channel activation, we performed the same experiments on DSM cells pretreated with the BK channel blocker IBTX for at least 10 min prior to NS-1619 application (Fig. 1B). The results show that NS-1619 did not change the whole cell outward K+ currents in the presence of 200 nM IBTX (n = 6, N = 3; P > 0.05; Fig. 1B). These data indicate that NS-1619 increases the whole cell outward K+ current in human DSM solely due to activation of the BK channel.

Pharmacological activation of the BK channels with NS-1619 hyperpolarized the resting membrane potential in freshly isolated human DSM cells. In this experimental series, the effect of NS-1619 on human DSM cell resting membrane potential was evaluated. The resting membrane potential recorded in current-clamp mode under control conditions was 38.1 ± 4.3 mV (n = 8, N = 7). Activation of the BK channels with 30 μM NS-1619 hyperpolarized the DSM cell resting membrane potential to 41.0 ± 4.6 mV (n = 8, N = 7, P < 0.05). This hyperpolarizing effect of NS-1619 was reversed by 200 nM IBTX to 39.2 ± 4.2 mV, which was not significantly different from the control value (n = 8, N = 7; P > 0.05; Fig. 2A). Moreover, 30 μM NS-1619 did not change the DSM resting membrane potential when BK channels were inhibited with 200 nM IBTX (n = 6, N = 5; P > 0.05; Fig. 2B). These findings support the concept that pharmacological activation of the BK channels can hyperpolarize the resting membrane potential in human DSM cells.

BK channel activation with NS-1619 decreased intracellular Ca2+ levels in freshly isolated human DSM cells. To study the effects of BK channel activation with NS-1619 on intracellular Ca2+ levels, real-time live-cell Ca2+ imaging was carried out using ratiometric dye fura 2-AM. The control resting Ca2+ level was measured for at least 10 min, then 30 μM NS-1619 was added into the bath solution, and recorded for at least 30 min. As shown in Fig. 3, the intracellular Ca2+ level was significantly decreased following NS-1619 application. Under control conditions, the fluorescent ratio (340/380 nm) was 0.90 ± 0.06, and in the presence of NS-1619 the fluorescent ratio (340/380 nm) decreased to 0.78 ± 0.08 (n = 11, N = 5; P < 0.01). Thus, activation of BK channels with NS-1619 (30 μM) decreased intracellular Ca2+ levels in freshly isolated human DSM cells.

BK channel activation with NS-1619 reduced spontaneous phasic and tonic contractions in human DSM isolated strips. In this experimental series, the functional effect of the BK channel pharmacological activation on DSM contractility was investigated. Here, we addressed how the pharmacological activation of BK channel functionally affects DSM contractility utilizing human DSM isolated strips. This experimental series was carried out in the presence of the neuronal Na+ channel blocker TTX (1 μM) to block any potential neurotransmitter release from the autonomic nerves located in DSM strips. NS-1619 (30 μM) significantly decreased the DSM spontaneous phasic contraction amplitude by 79.5 ± 2.4%, muscle force integral by 71.8 ± 4.1%, frequency by 67.4 ± 5.1%, duration by 74.7 ± 6.5%, and tone by 70.1 ± 7.3% (n = 9, N = 9; P < 0.01; Fig. 4, A and B). The inhibitory effect of NS-1619 on spontaneous contraction was completely reversed by the subsequent application of IBTX (200 nM), and all contraction parameters further increased above the control levels. This suggests that the inhibitory effect of NS-1619 on DSM spontaneous phasic contractions is mediated by the BK channel activation (Fig. 4B). To confirm the BK channel selectivity of NS-1619, the DSM strips were preincubated with...
IBTX (200 nM). As shown in Fig. 4, C and D, IBTX elicited a significant increase in spontaneous phasic contraction amplitude, muscle force integral, and muscle tone \((n = 6, N = 5; P < 0.05)\), consistent with our recent studies (20). In the continuous presence of 200 nM IBTX, NS-1619 (30 μM) had no significant effect on the DSM contraction parameters (Fig. 4). These results provide strong evidence that NS-1619 relaxes DSM by selectively activating BK channels.

