BK Channel Activation by NS11021 Decreases Excitability and Contractility of Urinary Bladder Smooth Muscle

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Running Title: BK channel activation reduces phasic contractions

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Abstract

Large-conductance Ca$^{2+}$-activated potassium (BK) channels play an important role in regulating the function and activity of urinary bladder smooth muscle (UBSM), and the loss of BK channel function has been shown to increase UBSM excitability and contractility. However, it is not known whether activation of BK channels has the converse effect of reducing UBSM excitability and contractility. Here, we have sought to investigate this possibility by using the novel BK channel opener NS11021. NS11021 (3 µM) caused a ~3-fold increase in both single BK channel open probability ($P_o$) and whole cell BK channel currents. The frequency of spontaneous action potentials in UBSM strips was reduced by NS11021 from a control value of 20.9 ± 5.9 / min to 10.9 ± 3.7 / min. NS11021 also reduced the force of UBSM spontaneous phasic contractions (SPCs) by ~50%, and this force reduction was blocked by pre-treatment with the BK channel blocker iberiotoxin. NS11021 (3 µM) had no effect on contractions evoked by nerve-stimulation. These findings indicate that activating BK channels reduces the force of UBSM spontaneous phasic contractions, principally through decreasing the frequency of spontaneous action potentials.

Keywords: Detrusor contractility; urinary bladder smooth muscle; BK; NS11021; BK channel opener
Introduction

Large-conductance Ca\(^{2+}\)-activated potassium (BK) channels are found in a broad range of excitable and non-excitable tissues. In smooth muscle, BK channels play a central role in modulating excitability and contractility (1, 4, 18, 21, 28). Because of their ability to respond to membrane potential depolarization and increases in intracellular Ca\(^{2+}\) levels, BK channels provide a feedback mechanism that limits contractility of smooth muscle, such as urinary bladder smooth muscle (UBSM) (21, 22, 48). Block of BK channels in UBSM increases excitability (18), spontaneous phasic contractions (SPCs) (6, 22, 37), and nerve-induced contractions (21, 44, 48). Mice that lack the gene encoding the pore-forming subunit of the BK channel (\(KCNMA1\); Skele-/- mice) display significant disruptions in normal urinary bladder function, including an elevation in urination frequency, increased intravesicular pressure, elevated pressure oscillations, and urinary leakage (33, 44). These observations suggest that alterations in normal BK channel activity may contribute to the symptoms that are associated with urinary bladder disorders (33, 44).

UBSM exhibits two principle modes of contractility: nerve-induced contractions, triggered by the release of acetylcholine (ACh) and ATP from parasympathetic nerve fibers (1, 16, 17), and phasic contractions (4, 13-15). Nerve-induced contractions of the detrusor are triggered by binding of ACh and ATP to muscarinic acetylcholine receptors (mAChR) and purinergic receptors (P2X1R), respectively (1, 19, 31, 45, 46). Although the precise mechanisms underlying UBSM contractility are still not completely defined, it is currently believed that the activation of these receptors initiates contraction, in part, by
depolarizing the cell membrane, which, in turn, contributes to the opening of L-type voltage-dependent calcium channels (L-VDCCs), whose activity is essential to urinary bladder contractility (10, 21, 47).

Phasic contractions are initiated by spontaneous opening of L-VDCCs which causes myogenic action potentials (4, 13-15). The central role of L-VDCCs in mediating UBSM spontaneous phasic activity is highlighted by the fact that inhibition of L-VDCCs by dihydropyridine compounds, such as nifedipine, abolishes both spontaneous action potentials and SPCs in UBSM (6, 14, 15, 18, 22). The physiological role of SPCs in normal healthy bladder is still not fully understood, but in disease states, disruptions in normal SPC activity may give rise to some of the symptoms associated with bladder instability and overactivity (34).

Given the prominent role that BK channels play in modulating urinary bladder excitability and contractility, it is perhaps not surprising that the development of drugs that enhance the activity of these channels has been a focus of efforts to address urinary bladder dysfunction (7, 8, 12, 23, 35, 40). However, BK channel openers developed to date lack sufficient potency and specificity (24, 35, 36). Recently, a novel BK channel opener, NS11021 (1-(3,5-bis-trifluoromethyl-phenyl)-3-[4-bromo-2-(1H-tetrazol-5-yl)-phenyl]-thiourea), with an improved selectivity profile has been described (2). NS11021 is approximately 10-fold more potent than the widely used NS1619 and exhibits significant activation of BK channels at concentrations above 1 µM. NS11021 does not affect Kv, Na⁺ or Ca²⁺ channel currents at concentrations below 10 µM.

