Effects of Nitric Oxide in Cultured Prevertebral Sympathetic Ganglion Neurons

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ABSTRACT

The effects of the nitric oxide donor, S-nitrosoacetylpenicillamine (SNAP), were tested on cultured dissociated guinea pig celiac ganglion neurons using whole cell patch-clamp recordings. S-nitrosoacetylpenicillamine induced a concentration- and voltage-dependent inwardly directed shift in holding current (inward current shift) in 89% of neurons. The inward current shift was prevented by pre-treatment with the nitric oxide scavenger reduced hemoglobin and was abolished by intracellular cesium. The amplitude of the inward current shift was also sensitive to the extracellular potassium concentration. The S-nitrosoacetylpenicillamine-induced inward current shift was mediated by a decrease in calcium-dependent potassium currents (I_{AMP}); apamin (100 nM), charybdotoxin (10 nM) or tetraethylammonium (5 mM) reduced but did not abolish the amplitude of its inward current shift and a combination of apamin and tetraethylammonium abolished the S-nitrosoacetylpenicillamine-induced inward current response. In the presence of extracellular cobalt, SNAP produced an outward current that was concentration- and voltage-dependent, abolished by reduced hemoglobin and extracellular cesium and reduced by 4-AP (1 mM); in the absence of cobalt, 4-AP increased the SNAP-induced inward current shift. These data indicate that NO exerts dual opposing effects on neuronal potassium conductances, namely an inward current shift mediated through an inhibition of I_{AMP} and induction of an outward current mediated by activation of the potassium delayed rectifier.

Sympathetic neurons in prevertebral ganglia the celiac ganglia (CG), superior mesenteric ganglion (SMG) and inferior mesenteric ganglion (IMG) innervate the mesenteric vasculature and regulate intestinal activities. Functional studies have indicated that prevertebral ganglia act as complex neural networks that are capable of integrating efferent and sensory information and coordinating peripheral reflexes (Miolan and Niel, 1996).

Immunohistochemical studies demonstrate the presence of NOS-IR in nerve terminals in prevertebral ganglia arising from three distinct sources including colonic intestinofugal neurons (Furness and Anderson, 1994; Anderson et al., 1995; Mann et al., 1995), sympathetic preganglionic neurons (Furness and Anderson, 1994; Anderson et al., 1995) and primary sensory afferent neurons whose cell bodies lie in dorsal root ganglia (Zheng et al., 1997b). These distinct sources of NOS-IR in fibers innervating prevertebral ganglia may reflect involvement in the control and modulation of specific functions such as motility and secretory reflexes (Anderson et al., 1995) or blood flow (Zheng et al., 1997b).

With regard to the regulation of the mesenteric vasculature, colonic distention activates capsaicin-sensitive inhibitory reflex pathways that result in a hyperpolarization and relaxation of mesenteric arterial smooth muscle (Meehan and Kreulen, 1992). This may be mediated, at least in part, via the release of nitric oxide from dorsal root ganglion neurons. It is conceivable, therefore, that the NOS-IR primary sensory afferent neurons that provide a direct nitrergic innervation to mesenteric blood vessels also innervate prevertebral sympathetic neurons (Zheng et al., 1997b).

The action of NO on prevertebral ganglion neurons has not been fully characterized. In the mouse intact superior mesenteric ganglion, intracellular recordings have shown that the NO donor, sodium nitroprusside hyperpolarizes the majority of neurons, although a subpopulation of these showed a biphasic response, i.e., a hyperpolarization followed by a depolarization (Mazet et al., 1996). Thus, our purpose was to determine the action of NO on cultured dissociated celiac neurons using whole-cell patch-clamp recordings. Celiac neurons were chosen because of their well-characterized proper-

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ABBREVIATIONS: SNAP, S-nitrosoacetylpenicillamine; NO, nitric oxide; IMG, inferior mesenteric ganglion; CG, celiac ganglion; DRG, dorsal root ganglion; $V_{m}$, membrane holding potential; $E_{m}$, resting membrane potential; $R_{in}$, membrane input resistance; TEA, tetraethylammonium; 4-AP, 4-aminopyridine; ATP, adenosine 3’5’-triphosphate; MEM, minimal essential medium; NOS-IR, nitric oxide synthase-like immunoreactivity.
ties and response to dissociation and culture techniques (Coggan et al., 1991, 1994; Vanner et al., 1993). A preliminary report of this work has been published previously (Zheng et al., 1997a).

