Nitric oxide contracts longitudinal smooth muscle of opossum oesophagus via excitation–contraction coupling

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(Resubmitted 3 January 2001; accepted after revision 7 June 2001)

1. The effects of sodium nitroprusside (SNP) and diethylenetriamine/nitric oxide adduct (DETA/NO), putative nitric oxide (NO) donors, on opossum oesophageal longitudinal smooth muscle were investigated using isometric tension and intracellular micro-electrode recordings.

2. SNP produced concentration-dependent contractions of oesophageal longitudinal smooth muscle with an EC_{50} of 239.6 ± 78.2 μM (mean ± S.E.M., $n = 10$). Maximal contraction induced by SNP (1 mM) was about 75.5 ± 8.5% ($n = 10$) of the 60 mM KCl-induced contraction. The SNP-induced contraction was resistant to tetrodotoxin (TTX; 1 μM), but abolished by nifedipine (1 μM), as well as by niflumic acid (300 μM) and 9-anthroic acid (9-AC; 1 mM), Ca^{2+}-activated Cl^- channel blockers.

3. DETA/NO at concentrations of 100 and 500 μM induced 83.1 ± 24.4 and 104.1 ± 34.9% of the 60 mM KCl-induced contraction ($n = 4$), respectively, which was abolished by nifedipine (1 μM), niflumic acid (300 μM) and 9-AC (1 mM).

4. Pre-application of 1H-[1,2,4]oxidiazolo[4,3,-α]quinoxalin-1-one (ODQ) (10 μM), a guanylate cyclase inhibitor, significantly inhibited the SNP-induced contraction, whereas 8-bromo-cGMP (1 mM), a membrane-permeable analogue of cGMP, mimicked the SNP-induced contraction.

5. Intracellular recordings revealed that SNP (300 μM) depolarized resting membrane potentials (RMPs) and increased the frequency of spontaneous spike-like action potentials. However, these electrical alterations were eliminated by pretreatment with niflumic acid (300 μM).

6. These results suggest that NO produces an excitation–contraction coupling in opossum oesophageal longitudinal smooth muscle via a cGMP-dependent signalling pathway. This contraction depends on extracellular Ca^{2+} entry through activation of L-type Ca^{2+} channels.

Several lines of evidence point to NO as being a non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter in gastrointestinal smooth muscle (Bennett, 1997). Observations from mice lacking the neuronal nitric oxide (NO) synthase gene indicate that NO released from NANC nerve terminals in intestinal circular smooth muscle is responsible for slow inhibitory post-synaptic junction potentials (IJP), which leads to muscle relaxation (Mashimo *et al.* 1996; Kim *et al.* 1999). The slow IJP was suppressed by soluble guanylate cyclase inhibitors LY-83583 and 1H-[1,2,4]oxidiazolo[4,3,-α]quinoxalin-1-one (ODQ), suggesting the involvement of cGMP in the inhibitory effects of NO (Goyal & He, 1998). It was reported that these inhibitory effects of NO might be due to either opening of K^+ channels or closing of Ca^{2+}-activated Cl^- channels (Cayabyab & Daniel, 1995; Koh *et al.* 1995; Zhang *et al.* 1998). However, the effects of NO on gastrointestinal longitudinal smooth muscle are paradoxical. It has been reported that NO has different effects on gastrointestinal longitudinal smooth muscle, causing a small transient relaxation, followed by sustained contraction, that can be either TTX sensitive or resistant, depending on the tissue studied (Bartho & Lefebvre, 1994, 1995; Olgart *et al.* 1997). By contrast, Corvera *et al.* (1997) showed that sodium nitroprusside (SNP) markedly inhibited spontaneous contractions of longitudinal smooth muscle of rat colon. The aforementioned observations were based on tension recordings, which make it difficult to determine whether the effects of NO are attributable to pharmaco-contraction coupling or excitation–contraction coupling.

The purpose of present study was to investigate the actions of NO on opossum oesophageal longitudinal smooth muscle, as well as the possible mechanisms involved, by using standard isometric tension and intracellular micro-electrode recordings. Our studies demonstrate that NO induces contraction of oesophageal longitudinal smooth muscle by excitation–contraction coupling via a cGMP-dependent signalling pathway, possibly by opening Ca^{2+}-activated Cl^- channels.

