Nitric Oxide Modulates Ca\textsuperscript{2+} Channels in Dorsal Root Ganglion Neurons Innervating Rat Urinary Bladder

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INTRODUCTION

Nitric oxide (NO) has been identified as a transmitter at various sites in the neural pathways controlling the urogenital organs. In the peripheral nervous system, NO released by parasympathetic nerves mediates penile erection and urethral relaxation (Andersson 1993; Andersson and Persson 1995); whereas NO released in the spinal cord appears to facilitate the hyperactivity of the urinary bladder induced by noxious stimuli. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Yoshimura, Naoki, Satoshi Seki, and William C. de Groat. Nitric oxide modulates Ca\textsuperscript{2+} channels in dorsal root ganglion neurons innervating rat urinary bladder. J Neurophysiol 86: 304–311, 2001. The effect of a nitric oxide (NO) donor on high-voltage-activated Ca\textsuperscript{2+} channel currents (I\textsubscript{Ca}) was examined using the whole cell patch-clamp technique in L\textsubscript{6}–S\textsubscript{1} dorsal root ganglion (DRG) neurons innervating the urinary bladder. The neurons were labeled by axonal transport of a fluorescent dye, Fast Blue, injected into the bladder wall. Approximately 70% of bladder afferent neurons exhibited tetrodotoxin (TTX)-resistant action potentials (APs), and 93% of these neurons were sensitive to capsaicin, while the remaining neurons had TTX-sensitive spikes and were insensitive to capsaicin. The peak current density of nimodipine-sensitive L-type Ca\textsuperscript{2+} channels activated by depolarizing pulses (0 mV) from a holding potential of −60 mV was greater in bladder afferent neurons with TTX-resistant APs (39.2 pA/pF) than in bladder afferent neurons with TTX-sensitive APs (28.9 pA/pF), while the current density of \omega-conotoxin GVIA-sensitive N-type Ca\textsuperscript{2+} channels was similar (43–45 pA/pF) in both types of neurons. In both types of neurons, the NO donor, \textit{S}-nitroso-\textit{N}-acetylpenicillamine (SNAP) (500 \textmu M), reversibly reduced (23.4–26.6%) the amplitude of I\textsubscript{Ca} elicited by depolarizing pulses to 0 mV from a holding potential of −60 mV. SNAP-induced inhibition of I\textsubscript{Ca} was reduced by 90% in the presence of \omega-conotoxin GVIA but was unaffected in the presence of nimodipine, indicating that NO-induced inhibition of I\textsubscript{Ca} is mainly confined to N-type Ca\textsuperscript{2+} channels. Exposure of the neurons for 30 min to 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 \mu M), an inhibitor of NO-stimulated guanylyl cyclase, prevented the SNAP-induced reduction in I\textsubscript{Ca}. Extracellular application of 8-bromo-cGMP (1 mM) mimicked the effects of NO donors by reducing the peak amplitude of I\textsubscript{Ca} (28.6% of reduction). Action potential configuration and firing frequency during depolarizing current pulses were not altered by the application of SNAP (500 \textmu M) in bladder afferent neurons with TTX-resistant and -sensitive APs. These results indicate that NO acting via a cGMP signaling pathway can modulate N-type Ca\textsuperscript{2+} channels in DRG neurons innervating the urinary bladder.
In the present study, we have used dissociated bladder afferent neurons as a model system to evaluate the actions of NO on bladder sensory pathways. In a preliminary report, we showed that NO donors such as sodium nitroprusside and SNAP can suppress HVA Ca\(^{2+}\) channel currents in bladder afferent neurons of the rat (Ozawa et al. 1999; Yoshimura and de Groat 1997a). The present study was conducted to further evaluate the effects of NO on specific types of HVA Ca\(^{2+}\) channels in the two subtypes of bladder afferent neurons. Our results demonstrate that NO can inhibit HVA Ca\(^{2+}\) channels in A\(_\beta\)- and C-fiber bladder afferent neurons, which were classified according to TTX and capsaicin sensitivity. This effect was primarily confined to N-type Ca\(^{2+}\) channels and mediated through a cyclic GMP (cGMP)-dependent mechanism.

