Biphasic effect of SNP on opossum esophageal longitudinal muscle: involvement of cGMP and eicosanoids

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Saha, Joy K., Ikuo Hirano, and Raj K. Goyal. Biphasic effect of SNP on opossum esophageal longitudinal muscle: involvement of cGMP and eicosanoids. Am. J. Physiol. 265 (Gastrointest. Liver Physiol. 28): G403-G407, 1993.—Effects of nitric oxide (NO)-containing compounds on opossum esophageal longitudinal smooth muscle in vitro were examined. Sodium nitroprusside (SNP) and authentic NO produced a biphasic concentration-dependent relaxation-contraction sequence in the esophageal longitudinal muscle (ELM) but only a concentration-dependent relaxation of the lower esophageal sphincter (LES) but no effect in the esophageal circular muscle. A cell membrane-permeable analogue of guanosine 3',5'-cyclic monophosphate (cGMP), 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) also produced relaxation-contraction sequence in the ELM and relaxation of the LES. The guanylate cyclase inhibitors methylene blue (MB) and LY-83583 increased resting tone and had no significant effect on SNP-induced relaxation of ELM. However, they abolished the SNP- and NO-induced contraction of ELM. The cyclooxygenase inhibitor indomethacin had no effect on ELM relaxation and abolished the contractions due to SNP, NO, and 8-BrcGMP. These studies show that in the ELM 1) SNP, authentic NO, and 8-BrcGMP cause a biphasic relaxation-contraction sequence; 2) MB and LY-83583 blocked contraction but not the relaxation associated with SNP and NO; and 3) indomethacin blocked contractions but not the relaxation due to SNP, NO, and 8-BrcGMP. These results suggest that in the ELM, NO donors exert an inhibitory action that is largely cGMP independent and an excitatory action via a cGMP-dependent pathway involving endogenous eicosanoids of the cyclooxygenase pathway.

smooth muscle; nitric oxide; 8-bromoguanosine 3',5'-cyclic monophosphate; methylene blue; LY-83583; indomethacin; lower esophageal sphincter; cyclooxygenase; prostaglandins; guanylate cyclase; smooth muscle tone

NITRIC OXIDE DONORS such as sodium nitroprusside (SNP) are known to cause relaxation of a wide variety of smooth muscles including vascular and gastrointestinal smooth muscles (10, 18). SNP and authentic nitric oxide (NO) have been shown to relax the lower esophageal sphincter (LES; 9), inhibit contraction, and produce hyperpolarization of the esophageal circular muscle (ECM; 1, 5). In the circular muscle of the esophagus, SNP-induced hyperpolarization is blocked by guanylate cyclase inhibitors, such as methylene blue (MB) or cys-
tamine, indicating the involvement of cGMP in the inhibitory effect (1). However, recent studies have shown that the relaxant effect of NO donors on the LES was not antagonized by guanylate cyclase inhibitors, suggesting a guanosine 3',5'-cyclic monophosphate-independent mechanism for the LES relaxation produced by NO donors (9). There is no available information on the action of SNP or NO on the esophageal longitudinal muscle (ELM).

We report here our findings that in contrast to the purely inhibitory actions of SNP and authentic NO on the LES and ECM, these agents cause an unusual biphasic response consisting of a transient inhibition followed by a prominent excitation of the ELM. The transient inhibitory effect of NO donors on the ELM is largely cGMP independent, whereas its excitatory effect is dependent on increases in intracellular cGMP. Moreover, NO and cGMP act via an endogenous arachidonic acid product of the cyclooxygenase pathway to cause contraction of the ELM.

MATERIALS AND METHODS

Experiments were performed in 28 opossums (Didelphis virginiana) of either sex weighing 1.5–3.5 kg. The animals were anesthetized with pentobarbital sodium (40 mg/kg ip), and the abdomen was opened by midline incision. A portion of esophagus (~0.5 cm above the sphincter was removed cleanly and gassed with 95% O2-5% CO2. The modified Krebs solution contained (in mM) 118.0 sodium chloride, 4.69 potassium chloride, 2.52 calcium chloride, 0.57 magnesium sulfate, 1.18 bicarbonate, 1.18 sodium phosphate, 25 sodium bicarbonate, and 11.1 glucose. The esophagus was then opened longitudinally and pinned flat over a Sylgard (Dow Corning)-base Petri dish. Longitudinally oriented unstretched muscle strips ~1.5 mm in width and 3.4 mm in length were prepared for the study. Circular muscle strips of identical dimensions were prepared by cutting the tissues in a direction perpendicular to the long axis of the esophagus. One end of each strip was tied to a stainless steel tissue holder while the other was connected to an isometric force displacement transducer (Gould-Statham U1C2 and Grass FT O3) by a silk thread. The transducers were mounted on a two-directional manipulator. The muscle contraction was recorded on a rectangular polygraph (Beckman R-711) after an initial amplification through a low-noise bioamplifier (Beckman 9853H). Muscle strips were suspended in 2 ml double-jacketed organ baths (Radnoti Glass, CA) containing Krebs solution gassed with 95% O2-5% CO2 mixture through a porous sintered disk at the bottom of the baths. Prewarmed water (37°C) was circulated through the outer jacket of the tissue bath via a constant temperature circulator (Haake FE2). The temperature of the Krebs solution in the organ bath was maintained within ±0.5°C. Both circular and longitudinal muscle strips were equil-

