Activation of Neurokinin-1 Receptors Increases the Excitability of Guinea Pig Dorsal Root Ganglion Cells

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ABSTRACT

The suppression of overactive bladder symptoms in patients and overactive bladder reflexes in animal models by neurokinin (NK)-1 receptor antagonists raises the possibility that these drugs target sensory neurons. This mechanism was evaluated by examining the interactions between a specific NK-1 agonist, [Sar⁹,Met(O₂)(11)]-substance P (Sar-Met-SP), and a potent NK-1 antagonist, netupitant (NTP), on small size (20–30 μ m) dissociated L6 and S1 dorsal root ganglion (DRG) neurons from female guinea pigs. Current-clamp recording revealed that Sar-Met-SP (1 μ M) elicited membrane depolarization (average 8.05 ± 1.38 mV) in 27% (18 of 65) of DRG neurons. In 74% of the remaining neurons (35 of 47) Sar-Met-SP decreased the rheobase for action potential (AP) generation and increased the response to a suprathreshold stimulus (3 times rheobase) with-

out changing the membrane potential. Sar-Met-SP also induced changes in the action potential (AP) wave form, including 1) an increase in overshoot (average 5 mV, $n = 35$ neurons), 2) a prolongation of AP duration (from 4.64 to 5.29 ms, $n = 34$), and 3) a reduction in the maximal rate of AP repolarization. NTP (200 nM) reversed the Sar-Met-SP-induced changes. Ca²⁺ imaging showed that application of Sar-Met-SP (1 μ M) decreased the tachyphylaxis induced by repeated application of capsaicin (0.5 μ M), an effect blocked by pretreatment with NTP (200 nM). These results raise the possibility that activation of NK-1 receptors in primary sensory neurons plays a role in the generation of overactive bladder and that block of NK-1 receptors in these neurons may contribute to efficacy of NK-1 antagonists in the treatment of overactive bladder symptoms.

Introduction

Considerable attention has been focused on the role of substance P (SP) and related neuropeptides in the neural control of the urinary bladder (Lecci and Maggi, 2001; Candenas et al., 2005). These substances are expressed in bladder afferent neurons and are released in the spinal cord or in the bladder by capsaicin-induced stimulation of nociceptive afferent nerves (Lecci and Maggi, 2001). SP can modulate bladder activity by acting on three subtypes of neurokinin receptors (NK-1, NK-2, and NK-3) expressed by bladder smooth muscle cells, urothelial cells, afferent nerves, or neurons in the spinal cord (Morrison,

1999; Lecci and Maggi, 2001; Morrison et al., 2002; Candenas et al., 2005; de Groat and Yoshimura, 2009).

The contribution of NK-1 receptors to the pathophysiology of bladder dysfunction, has attracted the most interest because clinical studies revealed that NK-1 receptor antagonists, aprepitant (Green et al., 2006) and serlopitant (Frenkl et al., 2010), reduce overactive bladder (OAB) symptoms. These agents also reduce normal bladder reflexes in guinea pig, rat, or cat (Doi et al., 1999, 2000; Kamo and Doi, 2001) as well as hyperactive bladder reflexes induced by spinal cord injury (Doi et al., 2000), systemic administration of L-DOPA (Ishizuka et al., 1995), or application of irritant chemicals to the bladder in the rat (Abdel-Gawad et al., 2001) or guinea pig (Doi et al., 1999, 2000). Various animal studies indicate that these effects are due at least in part to actions on peripheral or central bladder sensory pathways (Lecci and Maggi, 1995, 2001; Seki et al., 2005).

In the bladder, SP released from afferent nerves can influence afferent nerve activity indirectly by inducing smooth muscle contractions (Lecci and Maggi, 2001) or directly by acting in an autofeedback manner to regulate afferent nerve excitability (Morrison et al., 2002; Sculptoreanu and de

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ABBREVIATIONS: SP, substance P; NK, neurokinin; OAB, overactive bladder; DRG, dorsal root ganglion; TRPV1, transient receptor potential vanilloid; Sar-Met-SP, [Sar⁹,Met(O₂)(11)]-substance P; NTP, netupitant; APD, action potential duration; AHP, afterhyperpolarization; AP, action potential; PKC, protein kinase C.

Groat, 2007; Sculptoreanu et al., 2008, 2009). Different neurokinin receptor subtypes contribute to the regulation of bladder activity, depending on the species and site of action (Lecci and Maggi, 2001; Candenas et al., 2005). In the rat, all three receptor subtypes are involved in SP-induced bladder contractions; however, NK-2 receptors are primarily responsible for the facilitatory effect of intravesically administered SP on bladder afferent nerve firing (Morrison, 1999). Likewise, activation of NK-2 receptors by SP in rat dissociated dorsal root ganglion (DRG) neurons enhances firing to depolarizing current pulses (Sculptoreanu and de Groat, 2007), enhances L- and N-type Ca^{2+} currents (Sculptoreanu and de Groat, 2003) and the excitatory effect of capsaicin on TRPV1 channels (Sculptoreanu et al., 2008), and reduces A-type K^{+} currents (Sculptoreanu et al., 2009). On the other hand, activation of NK-1 receptors on dissociated DRG neurons in rat induces an inward current (Li and Zhao, 1998) and enhances N-methyl-D-aspartic acid (Wu et al., 2004), Nav1.8 (Cang et al., 2009), and TRPV1 currents (Zhang et al., 2007). Thus, the rat may not be a good model for testing NK-1 receptor antagonists because neurokinin mechanisms in rat sensory neurons are complicated and neurokinin receptors have a different pharmacology in rats and humans. The guinea pig may be a better model (Palea et al., 2010a) because the pharmacology of NK-1 receptors in guinea pig and human is very similar (Saria, 1999). Unfortunately, the effect of neurokinin receptor activation in DRG neurons in the guinea pig has not been studied.

In the present experiments we examined the interactions between a selective NK-1 agonist (Sar-Met-SP) and a selective NK-1 antagonist, netupitant (NTP) (Hoffmann et al., 2006) on dissociated L6 and S1 DRG cells from female guinea pigs to determine whether the depressant effects of NTP on reflex bladder overactivity observed in anesthetized guinea pigs (Palea et al., 2010a,b) might be due to action on sensory neurons. Our experiments revealed that NTP blocks the various excitatory effects elicited by activation of NK-1 receptors in primary sensory neurons, raising the possibility that NK-1 receptors have a role in the generation of OAB symptoms and that block of these receptors may contribute

to efficacy of NK-1 antagonists in the treatment of OAB. A preliminary report of some of these observations has appeared in an abstract (Zhang et al., 2011).