**BK channel activation with NS-1619 decreases pharmacologically induced DSM contractions.** We examined the effect of BK channel opener NS-1619 on carbachol or KCl-precontracted DSM strips. These experiments were also performed in the presence of 1 μM TTX. In the first experimental series, DSM strips were stimulated with 0.1 μM carbachol, a cholinergic agonist. Exposure to NS-1619 (30 μM) significantly decreased the phasic contraction amplitude by 77.2 ± 3.1%, muscle force integral by 74.4 ± 7.4%, frequency by 64.9 ± 2.9%, duration by 50.9 ± 9.2%, and tone by 74.2 ± 4.3% of carbachol-induced contractions \((n = 8, N = 7; P < 0.01; \text{Fig. } 5)\).

Next, we used 20 mM KCl to induce sustained membrane depolarization and increase the phasic and tonic contractions in human DSM isolated strips. As illustrated in Fig. 6, exposure to NS-1619 (30 μM) caused a significant inhibition of the 20 mM KCl-induced phasic contraction amplitude by 64.5 ± 2.1%, muscle force integral by 59.5 ± 6.3%, frequency by 41.0 ± 5.5%, and muscle tone by 55.0 ± 4.8% \((n = 8, N = 6; P < 0.01; \text{Fig. } 6B)\). To determine whether the inhibitory effects of NS-1619 were mediated through BK channel activation, an additional series of experiments was carried out in the presence of higher extracellular K⁺ (60 mM). An increased extracellular KCl concentration reduces further the electrochemical driving force for K⁺ and hence should inhibit the relaxant effects of K⁺ channel activators. KCl at 60 mM induced a sustained tonic contraction (Fig. 6C). As shown in Fig. 6D, 60 mM KCl-induced DSM tone was relaxed to a smaller extent by NS-1619 in contrast to the relaxation of the DSM strips precontracted by 20 mM KCl. NS-1619 inhibited DSM tone by 12.4 ± 10.5% in 60 mM K⁺ solution \((n = 5, N = 5; P > 0.05)\), whereas the DSM tone was inhibited by 55.0 ± 4.8% in 20 mM K⁺ \((n = 8, N = 6; P < 0.01)\). These results further support the concept that pharmacological activation of BK channels with NS-1619 relaxes the human DSM depending on the K⁺ driving force.

**BK channel activation with NS-1619 reduces the nerve-evoked contractions in human DSM isolated strips.** Activation of parasympathetic nerves initiates and maintains DSM contractions during voiding. Hence, we investigated whether BK channel activation with NS-1619 regulated neurogenic contractions of DSM strips induced by 20 Hz EFS. Figure 7 shows that NS-1619 (30 μM) had significant inhibitory effects on all parameters of the nerve-evoked contractions. NS-1619 inhibited the nerve-evoked contraction amplitude, muscle force integral, duration, and tone by 72.4 ± 5.8%, 47.6 ± 3.2%, 51.8 ± 2.2%, and 38.9 ± 10.7% respectively \((n = 10, N = 8; P < 0.01)\).
Following the washout of NS-1619 with fresh PSS, a partial but significant recovery of the DSM contractions was observed. Washout of NS-1619 increased the EFS-induced contraction amplitude to 62.1 ± 7.7%, muscle integral force to 82.8 ± 5.8%, and duration to 78.2 ± 11.7% (n = 6, N = 6; P < 0.05, Fig. 7).

In another group of experiments, increasing EFS frequencies (0.5–50 Hz) were applied to DSM isolated strips. The amplitude of the EFS-induced contraction at a given stimulation frequency (0.5–50 Hz) was measured and frequency-response curves were generated in the absence, presence, and following washout of NS-1619 (30 μM). The frequency-response curves presented in Fig. 8B demonstrate that NS-1619 significantly decreased the contraction amplitude at 7.5–50 Hz EFS frequencies. Addition of NS-1619 (30 μM) to the bath solution inhibited the EFS-induced contraction amplitude by 57.8 ± 5.9% at 50 Hz (n = 11, N = 10; P < 0.01), when compared with control responses. Washout of NS-1619 from the tissue bath with fresh PSS led to a significant recovery in EFS-induced contraction amplitude that increased to 75.0 ± 4.9% at 50 Hz (n = 8, N = 7; P < 0.05). This experimental series further indicates that BK channel pharmacological opening can reduce nerve-evoked contractions in human DSM.