**Methods**

**Sympathetic neuronal dissociation.** CG neurons were dissociated and cultured as previously described (Coggan et al., 1991). Briefly, female Duncan-Hartley guinea pigs (200–350 g) were anesthetized with halothane, decapitated and exanguinated. The CG was excised, enzymatically dissociated (papain 9 mg/ml; collagenase 1 mg/ml; dispase 4 mg/ml) and plated as a monolayer on to polylysine coated glass-bottomed 35-mm culture dishes. Cells were maintained in feeding medium (see below) at 37°C in a 5% CO₂ humidified incubator. Two-thirds of the feeding medium was replaced every 3 days.

**Electrophysiological recordings.** Culture dishes containing CG neurons were transferred to the stage of an Olympus IMT-2 inverted microscope and perfused with Krebs’ solution of the following composition (mM): NaCl 118.5; NaHCO₃ 23.8; KCl 4.7; MgCl₂ 1.2; CaCl₂ 2.5; KH₂PO₄ 1.2; glucose 5.5; maintained at pH 7.4 by bubbling with O₂/CO₂ (95%/5%). The temperature was held constant at 37°C. Whole cell patch recordings were made using borosilicate glass pipettes of resistance 3–8 MΩ with the following intracellular solution composition (mM): KCl 144.5; MgCl₂ 2; EGTA (ethylene glycol-bis(β-aminoethylether)N,N,N',N'-tetraacetic acid) 0.5; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 5; ATP (adenosine 5'-triphosphate) 4; GTP (guanosine 5'-triphosphate) 0.25; pH adjusted to 7.35 with KOH. When cesium was used as the current carrier, the following pipette solution was used (mM): Cs-gluconate 140; HEPES 10; EGTA 1; NaCl 16; CaCl₂ 0.3; TEA 20; ATP 4; GTP 0.25; pH adjusted to 7.35 with NaOH.

Electrophysiological recordings were made using either an Axopatch1D or an Axoclamp2A amplifier (Axon Instruments, Foster City, CA). Data were filtered at 2 Hz, digitized via a TL-1 DMA interface (Axon Instruments) and stored on a PC utilizing the Interface software (Axon Instruments). Data analysis was performed using the pClamp6 software (Axon Instruments). All values are quoted as mean ± S.E.M.; significance was set at P < .05.

**Drug delivery.** Drugs were applied by a U-shaped suction-ejection tube (‘cloud-burst’ method; Krishnal and Pidoplichko, 1980; see also Coggan et al., 1994) for time periods sufficient to allow a steady-state response to be obtained. The cloudburst method allows rapid application and removal of drugs to a discrete area, limited to the state response to be obtained. The cloudburst method allows rapid application and removal of drugs to a discrete area, limited to the state response to be obtained. The cloudburst method allows rapid application and removal of drugs to a discrete area, limited to the state response to be obtained. The cloudburst method allows rapid application and removal of drugs to a discrete area, limited to the state response to be obtained. The cloudburst method allows rapid application and removal of drugs to a discrete area, limited to the state response to be obtained.

**Materials.** SNAP was purchased from Molecular Probes, Inc. (Eugene, OR). Papain and collagenase were purchased from Worthington Biochemical Corp. (Freehold, NJ), dispase from Boehringer Mannheim GmbH. (Mannheim, Germany), minimal essential medium and penicillin-streptomycin from Life Technologies (Grand Island, NY), and 6,7-dimethyl-5,6,7,8-tetrahydropyridine from Calbiochem (San Diego, CA). All other cell culture reagents and chemicals were from Sigma Chemical Co. (St. Louis, MO).