Materials and Methods

Animals. Adult female guinea pigs (300–450 g; Harlan, Indianapolis, IN) were used in this study. All experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were consistent with the guidelines of the National Institutes of Health and the International Association for the Study of Pain.

DRG Neuron Culture. L6 and S1 DRGs, which contain sensory neurons that innervate the urinary bladder were removed bilaterally after laminectomy under isoflurane anesthesia. DRGs were enzymatically treated (collagenase type 4 and trypsin; Worthington Biochemical, Lakewood, NJ) and mechanically dissociated as described elsewhere (Dang et al., 2005). The cells were plated on poly-L-lysine-coated (Sigma-Aldrich, St. Louis, MO) glass coverslips and incubated at 37°C in 5% CO_2 and 90% humidity for at least 2 to 3 h to allow recovery from the dissociation procedure before Ca^{2+} imaging or electrophysiological studies. Cells were studied within 2 to 3 days after dissociation.

Patch-Clamp Recording. Current-clamp recordings were performed using an Axopatch 200B (Molecular Devices, Sunnyvale, CA) controlled with pClamp (version 8.2). Data were low pass-filtered at 5 to 10 kHz with a four-pole Bessel filter and digitally sampled at 25 to 100 Hz. Hanks' balanced salt solution (HBSS) containing 138 mM NaCl, 5 mM KCl, 0.3 mM KH_2PO_4 , 4 mM NaHCO_3 , 2 mM CaCl_2 , 1 mM MgCl_2 , 10 mM HEPES, and 5.6 mM glucose, pH 7.4, 320 mOsm, was used as bath solution. Electrode solution contained 140 mM KCl, 5 mM NaCl, 0.1 mM CaCl_2 , 2 mM MgCl_2 , 11 mM EGTA, 10 mM HEPES, 2 mM Mg-ATP, and 1 mM Li-GTP; pH was adjusted to 7.2 with Tris base, and osmolality was adjusted to 310 mOsm with sucrose. All salts were obtained from Sigma-Aldrich. After formation of a tight seal ($>3 \text{ G}\Omega$) and compensation of pipette capacitance with amplifier circuitry, whole-cell access was established, and a -60 mV holding potential was applied. Cell capacitance was determined with four hyperpolarizing pulses (10-ms duration) ranging from -60 to -80 mV . Then recording was switched to current-clamp configuration. A single action potential was evoked with depolarizing current injection (4-ms duration rectangular pulse) (Fig. 1A) to assess prop-

Fig. 1. Analysis of the electrophysiological properties of guinea pig DRG neurons using whole-cell current-clamp recording. A, AP was evoked with 4-ms depolarizing current injection through the recording electrode. AP overshoot above 0 mV, APD at 0 mV, and amplitude of AHP below resting membrane potential (REM) were measured. Rate of decay of AHP was determined with a single exponential fitted to the voltage trace (shown in inset). B, AP was evoked with a prolonged depolarizing current injection (750 ms). AP threshold was the largest membrane depolarization before AP generation (e.g., -24 mV), rheobase (R) was defined as the least amount of depolarizing current needed to evoke a single AP (e.g., 80 pA). C, Once R was determined; responses to 2 and 3 times R stimuli were measured using the same current injection protocol as in B.

erties of the action potential wave form (duration, overshoot, rate of rise and decay, and the afterhyperpolarization magnitude). A 750-ms depolarizing current injection was used to assess excitability (action potential threshold, rheobase, and suprathreshold responses) (Fig. 1, B and C). After establishing the baseline action potential wave form, action potential threshold, rheobase, and the response to suprathreshold stimuli, a depolarizing stimulus with rheobase intensity (750 ms) was applied every 10 s to monitor the changes in membrane potential and firing. After a stable baseline (6–8 sweeps) was recorded, 1 μ M Sar-Met-SP was bath applied for 30 s to 1 min and then action potential wave form and excitability data were collected 2 to 3 min after Sar-Met-SP application. In some neurons, membrane potential was recorded without a depolarizing stimulus (as shown in Fig. 6A). The effects of Sar-Met-SP on excitability and action potential wave form were only assessed in neurons demonstrating stable membrane potential. A neuron was considered excited by Sar-Met-SP if there was membrane depolarization, a decrease in threshold or rheobase or an increase in spike number of at least 2 SD from baseline mean.

Ca²⁺ Imaging. Fura 2 Ca²⁺ imaging was performed as described previously (Zhang et al., 2010). In brief, DRG cells were loaded with Fura 2-AM (2 μ M; Invitrogen, Carlsbad, CA) for 30 min at 37°C in an atmosphere of 5% CO₂. Fura 2-AM was dissolved in HBSS solution to which BSA was added (5 mg/ml; Sigma-Aldrich) to promote loading. Cover slips were placed on an upright epifluorescence microscope (Olympus IX70) and continuously superfused (3–4 ml/min) with HBSS. Fura 2 was excited alternately with ultraviolet light at 340 and 380 nm; and the fluorescence emission was detected at 510 nm using a computer-controlled monochromator. Image pairs were acquired every 1 to 30 s using illumination periods between 20 and 50 ms. Wavelength selection, timing of excitation, and the acquisition of images were controlled using the program C-Imaging (Compix, Cranberry Township, PA) running on a personal computer. Digital images were stored on hard disk for off-line analysis. One coverslip usually contained 20 to 40 DRG neurons/microscopic field. On each experimental day, we first collected the control data from four to five coverslips and then collected data from another four coverslips after Sar-Met-SP or SP application, followed by another four coverslips to test the effect of NTP on agonist responses.

Data Analysis. In Ca²⁺ imaging studies, data were analyzed using C-Imaging. Background was subtracted to minimize camera dark noise and tissue autofluorescence. An area of interest was drawn around each cell, and the average value of all pixels included in this area was taken as one measurement. The ratio of fluorescence signal measured at 340 nm divided by the fluorescence signal measured at 380 nm was used to measure the increase in intracellular Ca²⁺.