METHODS

Animals and tissues

The protocols were approved by the Animal Care Committee of Queen's University. Opossums (*Didelphis virginiana*) of either sex and weighing 2.5–5.0 kg were anaesthetized by tail vein injection of sodium pentobarbital (40 mg kg^{-1}). The distal oesophagus and a short segment of attached stomach were then excised and placed in pre-oxygenated modified Krebs solution, following which the animals were killed by intracardiac injection of sodium pentobarbital. The oesophagus was opened longitudinally and pinned out with the mucosa side up in a dissecting dish and the mucosal layer was carefully removed by sharp dissection. As a standard preparation, a strip of longitudinal smooth muscle (with attached submucosa and circular muscle layers) of about 3 mm × 15 mm was cut along the long axis of the oesophagus for tension studies and a sheet of longitudinal smooth muscle of about 5 mm × 10 mm was excised for intracellular recordings. In some tension studies, special preparations of longitudinal smooth muscle were made as follows, in order to exclude the involvement of other tissue layers in the excitatory effect of SNP: (1) intact preparation including mucosal, submucosal, circular and longitudinal smooth muscle layers; (2) longitudinal and circular smooth muscle preparation (i.e. both mucosa and submucosa dissected free); and (3) isolated longitudinal smooth muscle preparation.

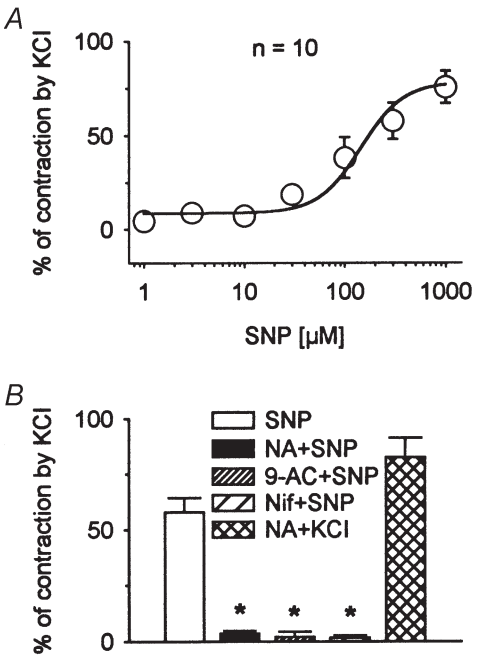

Figure 1. Effects of SNP on standard longitudinal smooth muscle preparations of opossum oesophagus

A, single concentration–response curve demonstrating concentration-dependent SNP-induced contraction. Curve was fitted with a Hill equation, which yielded $EC_{50} = 239.6 \pm 78.2$ μM ($n = 10$). *B*, histogram of effects of niflumic acid (NA, 300 μM), 9-AC (1 mM) and nifedipine (Nif, 1 μM) on the contraction produced by SNP (300 μM). Niflumic acid, 9-AC and nifedipine abolished the SNP-induced contraction ($n = 7$). However, in the presence of niflumic acid (300 μM) application of 60 mM KCl produced contraction to 82.7 ± 8.7 % of control induced by 60 mM KCl ($n = 3$), suggesting that inhibition of SNP-induced contraction was not due to the non-specific actions of niflumic acid on L-type Ca^{2+} channels or contractile protein processes. *$P < 0.05$.

Isometric tension and micro-electrode intracellular recordings

Strips were suspended in a water-jacketed tissue bath containing 10 ml Krebs solution gassed with 5% CO_2–95% O_2 at 35 °C. Mechanical responses were recorded using standard isometric tension recordings as described previously (Saha *et al.* 1993; Uc *et al.* 1999). Silver wire electrodes, placed on either side of the muscle strip, were used to electrically stimulate intramural nerves using square-wave pulses generated by a Grass S88 stimulator (Grass Instrument Co., MA, USA). Conventional intracellular microelectrode techniques were used to record electrical responses as described previously (Zhang & Lang, 1994).

Solutions and drugs

The modified Krebs solution contained (mM): NaCl 118.07, $NaHCO_3$ 25.00, D(+)-glucose 11.10, KCl 4.69, $CaCl_2$ 2.52, $MgSO_4$ 1.00 and NaH_2PO_4 1.01. Niflumic acid was purchased from ICN Biochemicals Inc., tetrodotoxin (TTX) from Research Biochemicals Inc., and all other chemicals from Sigma. Niflumic acid and ODQ were dissolved in dimethyl sulphoxide as stock solutions. Nifedipine and 9-anthroic acid (9-AC) were dissolved in ethyl alcohol (100%) and other chemicals in distilled water. These were diluted to final concentrations with the modified Krebs solution. SNP and DETA/NO were freshly dissolved in the modified Krebs solution and used within 5 min. The modified Krebs solution containing the drugs was gassed with 5% CO_2–95% O_2 to restore the pH to 7.4 prior to application.