**Reduced hemoglobin.** Hemoglobin was reduced in our laboratory with sodium dithionite following the method of Martin et al. (1985). The resulting solution was kept (−20°C) for a period of not longer than 2 wk.

**Results**

Electrophysiological recordings were made from 340 neurons from 46 guinea pigs between one and 6 days after dissociation.

**Neuronal Classification**

Neurons were classified on the basis of their responses to the passing of depolarizing pulses at three times rheobasic strength (holding potential −60 mV; depolarization duration 1 sec, frequency 0.2 Hz). Neurons that fired action potentials only at the onset of the depolarizing current pulses were defined as phasic, whereas tonic neurons fired action potentials throughout the duration of the depolarizing stimulus (Crowcroft and Szurszewski, 1971; Kreulen and Szurszewski, 1979). The passive and active properties of phasic and tonic neurons are summarized in table 1.

**The NO donor, SNAP, induces an inward current shift in the majority of CG neurons.** In neurons that were current clamped at potentials of, or more positive to, −50 mV, application of SNAP produced a 3.0 ± 1.4 mV membrane depolarization (n = 10; range 0–11 mV) that persisted throughout the drug application and decayed slowly after drug removal. Neurons voltage clamped at potentials >−60 mV were found to have a standing outward current. Application of SNAP produced an inward current shift in this holding current, defined as an inward current shift.

SNAP (0.03–10 mM) was applied to 340 neurons (159 phasic, 181 tonic). SNAP produced an inward current shift in 303 (89%) neurons (148 phasic, 155 tonic). A larger proportion of phasic neurons (93%) compared to tonic neurons (86%) responded to SNAP with an inward current shift (Fisher exact test). The responses to SNAP were not found to differ quantitatively between phasic and tonic neurons, however, and were not analyzed separately. Tachyphylaxis occurred to the excitatory effect of SNAP; after washout of SNAP and return of the holding current to pre-drug levels, reexposure of the CG neurons to SNAP within 10 min resulted in a current shift smaller than the initial response. Inward current shifts in response to cloudburst application of high potassium Krebs’ solution were unaffected, indicating that the tachyphylaxis was selective for SNAP. If the interval between exposures to SNAP was 10 min or longer, SNAP induced the same response observed initially. Desensitization to the effect of SNAP was also observed. If the period of SNAP application was longer than 30 sec, the magnitude of the inward current shift declined although SNAP was still present.

**TABLE 1**

<table>
<thead>
<tr>
<th>Basic properties of cultured dissociated celiac ganglion neurons</th>
<th>Phasic (n = 25)</th>
<th>Tonic (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eₘₑ (mV)</td>
<td>−54 ± 1.6</td>
<td>−48 ± 1.0ₐ</td>
</tr>
<tr>
<td>Rₑ (MΩ)</td>
<td>242 ± 36</td>
<td>636 ± 56ₐ</td>
</tr>
<tr>
<td>Action potential amplitude (mV)</td>
<td>75 ± 2.5</td>
<td>82 ± 1.8ₐ</td>
</tr>
<tr>
<td>Action potential threshold (mV)</td>
<td>−34 ± 1.1</td>
<td>−37 ± 0.8ₐ</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. ₐ Significantly different from phasic neurons.
Characterization of the Response to SNAP

Concentration dependency. The steady-state inward current shifts to SNAP were concentration-dependent (fig. 1). A maximum response to SNAP could not, however, be obtained because at concentrations higher than 10 mM, SNAP-induced an irreversible current shift with a concomitant loss of seal integrity. The response to 10 mM SNAP was taken, therefore, as maximal and the other data points normalized to this point.

Current/voltage relationship. The inward current shifts to SNAP were voltage dependent within the range −60 mV to 0 mV (fig. 2). No reversal of the SNAP-induced current shift was observed upon hyperpolarization, even at potentials of up to −100 mV.