Action potential duration (APD) was determined to be at 0 mV (Fig. 1A). Action potential overshoot was measured as the maximal deflection greater than 0 mV (Fig. 1A). Maximum rates of rise and fall of the action potentials were determined by taking the first derivative (dV/dt) of the action potential wave form. Magnitude of the afterhyperpolarization (AHP) was determined relative to the resting membrane potential at the largest potential change after the action potential (Fig. 1A). The AHP was fitted with a single exponential equation to estimate the decay rate (Fig. 1A, inset). Action potential threshold was defined as the maximum depolarization obtained in the absence of an action potential (Fig. 1B). Rheobase was defined as the minimum depolarizing current injection necessary to evoke an action potential with a long depolarizing current pulse (750 ms) (Fig. 1B). The response to suprathreshold stimuli was assessed by stimulating neurons with depolarizing current injection equal to 1, 2, and 3 times rheobase (Fig. 1C) or with a maximum intensity (420 pA). A paired *t* test was used to assess the statistical significance of Sar-Met-SP-induced changes in action potential wave form and excitability. One-way analysis of variances followed by Dunnett's post hoc test were used to assess the blocking effect of NTP

on Sar-Met-SP induced enhancement of capsaicin response and the increase in excitability. All data are expressed as means $\pm$ S.E.

Drugs. Sar-Met-SP and SP (Tocris Bioscience, Ellisville, MO) were prepared as 1 mM stocks in HBSS solutions and stored at -20°C . NTP (2-[3,5-Bis(trifluoromethyl)phenyl]-*N*-[6-(4-methylpiperazin-1-yl)-4-(*o*-tolyl)pyridin-3-yl]-*N*-methylisobutyramide) (provided by Helsinn Therapeutics, Bridgewater, NJ) was freshly prepared in a 1 mM solution in dimethyl sulfoxide on the day of the experiment and applied to the external bathing solution in a volume so that the dimethyl sulfoxide concentration was less than 1%. Capsaicin (from Sigma-Aldrich) was prepared as a 1 mM stock solution in 100% ethanol. All drugs were delivered via bath application using a gravity-driven application system.

Results

General Electrophysiological Properties of DRG Neurons. Because NK-1 receptors are mainly expressed in small-size DRG neurons (Li and Zhao, 1998; Segond von Banchet et al., 1999; Szucs et al., 1999), L6 and S1 DRG neurons ranging from 20 to 30 μ m in diameter were selected for study using current-clamp recording. The neurons ($n = 65$) had an average resting membrane potential of -57.7 ± 0.6 mV, cell capacitance of 26.4 ± 0.7 pF, and APD at 0 mV of 4.6 ± 0.2 ms. A 750-ms duration, 420-pA (near-maximal stimulus intensity) depolarizing current pulse evoked an average of 1.44 ± 0.11 action potentials, indicating that these neurons are phasic neurons according to our previous criteria (Sculptoreanu and de Groat, 2007).

Sar-Met-SP-Induced Membrane Depolarization. Sar-Met-SP applied for 30 to 60 s at 1 μ M concentration, which was used in our previous experiments (Sculptoreanu and de Groat, 2007), did not change membrane potential (Fig. 2A, top figure) in 47 of 65 neurons. These neurons are classified as type I neurons in this article. In the remaining 18 neurons, Sar-Met-SP induced a membrane depolarization (Fig. 2, B and C) after a delay ranging from 10 s to 2 min (average 40 ± 6 s). The amplitude of the depolarization ranged from 4 to 25 mV (average 8.05 ± 1.38 mV, $n = 18$). Four of these neurons (type II) exhibited a transient depolarization lasting less than 1 min even during continuous exposure to Sar-Met-SP (Fig. 2B). In the other 14 neurons (type III), Sar-Met-SP induced a transient membrane depolarization followed by a low amplitude, prolonged long-lasting depolarization, which lasted 5 to 20 min after washout of Sar-Met-SP (Fig. 2C). The depolarization induced by Sar-Met-SP was repeatable in both types of neurons 5 min after recovery from the first response, but the amplitude was usually smaller in type III neurons compared with the first depolarization (data not shown). The Sar-Met-SP-induced depolarizations did not induce action potentials (APs). Resting membrane potential and the threshold for eliciting AP with depolarizing current pulses were not different in the three types of neurons.

Sar-Met-SP-Induced Increase in Excitability. In type I neurons, even though Sar-Met-SP did not elicit membrane depolarization, it still produced a change in the excitability in 35 of 47 neurons (74%) (Fig. 3) and/or a change in action potential wave form (Fig. 4) within 2 to 3 min after application. Although Sar-Met-SP did not alter the firing to stimuli at rheobase intensity (Fig. 2A, bottom figures), it significantly decreased the rheobase (Fig. 4C) and increased the number of action potentials elicited by stimuli 3 times the rheobase current (Fig. 3B) or by a maximum stimulus inten-

Fig. 2. Variability of Sar-Met-SP ($1 \mu\text{M}$)-induced changes in membrane potential and excitability in three guinea pig DRG neurons. A prolonged depolarizing current pulse (750-ms duration) was applied at rheobase intensity every 10 s to evoke APs (bottom figures) before (a) and during (b) application of Sar-Met-SP and after washout (c). Membrane potential (indicated by dots in top figures) was measured every 10 s just before each depolarizing current pulse. Sar-Met-SP was applied for 30 to 60 s. A, in this neuron, application of Sar-Met-SP ($1 \mu\text{M}$) did not change membrane potential or the number of evoked APs (a, b, and c). B, in this neuron, Sar-Met-SP induced a transient 15-mV membrane depolarization and a marked increase in evoked firing (b). C, in this neuron, Sar-Met-SP induced a transient 20-mV depolarization followed by a long-lasting (>10 min) depolarization. More firing was elicited during depolarizations (b and c). The number above each graph indicates the number of neurons of each type. Resting membrane potentials were -60 mV (A), -58 mV (B), and -62 mV (C).

Fig. 3. Sar-Met-SP ($1 \mu\text{M}$) increased the response to suprathreshold stimuli in guinea pig type I DRG neurons. A, APs were evoked by 1, 2, and 3 times rheobase (R) before (left traces) and after (right traces) Sar-Met-SP application. B, pooled data from 33 DRG neurons demonstrating increased response to suprathreshold stimuli (3 times R stimuli) after Sar-Met-SP application (*, $p < 0.05$). Note that the rheobase intensity was reduced after Sar-Met-SP. C, pooled data from 15 DRG neurons showing that greater firing was evoked in response to a maximum depolarizing current pulse (420 pA) after Sar-Met-SP application (***, $p < 0.001$).

sity (420 pA) (Fig. 3C; Table 1) but did not induce a significant change in the voltage threshold for triggering an action potential (Fig. 4B; Table 1). In type I neurons Sar-Met-SP also increased the overshoot and the duration of the APD

(Fig. 4A; Table 1), and reduced the decay rate of the action potential (Table 1). However, it did not change the amplitude of AHP or the rate of AHP decay (Fig. 4A; Table 1). The effect of Sar-Met-SP on excitability lasted for more than 10 min

Fig. 4. Sar-Met-SP (1 μ M) changed action potential waveform and reduced the rheobase in guinea pig type I DRG neurons. A, Sar-Met-SP increased AP overshoot and AP duration (dashed trace) compared with control (solid trace). However, Sar-Met-SP did not change the AHP amplitude or decay rate. B, pooled data from 35 DRG neurons indicate that Sar-Met-SP did not significantly change AP threshold ($p > 0.05$). C, pooled data from 35 DRG neurons indicate that Sar-Met-SP reduced rheobase (***, $p < 0.001$).

