A Positive Modulator of $K_{Ca2}$ and $K_{Ca3}$ Channels, 4,5-Dichloro-1,3-diethyl-1,3-dihydro-benzoimidazol-2-one (NS4591), Inhibits Bladder Afferent Firing in Vitro and Bladder Overactivity in Vivo


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ABSTRACT

Calcium-activated potassium channels are attractive targets for the development of therapeutics for overactive bladder. In the current study, we addressed the role of calcium-activated potassium channels of small (SK; $K_{Ca2}$) and intermediate (IK; $K_{Ca3}$) conductance in bladder function pharmacologically. We identified and characterized a novel positive modulator of SK/IK channels, 4,5-dichloro-1,3-diethyl-1,3-dihydro-benzoimidazol-2-one (NS4591). In whole-cell patch-clamp experiments, NS4591 doubled IK-mediated currents at a concentration of 45 ≤ 6 nM (n = 16), whereas 530 ≤ 100 nM (n = 7) was required for doubling of SK3-mediated currents. In acutely dissociated bladder primary afferent neurons, the presence of SK channels was verified using apamin and 1-ethyl-2-benzimidazol-2-one. In these neurons, NS4591 (10 μM) inhibited the number of action potentials generated by suprathreshold depolarizing pulses. NS4591 also reduced carbachol-induced twitchs in rat bladder detrusor rings in an apamin-sensitive manner. In vivo, NS4591 (30 mg/kg) inhibited bladder overactivity in rats and cats induced by capsaicin and acetic acid, respectively. In conclusion, the present study supports the involvement of calcium-activated potassium channels in bladder function and identifies NS4591 as a potent modulator of IK and SK channels that is effective in animal models of bladder overactivity.

Bladder overactivity affects a large fraction of the population, significantly impairing the quality of life of those individuals affected. Different mechanisms may be involved in the pathogenesis of overactive bladder, including myogenic factors, neurological factors, or a combination. The most widely used therapeutics for overactive bladder are the anti-muscarinics oxybutynin and tolterodine, which generally are widely used therapeutics for overactive bladder are the anti-muscarinics oxybutynin and tolterodine, which generally are widely used therapeutics for overactive bladder because several classes of potassium channels are expressed in the bladder as well as in nerve fibers controlling bladder function, and they have been shown to be involved in the regulation of nerve and muscle cell excitability in these tissues. Potassium channel openers may hyperpolarize smooth muscle cells and affect contractility and thus have a relaxing effect on the bladder wall. In addition, positive potassium channel modulators may have a silencing effect on overactive afferent nerve fibers from the bladder while interfering minimally with normal bladder function, either by inhibiting afferent firing or by acting on potassium channels expressed in the urothelium, thereby influencing the local release of various mediators.

**ABBREVIATIONS:** $K_{ATP}$, ATP-sensitive K⁺; SK, calcium-activated potassium channels of small conductance; IK, calcium-activated potassium channels of intermediate conductance; BK, calcium-activated potassium channels of large conductance; HEK, human embryonic kidney; DC-EBIO, 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzoimidazol-2-one; 1-EBIO, 1-ethyl-2-benzimidazolone; DMSO, dimethyl sulfoxide; OxyHb, oxyhemoglobin; h, human; DRG, dorsal root ganglion; IB4, isolecitin B4; Fast DiI, 1,1-dilinoleyl-3,3,3',3'-tetramethylinocarbocyanine perchlorate; AP, action potential; RMP, resting membrane potential; $R_{in}$, input resistance; ANOVA, analysis of variance; PE, polyethylene; CMG, continuous cystometry; MP, maximal micturition pressure; MV, micturition volume; ICI, intercontraction interval; BP, basal pressure; TP, threshold pressure; AHP, afterhyperpolarization; CTL, control; ZD6186, (S)-N-(4-benzoylphényl)-3,3,3-trifluoro-2-hydroxy-2-methy1propionamide; NS-8, 2-amino-3-cyano-5-(2-fluorophenyl)-4-methylpyrrole.
Calcium-activated potassium channels of small (SK; KCa2), intermediate (IK; KCa3), and large (BK; KCa1) conductance have also attracted considerable interest as putative targets for therapeutics to treat overactive bladder. Due to their calcium dependence, calcium-activated potassium channels may inhibit the firing of afferent pathways, bladder smooth muscle cells, or both in an activity-dependent manner. BK channels are thought to be important for regulating contractility of bladder smooth muscle cells by contributing to membrane repolarization following action potentials (Heppner et al., 1997; Buckner et al., 2002; Herrera and Nelson, 2002; Hashitani and Bradin, 2003b). It has been shown that mice lacking functional BK channels, due to the deletion of the slo1 gene encoding the pore-forming unit of BK, exhibit enhanced bladder contractions and urinary frequency (Meredith et al., 2004; Thorneloe et al., 2005). Moreover, the BK-opener NS8 has been demonstrated to increase bladder capacity and to decrease afferent nerve firing, thereby suppressing the micturition reflex in rats (Tanaka et al., 2003; Malysz et al., 2004).

In addition to BK channels, SK and IK channels have been implicated in bladder function. IK channels have been demonstrated in mouse bladder myocytes (Ohyo et al., 2002), but in general, IK channels are mainly expressed in nonexcitable cells, and the putative role of IK in bladder function has not been studied in detail. In contrast, numerous studies have demonstrated an important role of SK channels in regulating detrusor contractility (Heppner et al., 1997, 2003; Herrera et al., 2000) in bladder myocytes and strips. In pig and human bladder smooth muscle, SK channels also seem to play an important role (Buckner et al., 2002; Hashitani and Bradin, 2003a; Darblade et al., 2006). In mice with conditionally suppressed SK3 expression (Herrera et al., 2003), an involvement of SK3, and possibly other SK forms, in mouse bladder contractility was shown in experiments on bladder strips as well as by in vivo cystometry. Later, when SK2-deficient mice were analyzed, an important role for SK2 in regulation of detrusor contractility was demonstrated (Thorneloe et al., 2008). The SK2 subunit was shown to be required for the apamin-sensitive regulation of bladder smooth muscle; however, both the actual SK channel composition in detrusor smooth muscle cells and possible species differences still remain to be further explored.