The inward current shift obtained on SNAP perfusion was of similar magnitude independently of the protocol, i.e., ramp depolarization or cloudburst application to neurons voltage clamped at specified potentials.

The absolute amplitude of the SNAP-induced inward current shifts varied widely between neurons; the mean inward shift of cells voltage-clamped at 0 mV to applications of 10 mM SNAP was −314 ± 34.5 pA (range −132 to −589 pA; n = 37). The amplitude of the response was correlated, however, with the slope conductance of the cells in control conditions calculated between −30 mV and −60 mV (First order regression correlation co-efficient $r^2 = 0.89$).

SNAP effect is prevented by hemoglobin. Pretreatment with the NO-scavenger, reduced hemoglobin (20 μM), attenuated the SNAP-induced inward current shift ($n = 8$; fig. 3A) implying that the SNAP-induced response is mediated via NO generation. The average reduction of the inward current shift induced to application of 10 mM SNAP was 72 ± 9% ($n = 6$; range 62–100%).

SNAP-induced current is mediated by alterations of neuronal potassium currents. Decreasing the extracellular potassium from 5.9 to 2.5 mM increased the amplitude of the maximum inward current shift at 0 mV from $−277 ± 18$ pA to 10 mM SNAP in control (5.9 mM extracellular potassium concentration; $n = 3$; range $−242$ to $−300$ pA) to $−394 ± 5.0$ pA in 2.5 mM extracellular potassium ($n = 3$;

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**Fig. 1.** SNAP-induced inward current shift is concentration-dependent. A, Cloudburst applications of SNAP produced a concentration-dependent inward current shift in a neuron voltage clamped at −30 mV. The effect was reversible after wash-out of SNAP. B, Concentration-response curve for the SNAP-induced inward current shift in neurons voltage-clamped at −30 mV. Each point represents data from 4 to 24 neurons. For data to be used each neuron was tested with at least three concentrations of SNAP, one of which was 10 mM; a minimum of 10 min elapsed between successive drug applications. All points were normalized to the response produced to 10 mM SNAP.

**Fig. 2.** SNAP-induced inward current shift is dependent on voltage and neuronal conductance. A, Steady-state current/voltage plot obtained from a ramp depolarization from −100 to 0 mV. In the presence of 10 mM SNAP, the amplitude of the steady-state current was reduced. The net SNAP-induced current shift was obtained by subtracting the control current from the SNAP current (see Ba). All subsequent current/voltage plots of the SNAP-induced current shift were obtained with this subtraction method. Ba, Net inward current shift induced by SNAP using a ramp depolarization protocol. Bb, Inward current shift induced by SNAP at different holding potentials. The traces have been obtained from the same neuron as in A. Note that the amplitude of the current shifts obtained with the ramp depolarization protocol (Ba) are similar to the amplitudes obtained with the cloudburst applications at different holding potentials (Bb). C, Neuronal conductance (nS; calculated between −60 and −30 mV in control conditions) plotted against the amplitude of the inward current induced by 10 mM SNAP applied to neurons voltage clamped at −30 mV. First order regression correlation co-efficient $r^2 = 0.89$. 
range –384 to –400 pA). Conversely, increasing the potassium concentration to 10 mM decreased the inward current shift to –168 ± 21.1 pA (n = 3; range –143 to –210 pA). Addition of cesium (5 mM) to the superfusing Krebs’ solution abolished the SNAP-induced current shift (fig. 3Ba). This indicated that the SNAP-induced inward current was mediated via inhibition of neuronal potassium conductance(s).

The actions of SNAP to alter neuronal potassium conductances were confirmed in a series of experiments where the nonselective potassium channel blocker, cesium, was substituted for potassium as the intracellular ion carrier. The SNAP-induced inward current shift was produced while recording with a KCl electrode. The cell was allowed to recover, then Cs-glucuronate was exchanged for KCl in the patch pipette. The electrode was allowed to settle (10–30 min) before the response to SNAP was retested. Replacement of KCl with Cs-glucuronate prevented the inward current shift to SNAP in 6/6 neurons tested (fig. 3Bb)

**Characterization of SNAP effects on ionic currents.** To determine the ionic currents involved in the SNAP-induced inward current shift, the effects of SNAP were studied under both current- and voltage-clamp conditions.