TABLE 1
Effect of Sar-Met-SP on AP waveform and excitability in type I neurons
AP overshoot is amplitude greater than 0 mV; AP duration is at 0 mV; AP rate of rise and fall are maximal velocities. AHP amplitude is measured from resting potential; τ_{AHP} is time constant of decay of the AHP. Rheobase is the minimum depolarizing current injection (picoamperes) to evoke an AP. Comparisons between control and during Sar-Met-SP application were performed with paired t tests. Number of neurons for each comparison is indicated in parentheses.

AP and Excitability Parameters	Control	Sar-Met-SP (1 μ M)
AP overshoot, mV	34.7 $\pm$ 0.96	39.4 $\pm$ 1.2 (35)***
AP duration, ms	4.65 $\pm$ 0.19	5.29 $\pm$ 0.17 (34)***
AP rate of rise, mV/ms	41.9 $\pm$ 0.68	39.7 $\pm$ 0.18 (35)
AP rate of fall, mV/ms	26.3 $\pm$ 0.47	24.6 $\pm$ 0.48 (35)***
Amplitude of AHP, mV	8.69 $\pm$ 0.61	8.24 $\pm$ 0.66 (33)
τ_{AHP} , ms	131.7 $\pm$ 12.9	150 $\pm$ 45 (28)
AP threshold, mV	-22.5 $\pm$ 0.7	-22.3 $\pm$ 0.9 (35)
Rheobase, pA	113.2 $\pm$ 10.2	94.6 $\pm$ 8.8 (35)***
Number of AP (1 $\times$ R)	1.03 $\pm$ 0.02	1.03 $\pm$ 0.02 (34)
Number of AP (2 $\times$ R)	1.06 $\pm$ 0.04	1.12 $\pm$ 0.05 (33)
Number of AP (3 $\times$ R)	1.3 $\pm$ 0.17	2.08 $\pm$ 0.34 (14)**
Number of AP (420 pA)	2.15 $\pm$ 0.40	2.84 $\pm$ 0.45 (15)***

R, rheobase.
** $P < 0.01$.
*** $P < 0.001$.

(three neurons tested). In type II and type III neurons, a depolarizing current injection at rheobase intensity elicited more firing during the Sar-Met-SP-induced depolarization (Fig. 2, B and C, bottom figures).

NTP Reverses Sar-Met-SP Induced Changes in Membrane Potential, Excitability, and AP Wave Form. NTP, which is a selective and potent NK-1 receptor antagonist with an EC_{50} of approximately 10 nM (Hoffmann et al., 2006; Campi et al., 2010; Palea et al., 2010a,b), was tested at 200 and 500 nM concentrations. In bladder smooth muscle preparations, NTP in concentrations between 10 and 100 nM

Fig. 5. Firing induced by a prolonged stimulus pulse (750 ms) at rheobase intensity in a guinea pig type I DRG neuron was increased by a high concentration of NTP (500 nM) but not by a lower concentration (200 nM). A, control before NTP. B, during application of NTP (500 nM). C, after washout. D, NTP (200 nM) had no effect in the same neuron.

selectively suppresses the contractions evoked by an NK-1 agonist, but a high concentration (1 μ M) reduces the responses evoked by carbachol or KCl (Palea et al., 2010a,b). In our experiments, NTP at 200 nM did not affect neuronal excitability of untreated neurons (Fig. 5D); however, at 500 nM it enhanced the firing induced by depolarizing current pulses ($n = 3$ neurons) (Fig. 5B). Thus, in the remaining experiments 200 nM NTP was used as an antagonist against Sar-Met-SP. In type III neurons ($n = 3$), NTP (200 nM)

Fig. 6. NTP (200 nM) reversed the Sar-Met-SP induced depolarization in type III neurons, action potential waveform changes and increased excitability in type I DRG neurons. **A**, the reduction in the resting membrane potential after application of Sar-Met-SP was reversed by 200 nM NTP (// indicates a 4-min gap in the recording). **B**, the Sar-Met-SP induced reduction in the rheobase ($n = 10$, **, $p < 0.01$) is reversed by NTP. NTP reversed the Sar-Met-SP increased firing at 3 times R stimulus **, $p < 0.01$ (**C**) as well as the Sar-Met-SP induced changes in AP shape (**D**).

TABLE 2

Reversal of Sar-Met-SP effect on AP waveform and excitability by NTP in type I neurons

Summary of data from 10 DRG neurons. All parameters were recorded before and during Sar-Met-SP and after NTP in the presence of Sar-Met-SP. One-way analyses of variance followed by Dunnett's post hoc test were used to assess the effect of Sar-Met-SP and NTP. Data in the NTP and control groups were not different.

AP and Excitability Parameters	Control	Sar-Met-SP (1 μ M)	NTP 200 nM
AP overshoot, mV	38.6 $\pm$ 1.4	46.3 $\pm$ 1.7**	40.2 $\pm$ 1.5
AP duration, ms	4.6 $\pm$ 0.2	5.9 $\pm$ 0.3**	4.82 $\pm$ 0.25
AP rate of rise, mV/ms	45.8 $\pm$ 1.0	42.8 $\pm$ 1.7	44.7 $\pm$ 0.9
AP rate of fall, mV/ms	26.7 $\pm$ 0.5	23.3 $\pm$ 0.9**	26.6 $\pm$ 0.8
Amplitude of AHP, mV	6.4 $\pm$ 1.0	5.8 $\pm$ 1.1	7.9 $\pm$ 1.3
τ_{AHP} , ms	135 $\pm$ 21	127.4 $\pm$ 24	142.6 $\pm$ 28
AP threshold, mV	-24.3 $\pm$ 0.8	-24.2 $\pm$ 1.1	-22.7 $\pm$ 0.4
Rheobase, pA	79 $\pm$ 12	64 $\pm$ 1*	87 $\pm$ 11.7
Number of AP (1 $\times$ R)	1.0 $\pm$ 0	1.0 $\pm$ 0	1.0 $\pm$ 0
Number of AP (2 $\times$ R)	1.0 $\pm$ 0	1.3 $\pm$ 0.2	1.16 $\pm$ 0.16
Number of AP (3 $\times$ R)	1.7 $\pm$ 0.3	3.0 $\pm$ 0.5*	1.67 $\pm$ 0.49
Number of AP (420 pA)	2.3 $\pm$ 0.4	3.16 $\pm$ 0.4*	2.5 $\pm$ 0.9

R, rheobase.