While it has been established that BK channel block increases UBSM contractility (21, 22) and excitability (18), it is unclear whether activation of BK channels has the
converse effect of reducing detrusor excitability and contractility. Thus, the goal of the present study was to determine if activation of BK channels using the novel BK channel opener NS11021 could decrease UBSM contractility. We found that NS11021 significantly decreased the force of spontaneous phasic contractions. This response appears to be largely attributable to an activation of BK channels. Furthermore, NS11021 significantly decreased the frequency of spontaneous action potentials that trigger phasic contractions. Interestingly, NS11021 had no effect on nerve-evoked contractions at the same concentration (3 µM) that significantly reduced SPC force.

Methods

NS11201

NS11021 was synthesized and kindly provided by NeuroSearch A/S (Ballerup, Denmark). Concentrations from 100 nM to 30 µM (100 nM, 300 nM, 1 µM, 3 µM, 10 µM, and 30 µM) were used on spontaneous phasic contractions to generate concentration-response curves. A concentration of 3 µM NS11021 was used for all other experiments because this concentration had been shown to cause a significant activation of BK channels and did not affect a number of other types of ion channels (2).

Tissue preparation

Juvenile male guinea pigs (250-400 g) were euthanized using isoflurane, and then exsanguinated according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Vermont. The urinary bladders were removed and placed
into ice-cold dissection solution (DS; 80 mM monosodium glutamate, 55 mM NaCl, 6 mM KCl, 10 mM glucose, 10 mM Hepes, and 2 mM MgCl₂, adjusted to pH 7.3). Each bladder was sliced open and rinsed free of urine, and the urothelial layer, including lamina propria and the majority of submucosa and muscularis mucosae, was removed by careful dissection with scissors.

**Contractility studies**

Force generation by UBSM strips was measured as previously described (21, 48). Briefly, detrusor strips (~ 2 mm x 5 mm) were suspended from force transducers in temperature-controlled (37°C) jacketed water baths containing physiological saline solution (PSS; 119 mM NaCl, 4.7 mM KCl, 24 mM NaHCO₃, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 1.2 mM MgSO₄, and 11 mM glucose) and force was measured and recorded using a MyoMed myograph system (MED Associates Inc., Georgia, VT, USA). Detrusor strips were pre-stretched to 10 mN of tension. For electrical field stimulation (EFS) studies, after the pre-stretch and relaxation period, detrusor strips were subjected to repetitive stimulation (0.2 ms pulse width, 20 Volts, 20 Hz frequency, 1 sec duration) at 2-min intervals. The BK channel opener, NS11021 (NeuroSearch, A/S, Ballerup, Denmark), was prepared in DMSO and added directly to the bath solutions. Time controls, consisting of an equivalent volume of DMSO lacking NS11021, were run in parallel. Where applicable, iberiotoxin (IBTX; 100 nM; Peptides International, Japan) was added directly to the bath approximately 30 min prior to the addition of NS11021. EFS-induced and spontaneous phasic contractions were analyzed using the MiniAnalysis software package (Synaptosoft, Fort Lee, NJ, USA).

**Electrophysiology**
Urinary bladder smooth muscle cells were isolated by placing sections of guinea pig detrusor (~ 2 mm x 2 mm) into a 1 mg/ml papain solution (Worthington Biochemical Corp., Freehold, NJ, USA), prepared in DS containing 1 mg/ml dithioerythritol, and incubating for 20 min at 37°C. Following a brief rinse in ice-cold DS, tissue sections were transferred to a vial containing 1 mg/ml collagenase (Type II) and 100 µM CaCl₂ and incubated for 6 min at 37°C. After a series of rinses in ice-cold DS, tissue sections were gently trituated using a fire-polished Pasteur pipette to release smooth muscle cells.