**SNAP does not affect the M-current:** Cells were voltage clamped at –30 mV then step hyperpolarized by increments of 10 mV for a duration of 2.3 sec every 2 to 18 sec until a final voltage of –110 mV was reached. These step hyperpolarizations and depolarizations back to the holding potential produced the inward- and outward-going current relaxations characteristic of the M-current (Vanner et al., 1993; Coggan et al., 1994). SNAP (10 mM) was not found to affect the amplitude of the inward current relaxation associated with IM. Measurement of IM from –30 to –10 mV (this voltage step induced the maximum IM amplitude in our experimental protocol) indicated that in the presence of SNAP, IM was not significantly different from control values (control = 80.5 ± 8.1 pA, range 25.1 to 99.3 pA; SNAP = 77.9 ± 10.4, range 20.0 to 103.2; P > .05; n = 6). In contrast, however, addition of 2 mM barium to the superfusing Krebs’ solution reduced the inward current relaxation from 62.6 ± 9.9 pA (range 31.2 to 70.2 pA) to 25.7 ± 4.2 pA (range 21.2 to 29.3 pA; P < .05; n = 4) confirming the inward current relaxation as IM (data not shown).

**SNAP does not affect the A-current:** SNAP does not affect the A-current (IA) because cloudburst applications of SNAP (0.1–10 mM) in cells voltage clamped between –30 and 0 mV induced large inward current shifts (see fig. 1); under these conditions IA would be inactivated. In addition, substitution of Cs+ for K+ as the ion carrier in the patch pipette completely abolished the SNAP-induced inward current shift; [Cs+]i does not block IA (Hille, 1992).

**SNAP decreases the after hyperpolarization:** The effect of SNAP was tested on the calcium-dependent after-hyperpolarization after action potential firing. Neurons were current clamped at –50 mV and action potential firing was induced by injection of direct depolarizing current. SNAP was then applied for a sufficient period to evoke a steady state membrane depolarization; current was injected to return the holding potential to –50 mV before action potentials were induced as before. SNAP (10 mM) decreased both the amplitude and the duration of the after-spike hyperpolarization (fig. 4A). Because the number of action potentials that are fired in response to a depolarizing stimulus varied from neuron to neuron, measurements on the amplitude and duration of the after-spike hyperpolarization were made between two arbitrarily chosen action potentials, in this instance the fifth and sixth action potentials. Because the action potentials did not always undershoot –50 mV, the potential at which the neuron was current clamped, the amplitude of the spike repolarization was measured from the peak of the action potential to the point at which it began to depolarize. In the presence of SNAP (10 mM) the amplitude of this action potential repolarization decreased by an average of 23% from 96 ± 6 mV (range 76 to 100 mV) to 75 ± 11 mV (range 57 to 98 mV; n = 5), an effect known to be mediated, at least in part, by BK, charybotoxin sensitive potassium channels (Sah and McLachlan, 1992). At the same
time, SNAP (10 mM) decreased the interspike interval by an average of 7% from 61.8 ± 12.8 msec (range 41 to 69 msec) to 56.4 ± 10.9 msec (range 38 to 63 msec; n = 6; fig. 4B).

In 20/20 neurons SNAP reduced the time constant of the after-burst hyperpolarization from 365.4 ± 39.7 msec (range 176 to 650 msec) in control to 253.6 ± 32.9 msec (range 70 to 554 msec) in 10 mM SNAP (P < .05; fig. 4B). Conversely, in only 11 of these neurons SNAP (10 mM) reduced the amplitude of the after-burst hyperpolarization from 10.7 ± 0.9 mV (range 6.7 to 17.1 mV) to 8.0 ± .8 mV (range 3.4 to 13.6 mV) (P < .05). Application of apamin (100 nM; n = 5) abolished the after-hyperpolarization after trains of action potentials (after-burst hyperpolarization) identifying this as the intermediate IAHP (data not shown; Sah and McLachlan, 1991; Vanner et al., 1993).