*, $P < 0.05$; **, $P < 0.01$, differences between the Sar-Met-SP group and control.

rapidly reversed the membrane depolarization evoked by Sar-Met-SP (Fig. 6A). In type I neurons the reduction in the rheobase (Fig. 6B; Table 2), and the increased firing to suprathreshold stimuli (Table 2) elicited by Sar-Met-SP was rapidly reversed by NTP (200 nM) (Table 2; Fig. 6, B and C). NTP also reversed the Sar-Met-SP induced changes in action potential wave form (Fig. 6D; Table 2).

Pretreatment with 200 nM NTP also blocked the Sar-Met-SP-induced excitation in type I neurons. In five type I neurons pretreated with 200 nM NTP, Sar-Met-SP did not induce changes in rheobase, suprathreshold responses, or action potential wave form (data not shown). On the basis of our control data, three to four neurons among these five neurons (74%) would have been expected to exhibit changes in these properties after application of Sar-Met-SP.

Sar-Met-SP Enhancement of Capsaicin-Induced Ca^{2+} Responses in DRG Neurons Is Blocked by NTP.

Activation of NK-1 or NK-2 receptors by SP or Sar-Met-SP has been reported to enhance TRPV1 activation by capsaicin and decrease TRPV1 desensitization in rat DRG neurons (Zhang et al., 2007; Sculptoreanu et al., 2008). To determine whether NK-1 receptors mediate a similar interaction between capsaicin and Sar-Met-SP in guinea pig DRG neurons, the Ca^{2+} imaging method was used to record the increase in intracellular Ca^{2+} elicited by repeated applications of capsaicin (0.5 μ M, 15-s duration, at 15-min intervals) that induce tachyphylaxis. Capsaicin elicited Ca^{2+} increases in 21% of small- to medium-size (286 of 1339) DRG neurons obtained from four guinea pigs. This percentage of capsaicin-responsive neurons was much lower than that reported in rat DRG neurons (60–70%) (Sculptoreanu et al., 2010a). Using this protocol, the second capsaicin response was significantly smaller in amplitude than the first response (Fig. 7A). However, when 1 μ M Sar-Met-SP was applied in the bath 3 min before the second capsaicin application (as shown in Fig. 7B), the amplitude of the second capsaicin response was significantly larger than that of the second response in control experiments (Fig. 7A), indicating a facilitation of the capsaicin response or a reduction of tachyphylaxis. SP (1 μ M) applied in the same manner also enhanced the second capsaicin response (the ratio of the second to the first response peak was 0.98 ± 0.15 , $n = 28$ cells from 2 guinea pigs). Neither SP or Sar-Met-SP alone evoked a detectable increase in intracellular Ca^{2+} .

When neurons were pretreated with NTP (200 nM) 2 min before Sar-Met-SP application (Fig. 7C), the second capsaicin responses were comparable in amplitude with the responses in the control untreated group ($p > 0.05$). The pooled data obtained from DRG neurons from 4 guinea pigs revealed that NTP completely blocked the enhancement of capsaicin responses induced by Sar-Met-SP (Fig. 7D). NTP (200 nM)

Fig. 7. Sar-Met-SP enhanced the capsaicin (Cap)-induced increase in intracellular Ca^{2+} responses, and this effect was blocked by NTP (200 nM). A, capsaicin increases the F340/F380 ratio, indicating an increase in intracellular Ca^{2+} in a DRG neuron. A second capsaicin application (0.5 μ M for 15 s) (15 min after the first) elicited a reduced response (tachyphylaxis). B, application of Sar-Met-SP (1 μ M) 3 min before the second capsaicin application reduced tachyphylaxis. C, pretreatment with NTP (200 nM) 2 min before the application of Sar-Met-SP suppressed the Sar-Met-SP facilitating effect. D, pooled data from four guinea pigs. The magnitude of tachyphylaxis is indicated by the ratio of the second Ca^{2+} peak vs the first peak. Sar-Met-SP application reduced tachyphylaxis (**, $p < 0.01$), and NTP pretreatment suppressed Sar-Met-SP effect ($p > 0.05$). n is the number of DRG neurons in each group.

alone did not change the amplitude of the capsaicin response (F340/F380 ratios: 1.43 ± 0.05 in the control group, $n = 22$ cells and 1.38 ± 0.04 in the presence of NTP, $n = 20$ cells) or the extent of tachyphylaxis (the ratio of the second to the first response peak: 0.52 ± 0.03 in control, $n = 25$ cells and 0.50 ± 0.03 in the presence of NTP, $n = 17$ cells). We also compared the effect of NTP with that of another selective NK-1 antagonist, aprepitant (Green et al., 2006). Aprepitant (1 μ M) blocked the Sar-Met-SP enhancement of capsaicin responses (the ratio of the second to the first response peak: 0.56 ± 0.04 in control, $n = 14$ cells and 0.82 ± 0.05 , $n = 12$ cells after Sar-Met-SP and 0.58 ± 0.03 , $n = 12$ cells, after Sar-Met-SP when pretreated with 1 μ M aprepitant).

Discussion

This study revealed that Sar-Met-SP, a selective NK-1 receptor agonist increases the excitability of a large percentage of small- to medium-size DRG neurons in the guinea pig and reduces the tachyphylaxis of TRPV1 receptors induced by repeated application of capsaicin. NTP, a selective and potent NK-1 receptor antagonist (Hoffmann et al., 2006), suppressed these effects of Sar-Met-SP. The results suggest that NK-1 receptors in sensory nerves, in addition to those at other sites such as the spinal cord and bladder smooth muscle may play an important role in the generation of overactive bladder and that block of these receptors may contribute to efficacy of NK-1 antagonists in the treatment of OAB.