Based on these studies using apamin and bladder detrusor strip preparations from mice deficient in specific SK forms, positive modulation of SK channels may have a beneficial effect in conditions associated with bladder overactivity (Herrera et al., 2003). However, it has been difficult to study the involvement of SK and IK channels in bladder function and the putative beneficial action of channel activators pharmacologically, due to the lack of potent openers of the channels. Recently, we identified a potent positive modulator of SK and IK channels, NS309 (Strøbæk et al., 2004) and we observed that this compound increased bladder capacity and decreased voiding frequency in rats when administered directly into the bladder (Pandita et al., 2006). To pursue this finding, we searched for positive modulators of SK/IK channels with improved bioavailability.

Here, we describe a new, potent, positive modulator of IK and SK channels, NS4591, which was characterized in patch clamp experiments using HEK293 cells expressing recombinant SK and IK channels as well as in acutely dissociated primary bladder afferent neurons. We further used this positive SK/IK modulator to study the role of SK/IK channels in rat and cat models of bladder overactivity in vivo.

Materials and Methods

Materials. NS4591 ZD6169, and 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benimidazol-2-one (DC-EBIO) were synthesized at NeuroSearch A/S (Ballerup, Denmark). 1-EBIO and apamin were purchased from Sigma-Aldrich (Vallensbæk Strand, Denmark). NS4591, apamin, DC-EBIO, and 1-EBIO were dissolved in DMSO and diluted at least 1000 times in the experimental solutions. For oral or intraduodenal administration, NS4591 was dissolved in a vehicle composed of 10% Tween 80 in saline. For intravesical administration during continuous bladder irrigation in anesthetized rats, NS4591 was first dissolved in DMSO at 30 and 10 mg/ml, respectively, and diluted in 0.25% acetic acid/0.9% saline to a final concentration of 30 and 10 μg/ml in 0.1% DMSO/0.25% acetic acid/0.9% saline. Oxyhemoglobin (OxyHb) was prepared from human hemoglobin with a 10-fold molar excess of sodium dithionite. In brief, a 10 μM solution of human hemoglobin (Sigma-Alrich) was mixed with a 100 μM solution of sodium hydrosulfite, dialyzed against water, and filtered. The OxyHb solution was kept at −20°C for a maximum of 7 days before use. Capsaicin was dissolved in a stock solution of DMSO and subsequently diluted to a concentration of 6 μM in 0.9% saline.

Animals. All experiments were performed in accordance with guidelines and regulations from the respective institutional and national committees for animal experimentation.

Electrophysiological Analysis of KCa Channels Modulators on Recombinant KCa Channels Expressed in HEK293 Cells. The establishment of hSK1-, hSK2-, hSK3- as well as hIK-expressing HEK293 cells has been described previously (Strøbæk et al., 2004). Cells were cultured in Dulbecco’s modified Eagle’s medium (BioWhittaker, Walkersville, MD) supplemented with 10% fetal calf serum (Sigma-Alrich) at 37°C and 5% CO2. At approximately 80% confluence, the cells were washed with phosphate-buffered saline and harvested by trypsin/EDTA (BioWhittaker) treatment. Subsequently the cells were transferred to Petri dishes containing coverslips (0.35 mm; custom made at VWR International, Herlev, Denmark).

Electrophysiological experiments were performed using an EPC-9 amplifier (HEKA, Lambrecht/Pfalz, Germany) connected to a Macintosh computer (Apple Computer, Cupertino, CA) by an ITC-16 interface. Membrane currents were recorded using the whole-cell or inside-out configuration of the patch-clamp technique. Patch pipettes (~ 2 MΩ) were pulled from borosilicate tubes with an outside diameter of 1.32 mm (Modulohm, Copenhagen, Denmark) using a horizontal electrode puller (Zeitri Instruments, Augsburg, Germany). Cells seeded on coverslips were transferred to a 15-μl recording chamber continuously perfused at 1 ml/min at room temperature. An electronically controlled micromanipulator (Eppendor) was used for the positioning of pipettes. A Ag/AgCl pellet electrode was fixed in the chamber serving as a reference electrode. Data were filtered at 3 kHz. Currents were elicited by applying a 200-ms linear voltage ramp from −80 to +80 mV every 5 s from a holding potential of 0 mV.
In whole-cell experiments, the cell capacitance (below 12 M\(\Omega\)) and series resistance (80\% compensation) were updated before each voltage ramp.

A high K\(^+\) solution was applied to the extracellular side of the membrane: 154 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM HEPES, pH adjusted to 7.4 with KOH. The intracellular solutions contained 154 mM KCl and 10 mM HEPES as well as 10 mM EGTA or a combination of EGTA and nitrilotriacetic acid (1 and 9 mM, respectively). MgCl\(_2\) and CaCl\(_2\) were added in concentrations calculated (EqCal, Cambridge, UK) to give a free Mg\(^{2+}\) concentration of 1 mM and a free Ca\(^{2+}\) concentration of 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 3, or 10 \(\mu\)M. Intracellular solutions were adjusted to pH 7.2 with concentrated HCl or KOH. Osmolarities of the extra- and intracellular solutions were in the range 290 to 300 mOsm.