In 9/9 neurons, however, apamin (100 nM) reduced, but did not abolish, the maximal inward current shift induced by SNAP (10 mM) in the ramp protocol (fig. 4D). Perfusion with the calcium-dependent potassium channel (BK) antagonist, charybdotoxin (10 nM; n = 7), antagonized the SNAP-induced inward current shift to the same extent as TEA (5 mM; n = 7; fig. 4C). These data thus suggest that the inward current shift induced by SNAP was via inhibition of both the intermediate apamin-sensitive after-hyperpolarization and the TEA- and charybdotoxin-sensitive BK after-hyperpolarization. In fact, pretreatment with a combination of apamin and TEA abolished completely the SNAP-induced inward current shift (n = 4; fig. 4D).

**SNAP-induced outward current.** The addition of the nonselective voltage-dependent calcium channel blocker, cobalt (2 mM), to the superfusing Krebs’ solution was expected to mimic the response seen with TEA and apamin but, in

Fig. 4. SNAP reduces the after-hyperpolarization currents. A, Perfusion of SNAP (10 nM) reduced the duration and amplitude of the after-hyperpolarization following bursts of action potential firing. Previous exposure to apamin (100 nM) abolished this after-burst hyperpolarization identifying it as the intermediate after-hyperpolarization current (data not shown). The depolarization observed after SNAP application was offset by injection of DC current necessary to return the holding voltage to the control value (–50 mV). Control = black trace; SNAP = gray trace. B, Expanded trace from A. Perfusion of SNAP (10 nM) reduced the amplitude of the hyperpolarization following individual action potentials, an effect mediated by charybdotoxin-sensitive BK channels, as well as reducing the inter-spike interval, an effect mediated by apamin-sensitive SK channels. Control = black trace; SNAP = gray trace. C, Previous exposure to TEA (5 mM) reduced but did not abolish the inward current shift induced by SNAP (10 mM; n = 7). The magnitude of this reduction in the SNAP-induced current shift by TEA was similar to that in the presence of charybdotoxin (10 nM; n = 7). D, Previous exposure to apamin (100 nM) reduced but did not abolish the inward current shift induced by SNAP (10 mM; n = 5). A combination of apamin (100 nM) and TEA (5 mM) was required to completely abolish the SNAP-induced inward current shift (n = 4). *Significantly different from control; P < .05.

Fig. 5. In the presence of cobalt, SNAP induces an outward current shift that is mediated by NO and gK. A, Left panel: Inward current shift induced by application of 10 mM SNAP. Right panel: In the same neuron, in the presence of extracellular cobalt (2 mM), cloudburst application of SNAP induced an outward current shift (Vh = –30 mV). B, The outward current shift to SNAP (10 mM) was voltage-sensitive and abolished by perfusion of extracellular cesium (5 mM; n = 3); C, The outward current shift to SNAP (10 mM) in the presence of cobalt was abolished by prior exposure to reduced hemoglobin (20 μM; n = 5). *Significantly different from control; P < .05.
stead, in 26/26 neurons to which cobalt was applied, the application of SNAP resulted in the induction of an outward current (fig. 5A). In the presence of cobalt, at 0 mV, the amplitude of the outward current shift was $211 \pm 40\ pA$ (range 62 to 558 pA; $n = 26$).

This SNAP-induced outward current was abolished by addition of cesium (5 mM) to the superfusing Krebs solution (fig. 5C). This implied that the outward current induced by SNAP in the presence of cobalt was mediated via NO-release acting to alter neuronal potassium conductances ($gK$s).