**METHODS**

**Animal preparation**

Experiments were performed on adult female Sprague-Dawley rats (150–250 g). Care and handling of animals were in accordance with institutional guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

As previously described (Yoshimura and de Groat 1997b, 1999; Yoshimura et al. 1998), the population of DRG neurons that innervate the urinary bladder were labeled by retrograde axonal transport of a fluorescent dye, Fast Blue (4% wt/vol) (Polyloy, Gross Umstadt, Germany), injected into the wall of the bladder in halothane-anesthetized animals 7 days prior to the dissociation. The dye was injected with a 29 G needle at three to six sites on the dorsal surface of the bladder (5–6 \(\mu\)l per site, total volume of 20–30 \(\mu\)l). Each injection site was washed with saline to minimize contamination of adjacent organs with dye. Particular care was taken to avoid injections into the lumen, major blood vessels, or overlying fascial layers to minimize nonspecific labeling due to dye leakage. No apparent leakage was observed.

**Cell dissociation**

Freshly dissociated neurons from DRG were prepared from halothane anesthetized animals (Yoshimura et al. 1996). Briefly, L\(_4\) and S\(_1\) DRG were dissected and then dissociated in a shaking bath for 25 min at 35°C with 5 ml of Dulbecco’s modified Eagle’s medium (Sigma) containing 0.3 mg/ml trypsin (Type 3, Sigma), 1 mg/ml collagenase (Type 1, Sigma), and 0.1 mg/ml deoxyribonuclease (Type 4, Sigma). Trypsin inhibitor (Type 2a, Sigma) was then added to neutralize the activity of trypsin. Individual DRG cell bodies were isolated by trituration and then plated on poly-L-lysine-coated 35-mm petri dishes.

**Electrical recording**

Dye-labeled primary afferent neurons that innervate the urinary bladder were identified using an inverted phase contrast microscope (Nikon, Tokyo) with fluorescent attachments (UV-1A filter; excitation wave length, 365 nm). Gigahm-seal whole cell recordings were performed at room temperature (20–22°C) on each freshly dissociated labeled neuron in a culture dish that usually contained two to five labeled cells among a few hundred unlabeled neurons. The internal solution used during current-clamp recordings of action potentials contained (in mM) 140 KCl, 5 KCl, 2.5 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES and 10 \(\alpha\)-glucose, adjusted to pH 7.4 with NaOH (340 mOsm). All recordings were made with an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA), and data were acquired and analyzed by pClamp software (Axon Instruments). Cell membrane capacitances were obtained by reading the value for whole cell input capacitance neutralization directly from the amplifier. Durations of action potentials were measured at 50% of the spike amplitude. Thresholds for action potential activation were determined by injection of depolarizing current pulses in 20-pA steps. In current-clamp recordings, data are presented from neurons that exhibited resting membrane potentials more negative than ~40 mV and action potentials that overshoot 0 mV.

After current-clamp recordings, Ca\(^{2+}\) channel currents conducted by Ba\(^{2+}\) ions were isolated by switching the external solution containing (in mM) 5 BaCl\(_2\), 155 tetraethylammonium (TEA)-Cl, 5 4-aminopyridine, and 10 HEPES adjusted to pH 7.4 with TEA-OH (340 mOsm). Using this external solution, high-threshold inward currents [mean peak amplitude; 3.5 ± 0.3 (SE) nA, \(n = 44\) cells] elicited by depolarizing pulses to 0 mV from a holding potential of ~60 mV were almost completely suppressed by 400 \(\mu\)M CdCl\(_2\) with a residual outward current of 21.6 ± 3.9 pA (\(n = 11\)), indicating that contamination by K\(^{+}\) currents was minimal under this condition. Cells in which excessive rundown was observed in a control period ranging up to 5 min were excluded from the experiments. In voltage-clamp recordings, the filter was set to ~3 dB at 2,000 Hz. Leak currents were subtracted by P/4 pulse protocol and the series resistance was compensated by 60–80%. All recordings were performed within 12 h after dissociation.

TTX, nimodipine, and SNAP were applied to neurons by superfusion of the external solution containing each drug. Capsaicin (1 \(\mu\)M) and \(\omega\)-conotoxin GVIA (3 \(\mu\)M) were directly applied to the cells by pressure ejection (Picospitzer, General Valve, Fairfield, NJ) through a glass pipette (10–20 \(\mu\)m tip diameter, 500 ms at 5–10 psi). Inward shift of holding currents in voltage-clamp recordings was observed in capsaicin-sensitive cells. Capsaicin (Sigma) was dissolved in the normal external solution containing 10% alcohol and 10% Tween 80 at a concentration of 5 mM and then diluted in the external solution prior to experiments. No effects were detected by application of alcohol and Tween 80 in concentrations as high as 0.2%. SNAP (Sigma) and nimodipine (RBI) were first dissolved in 100% DMSO at a concentration of 500 mM, diluted in the external solution (final DMSO concentration; 0.1%). DMSO alone up to 0.2% had no effects on Ca\(^{2+}\) channel currents. SNAP and nimodipine were protected from light during the experiments.