Isolated cells were allowed to adhere to a glass-plated chamber in DS for 20 min, and then rinsed with a bath solution consisting of 134 mM NaCl, 6 mM KCl, 10 mM glucose, 1 mM MgCl₂, 2 mM CaCl₂, and 10 mM Hepes, adjusted to pH 7.3. Whole-cell currents were recorded using the amphotericin B-perforated patch clamp technique. The pipettes (tip resistances of ~ 4-5 MΩ) contained a solution consisting of 110 mM potassium aspartate, 30 mM KCl, 10 mM NaCl, 1 mM MgCl₂, 0.05 mM EGTA, 200 µg/ml amphotericin B, and 10 mM Hepes, adjusted to pH 7.3. All reagents were from Sigma (Milwaukee, WI, USA) unless specifically indicated otherwise. Whole-cell currents were elicited by depolarizing steps (250 ms each) from an initial holding potential of -70 mV up to +50 mV in 10 mV increments. To block BK currents, IBTX was added to a final concentration of 100 nM. Currents were normalized to cell capacitance. All patch-clamp experiments were performed at room temperature.

Single-channel recordings of BK currents were obtained from excised inside-out patches as detailed previously (29). Guinea pig detrusor myocytes were obtained as detailed above. The bath and pipette solution consisted of 140 mM KCl, 1.08 mM MgCl₂, 5 mM EGTA, and 3.16 mM CaCl₂, adjusted to pH 7.2 with NaOH. The free Ca²⁺
concentration was calculated to be 300 nM (WEBMAXC Standard, www.stanford.edu/~cpatton/maxc.html, Chris Patton). The fire-polished pipettes used in the experiments had tip resistances of ~ 8-10 MΩ. Single-channel currents were recorded for 1-3 min at membrane potentials indicated in the text. The number of channels (N) present in each patch was determined at +60 mV. Data were analyzed using pCLAMP software (MDS Analytical Technologies, Sunnyvale, CA, USA). Channel open probability ($P_o$) was determined by dividing the open probability of each patch ($NP_o$) by the number of ion channels present in the patch (N).

**Intracellular recordings**

Small detrusor strips, containing only a few muscle bundles, were carefully dissected from the serosal surface of the bladder and pinned onto the Sylgard bottom of a recording chamber. The tissue was superfused with warm PSS (35 - 37°C, 3 ml/min) that was bubbled with 95% O₂ and 5% CO₂ to maintain physiological pH. After an equilibration period of about 20 min, individual smooth muscle cells were impaled with sharp microelectrodes filled with 0.5 M KCl. The electrode resistance was ~300 MΩ (294 ± 38 MΩ). Membrane potential and action potentials were recorded at a sampling rate of 2 kHz and the signal was amplified with an Axoclamp 2A amplifier (MDS Analytical Technologies, Sunnyvale, CA, USA). pCLAMP (MDS Analytical Technologies, Sunnyvale, CA, USA) and MiniAnalysis (Synaptosoft, Fort Lee, NJ, USA) software were used to record and analyze the data.

**Statistics**

Graphs and statistical analyses were performed using GraphPad Software (San Diego, CA, USA) and SigmaStat, Version 2.0 (SYSTAT Software Inc., Chicago, IL,
USA). Where applicable, comparisons of the NS11021-treated tissues were made against control tissue exposed to an equivalent volume of vehicle (DMSO). Data are reported as mean ± standard error of the mean (SEM) and \( n \) represents the number of tissue strips (for intracellular recordings and contractility experiments) or isolated myocytes (for the electrophysiology experiments). Statistical comparisons were made using the Student's \( t \)-test with \( P < 0.05 \) being considered significant.

**Results**

*NS11021 increases BK channel open probability*

It has recently been reported that the novel BK channel opener NS11021 (3 \( \mu \)M) was able to significantly increase (~ 2-fold) BK currents in heterologous expression systems (*Xenopus laevis* oocytes and HEK293 cells) (2). This activation was due to an increase in channel open probability and was independent of the level of intracellular calcium (2). To determine if NS11021 has a similar effect on BK channels in native tissues, the effect of 3 \( \mu \)M NS11021 on single BK channels in inside-out patches excised from freshly isolated UBSM cells was tested. A NS11021 concentration of 3 \( \mu \)M was chosen because it had been shown to have a significant effect on BK channels in expression systems (2), and would likely minimize non-specific effects. Representative recordings obtained in the absence and presence of 3 \( \mu \)M NS11021 are presented in Figure 1A. At +40 mV, 3 \( \mu \)M NS11021 significantly increased the single channel open probability (\( P_o \)) from an initial value of 0.15 ± 0.02 to 0.43 ± 0.05, a ~3-fold increase
(Figure 1B; $P < 0.05$, $n = 16$). However, NS11021 did not affect the single channel slope conductance (244 pS) (Figure 1C; $n = 5$). These results are consistent with those of Grunnet and colleagues (2) and indicate that in both native tissue and heterologous expression systems, NS11021 increases the open probability of BK channels without affecting single-channel conductance.