Concentration-response relationships for NS4591, DC-EBIO, and 1-EBIO were fitted to the Hill equation (see below) to obtain EC\(_{50}\) values:

\[
I(C) = \frac{\frac{I_{\text{max}}}{C^n}}{EC_{50} + C^n} + I(0)
\]

where \(I(C)\) is the current at the concentration \(C\); \(I_{\text{max}}\) is the maximal current fitted at saturating concentrations, \(I(0)\) is the current at zero concentration, and \(n\) is the Hill coefficient.

**Electrophysiological Studies of K\(_{\text{cat}}\) Channel Modulators in Primary Cultures of Bladder Afferent Dorsal Root Ganglion Neurons.** In adult female Sprague-Dawley rats (175–200 g), DRG neurons innervating the urinary bladder were labeled by retrograde axonal transport of the fluorescent dye 1,1',3-tetramethyl-3,3',3'-tetramethylnicotinamide perchlorate (Fast DiI, 25\% (w/v) in DMSO; Molecular Probes, Carlsbad, CA) 5 to 8 days before dissociation. Then, 10 \(\mu\)l of fluorescent dye was injected using a 32-gauge needle into the bladder wall of anesthetized animals in three to five sites around the bladder neck. The bladder exterior surface was rinsed thoroughly with sterile saline following injections to minimize dye contamination of surrounding tissue.

**Ex Vivo Bladder Myography.** Female Sprague-Dawley rats (175–225 g; Taconic, Ry, Denmark) were allowed to habituate to the housing facilities for a minimum of 5 days before experiments or tissue harvesting. The animals were housed in groups (three to five/cage according to weight) with food and water ad libitum in a temperature-controlled environment, with a light/dark cycle of 12:12 h. Rats were euthanized with isoflurane followed by cervical dislocation. Bladders were isolated and placed in dissection/experimental buffer: 114 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25 mM NaHCO\(_3\), and 11.7 mM glucose). Then, 1-mm rings of bladder were transversally cut and mounted in the organ baths (5 ml) of a myograph 601 (DMT A/S, Copenhagen, Denmark), heated to 37°C, and bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. Two rings were prepared from the upper half of the bladder dome of each animal. The bladder rings were allowed to acclimatize for 30 min before starting the experiment by application of 0.2 \(\mu\)M carbachol or 0.2 \(\mu\)M carbachol + 0.1 \(\mu\)M apamin. After 20-min baseline measurements, NS4591 or vehicle was added. The analog signal output was digitized (10-Hz sampling rate) using a PowerLab data acquisition system, and output was visualized and measured using Chart 5 software (both from ADInstruments, Colorado Springs, CO). The mean force and amplitude were determined over the last minute before every drug administration and 15 min after the last drug administration. The effects of NS4591 on mean tension and twitch amplitude where normalized to pretreatment values and to the respective vehicle and time controls. Overall two-way repeated measures ANOVA (NS4591 treatment \(\times\) apamin treatment) followed by Bonferroni’s post test was performed using GraphPad Prism 4.03 (GraphPad Software Inc., San Diego, CA).

**Recording of Basal Bladder Pressure and Micturition in Anesthetized Rats.** Female rats (250–275 g b.wt.) were anesthetized with urethane (1.2 g/kg), and a saline-filled catheter (PE-50) was inserted into the lumen of the proximal duodenum for intraduodenal drug administration. A heparinized (100 units/ml) saline-filled carotid catheter (PE-50) was also inserted for blood pressure monitoring. Via a midline lower abdominal incision, a flared-tipped PE-50 catheter was inserted into the bladder dome for bladder filling and pressure recording. The abdominal cavity was moistened with saline and closed by covering with a thin plastic sheet to maintain access to the bladder for emptying purposes. Fine silver or stainless steel wire electrodes were inserted into the external urethral sphincter percutaneously for electromyography.

Saline was continuously infused into the bladder of urethane anesthetized rats (\(n = 8\)) at a rate of 0.1 ml/min for 60 min to obtain a baseline of lower urinary tract activity (continuous cystometry; CMG). After the control period, three vehicle intraduodenal injections were made at 20-min intervals to determine whether administration of vehicle had any effect. Subsequently, increasing doses of NS4591 (3, 10, and 30 mg/kg) were administered intraduodenally at 60-min intervals to construct a cumulative dose-response relationship. At the end of the control saline cystometry period, after the third vehicle administration, and 20 and 50 min following each subsequent treatment, the infusion pump was stopped, the bladder was emptied by fluid withdrawal via the infusion catheter, and a single filling cystometrogram was performed at the same flow rate to determine changes in bladder capacity caused by drug administration. In four animals, 0.25% acetic acid was infused into the bladder for 90 min following the final measurement at the high dose.
Bladder Overactivity Induced by Acetic Acid. After a control period (60 min; flow rate, 0.055 ml/min), a 0.25% acetic acid solution in saline was infused into the bladder to induce bladder irritation. In studies of intraduodenal NS4591, 30 min of acetic acid infusion was followed by three vehicle injections and subsequently, increasing doses of NS4591 (3, 10, and 30 mg/kg) at 30-min intervals for a cumulative dose-response relationship (n = 7), or the high dose (30 mg/kg) only was administered intraduodenally (n = 3). In studies of intravesical NS4591, 30 min of acetic acid infusion was followed by infusion of 0.1% DMSO/0.25%. Subsequently, increasing doses of NS4591 (1, 3, 10, and 30 μg/ml; n = 4) in 0.1% DMSO/0.25% acetic acid were infused intravesically at 30-min intervals.

