BK channel activation by NS11021 decreases excitability and contractility of urinary bladder smooth muscle

Jeffrey J. Layne, Bernhard Nausch, Søren-Peter Olesen, and Mark T. Nelson

1Department of Pharmacology, University of Vermont, Burlington, Vermont; and 2NeuroSearch, Ballerup, Denmark

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LARGE-CONDUCTANCE Ca2+-ACTIVATED potassium (BK) channels are found in a broad range of excitable and nonexcitable 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 Ca2+ levels, BK channels provide a feedback mechanism that limits contractility of smooth muscle, such as urinary bladder smooth muscle (UBSM; Refs. 21, 22, 48). Block of BK channels in UBSM increases excitability (18), spontaneous phasic contractions (SPCs; Refs. 6, 22, 37), and nerve-induced contractions (21, 44, 48). Mice that lack the gene encoding the pore-forming subunit of the BK channel (KCNA1; Slo−/− 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 ~10-fold more potent than the widely used NS1619 and exhibits significant activation of BK channels at concentrations >1 μM. NS11021 does not affect voltage-dependent K+ (Kv). Na+, or Ca2+ channel currents at concentrations <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 SPCs. 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 evoked by nerve stimulation. These findings indicate that activating BK channels decreases excitability and contractility of UBSM strips was reduced by NS11021 from a control value of 20.9 ± 5.9 to 10.9 ± 3.7% per minute. NS11021 also reduced the force of UBSM spontaneous phasic contractions by ∼50%, and this force reduction was blocked by pretreatment 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.

detrusor contractility; urinary bladder smooth muscle; calcium-activated potassium channel opener
contractions at the same concentration (3 μM) that significantly reduced SPC force.

METHODS

NS11201. NS11021 was synthesized and kindly provided by NeuroSearch. Concentrations from 100 nM to 30 μM (100 nM, 300 nM, 1 μM, 3 μM, 10 μM, and 30 μM) were used on SPCs 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. Male juvenile 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 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 and lamina propria were removed by careful dissection with scissors.

Contractility studies. Force generation by UBSM strips was measured as previously described (21, 48). Briefly, detrusor strips (~2 × 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, Georgia, VT). Detrusor strips were pretreated to 10 mM of tetrodotoxin (TTX). To study repetitive stimulation (0.2-ms pulse width, 20 V, 20-Hz frequency, and 1-s duration) at 2-min intervals. The BK channel opener NS11021 (NeuroSearch) 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, ibotenic acid (IBTX; 100 nM; Peptides International, Japan) was added directly to the bath ~50 min before the addition of NS11021. EFS-induced and SPCs were analyzed using the MiniAnalysis software package (Synaptosoft, Fort Lee, NJ).

Electrophysiology. UBSM cells were isolated by placing sections of guinea pig detrusor (~2 × 2 mm) into a 1 mg/ml papain solution (Worthington Biochemical, Freehold, NJ), prepared in DS containing 1 mg/ml dithioerythritol, and incubated for 20 min at 37°C. After 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 triturated 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 (St. Louis, MO) 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). 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% O₂-5% 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). pCLAMP (MDS Analytical Technologies) software and MiniAnalysis (Synaptosoft) software were used to record and analyze the data.