**DISCUSSION**

Recently our group provided the first systematic identification and characterization of the BK channel in native human DSM and showed that, under physiological conditions, blocking the BK channels with IBTX inhibits the whole cell outward K⁺ current, depolarizes the cell resting membrane potential, and increases contractility of isolated human DSM strips (20). Here we extended our studies to elucidate the effect of BK channel pharmacological activation with NS-1619 on human DSM excitability and contractility under physiological conditions. From our data we have three important findings. First, we demonstrated that activation of the BK channels with NS-1619 decreases cell excitability and causes membrane potential hyperpolarization in freshly isolated human DSM cells. Second, NS-1619 reduces intracellular Ca²⁺ levels in isolated human DSM cells. Third, BK channel activation with NS-1619 decreases spontaneous, pharmacologically induced, and nerve-evoked contractions in human DSM isolated strips.

The patch-clamp experiments showed that the BK channel activator NS-1619 (30 μM) significantly increased the whole cell K⁺ current (Fig. 1). This stimulatory effect was completely blocked by IBTX, suggesting that the increase in the whole cell K⁺ current is mediated solely by the activation of the BK channels. In guinea pig, rat, and mouse DSM, the...
BK channels contribute to the repolarization phase of the action potential and to the setting of the resting membrane potential (11, 12, 15, 21). Our recent study showed that the BK channels control the resting membrane potential in human DSM cells as well (20). Our current-clamp data showed that NS-1619 significantly hyperpolarized the resting membrane potential in human DSM cells, and the effect was inhibited by IBTX (200 nM) (Fig. 2). Collectively, these findings support the hypothesis that pharmacological activation of the BK channels can hyperpolarize the resting membrane potential in human DSM cells.

Furthermore, we demonstrated that BK channel activation with NS-1619 significantly decreases intracellular Ca\(^{2+}\) levels in freshly isolated human DSM cells (Fig. 3). This decrease of intracellular Ca\(^{2+}\) is caused by activation of the BK channel, membrane hyperpolarization, and subsequent inhibition of the L-type voltage-gated Ca\(^{2+}\) channels. These findings are also supported by earlier studies showing that NS-1619 decreases rat and guinea pig DSM contractility by activation of the BK channel and inhibition of L-type voltage-gated Ca\(^{2+}\) channels (36, 38).

Human DSM exhibits two types of contractions: spontaneous contractions and nerve-evoked contractions. Our results demonstrated that NS-1619 significantly inhibits the spontaneous phasic contraction amplitude, muscle force integral, frequency, duration, and muscle tone (Fig. 4). A major advantage of our work compared with those conducted previously on human DSM is that herein we evaluated the effects of BK channel pharmacological activation on human DSM strips exhibiting spontaneous phasic contractions. The advantage of using these spontaneously contracting DSM strips is their direct physiological relevance compared with those in which contractions have been artificially induced following depolarization with KCl (31). Furthermore, our study showed that IBTX completely reversed the inhibitory effects of NS-1619 on DSM contractility (Fig. 4). This indicates that NS-1619 inhibitory effects are mediated by the BK channels and that pharmacological activation of BK channel by selective openers can reduce the myogenic activity of DSM (31, 38).

During the normal voiding cycle, neuronal release of acetylcholine activates muscarinic receptors in DSM and evokes phasic contractions (1). To study the effect of BK channel activation on cholinergic DSM contractions, we stimulated...
muscarinic receptors with carbachol, a chemically stable analog of acetylcholine that is resistant to acetylcholinesterase. NS-1619 significantly inhibited carbachol-induced phasic contraction amplitude, muscle force integral, frequency, duration, and tone of human DSM strips (Fig. 5). Our data on human DSM are consistent with the earlier findings that NS-1619 causes a decrease in the carbachol-induced contractions in rat DSM (30, 38).