Recording of Basal Bladder Pressure and Micturition in Conscious Rats. Female Sprague-Dawley rats (200–300 g; M&B Breeding and Research Centre, Tornbjerg, Ll. Skensved, Denmark) were habituated to the surroundings for at least 5 days before the experiments were conducted. For surgery, rats were placed on a heating pad and anesthetized with 1.5 to 2.5% halothane in a 2:1 N2O/O2 mixture using a face mask, with the rectal temperature being kept between 37.5 and 38°C. A saline-filled PE-50 catheter was inserted into the bladder dome, tunneled subcutaneously to the neck, and exteriorized. Rats were treated with Rimadyl (5 mg/kg s.c. 30 min before surgery), locally with lidocaine (Xylocaine, 5%) and prophylactically with antibiotics (streptipenprokain 40 IU s.c. during and 24 h after surgery). Rats were allowed to recover for 3 days before experiments were undertaken.

For cystometry, rats were placed in metabolic cages and allowed to adjust to the surroundings for at least 30 min. The bladder catheter was connected to a calibrated pressure transducer (P10EZ; Ohmeda PD, Liberty Corner, NJ) and a pump (Ole Dich 4 channel peristaltic pump (Ole Dich, Hvidovre, Denmark) or CMA100 microinjection pump (CMA/Microdialysis, Solna, Sweden) via a T-tube. Urine was collected and monitored by means of an electronic scale (Mettler Toledo PG-S; Mettler Toledo, Glostrup, Denmark) modified to output a real-time analog signal corresponding to the weight of the excreted urine. The transducer signal and the analog weight signal were recorded from four rats simultaneously and analyzed using a Macintosh PowerPC equipped with a Mac Lab 4S recording unit, four bridge amplifiers, and the multichannel recording and analysis Chart software (ADInstruments). The bladder was constantly perfused with 0.9% saline at a constant rate of 10 ml/h. NS4591 and reference compounds were tested after intravesical, intraperitoneal, or oral administration. For intravesical administration, up to three cumulative doses of test substance were infused through the bladder catheter for 45 min per dose following a stable 45-min baseline recording. In separate experiments, saline or vehicle was administered for at least 3 h under similar conditions. For analysis of cystometric data, peaks in bladder pressure occurring simultaneously with changes in volume were identified as bladder contractions related to micturition: maximal micturition pressure (MP), micturition volume (MV), intercontraction interval (ICI), and in some experiments, basal pressure (BP) and threshold pressure (TP), the highest bladder pressure immediately before a micturition event, were determined using the Chart software (ADInstruments).

Bladder Overactivity Induced by Oxyhemoglobin or Capsaicin. Following a 60-min stable baseline recording, capsaicin (6 μM in 0.9% NaCl) was infused for approximately 20 min followed by 60-min saline infusion. OxyHb was infused for 60 min followed by 60-min saline infusion. NS4591 or vehicle was administered orally before infusion of capsaicin or OxyHb. Animals were randomized to the treatment groups.

Cystometry in Anesthetized Cats. Female cats (2.3–3 kg b.w.; Harlan, Indianapolis, IN) were anesthetized with 5% isoflurane for induction and 2 to 3% for surgical maintenance. Isoflurane anesthesia was discontinued and replaced by intravenous administration of o-chloralose (70–75 mg/kg; Thermo Fisher Scientific, Waltham, MA) for experiment anesthesia maintenance. A heparinized (100 units/ml), saline-filled catheter (PE-90) was inserted into the carotid artery to monitor blood pressure and heart rate. A trachea tube was placed to monitor respiration. Through a midline lower abdominal incision, a modified PE-240 catheter was inserted into the proximal duodenum for drug administration. The distal portion of the stomach was loosely ligated to prevent reflux into the stomach. A modified 16-gauge intravenous catheter was inserted into the bladder dome for bladder filling and pressure recording. The abdominal cavity was moistened with saline and closed by covering with a thin plastic sheet to maintain access to the bladder for emptying purposes. Fine platinum wire electrodes were inserted into the external urethral sphincter for electromyography.

Saline was continuously infused at a rate of 0.5 to 1 ml/min via the bladder catheter for approximately 60 min to obtain baseline of lower urinary tract activity (CMG) and bladder capacity. After the control period, a 0.5% acetic acid solution, pH 2.8, in saline was infused into the bladder at the same flow rate to induce bladder irritation. After approximately 30 min of acetic acid infusion, one to two vehicle injections were given at 30-min intervals to determine whether administration of vehicle had any effect. Subsequently, increasing doses of NS4591 were administered intraduodenally at 30 to 60-min intervals to construct a cumulative dose-response relationship (n = 5–7). At the end of the control saline cystometry period, the vehicle, and 15 to 20 min following each subsequent treatment, the infusion pump was stopped, the bladder was emptied by fluid withdrawal via the infusion catheter, and a single-filling CMG was performed at the same flow rate to determine changes in bladder capacity caused by the irritation protocol and subsequent drug administration. Data were analyzed by nonparametric ANOVA for nonrepeated measures (Kruskal-Wallis) with Dunn’s multiple comparison test for cumulative dose-response studies. All comparisons were made from last vehicle measurement. P < 0.05 was considered significant.

Results

NS4591. Based on previously reported observations of the SK/IK channel modulator NS309 (Strøbek et al., 2004; Pandita et al., 2006), we searched for novel positive modulators of SK/IK channels with improved bioavailability, which lead to the identification of NS4591. The chemical structure of NS4591 is shown in Fig. 1A, along with the structures of NS309, DC-EBIO, and 1-EBIO. In rats, the bioavailability of NS4591 after oral dosing was estimated to 97% (data not shown). NS4591 was characterized in vitro by patch-clamp recordings in HEK293 cells expressing recombinant SK and IK channels and in primary bladder afferent neurons, as well as in bladder detrusor rings and in vivo in animal models of overactive bladder.