Since the first report that SP depolarizes rat DRG neurons (Dray and Pinnock, 1982), many studies have focused on the actions of neuropeptides on sensory neurons (Spigelman and Puil, 1990; Inoue et al., 1995; Akasu et al., 1996; Li and Zhao, 1998; Abdulla et al., 2001) and on firing in bladder afferent nerves (Morrison, 1999). In general, SP elicits excitatory actions including membrane depolarization (Spigelman and Puil, 1990; Li and Zhao, 1998), inward current (Inoue et al., 1995; Akasu et al., 1996; Yang et al., 2003), or enhanced

excitability in the absence of a membrane potential change (Abdulla et al., 2001). However, the effects have been variable. In some experiments, SP induced a membrane depolarization in rat DRG neurons (Li and Zhao, 1998), whereas in others studies it did not change membrane potential (Abdulla et al., 2001). Some articles described transient depolarizations (Spigelman and Puil, 1990; Yang et al., 2003), whereas others reported long-lasting depolarizations (Li and Zhao, 1998), which seem to be mediated by an opening of nonselective cation channels or by an inhibition of K^+ channels (Spigelman and Puil, 1990; Inoue et al., 1995). These inconsistencies may depend on many factors including the following: 1) different actions of the three tachykinin receptors (NK-1, NK-2, and NK-3); 2) different species (rat versus guinea pig); and 3) different recording conditions (intracellular versus whole-cell patch-clamp).

Our study indicates that a large percentage (74%) of small-to-medium size L6 to S1 DRG neurons from female guinea pigs express functional NK-1 receptors and that activation of these receptors can increase electrical excitability evident as 1) decreased rheobase, 2) enhanced responses to suprathreshold stimuli, 3) prolonged AP duration and increased AP overshoot and 4) enhanced capsaicin-induced increase in intracellular Ca^{2+} . However, these effects were associated with membrane depolarization in only a subpopulation (<30%) of the neurons. This is different from the effects in guinea pig trigeminal ganglion neurons, of which 75% exhibit a SP-induced depolarization (Spigelman and Puil, 1990). While the Sar-Met-SP-induced depolarization would be expected to enhance the firing of afferent nerves elicited by other stimuli, it alone was not of sufficient magnitude to induce firing or a detectable increase in intracellular Ca^{2+} , suggesting that the depolarization is induced by an unusual ionic mechanism such as the opening of a nonselective cation channel as proposed for the SP-induced depolarization in rat DRG neurons (Inoue et al., 1995).

The enhancement of DRG neuron excitability that occurs in the absence of depolarization must be mediated by another mechanism. In rat DRG neurons, voltage- and current-clamp recording revealed that SP increases the number of action potentials evoked by depolarizing current pulses (i.e., conversion of phasic firing to tonic firing) and that this effect is mediated by suppression A-type K^+ channels (Sculptoreanu and de Groat, 2007). Similar increases in firing were elicited by traditional A-type K^+ channel blockers (4-aminopyridine or heteropodotoxin) and reversed by an A-type K^+ channel opener Sculptoreanu et al., 2008, 2009). However, in the rat DRG neurons, this effect was dependent on the activation of NK-2 receptors (Sculptoreanu et al., 2009). Activation of NK-2 receptors also enhances the firing of mechano-sensitive bladder afferent nerves in the rat (Morrison, 1999), indicating that NK receptors present in dissociated DRG cells are also expressed in peripheral afferent terminals. A reduction in AP threshold and an increase in firing to suprathreshold current pulses has been detected in sensory neurons innervating the bladder (Yoshimura and de Groat, 1999), ileum (Moore et al., 2002), or masseter muscle (Harriott et al., 2006) after inflammation of the target organ. Reductions in A-type K^+ currents contribute to these pathology-induced increases in afferent excitability.

It seems likely that an inhibition of A-type K^+ channels also underlies the Sar-Met-SP-induced increase in excitability in the guinea pig DRG neurons, but the receptors are different in rat and guinea pig. The increased duration of the action potential could be mediated by a similar suppression of A-type K^+ channels or by inhibition of Ca^{2+} -activated K^+ channels (Spigelman and Puil, 1990; Sculptoreanu et al., 2009). However, because Sar-Met-SP did not change the amplitude or the decay of the AHP, both of which depend on Ca^{2+} -activated K^+ channels, it seems likely that inhibition of A-type K^+ channels plays an important role in the effects of NK-1 receptors on the action potential in guinea pig DRG neurons.

The increase in action potential overshoot by Sar-Met-SP may be due to a third mechanism, i.e., enhancement of voltage-gated Na^+ channels. Consistent with this idea, it has been shown in small DRG neurons of the rat that activation of NK-1 receptors increases the amplitude of the tetrodotoxin-resistant sodium current, the main contributor to the upstroke of the AP (Cang et al., 2009). In addition, our preliminary studies using whole-cell voltage-clamp methods in dissociated small- to medium-size guinea pig DRG neurons have revealed that Sar-Met-SP also increases the amplitude of tetrodotoxin-resistant sodium currents and that this effect is blocked by NTP (Sculptoreanu et al., 2010b).

Enhancement of capsaicin-induced opening of TRPV1 channels is a fourth mechanism that might underlie the facilitating effect of Sar-Met-SP on guinea pig DRG neurons. TRPV1 channels are expressed on C-fiber afferent nerve terminals in the bladder wall, and activation of these channels with capsaicin or acidic conditions, such as intravesical acetic acid infusion in vivo experiments (Palea et al., 2010a), can increase afferent firing and induce bladder overactivity (de Groat and Yoshimura, 2009). Thus, activation of NK receptors on primary afferent terminals in the bladder should enhance TRPV1 channel activity. Previous studies in rat DRGs have shown that pretreatment with SP enhances capsaicin-induced currents and that this effect is mediated

by NK-2 receptors and an intracellular signaling mechanism involving protein kinase C (PKC) (Sculptoreanu et al., 2008). On the other hand, in rat DRG neurons PKC has not been linked with the SP suppression of K^+ channels and increased firing evoked by depolarizing current pulses, indicating that multiple intracellular signaling pathways as well as multiple receptors are involved in the actions of neurokinins (Sculptoreanu et al., 2009). Further experiments are needed to determine whether PKC signaling mechanisms are also involved in the NK-1 facilitation of TRPV1 channels in guinea pig DRG.

Application of NTP suppresses bladder hyperactivity induced by intravesical infusion of acetic acid in the guinea pig (Palea et al., 2010a), indicating that NK-1 receptors either in the peripheral or central nervous system play a role in the increased reflex voiding frequency. Because NTP reduces voiding frequency without changing the amplitude of voiding contractions (Palea et al., 2010a), it is likely that the drug acts on the afferent rather than the efferent limb of the micturition reflex. These findings are consistent with previous reports that NK-1 antagonists decrease reflex bladder activity in anesthetized guinea pigs (Yamamoto et al., 2003) and increase bladder capacity without changing voiding efficiency in decerebrate cats (Kamo and Doi, 2001).