To further investigate the mechanism of BK channel activation in decreasing DSM contractility, we exposed DSM isolated strips under conditions of moderate (20 mM) to high (60 mM) extracellular K⁺. A major characteristic of K⁺ channel openers is that they can still inhibit the contractions induced by lower concentrations (20 mM) of KCl, whereas they are mostly ineffective in reducing contractions induced by higher concentrations (60 mM) of KCl. It has been demonstrated that in rat DSM, activation of the BK channel with NS-1619 causes a decrease in contractions at a concentration of 20 mM KCl (25, 38). Our results in humans are consistent with these findings and indicate that NS-1619 decreases amplitude, force, frequency, and tone of 20 mM KCl-induced contraction (Fig. 6). A higher concentration of KCl (60 mM) was used to provide evidence that the inhibitory effect of NS-1619 is mediated through K⁺ channel activation. Raising the extracellular K⁺ concentration changes the equilibrium potential for K⁺ toward a more depolarized value and reduces the relaxant effects of K⁺ channel activators (32, 38). We demonstrated that the effect of NS-1619 was significantly weaker in 60 mM KCl compared with the effect on 20 mM KCl-induced contractions (Fig. 6). These findings strongly suggest that activation of BK channels with NS-1619 causes relaxation in human DSM, which is dependent on the K⁺ gradient.

Nerve stimulation plays an important role in the micturition process during which acetylcholine and ATP are released from parasympathetic nerve terminals and act on DSM muscarinic and purinergic receptors to generate DSM contractions (1, 13).
Pharmacological inhibition of the BK channels as well as BK channel genetic deletion significantly increases nerve-evoked contractions in DSM (16, 20, 28, 42). Our results showed that pharmacological activation of the BK channel significantly decreased the amplitude of nerve-evoked contractions in human DSM strips over a wide range of stimulation frequencies (Figs. 7 and 8).

Injection of BK channel naked DNA (hSlo cDNA) has been demonstrated to ameliorate DSM hyperactivity (7). To date, a clinical phase 1 safety trial of gene therapy using hSlo DNA has been completed for humans with erectile dysfunction, and a future clinical trial is planned for OAB treatment (26, 27). Also, targeted deletion of the BK channel regulatory β1-subunit or pore-forming α-subunit resulted in increased DSM activity in mouse models (2, 28, 34). These studies demonstrate a prominent link between BK channels and DO. Herein, our data on human DSM suggest that BK channel activation may represent a novel approach for OAB treatment. Because the BK channels appear to be restricted to DSM cells with no detectable expression in nerves or other components of the detrusor (42), the effects of NS-1619 seen in this study are due to a direct effect of this compound on the DSM BK channels. Prior clinical studies investigating nonselective openers of the ATP-sensitive K+ channels for the treatment of bladder dysfunction have demonstrated considerable effects on blood pressure at doses that have negligible effects on bladder tissue (14, 22). However, such studies have not utilized BK channel-specific agents. Therefore, further exploration and clinical investigation of specific BK channel openers are warranted as they represent a potentially promising and novel therapeutic avenue for treating at least some types of bladder dysfunction in humans. Indeed, there are currently ongoing efforts by both the pharmaceutical industry and academic institutions to develop new class of more potent and selective BK channel openers (29).

In animal models, the BK channel is a key element underlying the β-adrenergic relaxation of DSM (2, 21, 35). In rat DSM, the β3-adrenoceptor subtype and the BK channel are functionally coupled at the RyRs level to mediate DSM relaxation (21). It has been suggested that β-adrenoceptor agonists can modulate K+ conductance in human DSM (40). However, future studies are needed to define the role of the BK channel in β-adrenergic relaxation of human DSM and its clinical implications. Our data reported here provide a foundation for future studies to define the role of the BK channels in human DSM and its clinical implications.

In conclusion, our results demonstrate that BK channel pharmacological activation with NS-1619 significantly increases the whole cell BK current, causes membrane potential hyperpolarization, decreases the level of global intracellular Ca2+, and inhibits myogenic and nerve-evoked contractions in native human DSM. Our data support the concept that pharmacological activation of the BK channel may represent an attractive therapeutic strategy to control DO associated with OAB syndrome.

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