NS4591 Is a Potent Positive Modulator of Calcium-Activated Potassium Channels of Intermediate and Small Conductance. The effects of NS4591 on IK (Fig. 1B) and SK3 (Fig. 1C) calcium-activated K+ channels expressed in HEK293 cells were studied in whole-cell patch-clamp experiments. The figures illustrate that application 0.03 μM NS4591 augmented the IK current to the same extent (a factor of 1.8) as 0.3 μM NS4591 did on the SK3 current, reflecting that the IK channels are approximately 10-fold more sensitive to NS4591 than SK3 channels. The concentration of compound required to double the control current level was 0.045 ± 0.006 μM (n = 16) for IK and 0.53 ± 0.10 μM (n = 7) for SK3, respectively.

To compare the potency of NS4591 to that of the reference compounds DC-EBIO and 1-EBIO, inside-out patch-clamp experiments were performed, since this allows full control of [Ca2+]i, and thereby construction of concentration-response curves at a fixed degree of Ca2+-induced response. In Fig. 2A,
SK3 current measured at −75 mV is depicted as a function of time. The inside-out patch was first exposed to 10 μM Ca2⁺ to measure the maximal SK3 current in the patch and, subsequently, to an [Ca2⁺]i of 0.2 μM (approximately 5% of the maximal channel activity). At 0.2 μM Ca2⁺, the patch was exposed to increasing [NS4591] as indicated by the bars. B, concentration-response relationship for NS4591, DC-EBIO, and 1-EBIO obtained from experiments similar to the one illustrated in A. Currents were normalized with respect to maximal current obtained at 10 μM Ca2⁺, and data points represent mean ± S.E.M. of six to seven experiments. The solid lines are the fit of the averaged data to the Hill equation. C, current at −75 mV measured from an inside-out patch. The patch was exposed to a [Ca2⁺]i of 0.01, 0.2, or 10 μM as indicated, and NS4591 (10 μM) was applied at the different [Ca2⁺]i as shown by the bars. D, [Ca2⁺]-response relationships for hSK3 in the absence or presence of 10 μM NS4591. Currents from individual patches were normalized with respect to the effect of 10 μM Ca2⁺ (in the absence of NS4591). Data points represents mean ± S.E.M. of 19 experiments in the absence and seven in the presence of 10 μM NS4591, and the solid lines are the fit of the averaged data to the Hill equation.

Fig. 1. A, structures of NS4591, NS309, DC-EBIO, and 1-EBIO. B and C, whole-cell current-voltage relationships, measured in HEK293 cells stably expressing hIK (B) or rat SK3 (C) upon application of 200-ms long voltage ramps (−80 to +80 mV), elicited every 5 s from a holding potential of 0 mV. The experiments were conducted with symmetrical [K⁺] (150/150 mM) and 0.2 μM free Ca2⁺ in the pipette solution. Current-voltage relationships were measured in the absence of compound (Ctrl) or in the presence of NS4591 (0.03 μM for hIK and 0.3 μM for rat SK3 experiments, respectively).

SK3 current measured at −75 mV is depicted as a function of time. The inside-out patch was first exposed to 10 μM Ca2⁺ to define the maximal current and subsequently to 0.2 μM Ca2⁺ in which the concentration-response relationship for NS4591 was then determined. In Fig. 2B, the SK3 currents measured at the steady-state level of activation is plotted as a function of [NS4591], together with the values for DC-EBIO and 1-EBIO obtained from similar experiments. The order of potency was (EC50 values) NS4591 (4.4 ± 0.6 μM; n = 6) < DC-EBIO (20 ± 2 μM; n = 7) < 1-EBIO (589 ± 75 μM; n = 6). Furthermore, all compounds were found to have an efficacy of close to 100% compared with the effect of saturating [Ca2⁺].
From whole-cell experiments, NS4591 was found to be an order of magnitude more potent on IK than on SK3. To investigate whether NS4591 is more potent on either of the SK1–3 subtypes, EC₅₀ values for NS4591 were obtained on SK1 and SK2 from experiments similar to that illustrated for SK3 in Fig. 2. NS4591 was found to stimulate the currents, with EC₅₀ values of 3.8 ± 0.5 μM (SK1; n = 6) and 4.5 ± 0.4 μM (SK2; n = 5), respectively, i.e., not significantly different from the EC₅₀ value obtained on SK3.

Previously described positive modulators augment IK/SK channel activity by increasing the apparent Ca²⁺ sensitivity of the channels, and the effect of NS4591 on the Ca²⁺ dependence of SK3 was thus elucidated. Figure 2C shows the time course of an inside-out experiment were the effect of 10 μM NS4591 was tested at [Ca²⁺]ᵢ values of 0.01, 0.2, and 10 μM. No stimulation of the SK3 current by NS4591 was observed at very low (0.01 μM Ca²⁺) or very high (10 μM Ca²⁺) degree of channel activation, whereas a substantial stimulation was seen at the intermediate Ca²⁺ concentration. Figure 2D shows the [Ca²⁺]-response curves for SK3 in the absence and presence of 10 μM NS4591. The main effect of NS4591 is clearly to induce a leftward shift in the [Ca²⁺]-response curve, which is reflected in a reduction in the EC₅₀ value for Ca²⁺-induced current activation from 0.42 ± 0.01 μM (n = 19) under control conditions to 0.16 ± 0.01 μM (n = 7) in the presence of 10 μM NS4591.

The plasma concentration peaks at 10 μM after a 30 mg/kg dose administered orally to rats, and the selectivity of NS4591 was initially investigated by testing the compound at 10 μM in a variety of radioligand binding assays at MDS Pharma Services (SpectrumScreen; Taipei, Taiwan). Inhibitors, with EC₅₀ values of 3.8 ± 0.5 μM (SK1; H9262) and 4.5 ± 0.4 μM (SK2; n = 5), respectively, i.e., not significantly different from the EC₅₀ value obtained on SK3.