All data are expressed as means ± SE. The data were analyzed by the Mann-Whitney \(U\) test. A level of \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

**L- and N-type Ca\(^{2+}\) channel currents in bladder afferent neurons**

As noted in previous experiments (Yoshimura 1999; Yoshimura and de Groat 1997a; Yoshimura et al. 1996), bladder afferent neurons could be divided into two populations according to the sensitivity of their action potentials (APs) to TTX (1 \(\mu\)M). Approximately 73% of bladder afferent neurons (30 of 41 neurons) exhibited long-duration (7.9 ± 0.5 ms) APs that were resistant to TTX in a concentration up to 6 \(\mu\)M; while the remaining 11 bladder afferent neurons exhibited APs that were reversibly blocked by 1 \(\mu\)M TTX (Fig. 1) (Yoshimura 1999; Yoshimura et al. 1996). In the neurons with TTX-resistant APs, the mean threshold of APs activated by depolarizing current pulses was ~20.4 ± 0.9 mV. This type of neuron was small in size with mean diameter and cell input capacitance of 23.6 ± 1.3 \(\mu\)m and 28.2 ± 1.8 pF, respectively. In 30 bladder...
afferent neurons with TTX-resistant APs, capsaicin application (1 mM) produced inward currents (1.26 ± 0.12 nA, range; 0.4 – 2.8 nA) at the holding potential of -60 mV in 28 neurons (93%), while the remaining 2 neurons were not sensitive to capsaicin. In contrast, the second population of bladder afferent neurons which exhibited TTX-sensitive APs was significantly (P < 0.01) larger in size (diameter, 30.6 ± 1.8 μm and cell input capacitance, 38.2 ± 2.0 pF, n = 11) and had shorter (P < 0.05) duration (5.4 ± 0.5 ms) APs that were activated at significantly (P < 0.05) lower thresholds (-226.1 ± 0.9 mV) than those in TTX-resistant neurons. Only one of these neurons was sensitive to capsaicin (1.6 nA).

After examining action potential properties, HVA Ca²⁺ channel currents carried by Ba²⁺ ions were evaluated in voltage-clamp recordings by switching to an external solution (described in METHODS) that suppressed Na⁺ and K⁺ currents. In bladder afferent neurons with TTX-resistant spikes (n = 30), L- and N-type Ca²⁺ channel blockers [nimodipine (3 μM) and ω-conotoxin GVIA (3 μM), respectively] inhibited 35.6 ± 3.2 and 39.2 ± 2.9% of the total HVA Ca²⁺ channel currents evoked by depolarizing pulses to 0 mV from the holding potential of -60 mV (Fig. 1). In TTX-sensitive bladder afferent neurons (n = 11), the suppression of HVA Ca²⁺ channel currents by nimodipine and ω-conotoxin GVIA averaged 25.8 ± 3.0 and 40.3 ± 2.9%, respectively (Fig. 1). In addition, the peak current density of L-type channels at 0 mV in TTX-sensitive bladder neurons was significantly (P < 0.05) smaller (28.9 ± 4.1 pF/pA) than in TTX-resistant bladder neurons while there was no significant difference in N-type Ca²⁺ channel current density in bladder afferent neurons with TTX-resistant and -sensitive spikes (43.2 ± 3.6 and 45.1 ± 4.8 pF/pA, respectively).

Effects of SNAP on HVA Ca²⁺ channel currents

When an NO donor, SNAP (500 μM), was applied in the external solution, the HVA Ca²⁺ channel currents evoked by depolarizing pulses to 0 mV from the holding potential of -60 mV was reversibly suppressed in all bladder afferent neurons tested (range; 10 – 40% of the peak current amplitude, n = 22 cells). This concentration of SNAP was selected based on a previous study that showed that concentrations ranging 100 – 500 μM produced significant effects on neuronal Ca²⁺ channels (Chen and Schofield 1995; Pehl and Schmid 1997). The suppression of Ca²⁺ channel currents started within 20 s after SNAP application, reached a plateau in 4 – 5 min, and recovered after washout (Fig. 2). The reduction of peak amplitude of Ca²⁺ channel currents did not differ statistically between TTX-resistant (23.4 ± 2.6%, n = 16) and TTX-sensitive neurons (26.6 ± 3.1%, n = 6). SNAP did not change the current-
voltage relationship of HVA Ca\(^{2+}\) channel currents in all 22 neurons (Fig. 2C). The reproducibility of SNAP-induced suppression of Ca\(^{2+}\) channel currents was tested in six neurons, in which a second application of SNAP induced a suppression of Ca\(^{2+}\) channel currents similar to that evoked by the first application.