*NS11021 increases whole-cell BK currents in UBSM cells*

The increase in single channel open probability induced by NS11021 suggests that a similar increase in whole cell BK currents should be observed. Whole-cell currents were measured using the perforated patch configuration of the patch-clamp technique. Currents were elicited by applying voltage steps (250 ms each) from a holding potential of -70 mV up to +50 mV in 10 mV increments. Representative whole-cell current traces recorded before and after the application of 3 µM NS11021 are presented in Figure 2A, with the resultant NS11021-induced difference current (current in presence of NS11021 minus initial current) in the last panel. The averaged current-voltage (I-V) plots of the end-pulse currents in the absence and presence of 3 µM NS11021 are presented in Figure 2B, and the NS11021-induced difference current is presented in Figure 2C. In isolated UBSM cells, NS11021 (3 µM) increased outward currents at all membrane potentials positive to +20 mV ($P < 0.05$; $n = 22$). At +40 mV, the outward current was increased approximately 3-fold by NS11021, from $4.9 \pm 0.6$ pA/pF to $14.8 \pm 2.0$ pA/pF. The difference current (Figure 2C) clearly shows that a large, voltage-dependent outward current was induced by 3 µM NS11021. The effect of NS11021 appears to be largely reversible as washing the cells with fresh bath solution returned the voltage-dependent
current to the initial conditions, as shown in the voltage-ramp recording (from -100 mV to +100 mV) in Figure 2D (representative of 5 separate recordings).

To determine if the NS11021-induced increase in outward current was specifically due to activation of BK channels, the BK channel blocker iberiotoxin (IBTX, 100 nM) was applied to block BK channels prior to NS11021 treatment. Representative current recordings obtained before IBTX treatment (left panel), after IBTX treatment (right top panel), and after IBTX + 3 µM NS11021 (right bottom panel) are presented in Figure 2E. The averaged I-V curves for the end-pulse currents are presented in Figure 2F. Treatment with IBTX led to a decrease in outward current consistent with blockade of BK channels, and this decrease was significant at all positive membrane potentials (P < 0.05; n = 9). At +40 mV, the current density was reduced from an initial value of 7.5 ± 1.5 pA/pF to 2.5 ± 0.8 pA/pF by IBTX treatment. In these IBTX-treated cells, the addition of 3 µM NS11021 did not significantly increase the outward current at any membrane potential (P > 0.05; n = 9). These results indicate that NS11021 (3 µM) increases BK channel currents ~3-fold (at +40 mV), without affecting voltage-dependent K⁺ (KV) channels.

NS11021 decreases the frequency of UBSM spontaneous action potentials

BK channels play an important role in braking UBSM excitability and blocking BK channels increases UBSM action potential frequency (18). We next tested the hypothesis that activation of BK channels should have the opposite effect and lead to a decrease in spontaneous action potentials.
Action potentials (APs) in smooth muscle cells were measured in intact UBSM strips using sharp microelectrodes. Representative recordings of action potentials before and ~10 min after exposure to NS11021 (3 µM) are presented in Figure 3A. NS11021 reduced the frequency of spontaneous APs approximately by ~50%, from 20.9 ± 5.9 APs / min to 10.9 ± 3.7 APs / min (Figure 3B, P < 0.05, paired t-test, n = 9). NS11021 had no significant effect on the resting membrane potential (control = -46.1 ± 1.4 mV; 3 µM NS11021 = -46.5 ± 1.0 mV; P > 0.05; n = 12). Thus, it appears that, at a concentration of 3 µM, the principle effect of NS11021 was to reduce the frequency of spontaneous action potentials.