Effect of SK/IK Openers on Action Potential Firing from Bladder Primary Afferent Neurons in Vitro. The roles of SK and IK channels on AP firing properties in bladder DRG neurons were examined using current-clamp techniques and the pharmacological tools apamin and 1-EBIO. Putative bladder L₆/S₁ DRG neurons were identified as retrogradely Fast DiI-labeled, IB4⁺ small diameter (<30-μm) neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified neurons as described under Materials and Methods. The SK channel blocker apamin was bath applied to five identified
bladder DRG neurons. All neurons fired a single AP in response to a threshold depolarization, and three neurons fired multiple APs in response to suprathreshold depolarization during control. Apamin (100 nM, n = 3; 300 nM, n = 2) increased the median number of APs from two (control) to seven (apamin) in response to suprathreshold depolarization (Fig. 3A) without producing consistent changes in RMP or $R_{in}$. Afterhyperpolarizations (AHPs) were measured following firing of individual APs. Apamin did not affect the AHP time course, but it produced a small decrease in the AHP peak amplitude in four of five neurons (87 ± 8.0% control; n = 5; $P > 0.05$, paired t test). The effects of apamin were rapid in onset but were not reversible.

To further elucidate the role of SK channels in bladder DRG firing, the SK/IK channel opener 1-EBIO was bath applied to five neurons. Four of five neurons fired multiple APs in response to a suprathreshold depolarizing pulse. In less than 1 min, 1-EBIO (300 μM) produced a reduction in the median number of APs from eight (control) to two (NS4591; Fig. 3D). Similar to the effects of 1-EBIO, NS4591 (10 μM) did not produce a change in either amplitude or duration of AHPs following single AP firing, or AHPs following action potentials generated by 500-ms suprathreshold depolarizing pulses. Although increased AP firing in response to apamin and decreased AP firing in response to 1-EBIO indicated that SK channels can modulate the excitability of bladder DRG neurons, this modulation does not seem to be due to changes in the AHP.

We next examined the ability of NS4591 to modulate neuronal excitability. Recordings were obtained from nine identified bladder DRG neurons. Table 1 lists membrane properties of neurons used in these experiments. Changes in RMP, $R_{in}$, and AP threshold were measured in response to increasing concentrations of NS4591. No significant changes in any parameter were observed following application of compound at concentrations of 1 and 10 μM. At the high concentration of 100 μM NS4591, the appearance of apparently nonspecific sustained plateau depolarizations and increases in input resistance were observed in two neurons. These effects were not investigated further.

NS4591 (10 μM) reduced tonic AP firing (Fig. 3C) in five of eight neurons tested. In the five neurons that responded to NS4591, the median number of APs elicited in response to a 500-ms depolarizing current pulse was reduced from seven (control) to 2 (NS4591; Fig. 3D). Similar to the effects of 1-EBIO, NS4591 (10 μM) did not produce a change in either amplitude or duration of AHPs following single AP firing, or AHPs following action potentials generated by 500-μs suprathreshold depolarizing pulses (n = 8). The overall effects of NS4591 were similar to the effect of 1-EBIO on bladder sensory neurons, and they were consistent with the opening of SK/IK channels resulting in a decrease in AP firing.

**Effect of SK/IK Openers on Bladder Detrusor Contractility in Vitro.** We next addressed whether in addition to the observed effect on bladder afferent nerves, NS4591 may also directly affect bladder detrusor contractility. Car-

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**TABLE 1**

<table>
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<tr>
<th>Basic electrical properties of neurons in response to NS4591</th>
<th>Number of neurons studied per group are indicated in parentheses.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RMP mV</td>
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<tr>
<td>Control</td>
<td>−50.6 ± 3.1 (5)</td>
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<tr>
<td>NS4591</td>
<td></td>
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<tr>
<td>1 μM</td>
<td>−53.3 ± 4.1 (3)</td>
</tr>
<tr>
<td>10 μM</td>
<td>−51.5 ± 3.6 (5)</td>
</tr>
<tr>
<td>100 μM</td>
<td>N.A.</td>
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</table>

N.A., not available.

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**Fig. 4.** Organ bath isometric force measurements of rat detrusor muscle strips. NS4591 effect on contractions induced by 0.2 μM carbachol or 0.2 μM carbachol + 0.1 μM apamin. A, twitch amplitude. Overall lower effect of NS4591 on carbachol-induced contractions in the presence of apamin compared with carbachol alone. Two-way repeated measures ANOVA: apamin effect, $F(60,1) = 7.916$, $P = 0.0184$ and NS4591 effect, $F(60,6) = 13.96$, $P < 0.0001$. Bonferroni’s post hoc test identified significant effects of apamin pretreatment for the effects of 0.3 μM NS4591 (*, $P < 0.01$) and 3 μM NS4591 (+, $P < 0.05$). B, mean tension. No significant effect of apamin. Significant effect of NS4591, $F(60,6) = 15.69$, $P < 0.0001$. C, representative traces demonstrating the effects of NS4591 (red) and vehicle (blue) on detrusor muscle strips stimulated with 0.2 μM carbachol (the two top traces) or 0.2 μM carbachol + 0.1 μM apamin (the two bottom traces). D, representative enlarged traces illustrating twitch amplitude and mean tension measurements.
bachol-induced contractions were studied in ring preparations of bladder detrusor with intact urothelium. Carbachol (0.2 μM) induced an increase in the mean tension as well as phasic contractions, “twitches,” measured as twitch amplitude (Fig. 4, C and D). In the presence of 100 nM apamin, a significant increase in twitch amplitude was observed (Fig. 4C), supporting an important role for SK in bladder contractility. In contrast, NS4591 was found to reduce the amplitude of detrusor twitches in a concentration-dependent manner. This effect of NS4591 was significantly reduced by pretreatment with 100 nM apamin (Fig. 4, A and C), indicating that NS4591 reduced twitch amplitude in an SK-dependent manner. NS4591 had only a marginal effect on the mean tension (Fig. 4B).