Effect of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ)

To test whether SNAP inhibition of Ca\(^{2+}\) channel currents is associated with activation of guanylyl cyclase and production of cGMP, the effect of SNAP on Ca\(^{2+}\) channel currents was tested in the presence of ODQ, an NO-selective guanylyl cyclase inhibitor (Garthwaite et al. 1995). ODQ was applied to bladder afferent neurons prior to SNAP application. Following a 30 min incubation with ODQ (10 \(\mu M\)), the peak current density of HVA-Ca\(^{2+}\) channel currents in ODQ-treated bladder afferent neurons (109.0 ± 9.5 pA/pF, \(n = 7\)) was not different from the current density in untreated bladder afferent neurons (112.0 ± 5.6 pA/pF; Fig. 3). In addition, in ODQ-treated bladder afferent neurons SNAP did not suppress HVA-Ca\(^{2+}\) channel currents activated at 0 mV from the holding potential of −60 mV, indicating that ODQ suppressed the inhibitory effects of SNAP on Ca\(^{2+}\) channel currents (Fig. 3).

Effect of 8-bromo-cGMP

Experiments were also performed to investigate whether 8-bromo-cGMP mimicked the effect of SNAP on HVA-Ca\(^{2+}\) channel currents in bladder afferent neurons. When 1 mM 8-bromo-cGMP, a membrane permeable agent, was applied in
the external solution, Ca\(^{2+}\) channel currents evoked at 0 mV from the holding potential of −60 mV were suppressed by 28.6 ± 1.1% (n = 7 bladder afferent neurons; Fig. 4). The suppression of Ca\(^{2+}\) channel currents started within 20 s after 8-bromo-cGMP application and reached a maximum in 3 min.

**Channel subtypes involved in NO-mediated suppression of Ca\(^{2+}\) channel**

SNAP-induced suppression of Ca\(^{2+}\) channel currents was examined in the presence of nimbodine or ω-conotoxin GVIA to block L- and N-type Ca\(^{2+}\) channels, respectively, in bladder afferent neurons with TTX-resistant spikes. Figure 5A shows that the amount of HVA Ca\(^{2+}\) channel currents reduced by SNAP (500 μM) was similar before and after inhibition of L-type channels by nimbodine (3 μM). A comparison of the currents in the presence of SNAP before and after nimbodine application, indicated that only a small proportion (4.9 ± 0.7%, n = 6) of L-type Ca\(^{2+}\) channel currents were suppressed by SNAP (Fig. 5B). On the contrary, the reduction of Ca\(^{2+}\) channel currents by SNAP was smaller after an application of ω-conotoxin GVIA (3 μM; Fig. 6). The suppression of N-type Ca\(^{2+}\) channel currents by SNAP estimated by subtraction of currents before and after application of ω-conotoxin averaged 54.2 ± 3.2% (n = 7 bladder afferent neurons; Fig. 6B), indicating that SNAP preferentially suppressed N-type Ca\(^{2+}\) channel currents.

**Effects of SNAP on AP characteristics**

Table 1 summarizes the effects of SNAP (500 μM) on AP characteristics in bladder afferent neurons. In neurons with TTX-resistant (n = 12) and TTX-sensitive spikes (n = 5), SNAP did not influence resting membrane potential, spike threshold, spike duration and amplitude, or firing frequency during long (500 ms) depolarizing current injection.

**DISCUSSION**

The present results indicate that application of an NO donor to bladder DRG cells can reversibly suppress HVA Ca\(^{2+}\) channel currents and that this effect is confined primarily to N-type Ca\(^{2+}\) channels. Furthermore the NO-induced suppression of Ca\(^{2+}\) currents appeared to be mediated by a cGMP-dependent mechanism since membrane permeable cGMP mimicked the effect of NO donors and NO-mediated suppression of Ca\(^{2+}\) channel currents was prevented by ODQ, an inhibitor of NO-dependent guanylyl cyclase.