NK-1 receptors on bladder afferent terminals may participate in complex sensory mechanisms that regulate bladder activity. The receptors could be activated in an autofeedback manner by SP or other neurokinins released from the afferent terminals but also by neurokinins released from urothelial cells (Birder et al., 2008). Because activation of the receptors induces membrane depolarization and hyperexcitability, the responses of the afferent terminals to mechanical (i.e., bladder stretch) or chemical stimuli (ATP, low pH, or TRPV1 or transient receptor potential ankyrin-1 agonists) would be enhanced. In addition, NK-1-induced facilitation of the opening of voltage-gated Ca^{2+} channels by protein kinase-induced phosphorylation (Sculptoreanu and de Groat, 2003) or by increased overshoot and duration of action potentials would enhance Ca^{2+} influx and further increase neurokinin release from the afferent terminals. Thus, NK-1 receptors could play a key role in a positive feedback, amplification mechanism that enhances sensory input from the bladder and contributes to abnormal reflex bladder activity in guinea pigs. Clinical studies showing that NK-1 antagonists (aprepitant and serlopitant) are effective in treating overactive bladder symptoms (Green et al., 2006; Frenkl et al., 2010) raise the possibility that similar mechanisms exist in humans.

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Authorship Contributions

Participated in research design: Zhang, Pietra, Lovati, and de Groat.

Conducted experiments: Zhang.

Performed data analysis: Zhang and de Groat.

Wrote or contributed to the writing of the manuscript: Zhang, Pietra, Lovati, and de Groat.

References

- Abdel-Gawad M, Dion SB, and Elhilali MM (2001) Evidence of a peripheral role of neurokinins in detrusor hyperreflexia: a further study of selective tachykinin antagonists in chronic spinal injured rats. *J Urol* **165**:1739–1744.