Statistical analysis

Data are presented as means ± S.E.M. For tension recording studies, n refers to the number of animals, whereas for intracellular recording studies, n refers to the number of cells from which successful recordings were obtained. In tension recordings, the amplitude of contraction was standardized as a percentage of the maximal contraction induced by 60 mM KCl. Pre- and post-drug comparisons were made using Student's paired t test. $P < 0.05$ was considered statistically significant.

RESULTS

Isometric tension studies

None of the muscle strips developed basal tone, but in 6 of 67 preparations, spontaneous phasic contractions were recorded with an amplitude of 19.7 ± 8.1 % of maximal KCl (60 mM)-induced contraction and a frequency of 2.04 ± 0.62 min^{-1}. Bath application of SNP produced a sustained concentration-dependent contraction with an EC_{50} of 239.6 ± 78.2 μM ($n = 10$; Fig. 1*A*). The maximum effective concentration of SNP (1 mM) evoked 75.5 ± 8.5% ($n = 10$) of the KCl-induced contraction. DETA/NO, another putative NO donor, at a concentration of 100 and 500 μM induced 83.1 ± 24.4 and 104.1 ± 34.9 % of the KCl-induced contraction ($n = 4$), respectively (Fig. 2). The effects of SNP and DETA/NO were reversed after washing for 5–7 min.

Theoretically, either blockade of outward currents or activation of inward currents in smooth muscle could

depolarize resting membrane potential (RMP). Membrane depolarization could activate voltage-gated, long-lasting Ca^{2+} channels, leading to excitation–contraction coupling (Sanders & Ozaki, 1994). In smooth muscle, the inward currents are usually carried by Ca^{2+}, non-selective cation, or Cl^- channels (Sanders & Ozaki, 1994; Large & Wang, 1996). These possibilities were tested using different channel blockers (Fig. 1*B*). Niflumic acid (300 μM) and 9-AC (1 mM), putative Ca^{2+}-activated Cl^- channel blockers, and nifedipine (1 μM), an L-type Ca^{2+} channel blocker, significantly inhibited the contraction induced by SNP (300 μM) from $57.9 \pm 6.7\%$ to 3.6 ± 1.2, 2.1 ± 2.3 and $1.7 \pm 1.0\%$, respectively, of maximal contraction ($P < 0.05$, $n = 7$). However, DETA/NO-induced contractions at a concentration of 100 and 500 μM were significantly reduced from 83.1 ± 24.4 and $104.1 \pm 34.9\%$ of the KCl-induced maximal contraction to 0.8 ± 0.6 and $2.6 \pm 0.9\%$ by nifedipine (1 μM), to 0.9 ± 0.8 and $9.6 \pm 6.9\%$ by niflumic acid (300 μM) and to 0.2 ± 1.4 and $1.2 \pm 2.5\%$ by 9-AC (1 mM; Fig. 2). These data suggest that the NO-induced contraction might be due to the opening of Ca^{2+}-activated Cl^- channels, which, in turn, results in the activation of L-type Ca^{2+} channels. Niflumic acid (300 μM) did not significantly affect the KCl-induced contraction (Fig. 1*B*). Nifedipine (1 μM) substantially reduced the 60 mM KCl-induced contraction to $26.6 \pm 4.4\%$ ($n = 4$). However, in the presence of 1 μM TTX in a control

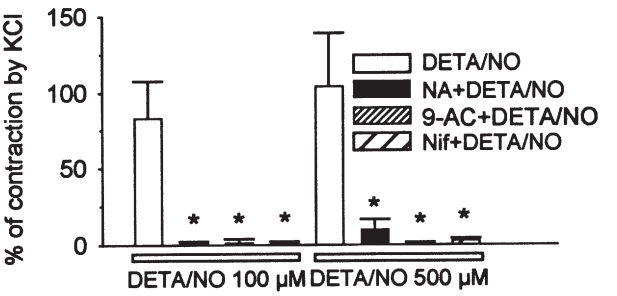

Figure 2. DETA/NO-induced contractions
DETA/NO, a putative NO donor, at 100 and 500 μM induced contraction of oesophageal longitudinal smooth muscle in a concentration-dependent fashion ($n = 4$). Pretreatment with niflumic acid (300 μM), 9-AC (1 mM) and nifedipine (1 μM), respectively, blocked the DETA/NO-induced contraction. $^*P < 0.05$.