It has been reported that NO exerts multiple actions on various types of voltage-sensitive ion channels through generation of cGMP and/or through mechanisms independent of cGMP. For example, NO donors enhance several types of Ca\(^{2+}\)-activated or ATP-dependent K\(^{+}\) currents in smooth muscle (Koh et al. 1995) but inhibit K\(^{+}\) currents in neurons from ciliary ganglia (Cetiner and Bennett 1993). It has also been reported that NO suppressed TTX-resistant and-sensitive Na\(^{+}\) channels in baroreceptor afferent neurons from the nodose ganglion by direct interaction of NO with the channels (Li et al. 1998). In other studies, NO donors increased voltage-dependent Ca\(^{2+}\) currents in rat sympathetic neurons (Chen and Schofield 1995) but inhibited Ca\(^{2+}\) currents in ciliary ganglia (Khurana and Bennett 1993). In DRG neurons from mice and embryonic chicks, NO donors such as sodium nitroprusside and SNAP (Ward et al. 1994) or a direct application of authentic NO (Kostyuk and Solovyova 1998) inhibited voltage-dependent Ca\(^{2+}\) currents, although these studies were performed in unidentified cells and the subtypes of HVA Ca\(^{2+}\) channels suppressed were not investigated. Our data support the latter results and further establish that the NO-evoked inhibition is mediated by a cGMP-dependent signaling pathway that targets N-type Ca\(^{2+}\) channels in a specific population of visceral afferent neurons innervating the urinary bladder.

Voltage-sensitive Ca\(^{2+}\) channels are divided into HVA and low-voltage-activated types according to their voltage thresholds for activation. HVA channels are further classified into L, N, P/Q, and R subtypes based on electrophysiological and pharmacological properties (Catterall 1998; Waterman 2000). Bladder afferent pathways consist of Aδ and C fibers that have different physiological and pharmacological properties (Yoshimura 1999; Yoshimura and de Groat 1997c). TTX resistance and capsaicin sensitivity occur primarily in small-sized C-fiber afferent neurons; whereas TTX-sensitive Na\(^{+}\) channels and capsaicin resistance are properties of the larger Aδ afferent cells (Yoshimura 1999; Yoshimura and de Groat 1999). The present study revealed that N- and L-type channels account for approximately 70% of the total HVA Ca\(^{2+}\) current in bladder afferent neurons and that the density of L-type channel currents in TTX-resistant bladder afferent neurons is larger than in TTX-sensitive neurons. Although P/Q channel subtypes sensitive to agonists were not investigated, it seems reasonable to conclude that N and L channels are major subtypes of HVA.
Ca\(^{2+}\) channels in both types of bladder afferent neurons, expression of L-type Ca\(^{2+}\) channels is greater in C-fiber bladder afferent neurons than in A\(\delta\)-fiber neurons, and the proportion of N-type channels is similar in the two types of neurons. This is in line with previous findings in unidentified DRG neurons in which L-type Ca\(^{2+}\) currents were more prominent in small-diameter DRG neurons than in medium/large diameter neurons (Scroggs and Fox 1992).

While it has been documented that low-voltage-activated T-type channels are important in controlling cell excitability, HVA Ca\(^{2+}\) channels are known to be involved in neurotransmitter release in nerve terminals (Catterall 1998; Waterman 2000). It has been postulated that Ca\(^{2+}\) entry via N-type channels mediates release from small vesicles containing classical transmitters such as norepinephrine while Ca\(^{2+}\) influx through L-type channels may favor release from large dense core vesicles containing neuropeptides (Hirning et al. 1988). Since C-fiber afferent nerves likely contain neuropeptides such
as substance P or CGRP, the prominence of L-type currents in C-fiber bladder afferent neurons with TTX-resistant spikes very likely contributes to peptide release from the nerve terminals. However, recent studies have demonstrated that N-type Ca$^{2+}$ channels in sensory nerves including bladder afferents are also important for the release of neuropeptides induced by electrical stimulation or application of bradykinin, whereas K$^+$-induced release was dependent on L-type channels (Maggi et al. 1989, 1990; Waterman 2000). Therefore NO-mediated suppression of N-type Ca$^{2+}$ channels raises the possibility that NO might modulate the release of various transmitters from afferent nerve terminals in the bladder. If this occurs, it seems likely that both A#$\Delta$- and C-fiber bladder afferent nerves would be affected because SNAP suppressed HVA Ca$^{2+}$ currents in both TTX-resistant and -sensitive neurons.