NS11021 decreases the force of spontaneous phasic contractions (SPCs) by activating BK channels

Spontaneous phasic contractions of the detrusor are driven by spontaneous action potentials (15). The upstroke of UBSM action potential is caused by calcium entry through voltage-dependent calcium channels (VDCCs), which is opposed by activation of BK channels (18). Blocking BK channels has been shown previously to increase SPC force in UBSM strips (14, 22, 27, 37). Given our observations that NS11021 decreases the frequency of spontaneous APs, NS11021 should have a similar effect on spontaneous phasic contractions.

NS11021 (3 µM) reduced the force of spontaneous phasic contractions by 52.7 ± 4.7 % (Figure 4A), a value similar to the reduction in action potential frequency. The BK channel blocker IBTX (100 nM) increased the force of SPCs but prevented the reduction in SPC force to NS11021 (3 µM) (Figure 4). NS11021 (from 100 nM to 30 µM) caused
a concentration-dependent reduction in SPC force, which was significant at concentrations of 1 \( \mu \text{M} \) and above (Figure 4B, \( P < 0.05; n = 6 \)). While IBTX completely blocked the effect of NS11021 from 100 nM to 3 \( \mu \text{M} \), IBTX only blocked about 50\% of the force reduction induced by 30 \( \mu \text{M} \) NS11021, indicating that, at high concentrations, a fraction of the effect of NS11021 is not mediated by BK channel activation.

\textit{NS11021 does not affect the force of nerve-induced contractions}

To void urine, parasympathetic nerves are activated to contract the detrusor (1). Therefore, the effects of NS11021 on nerve-evoked contractions of UBSM strips were also investigated. Electrical field stimulation (EFS) caused transient contractions of UBSM strips (representative myograph recording in Figure 5A) due to activation of UBSM purinergic and muscarinic receptors (19). As is evident from the representative recording (Figure 5A) and the summarized graph of the results (Figure 5B), NS11021 (3 \( \mu \text{M} \)) had no significant effect on nerve-evoked contractions (\( P > 0.05; n = 6 \)). NS11021 at 30 \( \mu \text{M} \) did cause an iberiotoxin-resistant reduction in force of spontaneous phasic contraction (Figure 4B). Consistent with this effect, NS11021 (30 \( \mu \text{M} \)) reduced the force of nerve-evoked contractions by 44\% \pm 4\% in mouse UBSM strips, and this action was not affected by iberiotoxin (\( P > 0.05, n = 14 \) (control) and 6 (IBTX)).
Discussion

In the present study, we sought to determine if BK channel activation could reduce UBSM excitability and contractility. We found that NS11021 (3 µM) increased the open probability of single BK channels, increased whole cell BK currents, decreased the frequency of spontaneous action potentials, and decreased the force of spontaneous phasic contractions.

*NS11021 activates BK channels in UBSM cells*

We found that exposure to 3 µM NS11021 increased whole cell BK currents ~3.7-fold at +40 mV in myocytes isolated from guinea pig urinary bladders. These results are consistent with a recently published electrophysiological characterization of NS11021 (2), in which it was reported that the compound was able to activate the pore-forming α-subunit of the BK channel expressed in heterologous expression systems (*Xenopus laevis* oocytes and HEK293 cells). It has also been reported that NS11021 (30 µM) did not affect Kv1.4, Kv1.5, Kv4.3, Kv7.1, Kv7.2/7.3 or inward-rectifying K+ (Kir) 2.2 channels expressed in oocytes, or voltage-dependent Na+ or Ca2+ currents in guinea pig myocytes (2). Thus, our observations on the effects of NS11021 on native UBSM currents and contractions are in accord with the selectivity data from cloned channels in expression systems.

Earlier reports have identified a number of both synthetic and naturally-occurring compounds that could activate BK currents, including NS1608 (25, 40, 42), NS004 (25, 26, 32, 49), NS1619 (11, 50, 51), as well as a number of other less well-characterized
compounds and their derivatives (3, 7, 8, 23, 38). However, to date, these synthetic BK channel openers have lacked selectivity, particularly on native tissue (35). For instance, NS1619 has been shown to block both Ca\(^{2+}\) currents and Kv currents over the same concentration range that activates BK channels (11, 39). Imaizumi and colleagues (50) have reported that the opening of BK channels triggered by NS1619 is at least partially due to direct actions of the drug on ryanodine receptors (RyRs) that trigger Ca\(^{2+}\) release from internal stores, an effect that could have potentially deleterious effects in other tissues, such as cardiac myocytes. Likewise, Malysz et al. (30) have shown that NS8, NS1619, and two other experimental BK channel openers reduced contractions induced by 80 mM K\(^+\), indicating that a significant component of the effect of these drugs was due to non-BK related mechanisms. Similarly, the BK channel opener NS004 was shown to strongly inhibit both Kv channels and ATP-sensitive K\(^+\) channels at concentrations that activated BK channels (49). In contrast, our results indicate that the BK channel opener NS11021 is effective and selective on intact tissue at concentrations less than 10 \(\mu\)M.