Statistics. Graphs and statistical analyses were performed using GraphPad Software (San Diego, CA) and SigmaStat, Version 2.0 (SYSTAT Software, Chicago, IL). 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 means ± SE, 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 μM) 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 μM NS11021 on single BK channels in inside-out patches excised from freshly isolated UBSM cells was tested. A NS11021 concentration of 3 μM was chosen because it had been shown to have a significant effect on BK channels in expression systems (2) and would likely minimize nonspecific effects. Representative recordings obtained in the absence and presence of 3 μM NS11021 are presented in Fig. 1A. At +40 mV, 3 μM NS11021 significantly increased the single channel open probability (P_o) from an initial value of 0.15 ± 0.02 to 0.43 ± 0.05, an approximately threefold increase (Fig. 1B; P < 0.05; n = 16). However, NS11021 did not affect the single channel slope conductance (244 pS; Fig. 1C; n = 5). These results are consistent with those of Bentzen et al. (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.
BK channel blocker IBTX (100 nM) was applied to block BK current. It was specifically due to activation of BK channels, the recordings obtained before IBTX treatment (left), after IBTX treatment (right top), and after IBTX + 3 μM NS11021 (right bottom) are presented in Fig. 2E. The averaged I-V curves for the end-pulse currents are presented in Fig. 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 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 approximately threefold (at +40 mV), without affecting 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 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 shown in Fig. 3A. NS11021 reduced the frequency of spontaneous action potentials approximately by ~50%, from 20.9 ± 5.9 to 10.9 ± 3.7 action potential/minute (Fig. 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 SPCs by activating BK channels. SPCs of the detrusor are driven by spontaneous action potentials (15). The upstroke of UBSM action potential is caused by calcium entry through VDCCs, which is opposed by activation of BK channels (18). Blocking BK channels has been previously shown to increase SPC force in UBSM strips (14, 22, 27, 37). Given our observations that NS11021 decreases the frequency of spontaneous action potentials, NS11021 should have a similar effect on SPCs.

NS11021 (3 μM) reduced the force of SPCs by 52.7 ± 4.7% (Fig. 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; Fig. 4). NS11021 (from 100 nM to 30 μM) caused a concentration-dependent reduction in SPC force, which was significant at concentrations of 1 μM and above (Fig. 4B; P < 0.05; n = 6). While IBTX completely blocked the effect of NS11021 from 100 nM to 3 μM, IBTX only blocked ~50% of the force reduction induced by 30 μM NS11021, indicating that, at high concentrations, a fraction of the effect of NS11021 is not mediated by BK channel activation.

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. EFS caused transient contractions of UBSM strips (representative myograph recording in Fig. 5A) due to activa-
tion of UBSM purinergic and muscarinic receptors (19). As is evident from the representative recording (Fig. 5A) and the summarized graph of the results (Fig. 5B), NS11021 (3 μM) had no significant effect on nerve-evoked contractions (P > 0.05; n = 6). NS11021 at 30 μM did cause an IBTX-resistant reduction in force of SPC (Fig. 4B). Consistent with this effect, NS11021 (30 μM) reduced the force of nerve-evoked contractions by 44 ± 4% in mouse UBSM strips, and this action was not affected by IBTX [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 SPCs.

**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 the voltage-dependent Na+ or Ca2+ currents in guinea pig myocytes (2). Thus our observations on the effects of NS11021 on native UBSM currents and contrac-
tions 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\(^{2+}\) and KV currents over the same concentration range that activates BK channels (11, 39). Yamamura et al. (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 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 <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 a 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 the only approved therapy for the treatment of overactive bladder, but they are not effective for all patients and can cause significant side effects. Therefore, there is a need for new therapeutic strategies for the treatment of bladder dysfunction. BK channel activators could be a potential therapeutic option for the treatment of bladder dysfunction, as they have been shown to reduce spontaneous phasic contractions and nerve-evoked contractions in vitro.

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**Fig. 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 \(\mu\)M NS11021, and 3 \(\mu\)M NS11021 applied after pretreatment 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 < 10 \(\mu\)M, this effect was blocked by pretreatment with IBTX. \(*P < 0.05, \text{statistically significant difference by } t\)-test \((n = 6)\). Data are means ± SE.

**Fig. 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 3 \(\mu\)M NS11021. B: NS11021 (3 \(\mu\)M) had no effect on the peak amplitude or force integral. \(P > 0.05\) by \(t\)-test \((n = 6)\). Data are means ± SE.
BK channels play an important role in the 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 NS11021 reduces the force of SPCs 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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DISCLOSURES

S.-P. Olsen is consultant for and owns stocks in NeuroSearch. M. T. Nelson is consultant for NeuroSearch.

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