Hence, the outward SNAP-induced current is present in cobalt-containing Krebs solutions, that is to say, when $I_{AHP}$ are not present. Such an outward current is still present at holding potentials between $-30$ and 0 mV ruling out involvement of $I_A$. Possible neuronal potassium conductances that SNAP could be acting to increase and that are blocked by TEA but not by cobalt include the delayed rectifier ($I_{K_V}$) and ATP-sensitive potassium currents ($I_{KATP}$; Hille, 1992).

**SNAP increases the delayed rectifier current.** The delayed rectifier current was studied in the presence of tetrodotoxin (100 nM) and cobalt (1 mM). Neurons were voltage clamped at $-50\ mV$ and then stepped (250 msec) between $-60\ mV$ and $+20\ mV$ before being returned to the holding voltage of $-50\ mV$.

In 8/8 neurons, SNAP increased the delayed rectifier current (fig. 6A) by $22.4 \pm 3.8\%$ ($222 \pm 51\ pA$; range 53 to 495 pA), a value similar to the SNAP-induced outward current ($211 \pm 40\ pA$). This SNAP-induced increase in the delayed rectifier current was partially attenuated by prior exposure to 4-AP at concentrations suggested to be selective for the potassium delayed rectifier in other sympathetic neurons (1 mM; Marsh and Brown, 1991). In the presence of 4-AP, the SNAP-induced increase in the delayed rectifier was reduced by approximately 75% (to $45 \pm 14\ pA$ range 7–64 pA; $n = 3$).

The outward current induced by SNAP in the presence of 1 mM extracellular cobalt was reduced by 4-AP in 7/7 neurons. Operating on the assumption that the inward current shift and the outward current opposed each other, we tested whether blockade of the outward current with 4-AP would increase the magnitude of the inward current shift. The SNAP-induced inward current shift at 0 mV was increased in the presence of 4-AP by $148 \pm 18\ pA$ (range 121–183 pA) suggesting that the magnitude of the inward current shift was attenuated by the activation of a 4-AP-sensitive outward current. This is further corroborated by the fact that the size of the increase in the amplitude of the inward current produced by 4-AP is similar to the size of the reduction in amplitude of the outward current produced by the same concentration of 4-AP ($95 \pm 16\ pA$ at $mV$; range 66 to 120 pA; fig. 6B).

**Discussion**

We report that the NO-donor SNAP produced an inward current shift in the majority (89%) of cultured dissociated guinea pig celiac ganglion neurons. This action of SNAP was mediated via NO because the response was abolished by prior exposure to the NO-scavenger, reduced hemoglobin and via a decrease in neuronal potassium conductances because of its block by intra- and extracellular cesium and its sensitivity to the extracellular potassium concentration. The SNAP-induced inward current shift was mediated through an inhibition of after-hyperpolarization currents because apamin, charybdotoxin or TEA attenuated the response; a combination of apamin and TEA was required to block the response. Blockade of $I_{AHP}$ by cobalt unmasked a SNAP-induced outward current. Abolition by hemoglobin indicated that this outward current was similarly mediated via NO, and sensitivity to extracellular cesium implied an involvement of potassium conductances. Application of 4-AP (1 mM) reduced this outward current. Low concentrations of 4-AP inhibit both the A-current (Belluzzi et al., 1985) and the delayed rectifier (Marsh and Brown, 1991). The A-current, however, is unlikely to have any role in the SNAP-induced responses since SNAP induced an inward current shift in conditions in which $I_A$ would be inactivated. Conversely, the outward current induced by SNAP in the presence of cobalt could be mediated through activation of $I_{K_V}$. In the absence of cobalt, 4-AP increased the SNAP-induced inward current shift implying that SNAP simultaneously inhibits $I_{AHP}$ and activates $I_{K_V}$.