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liberated at an applied tension of 1.5–2 g for 1 h. This tension stretched the tissues to ~150% of the original length and placed the tissues near the length where both the longitudinal and circular muscle strips were found to develop maximal active tension (14). The active tone was defined as the tone that was susceptible to loss when exposed to 0 Ca²⁺ and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (2 mM) containing Krebs. During the equilibration period, the Krebs solution was changed every 15 min.

All data are expressed as means ± SE of actual force values in grams or as percent changes in the force with treatments. Data were analyzed statistically by comparing individual values using the Student’s paired t test. P values of <0.05 were considered significant. The values for maximal response and half-maximal response (EC₅₀) were obtained graphically from percentage increase in tension vs. log molar concentration plot of SNP.

The following drugs were used. Arachidonic acid, 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP), atropine, MB, SNP, indomethacin, and tetrodotoxin were all purchased from Sigma Chemical; 6-anilino 5,8-quinolinedione (LY-83583) was from Calbiochem. LY-83583 was dissolved in dimethyl sulfoxide (DMSO) and indomethacin was dissolved in ethanol just before use. No vehicle effects were observed from the highest concentration of DMSO or ethanol required (0.02 µl in 2 ml bath). NO solution was prepared as described by Ignarro et al. The tissues near the length where both the longitudinal and circular muscle strips were found to develop maximal active tension (14). The active tone was defined as the tone that was susceptible to loss when exposed to 0 Ca²⁺ and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (2 mM) containing Krebs. During the equilibration period, the Krebs solution was changed every 15 min.

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RESULTS

General observations. Longitudinal muscle strips from the esophagus 0.5 cm above the LES developed spontaneous tone. This behavior was similar to that described for the circular muscle of the LES and was in contrast to the lack of spontaneous tone in the ECM. In the ELM preparations, the mean amplitude of spontaneous sustained active tone was 1.2 ± 0.1 g (n = 38). In addition, 29 of 38 strips also demonstrated spontaneous rhythmic contractions that had a mean amplitude of 1.5 ± 0.1 g. The values described for various drug treatments relate to sustained active tone.

Effects of SNP and NO. SNP produced a biphasic response consisting of an initial transient relaxation followed immediately by a longer lasting contraction of the ELM (Fig. 1A). The relaxation was concentration dependent (Fig. 1B) with a threshold concentration of 1 µM, EC₅₀ of 4.3 ± 1.2 µM, and maximum relaxation at 10 µM. Both the tonic and phasic components of the ELM were inhibited during the relaxation. In these tissues, the tonic force was 1.4 ± 0.2 g, and phasic force was 2.4 ± 0.3 g (n = 14). With SNP, the tonic force fell to a nadir of 0.75 ± 0.2 g (n = 14), and the phasic activity was abolished. The contractile response to SNP was also concentration dependent (Fig. 1B) with a threshold of 1 µM and EC₅₀ of 13.3 ± 2.8 µM. At 100 µM, SNP produced maximal contraction with a peak tension of 5.3 ± 0.6 g. SNP (100 µM) resulted in relaxation with a latency of 16 ± 2 s and duration of 30 ± 3 s and contraction lasting 5.8 ± 0.3 min (n = 11). The phasic contractions were also lost during the peak contraction. NO (100 µl), like SNP, produced a transient relaxation (0.7 ± 0.1 g) followed by a longer-duration contraction (3.6 ± 0.5 g, n = 8) from an average resting tension of 1.3 ± 0.2 g.

In contrast to its biphasic effect on the longitudinal muscle, SNP produced only a long-lasting concentration-dependent relaxation of the LES as described earlier (5, 9). The threshold concentration of SNP for relaxation was 10 nM, and EC₅₀ was 0.50 ± 0.02 µM (n = 17). The maximal LES relaxation was observed at 100 µM SNP. SNP had neither contractile nor relaxant effects on ECM.

![Figure 1](http://ajpgi.physiology.org/)

**Fig. 1.** Effects of sodium nitroprusside (SNP) and 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) on esophageal longitudinal smooth muscle. **A:** representative tracing showing effect of SNP (100 µM). Note that administration of SNP produced a transient relaxation followed by contraction. Tension returned to baseline after washout (w). **B:** concentration dependent response of SNP producing relaxation and contraction; results are means ± SE for 14 observations. **C:** representative tracing showing effect of 8-BrcGMP (1 mM). Note that administration of 8-BrcGMP also produced a biphasic effect consisting of initial relaxation followed by a contraction.
up to doses of 100 μM.