**Basal Urodynamic Effects of SK/IK Openers.** To address the putative role of IK and SK channels in bladder control, we tested the effect of NS4591 administered to conscious rats. When bladder pressure and micturition were recorded during constant intravesical infusion of saline, a regular pattern of bladder contractions occurring simultaneously with micturition was observed (Fig. 5). The effect of NS4591 was investigated after intravesical infusion. When administered in cumulative concentrations from 0.1 to 10 μg/ml, corresponding to 0.39 to 39 μM, NS4591 modulated bladder function in a concentration-dependent manner, resulting in a higher TP, a larger MV, a longer ICI, and a lower MP while leaving the BP unaffected (Table 2). At a concentration of 10 μg/ml (39 μM), NS4591 significantly increased ICI (+45%), MV (+24%), and TP (+24%), and it reduced MP (−32%), indicating a relaxing effect on the bladder. DC-EBIO similarly increased MV and ICI while decreasing MP in a concentration-dependent manner (1–100 μg/ml).

To test whether systemic administration of a positive IK/SK channel modulator may have beneficial effects on bladder function, bladder pressure and micturition were studied after oral dosage of NS4591 in conscious rats (Table 2). When cystometric parameters were studied after oral administration, an increase in the average TP was observed in the time interval 1 to 2 h after dosing of NS4591 (30 mg/kg). No significant change in other cystometric parameters was observed. No effect on cystometric parameters was observed after administration of 10 mg/kg p.o (Table 2).

NS4591 was also tested in urethane-anesthetized rats. In contrast to the observations in awake animals, no significant effect of the compound on cystometric parameters was observed after either intravesical (0.3–10 μg/ml) or intraduodenal (3–30 mg/kg) dosing (data not shown). It remains to be determined whether anesthesia affects the impact of SK/IK channels on bladder afferent activity, smooth muscle activity, or both. Taken together, the current observations indicate that positive modulation of SK/IK channels by NS4591 may have a modest relaxant effect on the rat bladder under basal conditions.

**Effect of NS4591 on Oxyhemoglobin or Capsaicin-Induced Bladder Overactivity.** To study the putative role of SK/IK channels in bladder overactivity in conscious, unrestrained animals, rats were pretreated systemically with NS4591 before intravesical infusion of OxyHb or capsaicin. As reported previously, both OxyHb and capsaicin induced a decrease in MV and ICI and an increase in MP (Fig. 6). This response was inhibited in rats pretreated with NS4591 orally (30 mg/kg) or intraperitoneally (10 mg/kg) 2 h and 45 min before instillation of capsaicin, respectively (Fig. 6), whereas lower doses of NS4591 had no effect. The response to OxyHb also seemed to be diminished in rats pretreated with NS4591 (30 mg/kg p.o.); however, no significant effect was demonstrated.

The effects of NS4591 were also tested on bladder function.

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**Fig. 5.** A, calculation of cystometric parameters. B, representative traces showing bladder pressure (mm Hg; black) and micturition (grams; red) before (control) and after intravesicular infusion of capsaicin in conscious rats pretreated with vehicle (top traces) or NS4591 (30 mg/kg p.o.).
When administered intraduodenally, NS4591 had a pronounced inhibitory effect on bladder overactivity induced by acetic acid. As can be seen in Fig. 7A, coordinated micturition contractions were evident following NS4591 administration. A dose-dependent increase in bladder capacity (P = 0.0259, Friedman test; n = 3) to approximately 66% of vehicle at 30 mg/kg was seen with cumulative dosing of intraduodenal NS4591 (Fig. 7B).

NS4591 was also administered to urethane-anesthetized rats with acetic acid-irritated bladders (n = 7). When cumulative doses of NS4591 were administered intraduodenal, 30 mg/kg modestly, but significantly, inhibited the acetic acid-induced reduction in bladder capacity (Fig. 7C). No effect of intravesical administration was observed in cats or rats (data not shown).

These observations show that NS4591 reduced acetic acid-induced bladder overactivity in the anesthetized cat and rat after systemic administration. The overall response to NS4591 was greater in the cat, suggesting that bladder function in this species is more sensitive to modulation of SK/IK channels than in the rat.

### Discussion

SK and IK channels have been implicated in the regulation of bladder function, and positive modulation of these channels may represent a novel treatment strategy for overactive bladder. In the current study, we have identified and characterized NS4591, a new positive modulator of SK/IK channels and used this compound to address the impact of SK/IK channels on firing properties of bladder C-fiber afferents and detrusor contractility in vitro and for bladder overactivity in vivo. NS4591 is shown to be a potent positive IK/SK modulator, with high bioavailability after oral administration; thus, it seems to be a useful pharmacological tool to study the involvement of SK/IK channels in models of overactive bladder in vivo.

Positive SK/IK channel modulators may theoretically alleviate bladder overactivity, either by a directly relaxing bladder smooth muscle cells or by reducing afferent or efferent nerve activity. In bladder detrusor smooth muscle cells, SK channels have been shown to be important regulators of contractility, which is markedly increased by the SK channel blocker apamin. Conversely, a positive SK channel modulator may be expected to reduce bladder smooth muscle contractions.