- Abdulla FA, Stebbing MJ, and Smith PA (2001) Effects of substance P on excitability and ionic currents of normal and axotomized rat dorsal root ganglion neurons. *Eur J Neurosci* **13**:545–552.
- Akasu T, Ishimatsu M, and Yamada K (1996) Tachykinins cause inward current through NK1 receptors in bullfrog sensory neurons. *Brain Res* **713**:160–167.
- Birder LA, de Groat WC, and Apodaca G (2008) Physiology of the urothelium, in *Textbook of the Neurogenic Bladder*, 2nd ed (Shick E and Corcos J eds) pp 19–39, Taylor and Francis, London.
- Campi B, Camarda V, Pietra C, Cantoreggi S, and Calo G (2010) Pharmacological characterization of the novel NK1 receptor ligand netupitant at human tachykinin receptors. *Neuropeptides* **44**:539–540.
- Candenas L, Lecci A, Pinto FM, Patak E, Maggi CA, and Pennefather JN (2005) Tachykinins and tachykinin receptors: effects in the genitourinary tract. *Life Sci* **76**:835–862.
- Cang CL, Zhang H, Zhang YQ, and Zhao ZQ (2009) PKCepsilon-dependent potentiation of TTX-resistant Nav1.8 current by neurokinin-1 receptor activation in rat dorsal root ganglion neurons. *Mol Pain* **5**:33.
- Dang K, Bielefeldt K, and Gebhart GF (2005) Differential responses of bladder lumbosacral and thoracolumbar dorsal root ganglion neurons to purinergic agonists, protons, and capsaicin. *J Neurosci* **25**:3973–3984.
- de Groat WC and Yoshimura N (2001) Pharmacology of the lower urinary tract. *Annu Rev Pharmacol Toxicol* **41**:691–721.
- de Groat WC and Yoshimura N (2009) Afferent nerve regulation of bladder function in health and disease. *Handb Exp Pharmacol* **194**:91–138.
- Doi T, Kamo I, Imai S, Okanishi S, Ikeura Y, and Natsugari H (2000) Effects of TAK-637, a tachykinin receptor antagonist, on the micturition reflex in guinea pigs. *Eur J Pharmacol* **395**:241–246.
- Doi T, Kamo I, Imai S, Okanishi S, Ishimaru T, Ikeura Y, and Natsugari H (1999) Effects of TAK-637, a tachykinin receptor antagonist, on lower urinary tract function in the guinea pig. *Eur J Pharmacol* **383**:297–303.
- Dray A and Pinnock RD (1982) Effects of substance P on adult rat sensory ganglion neurones in vitro. *Neurosci Lett* **33**:61–66.
- Frenkl TL, Zhu H, Reiss T, Seltzer O, Rosenberg E, and Green S (2010) A multicenter, double-blind, randomized, placebo controlled trial of a neurokinin-1 receptor antagonist for overactive bladder. *J Urol* **184**:616–622.
- Green SA, Alon A, Ianus J, McNaughton KS, Tozzi CA, and Reiss TF (2006) Efficacy and safety of a neurokinin-1 receptor antagonist in postmenopausal women with overactive bladder with urge urinary incontinence. *J Urol* **176**:2535–2540.
- Harriott AM, Dessem D, and Gold MS (2006) Inflammation increases the excitability of masseter muscle afferents. *Neuroscience* **141**:433–442.
- Hoffmann T, Bös M, Stadler H, Schnider P, Hunkeler W, Godel T, Galley G, Ballard TM, Higgins GA, Poli SM, et al. (2006) Design and synthesis of a novel, achiral class of highly potent and selective, orally active neurokinin-1 receptor antagonists. *Bioorg Med Chem Lett* **16**:1362–1365.
- Inoue K, Nakazawa K, Inoue K, and Fujimori K (1995) Nonselective cation channels coupled with tachykinin receptors in rat sensory neurons. *J Neurophysiol* **73**:736–742.
- Ishizuka O, Mattiasson A, and Andersson KE (1995) Effects of neurokinin receptor antagonists on L-dopa induced bladder hyperactivity in normal conscious rats. *J Urol* **154**:1548–1551.
- Kamo I and Doi T (2001) Effect of TAK-637, a tachykinin NK1-receptor antagonist, on lower urinary tract function in cats. *Jpn J Pharmacol* **86**:165–169.
- Lecci A and Maggi CA (1995) Spinal cord tachykinins in the micturition reflex. *Prog Brain Res* **104**:145–159.
- Lecci A and Maggi CA (2001) Tachykinins as modulators of the micturition reflex in the central and peripheral nervous system. *Regul Pept* **101**:1–18.
- Li HS and Zhao ZQ (1998) Small sensory neurons in the rat dorsal root ganglia express functional NK-1 tachykinin receptor. *Eur J Neurosci* **10**:1292–1299.
- Moore BA, Stewart TM, Hill C, and Vanner SJ (2002) TNBS ileitis evokes hyperexcitability and changes in ionic membrane properties of nociceptive DRG neurons. *Am J Physiol Gastrointest Liver Physiol* **282**:G1045–G1051.
- Morrison J (1999) The activation of bladder wall afferent nerves. *Exp Physiol* **84**:131–136.
- Morrison J, Steers WD, Brading A, Block B, Fry C, de Groat WC, Kakizaki H, Levin R, and Thor K (2002) Neurophysiology and neuropharmacology, in *2nd International Consultation on Incontinence*, pp 83–163, World Health Organization, Geneva, Switzerland.
- Palea S, Guilloateau V, Marc G, Liorens MA, Pietra C, Cantoreggi S, Lovati E, and Lluel P (2010a) Comparison of the effects of netupitant and tolterodine on overactive bladder induced by intravesical acetic acid infusion in anesthetized female guinea-pigs, in *Proceedings of the Meeting of the International Continence Society and the International Urogynecological Association*; 2010 Aug 23–27; Toronto, ON, Canada.
- Palea S, Rekik M, Pietra C, Lovati E, and Lluel P (2010b) Netupitant, a potent and highly selective NK-1 receptor antagonist in the guinea-pig isolated urinary bladder: comparison with aprepitant, in *Proceedings of the Meeting of the International Continence Society and the International Urogynecological Association*; 2010 Aug 23–27; Toronto, ON, Canada.
- Saria A (1999) The tachykinin NK1 receptor in the brain: pharmacology and putative functions. *Eur J Pharmacol* **375**:51–60.
- Sculptoreanu A, Artim DE, and de Groat WC (2009) Neurokinins inhibit low threshold inactivating K⁺ currents in capsaicin responsive DRG neurons. *Exp Neurol* **219**:562–573.
- Sculptoreanu A, Aura Kullmann F, and de Groat WC (2008) Neurokinin 2 receptor-mediated activation of protein kinase C modulates capsaicin responses in DRG neurons from adult rats. *Eur J Neurosci* **27**:3171–3181.
- Sculptoreanu A and de Groat WC (2003) Protein kinase C is involved in neurokinin receptor modulation of N- and L-type Ca²⁺ channels in DRG neurons of the adult rat. *J Neurophysiol* **90**:21–31.
- Sculptoreanu A and de Groat WC (2007) Neurokinins enhance excitability in capsaicin-responsive DRG neurons. *Exp Neurol* **205**:92–100.
- Sculptoreanu A, Kullmann FA, Artim DE, Bazley FA, Schopfer F, Woodcock S, Freeman BA, and de Groat WC (2010a) Nitro-oleic acid inhibits firing and activates TRPV1- and TRPA1-mediated inward currents in dorsal root ganglion neurons from adult male rats. *J Pharmacol Exp Ther* **333**:883–895.
- Sculptoreanu A, Zhang X, Pietra C, Lovati E, Daugherty S, and de Groat WC (2010b) The facilitatory function of neurokinin 1 receptors in rat and guinea pig sensory neurons, in *Proceedings of the Meeting of the International Continence Society and the International Urogynecological Association*; 2010 Aug 23–27; Toronto, ON, Canada.
- Segond von Banchet G, Petersen M, and Schaible HG (1999) Expression of neurokinin-1 receptors on cultured dorsal root ganglion neurons from the adult rat. *Neuroscience* **90**:677–684.
- Seki S, Erickson KA, Seki M, Nishizawa O, Igawa Y, Ogawa T, de Groat WC, Chancellor MB, and Yoshimura N (2005) Elimination of rat spinal neurons expressing neurokinin 1 receptors reduces bladder overactivity and spinal c-fos expression induced by bladder irritation. *Am J Physiol Renal Physiol* **288**:F466–F473.
- Spigelman I and Puil E (1990) Ionic mechanism of substance P actions on neurons in trigeminal root ganglia. *J Neurophysiol* **64**:273–281.
- Szucs P, Polgar E, Spigelman I, Porszasz R, and Nagy I (1999) Neurokinin-1 receptor expression in dorsal root ganglion neurons of young rats. *J Peripher Nerv Syst* **4**:270–278.
- Wu ZZ, Guan BC, Li ZW, Yang Q, Liu CJ, and Chen JG (2004) Sustained potentiation by substance P of NMDA-activated current in rat primary sensory neurons. *Brain Res* **1010**:117–126.
- Yamamoto T, Hanioka N, Maeda Y, Imazumi K, Hamada K, Matsuo M, Manda T, and Mutoh S (2003) Contribution of tachykinin receptor subtypes to micturition reflex in guinea pigs. *Eur J Pharmacol* **477**:253–259.
- Yang YL, Yao KH, and Li ZW (2003) Similarities of SP-, NKA- and NKB-induced currents in rat dorsal root ganglion neurons. *Brain Res* **991**:18–25.
- Yoshimura N and de Groat WC (1999) Increased excitability of afferent neurons innervating rat urinary bladder after chronic bladder inflammation. *J Neurosci* **19**:4644–4653.
- Zhang H, Cang CL, Kawasaki Y, Liang LL, Zhang YQ, Ji RR, and Zhao ZQ (2007) Neurokinin-1 receptor enhances TRPV1 activity in primary sensory neurons via PKCε: a novel pathway for heat hyperalgesia. *J Neurosci* **27**:12067–12077.
- Zhang X, Daugherty SL, and de Groat WC (2010) Activation of CaMKII and ERK1/2 contributes to the time-dependent potentiation of Ca²⁺ response elicited by repeated application of capsaicin in rat DRG neurons. *Am J Physiol Regul Integr Comp Physiol* **300**:R644–R654.
- Zhang X, Pietra C, Lovati E, and de Groat WC (2011) Netupitant, a selective NK-1 receptor antagonist, blocks the excitatory effect of an NK-1 receptor agonist, [Sar⁹,Met(O₂)(11)]-substance P, in guinea pig DRG neurons. *Proceedings of the Society for Neuroscience Annual Meeting*; 2011 Nov 12–16; Washington, DC. Society for Neuroscience, Washington, DC.

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