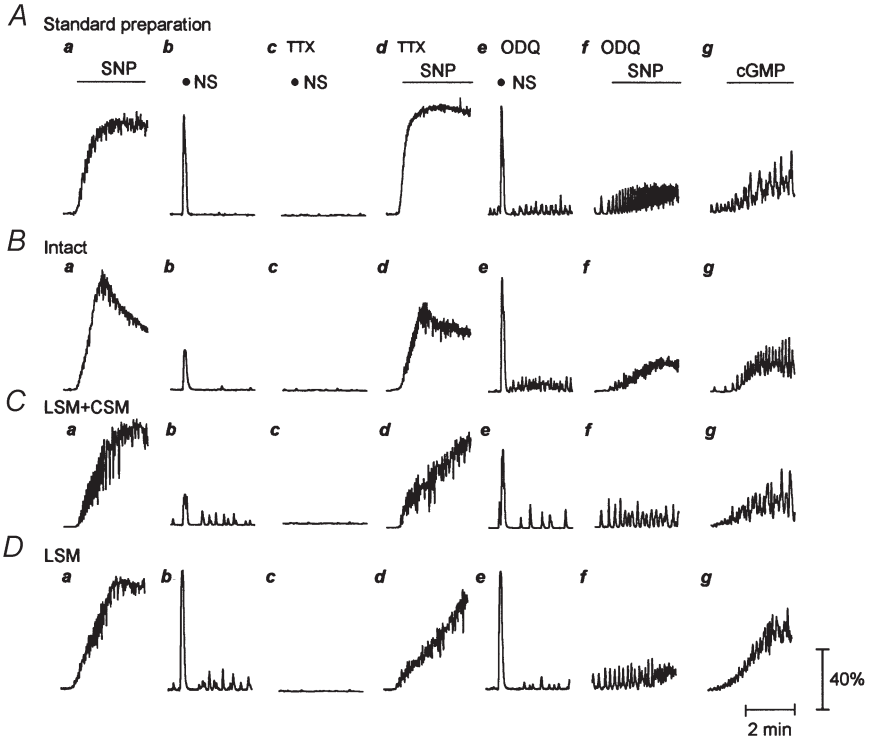

Figure 3. Examples of effects of TTX (1 μM) and ODQ (10 μM) on contractions induced by SNP (300 μM) of different longitudinal smooth muscle preparations
A, standard preparation. *B*, intact preparation. *C*, longitudinal smooth muscle (LSM) preparation with attached circular muscle (CSM) only. *D*, isolated longitudinal preparation. *a*, control contraction produced by SNP. *b–d*, TTX abolished nerve stimulation (NS)-induced contraction (20 Hz, 0.1 ms pulse duration, 60 V, 5 s), but failed to affect the contraction produced by SNP. *e* and *f*, ODQ significantly inhibited the SNP-induced contraction. However, in the presence of ODQ, nerve stimulation still evoked a contraction which was comparable to that without ODQ, implying that this inhibition was not due to the direct inhibition of ODQ on the longitudinal smooth muscle. *g*, 8-bromo-cGMP (1 mM) mimicked the SNP-induced contraction.

solution of 60 mM KCl and a solution of 60 mM KCl containing niflumic acid (300 μM), the KCl-induced contraction was unaffected by niflumic acid. The data establish that the KCl-induced contraction is largely mediated by L-type Ca^{2+} channels. They indicate that the inhibitory effect of niflumic acid was not via non-specific actions on L-type Ca^{2+} channels or contractile protein processes.

It was previously reported that SNP releases cyanide, which could produce toxic effects (Bates *et al.* 1991). To exclude the possibility that the contraction induced by SNP was due to the release of cyanide, we tested the effect of bath application of sodium cyanide on longitudinal muscle strips. At concentrations between 1 and 300 μM, no measurable contractions were evoked ($n = 3$; not shown).

To exclude the possibility that the SNP-induced contraction was due to mediators released from other layers of oesophageal wall, rather than via a direct action on the longitudinal smooth muscle, experiments were performed using longitudinal muscle strip preparations that included different components of the oesophageal wall (Fig. 3 and 4). SNP (300 μM) evoked virtually identical responses in four different preparations ($n = 5$). TTX (1 μM) failed to affect the SNP-induced contraction, whereas it abolished the contractile response induced by transmural nerve stimulation (20 Hz, 0.1 ms pulse duration, 60 V, 5 s). ODQ (10 μM), a guanylate cyclase inhibitor, markedly suppressed, whereas 8-bromo cGMP (1 mM), a membrane-permeable analogue of cGMP, mimicked the SNP-induced contraction. However, the contractile response evoked by nerve stimulation was not affected by ODQ, implying that the effects of ODQ on the SNP-induced contraction were not due to a non-specific direct inhibition of longitudinal smooth muscles.