**NS11021 decreases the force of phasic contractions by activating BK channels**

In the current study, we have shown that activating BK channels using NS11021 led to a significant decrease (~50%) in the frequency of spontaneous action potentials, and corresponding decrease in force of phasic contractions. Interestingly, although 3 \(\mu\)M NS11021 was able to reduce UBSM SPC force significantly, it did not affect nerve-evoked contractions (20 Hz stimulation frequency). However, loss of BK channel function does cause a substantial increase of nerve-evoked contractions (4, 21, 33, 44, 48). Nerve-evoked contractions reflect the activation of UBSM purinergic and
muscarinic receptors, which engage different mechanisms to enhance excitability and contractility. It is conceivable that nerve-evoked increases in excitability and intracellular calcium cause a very substantial activation of BK channels, such that further activation of BK channels has little effect.

**BK channels as a therapeutic target in the treatment of bladder dysfunction**

Urinary bladder dysfunction is increasingly prevalent among the aging population, and overactive bladder symptoms are estimated to affect over 33 million adults in the United States alone (41). Currently, antimuscarinic drugs are used mostly to treat overactivity, but they are not very effective and have unwanted side effects such as dry mouth (20). Because muscarinic receptors are essential for micturition (19), therapeutics that do not interfere with muscarinic receptors would provide alternative treatment options, especially in patients suffering from detrusor hyperactivity with impaired contractility (43), a condition affecting many nursing home residents. We have previously reported that mice lacking the pore-forming α-subunit of the BK channel exhibit voiding symptoms and a cystometric profile typically associated with overactive detrusor (33, 44), indicating that diminished BK channel function may be associated with urinary bladder dysfunction. It has also been reported that increasing BK channel activity may have the converse effect: reversing the deleterious effects associated with overactive bladders. Christ and colleagues (9) have shown that, after 6 weeks of surgically-induced partial bladder outlet obstruction, rat urinary bladders exhibited a cystometric profile typified by the presence of spontaneous, non-voiding bladder contractions, a hallmark of overactive bladder. A BK channel gene therapy strategy involving direct injections of
Slo cDNA into the bladders of these obstructed rats significantly reduced spontaneous, non-voiding contractions. Thus, in vivo evidence suggests that increasing BK channel activity may have a potential therapeutic benefit in the treatment of urinary bladder dysfunction.

In this study we provide evidence that NS11021 reduces the force of spontaneous phasic contractions in UBSM without affecting nerve-evoked contractions. Since SPCs are a hallmark of overactive bladder and might contribute to frequency and urge incontinence (5), BK channel openers like NS11021 could provide a novel treatment for overactive bladder without inhibiting micturition.

Conclusions

We have found that the BK channel opener NS11021 was able to significantly increase BK currents in isolated UBSM cells, an action that manifested at the tissue level as a decrease in spontaneous action potentials and SPC force. These findings strongly suggest that, at low concentrations (100 nM to 3 µM), NS11021 selectively activates BK channels in UBSM which reduces the force of phasic contractions. The potent inhibition of UBSM phasic contractions by NS11021 at concentrations that did not affect nerve-evoked contractions indicates that this compound may provide a therapeutic approach to control overactive bladder without compromising voiding.