### Table 2

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<th>MV (95% CI)</th>
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<th>TP (95% CI)</th>
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*P < 0.05, t test.
**P < 0.01, t test.
The expression of SK/IK channels in bladder afferent fibers and their possible role in nervous regulation of bladder function have not been explored in great detail. Positive modulation of SK/IK channels expressed in C-fiber afferents from the bladder may dampen excessive afferent activity and thereby stabilize the overactive bladder without compromising the capacity for voiding contractions. A recent study demonstrated the expression of IK as well SK1, SK2, and SK3 in rat DRG neurons, including in putative nociceptive neurons (Mongan et al., 2005). Studies on mice with altered levels of SK2/SK3 expression have supported a role for both SK2 and SK3 in bladder control (Herrera et al., 2003; Thorneroe et al., 2008). However, in these mouse studies SK2 was shown to be expressed in smooth muscle cells, but not in the urothelium, whereas SK3 expression was observed in bladder smooth muscle and urothelium, but neither in afferent nor efferent nerves as evaluated by immunohistochemical costainings with neuronal markers. We here show that NS4591 as well as 1-EBIO reduces the firing of multiple action potentials from small-diameter primary rat bladder afferent neurons, strongly suggesting that SK/IK channels are expressed in bladder afferent nerves and participate in the control of bladder afferent nerve firing, at least in rats. Bahia et al. (2005) demonstrated SK3 immunoreactivity in rat DRG neurons and revealed a role for SK channels in modulation of sensory input to the spinal cord. However, this study showed only minimal functional activation of SK channels in DRG neurons with EBIO. Whereas evidence exists for AHP-associated SK channel activation in brain neurons (Pedrazani et al., 2005), Bahia et al. (2005) were able to demonstrate only minimal effects of EBIO on DRG AHPs. These results support our findings that SK/IK channel modulators have little effect on DRG AHPs; yet, they can modulate DRG action potential firing. Whether these DRG SK channels contribute to processes other than AHP generation, or whether SK/IK channels in bladder afferent nerves are pharmacologically different from recombinant receptors remains to be determined.

Taken together, the in vitro observations in the current study indicate that pharmacologically positive SK modulators are capable of reducing the activity of bladder afferent nerves as well as of the bladder detrusor and thereby potentially have beneficial effect on myogenic and neurogenic bladder overactivity.

To address the putative role of SK/IK channels in bladder function in vivo, we infused capsaicin, oxyhemoglobin, or acetic acid into the bladder, agents that presumably cause bladder overactivity by activation of bladder C-fibers. We observed that systemic administration of NS4591 before the instillation of capsaicin inhibited this bladder overactivity response, suggesting that positive modulators of SK/IK channels may target bladder afferents and reduce action potential firing in rats in vivo. In addition a direct relaxing effect on detrusor smooth muscle may be speculated to contribute to the effect. Interestingly, only a minor effect of NS4591 was observed when bladder
overactivity was induced by OxyHb, although OxyHb has been suggested to induce bladder overactivity by scavenging NO and thereby possibly decrease the threshold for bladder afferent firing (Pandita et al., 2000). Likewise, NS4591 only had a minor effect on bladder overactivity induced by acetic acid in anesthetized rats, and no significant effect on bladder function was observed in spinal cord-transected rats, although increased C-fiber activation has been demonstrated in both conditions (Thor and Mulhhauser, 1999; Vizzardi, 2000; Mitsui et al., 2001). These observations support the idea that positive modulators of IK/SK channels may have beneficial effect on some forms of bladder overactivity. It should be noted that a significant reduction in blood pressure was observed after cumulative dosing of NS4591 to urethane-anesthetized rats at 30 mg/kg but not in lower doses (10%; \( P < 0.01 \), Friedman test, \( n = 7 \); data not shown), whereas no effect on blood pressure has been observed in awake rats in doses of NS4591 up to 100 mg/kg p.o. In cats, a possible nonsignificant reduction in blood pressure was observed at 30 mg/kg i.d. (17%, \( n = 3 \); data not shown). It remains to be determined whether positive modulators of SK/IK may have effect on blood pressure in conscious animals in some species. Furthermore, additional studies are needed to address the finding that NS4591 alleviates bladder overactivity in some but not all rat models tested.

Interestingly, in the cat NS4591 significantly alleviated bladder overactivity induced by acetic acid. Thus, at 30 mg/kg i.d. NS4591 almost restored bladder capacity to the same level as before exposure to acetic acid. It is plausible that the differentiated response to SK/IK modulation observed in the various animal models used in the current study reflects species differences with respect to the expression and functional properties of afferent fiber and smooth muscle ion channels. Moreover, different subpopulations of bladder afferent neurons may be activated and the overall level of afferent nerve activity may vary between the different animal models. In conditions of very pronounced bladder overactivity and afferent fiber activation, SK/IK channels may be endogenously activated to a high extent, leaving limited room for further activation by pharmacological positive modulators such as NS4591, whereas in other conditions, pharmacological SK/IK modulation may be of considerable importance. Thus, further studies are warranted to clarify the role of IK/SK channels in the regulation of bladder afferent function in normal bladder as well as under different pathological conditions in different species, including mice, rats, and cats.

In conclusion, we here report that a new positive modulator of SK/IK potassium channels, NS4591, inhibits the firing of action potentials in primary bladder afferent DRG neurons and also reduces carbachol-induced contractions of bladder rings. In vivo, NS4591 alleviates bladder overactivity induced by capsaicin in awake rats as well as by acetic acid in anesthetized cats. These findings support the hypothesis that SK/IK channels play an important role in bladder function and suggest that positive modulators of SK/IK channels may be of benefit for the treatment of overactive bladder.

Acknowledgments
We acknowledge the excellent technical assistance of Vibeke Mylelland-Smyth, Charlotte Heltoft, Peter Buhl, and Charlotte L. Petersen. We thank Palle Christophersen for critically reviewing the manuscript. We also thank Camilla Bank and Pia Weikop for NS4591 bioavailability analyses and Elsebet Østergaard Nielsen for organizing selectivity screening of NS4591.

References
Thor KB and Mulhhauser MA (1998) Vesicoanal, urethral, and urethrovulinal


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