Intracellular micro-electrode recordings

Intracellular micro-electrode recordings were employed to determine whether the SNP-induced contraction was due to pharmaco– or excitation–contraction coupling. Longitudinal smooth muscle exhibited spontaneous electrical activity consisting of slow-wave-like potentials with a RMP of -53.1 ± 1.6 mV, slow-wave-like potential amplitude of 4.4 ± 0.3 mV and slow-wave-like potential frequency of 2.32 ± 0.27 min^{-1}. Superimposed on the slow-wave-like potentials were spike-like action potentials with a frequency of 4.4 ± 0.4 per slow-wave-like potential and an amplitude of 17.6 ± 0.9 mV ($n = 26$; Fig. 5*A*). Bath application of nifedipine (1 μM) led to membrane depolarization of 3.3 ± 0.4 mV *vs.* control ($P < 0.05$, $n = 3$) and abolished spontaneous electrical activity (Fig. 6). SNP (300 μM) significantly depolarized RMP by 2.2 ± 0.6 mV *vs.* control ($P < 0.05$, $n = 6$) and induced a sustained burst of spike-like action potentials at a frequency of 22.02 ± 4.57 min^{-1} ($P < 0.05$ *vs.* control, $n = 6$) and with amplitude of 15.6 ± 3.0 mV ($P > 0.05$ *vs.* control, $n = 6$; Fig. 7*A*). Spontaneous electrical activity returned to the basal state 5–7 min after washout. Niflumic acid (300 μM) hyperpolarized RMP by -9.8 ± 1.6 mV *vs.* control ($P < 0.05$, $n = 4$), gradually decreased the amplitude of spike-like action potentials and finally abolished spontaneous action potentials. Effects of niflumic acid on spontaneous electrical activity were reversible.

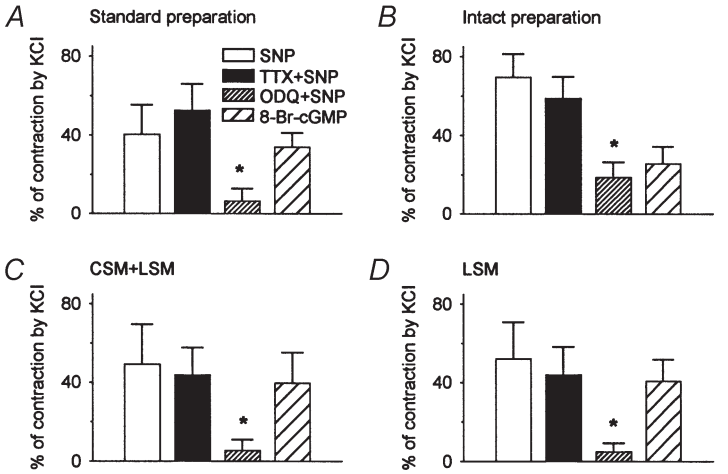

Figure 4. Histograms of effects of TTX (1 μM) and ODQ (10 μM) on the contraction induced by SNP (300 μM)

A, standard preparation. *B*, intact preparation. *C*, longitudinal smooth muscle preparation with attached circular muscle only. *D*, isolated longitudinal smooth muscle preparation. In each preparation, SNP produced a similar contraction, which was unaffected by pre-application of TTX, but significantly inhibited by ODQ. However, 8-bromo-cGMP (1 mM) mimicked the SNP-induced contraction. These data indicate that SNP has a direct excitatory effect on oesophageal longitudinal smooth muscle via a cGMP-dependent signalling pathway. $n = 5$, $^*P < 0.05$.

The spontaneous spike-like action potentials quickly recovered 5–10 min after washout (Fig. 7*Ba*). In the presence of niflumic acid, concomitant application of SNP (300 μM) slightly depolarized RMP (1.6 ± 0.7 mV; $P > 0.05$ *vs.* control, $n = 3$) and failed to induce a sustained bursting of spike-like action potentials ($n = 3$; Fig. 7*B*).

DISCUSSION

The results of our study show that NO produced a direct, TTX-resistant contraction of oesophageal longitudinal smooth muscle, which was abolished by blockade of either L-type Ca^{2+} channels or Ca^{2+}-activated Cl^- channels. This unique contraction was inhibited by a guanylate cyclase inhibitor, and mimicked by a cGMP analogue. Moreover, intracellular recordings demonstrated that the SNP-induced contraction was associated with membrane depolarization and sustained bursts of spike-like action potentials, which were abolished by a Ca^{2+}-activated Cl^- channel blocker. These data suggest that NO opens Ca^{2+}-activated Cl^- channels via a cGMP-dependent pathway, leading to extracellular Ca^{2+} entry through L-type Ca^{2+} channels and contraction of opossum oesophageal longitudinal smooth muscle.