Perspectives and Significance

BK channels play an important role in regulation of UBSM contractility. However, most of our knowledge stems from studies where BK channel function was disabled by
inhibitors or gene deletion. The current study investigates the effects of BK channel activation in UBSM by using the novel and selective BK channel opener NS11021. We were able to show that NS11201 reduces the force of spontaneous phasic contractions through activation of BK channels without affecting nerve-evoked contractions. The lack of effect on nerve-evoked contractions was not predictable based on data from previous studies using BK channel inhibitors, but it is a desirable feature for treatment of overactivity, because micturition should not be compromised. Furthermore, NS11021 provides a valuable tool to investigate the role of BK channels in healthy animals and in disease models, such as partial bladder outlet obstruction.
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Disclosure

Dr. Søren-Peter Olesen is consultant for and owns stocks in NeuroSearch. Dr. Mark T. Nelson is consultant for NeuroSearch.
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Figure Legends

Figure 1. NS11021 increases BK channel open probability in excised, inside-out patches from UBSM cells. A: Representative single-channel recordings of BK currents obtained under control conditions and after exposure to 3 µM NS11021. Currents were recorded in symmetrical 140 mM K⁺ and a free Ca²⁺ level of 300 nM. B: Mean BK channel open probability at +40 mV before (control) and after exposure to 3 µM NS11021. *Statistically significant difference; t-test; P < 0.05, n = 16. Data are expressed as mean ± SEM. C: Unitary current amplitudes, recorded under control conditions and after exposure to 3 µM NS11021, plotted as a function of membrane potential, n = 5. Because NS11021 had no effect on unitary current amplitude, the symbols for control and NS11021 coincide.

Figure 2. NS11021 increases BK currents in isolated UBSM myocytes. A: Representative patch-clamp recordings of voltage-dependent outward currents obtained before (control) and after exposure to 3 µM NS11021, with the resultant NS11021-induced current presented in the right panel. B: Summarized current-voltage (I-V) relationships for end-pulse currents obtained from isolated UBSM myocytes under control conditions and after exposure to 3 µM NS11021. *Statistically significant difference; t-test; P < 0.05, n = 22. Data are expressed as mean ± SEM. C: I-V relationship for the NS11021-induced difference current. D: Representative traces of UBSM outward currents induced by a voltage ramp from -100 mV to +100 mV over 250 ms. Currents were recorded under control conditions (black), after exposure to 3 µM
NS11021 (blue), and then after wash-out using the bath solution (red). Representative of five separate recordings. 

E: Representative patch-clamp recordings of voltage-induced outward currents under control conditions, after exposure to 100 nM IBTX, and after combined exposure to IBTX and NS11021. 

F: I-V plot of end-pulse currents of isolated UBSM myocytes obtained under control conditions, after exposure to IBTX, and after combined IBTX/NS11021 exposure. Because NS11021 had no effect in the presence of IBTX, the symbols for IBTX and IBTX + NS11021 coincide. *Statistically significant difference; t-test; \( P < 0.05 \), \( n = 9 \). Data are expressed as mean ± SEM.

**Figure 3.** NS11021 suppresses UBSM spontaneous action potentials. 

A: Representative microelectrode recordings of spontaneous action potentials obtained from intact detrusor muscle bundles under control conditions and after treatment with 3 μM NS11021. 

B: NS11021 (3 μM) significantly decreased the frequency of spontaneous action potentials after ~10 min of exposure. *Statistically significant difference; paired t-test; \( P < 0.05 \), \( n = 9 \). Data are expressed as mean ± SEM.

**Figure 4.** NS11021 decreases the force of spontaneous phasic contractions of guinea pig detrusor strips by activating BK channels. 

A: Representative myograph recordings obtained from guinea pig detrusor strips under control conditions (DMSO vehicle), after exposure to 3 μM NS11021, and 3 μM NS11021 applied after pre-treatment with IBTX to block BK channels. Application of IBTX greatly increases the force of spontaneous phasic contractions. Note the 10-fold difference in y-scales. 

B: NS11021 decreased the force of spontaneous phasic contractions (force integral) in a concentration-dependent
manner. At concentrations of NS11021 < 10µM, this effect was blocked by pre-treatment with IBTX. *Statistically significant difference; t-test; $P < 0.05$, $n = 6$. Data are expressed as mean ± SEM.

**Figure 5.** NS11021 has no effect on nerve-evoked contractions of guinea pig detrusor strips. *A:* Representative myograph recordings of nerve-evoked contractions obtained from guinea pig detrusor strips before and after the addition of 3µM NS11021. *B:* NS11021 (3 µM) had no effect on the peak amplitude or force integral; t-test, $P > 0.05$, $n = 6$. Data are expressed as mean ± SEM.
A

3 μM NS11021

10 min

5 mN

B

Amplitude (mN)

Force Integral (mN·s)

Control

NS11021

Amplitude

Force Integral