An action of NO on afferent nerve terminals is consistent with the results of our recent in vivo study, which revealed that NO donors administered into the bladder suppressed bladder hyperactivity elicited by CYP-induced cystitis in the rat (Ozawa et al. 1999). Because the bladder smooth muscle in the rat is relatively insensitive to NO (Andersson 1993), it was proposed that the depressant effect of the NO donor was due to an inhibitory action on afferent nerves that were sensitized by the cystitis. However, in the present experiments, SNAP did not alter the resting membrane potential or the threshold for initiation of AP, suggesting that NO may not directly affect firing properties of bladder afferent nerves but may act indirectly by inhibiting N-type Ca$^{2+}$ channels and in turn the release of afferent neuromodulators that amplify the inflammatory conditions in the bladder (Burnstock 1999; Maggi et al. 1989).

NO might be released from multiple sites in the bladder. The NO-synthesizing enzyme, nitric oxide synthase (NOS), and NADPH diaphorase, a chemical marker for NOS, are localized in afferent and efferent neurons innervating the urinary tract (Vizzard et al. 1993). At least three types of NOS have been identified. Neuronal (nNOS) and endothelial NOS (eNOS), which are constitutive and Ca$^{2+}$/calmodulin-dependent, release NO for short periods in response to stimulation. The other enzyme is inducible (iNOS), is Ca$^{2+}$-independent, and, once expressed, generates large amounts of NO for long periods (Moncada et al. 1991). NOS activity in the normal bladder is mainly due to the constitutive NOS isoforms (more than 95%). eNOS has been detected in urothelial cells. NO can also be released from these cells by various chemical stimuli (e.g., norepinephrine, acetylcholine, and capsaicin) (Birder et al. 1998). High basal release of NO mediated by iNOS has been detected in bladder strips from rats with CYP-induced cystitis (Birder et al. 1997). Previous studies in rats have also demonstrated that chronic bladder inflammation upregulates the expression of NOS in bladder afferent neurons (Vizzard et al. 1996) and that suppression of NO release in the spinal cord by a NOS inhibitor suppressed cystitis-induced bladder hyperactivity (Kakizaki and de Groat 1996; Rice 1995). Thus it seems likely that increased generation of NO in afferent nerve terminals in the spinal cord may be involved in enhancing reflex activity and/or inflammatory responses of the bladder.

However, NO is also proposed as a mediator that suppresses tissue inflammation or injury in visceral organs such as intestine and bladder. For example, experimental studies revealed that NO donors can reduce the severity of gastric and intestinal injury induced by ethanol application or ischemia (Muscara and Wallace 1999). Recent clinical studies also demonstrated that production of NO in the bladder is decreased in patients with interstitial cystitis, a painful bladder syndrome of unknown etiology (Smith et al. 1996), and that orally administered L-arginine, an NO precursor, was effective in reducing irritable bladder symptoms in these patients (Smith et al. 1997). Furthermore, as mentioned in the preceding text, NO donors injected into the bladder inhibited bladder hyperactivity elicited by CYP-induced inflammation, probably due to suppression of afferent pathways (Ozawa et al. 1999). Thus it is reasonable to assume that NO has a dual effect, possibly dependent on concentration or different sites of action (i.e., central or peripheral), to suppress or amplify nociceptive mechanisms (Colasanti and Suzuki 2000). The NO-mediated protective mechanism might involve suppression of N-type Ca$^{2+}$ channels and subsequent neuropeptide release from bladder sensory nerves as suggested from the present study. Therefore manipulation of NO levels or NO-cGMP signaling mechanisms could provide a new therapeutic modality for treating hyperactivity and/or painful sensations in visceral organs such as the urinary bladder.

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### REFERENCES


### Table 1. Effects of SNAP on action potential characteristics of bladder afferent neurons

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<th>TTX-Resistant Neurons</th>
<th>TTX-Sensitive Neurons</th>
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<td><strong>Control</strong> SNAP (500 μM)</td>
<td><strong>Control</strong> SNAP (500 μM)</td>
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<tr>
<td>Resting membrane potential, mV</td>
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<td>Spike threshold, mV</td>
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<td>Spike amplitude, mV</td>
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<td>Firing frequency, spikes</td>
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<td>Data were obtained before (control) and 2 min after application of S-nitroso-N-acetylpenicillamine (SNAP, 500 μM). Firing frequency was expressed as the number of spikes elicited by 500-nS depolarizing current pulses. Values are means ± SE. n = 12 and 5 for TTX-resistant and TTX-sensitive neurons, respectively.</td>
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INHIBITION OF Ca\(^{2+}\) CURRENTS BY NO IN DRG NEURONS


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