It was previously reported that NO elicited a biphasic response (a small relaxation followed by a marked contraction) in longitudinal smooth muscles of opossum oesophagus (Saha *et al.* 1993) and guinea-pig small intestine (Bartho & Lefebvre, 1994; Olgart *et al.* 1997). In the present study, no initial relaxation was observed, which is not surprising, because our longitudinal muscle strips did not develop resting tone. This might have occurred if additional stretch had been applied to the strips, although the preparations were stretched to 140% of their original length to determine the ideal pre-load in our experiments (Saha *et al.* 1993; Uc *et al.* 1997). However, intracellular recordings also failed to demonstrate an initial inhibitory effect of SNP, in that we never observed membrane hyperpolarization upon application of SNP; invariably membrane depolarization and continuous spiking were recorded.

Alterations in several different ionic conductances could result in excitation–contraction coupling in smooth muscle (Sanders & Ozaki, 1994; Bolton *et al.* 1999). Because K^+ conductance is a major contributor to the RMP, inhibition of K^+ channels by different agents could be a powerful mechanism for inducing membrane depolarization. However, activation of one or more inward currents, usually carried by Ca^{2+}, non-selective

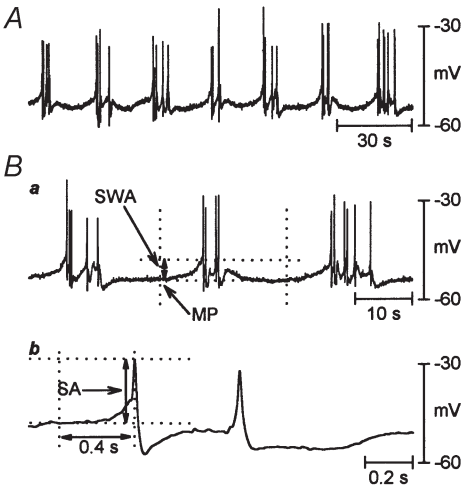

Figure 5. Spontaneous electrical activity of longitudinal smooth muscle of opossum oesophagus obtained by conventional intracellular recordings

A, intracellular micro-electrode recording in the basal state revealed 1–6 spike-like action potentials usually superimposed on slow-wave-like potentials ($n = 26$). *B*, measurement of electrical parameters: *a*: SWA, amplitude of slow-wave-like potentials; MP, resting membrane potential; *b*: SA, amplitude of spike-like action potentials.

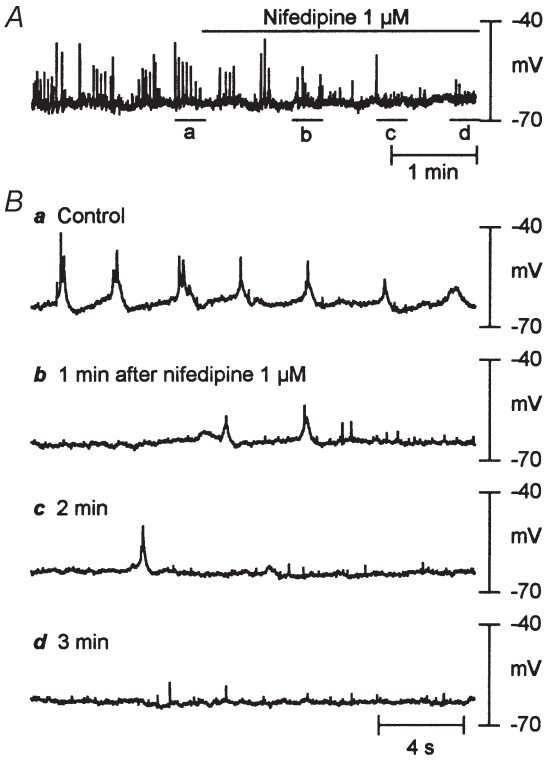

Figure 6. Effects of nifedipine on spontaneous electrical activity

A, bath application of nifedipine (1 μM) led to membrane depolarization of 3.3 ± 0.4 mV over control ($P < 0.05$, $n = 3$) and elimination of spontaneous action potentials. *B*, snapshots of original recordings from *A* on an expanded time scale before and after application of nifedipine. Note that small upward deflections in original recordings are due to perfusion pump noise.