An action of NO to inhibit calcium-dependent potassium currents has been reported in hippocampal slices, with a concomitant enhancement of neuronal excitability (Ertemli and Kranjcić, 1995). Actions of nitric oxide to activate the
potassium delayed rectifier current have been reported in the rat pulmonary artery (Zhao et al., 1997). To our knowledge, however, this is the first report of nitric oxide mediating a simultaneous activation (of IKV) and inhibition (of IAHP) of potassium conductances in the same neuron or tissue.

Both inhibitory and excitatory actions of NO-donors have been reported in spinal thermosensitive neurons where NO causes an inhibitory effect, observed as a decrease in firing rate, in the majority of neurons recorded from laminae I and II. In contrast, however, the majority of thermosensitive neurons in lamina X respond to NO-donors with an increase in excitability (Schmid and Pehl, 1996; Schmid et al., 1997). This biphasic effect is likely to be due to that the presence (laminae I and II) or absence (lamina X) of IAHP.

Few studies have been undertaken to assess the actions of NO in sympathetic ganglionic neurons. Intracellular recordings were made from the mouse superior mesenteric ganglion in vitro where NO is proposed to act to modulate slow synaptic transmission inducing a membrane hyperpolarization in the majority (64%) of neurons, although a small number of neurons (8%) respond with a membrane hyperpolarization followed by a depolarization (Mazet et al., 1996). The results of our study differ from these intracellular recordings that may reflect the influence that the synaptic inputs present in intact ganglia may have on neuronal excitability and behavior, reflected in the differing neurmodulatory actions of NO.

A similar neurmodulatory role for NO has been proposed in pancreatic ganglia of the cat, where both NO and the NO donor, sodium nitroprusside, evoked a neuronal membrane hyperpolarization as well as an initiating fast excitatory postsynaptic potentials in the majority of neurons recorded (Sha et al., 1995).

Our experiments indicated that SNAP exerted little influence upon neurons at resting membrane potentials. The mean resting membrane potential (Em) for all dissociated neurons, both phasic and tonic, was −50.1 ± 0.1 mV. This compares favorably with a value for Em of −54 ± 0.9 mV for intact celiac ganglion neurons (Kreulen and Szurszewski, 1979) implying that Em does not alter significantly after dissociation and culture. SNAP-induced an inward current shift only when neurons were depolarized to potentials less negative than −50 mV. At the average resting membrane potential (−50.1 ± 0.9 mV), the NO donor, SNAP, produced only a 24 ± 5.4 pA inward current shift. This would suggest that in the absence of any other depolarization, release of nitric oxide would have little effect on the prevertebral neurons.

If, however, the neuron was depolarized by any means other than inhibition of IAHP, then the nitricergic input would inhibit the after-hyperpolarization currents. These calcium-dependent potassium currents hyperpolarize the neuron after either a burst of action potentials or a single action potential of duration sufficient to raise intracellular calcium levels, and act to limit action potential firing frequency by increasing the refractory period (for review see Adams and Harper, 1995). Inhibition of these currents by NO decreased the after-hyperpolarization after bursts of action potentials as well as after individual action potentials, and decreased the refractory period resulting in an increased ability of the neuron to fire action potentials as well as an increased frequency of action potential burst firing.

If the prevertebral sympathetic neuron was depolarized but the IAHP already inhibited, then the predominant effect of the nitricergic input would be to activate IKV. Such a delayed outward rectifying potassium current terminates the action potential and may make a significant contribution to the after-hyperpolarization after the action potential (Marsh and Brown, 1991; for review see Adams and Harper, 1995). Activation of this current by NO would tend to offset the existing CG neuronal depolarization, thereby reducing its excitability.

Conclusions

The overall effect of NO on sympathetic postganglionic neurons will depend, therefore, on the excitability state of the neuron at the time it receives the nitricergic input that, in turn, may be dependent on the principal neurotransmitters involved in synaptic transmission in these neurons. In particular, an excitatory input of a neurotransmitter such as acetylcholine (which does not decrease IAHP) would be amplified by a concomitant nitricergic input, although an excitatory neurotransmitter such as substance P, which can decrease IAHP, (Vanner et al., 1993) would be antagonized.

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