cation, or Cl^- conductances (Benham *et al.* 1985; Vogalis & Sanders, 1990; Large & Wang, 1996) could also underlie the membrane depolarization. It is estimated that the reversal potential of the Cl^- channel is between −30 and −20 mV (Aickin & Brading, 1982, 1983). At the resting state, activation of Cl^- channels would allow outward movement of Cl^- ions to carry an inward current, resulting in membrane depolarization. Previous reports have implicated Cl^- channels in noradrenaline (norepinephrine)-induced constriction of vascular smooth muscle (Criddle *et al.* 1996; Lamb & Barna, 1998), and the myogenic tone of both cerebral arteries (Nelson *et al.* 1997) and tracheal smooth muscle (Janssen & Sims, 1994). Evidence also exists implicating the activation of Cl^- channels in agonist-induced contraction of colonic smooth muscle (Kolbel *et al.* 1998). Our studies are the first to implicate Ca^{2+}-activated Cl^- channels in the NO-induced contraction of gastrointestinal longitudinal smooth muscle. In addition, the membrane hyperpolarization induced by niflumic acid suggests that the activity of Ca^{2+}-activated Cl^- channels also contributes to the RMP of this muscle.

Appropriate interpretation of our results rests on the selectivity of niflumic acid. Some studies have suggested that this drug might have non-selective effects, by affecting either K^+ channels or Ca^{2+}-dependent contractile processes (Large & Wang, 1996; Kato *et al.* 1999). It is unlikely that such mechanisms explain the blocking effect of niflumic acid on SNP-induced contraction in our experiments, because: (1) the non-specific effect of niflumic acid demonstrated in other studies was very slow to reverse, whereas the effect of niflumic acid was rapidly reversible in our experiments (Fig. 7*B*); (2) niflumic acid had no significant effect on KCl-induced longitudinal smooth muscle contraction, indicating that it did not affect contraction via a non-specific effect on voltage-gated Ca^{2+} channels or contractile proteins (Fig. 1*B*); (3) niflumic acid abolished both the membrane depolarization and sustained bursts of spontaneous action potentials produced by SNP. It is known that niflumic acid opens large conductance Ca^{2+}-activated K^+ channels at a higher concentration (> 50 μM; Greenwood & Large, 1995). It is possible that the inhibition of NO donor-induced contraction and of spontaneous electrical activity could contribute to the opening of large conductance Ca^{2+}-activated K^+ channels by niflumic acid. However, our experiments do not support this possibility, because niflumic acid relaxed both oesophageal longitudinal smooth muscle strips (data not shown) and circular smooth muscle of the lower oesophageal sphincter (Zhang *et al.* 2000) contracted by 1 mM TEA.

Niflumic acid has been used as a non-steroidal anti-inflammatory agent (Kulmacz *et al.* 1991), raising the possibility that the action of niflumic acid might be mediated by a reduction in eicosanoid production. In the present studies, it appeared that the action of niflumic acid on the inhibition of cyclo-oxygenase was insignificant relative to its blockade of Ca^+-activated Cl^- channels. This is because the effect of niflumic acid reported here was dramatically different from the effects of indomethacin on spontaneous contractile and electrical activity of the proximal renal pelvis smooth muscle of the guinea-pig, as reported by Zhang & Lang (1994). In their work, indomethacin abolished both the spontaneous mechanical and the electrical activity of this muscle and depolarized the RMP. It took 25–30 min for indomethacin to achieve a maximal response and 30–60 min to recover

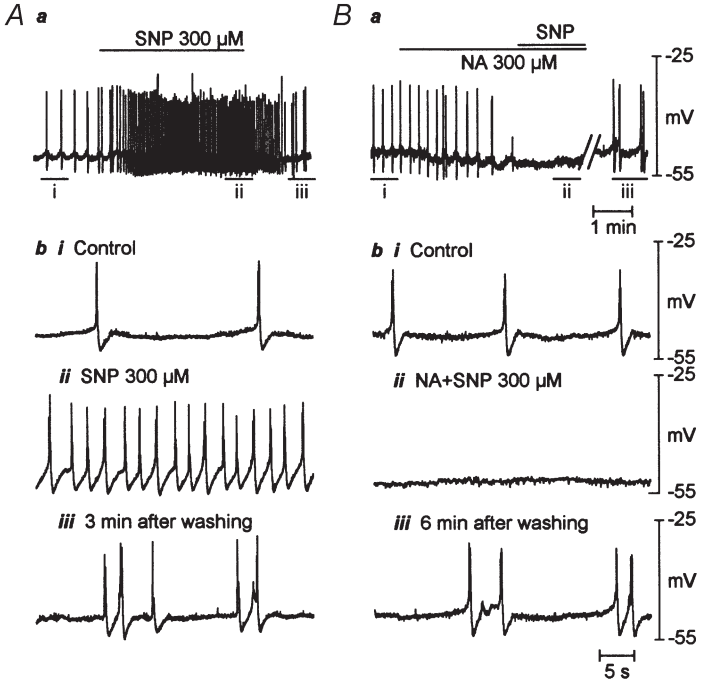

Figure 7. Effects of SNP (300 μM) on the electrical properties of the longitudinal smooth muscle

A and *B*, original intracellular micro-electrode recordings demonstrating effects of SNP in the absence and presence of niflumic acid (300 μM) from same cell. *Ab* and *Bb*, snapshots of original recordings from *Aa* and *Ba* on an expanded time scale. i, control. ii, drug application. iii, recovery after washout. SNP depolarized the resting membrane potential (RMP) and induced on-going spike-like action potentials ($n = 6$). Niflumic acid hyperpolarized RMP ($n = 4$), gradually decreased the amplitude of spike-like action potentials and finally abolished them. In the presence of niflumic acid, SNP failed to depolarize RMP and evoke on-going spike-like action potentials ($n = 3$).

to its control state upon washing out. By contrast, our data showed that niflumic acid suppressed spontaneous electrical activity, but hyperpolarized RMP. Furthermore, the onset of the action of niflumic acid in oesophageal longitudinal smooth muscle is rapid, reaching a maximal response within 5 min of bath application and returning to a control state 5–7 min after washout (Fig. 7*Ba*). This is not consistent with an effect mediated via cyclo-oxygenase inhibition.

Activation of L-type Ca^{2+} or other cation channels could represent an ionic mechanism for the SNP-induced contraction. Our experiments do not support this idea, because nifedipine and niflumic acid abolished the SNP-induced contraction, whereas the KCl-induced contraction in the presence of niflumic acid was no different from control KCl-induced contractions (Fig. 1*B*). Furthermore, there is no direct evidence to date that niflumic acid blocks cation channels. Nevertheless, the possible involvement of NO in the activation of cation channels cannot be completely excluded. Further patch-clamp studies in dispersed single longitudinal smooth muscle cells are required to help clarify these issues.

An intriguing question that arises from our study is how SNP can work via cGMP to elicit contraction of longitudinal smooth muscle but relaxation of circular smooth muscle (Saha *et al.* 1993; Hirano *et al.* 1997; Olgart *et al.* 1997). This suggests that there are fundamental differences between the two muscle types in the second messenger systems downstream of cGMP. Zhang *et al* (1998) showed that NO closed Ca^{2+}-activated Cl^- channels in oesophageal circular smooth muscle, although others (Cayabyab & Daniel, 1995; Koh *et al.* 1995) reported that NO opened K^+ channels through the elevation of cGMP to relax circular smooth muscle. Based on current studies in longitudinal smooth muscle, we propose that NO activates Ca^{2+}-activated Cl^- channels via a cGMP-dependent pathway, which in turn activates L-type Ca^{2+} channels, leading to extracelluar Ca^{2+} entry and contraction of the muscle. This seems paradoxical, because NO elevates the same cGMP messenger in the two muscle types. However, it is possible that cGMP acts through diverging cellular signalling pathways to affect multiple ion channel processes, such as activation of Ca^{2+}-activated Cl^- channels in longitudinal muscle and inhibition of Ca^{2+}-activated Cl^- channels in circular muscle. This would be analogous to the divergence of signal transmission that has been described at single nerve terminals for the control of the radula closer muscle in *Aplysia* (Brezina *et al.* 1996). However, further studies are required to clarify different signal transmission processes downstream of cGMP in the two muscle types.

In summary, the present study is the first to report NO-evoked contraction via excitation–contraction coupling in gastrointestinal longitudinal smooth muscle. This contraction depends on extracellular Ca^{2+} entry through L-type Ca^{2+} channels, possibly resulting from the activation of Ca^{2+}-activated Cl^- channels through the elevation of cGMP. This might be of physiological significance to the coordination of motility between circular and longitudinal smooth muscle layers during peristalsis.

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Acknowledgements

This work was supported by Medical Research Council of Canada grant no. MT9978. The authors would like to thank Mr David Miller for his technical assistance in conducting muscle strip studies.

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