Effect of 8-BrcGMP. The biological effects of NO donors generally involve increases in intracellular cGMP due to the stimulation of cytosolic guanylate cyclase. Therefore, the effects of 8-BrcGMP, a stable analogue of cGMP, on the ELM were examined. 8-BrcGMP (1 mM) produced a biphasic response with an initial relaxation followed by contraction (Fig. 1C). The relaxation occurred 30 ± 5 s after administration and had a duration of 125 ± 13 s; the contraction lasted 7.5 ± 0.5 min (n = 6). 8-BrcGMP resulted in a decrease in the resting tone from 1.4 ± 0.3 to 0.7 ± 0.2 g and an increase in resting tone to 3.9 ± 1.1 g (n = 7). The similarities of actions of SNP, NO, and 8-BrcGMP were consistent with the possibility that SNP and NO may act via increases in intracellular cGMP to produce the biphasic response.

Influence of guanylate cyclase inhibitors. To further examine the involvement of cGMP in the responses to SNP, the effects of MB and LY-83583, two known inhibitors of guanylate cyclase, on SNP-induced biphasic response were examined. MB (100 μM) produced significant (50–80%) increase in resting tone (Table 1). The effect reached a steady state after a period of 20 min. After MB treatment, SNP still produced significant fall in the resting tone (P < 0.05) but failed to produce a significant increase (P > 0.05, n = 7) in tone subsequent to the relaxation (Table 1). Because of the significant increase in the resting tone by MB, a quantitative difference in the fall in the tone by SNP with or without MB treatment was difficult to compare. The decrease in force by SNP before and after MB was not significantly different (P > 0.05). The residual tone (at the nadir of relaxation) with SNP was, however, higher after MB treatment compared with the value before MB treatment (P < 0.05). LY-83583 also caused increase in resting ELM tone, did not affect relaxation, and abolished SNP-induced contraction of the ELM (Table 1). MB and LY-83583 treatment also abolished NO-associated contraction without blocking the preceding relaxation (Fig. 2).

Influence of indomethacin. It was possible that the actions of SNP and cGMP were not due to direct actions on the longitudinal muscle but were due to indirect actions via intramuscular nerves or autacoids such as prostaglandins. NO donors may act to release neurotransmitters (6). However, neither the inhibitory nor the excitatory effects of SNP or NO were modified by tetrodotoxin. After treatment with tetrodotoxin (10 μM), which blocked the electrical field-stimulated responses, NO-induced relaxations were 124 ± 21% of the control value, and the contractions were 98 ± 4% of the control value, (P > 0.05; n = 6). It has

Table 1. Influence of guanylate cyclase inhibitors MB and LY-83583 on the action of SNP and NO and of indomethacin on SNP, NO, and 8-BrcGMP

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<td>Spontaneous Relaxation</td>
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<td></td>
<td>MB (100 μM)</td>
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<td></td>
<td>Control</td>
<td>1.1±0.2</td>
<td>0.7±0.2*</td>
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<td></td>
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<td></td>
<td>MB (100 μM)</td>
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<td>0.4±0.1NS2</td>
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<td>MB (100 μM)</td>
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<td></td>
<td>MB (100 μM)</td>
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<td>MB (100 μM)</td>
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<tr>
<td>8-BrcGMP (1 mM)</td>
<td>Control</td>
<td>1.4±0.3</td>
<td>0.7±0.2*</td>
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<td></td>
<td>MB (100 μM)</td>
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<td>0.6±0.3NS2</td>
<td>0.4±0.2NS1</td>
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Values are means ± SE. *P < 0.05, significant compared with resting tone; †P < 0.05, significant compared with treatment control. NS2 P > 0.05, not significant. NS1, not significant compared with resting tone; NS2, not significant compared with treatment control.
been reported that endogenous eicosanoids may be involved in NO-associated depolarization in the taenia coli and the proximal colon circular muscle (20). Indomethacin is a known inhibitor of cyclooxygenase and, therefore, acts to inhibit arachidonic acid-derived eicosanoids (2). Indomethacin itself had no effect on resting tone (P > 0.05, Table 1). Indomethacin (10 μM) treatment completely abolished the contractions of SNP or NO without affecting the relaxations (Table 1). These studies show that whereas ELM relaxations in response to SNP, NO, and 8-BrcGMP do not involve endogenous eicosanoids, the contractions do involve eicosanoids that are produced in an indomethacin-sensitive pathway (Figs. 2 and 3).

**DISCUSSION**

These studies show that the three major muscle groups of esophageal smooth muscle respond quite differently to NO-containing compounds. Whereas SNP and NO caused relaxation of the LES (5, 9, 21) and no mechanical response in the ECM (present study), they evoked a biphasic response consisting of relaxation followed by contraction in the ELM. A contractile effect of NO donors has not been described before in any smooth muscle system. Based on the present findings we suggest that in the ELM, NO donors produce relaxation via a largely cGMP-independent pathway and contraction via a cGMP-dependent pathway that involves eicosanoids (Fig. 3).

Many of the biological actions of SNP and other NO compounds have been shown to be mediated by cGMP (7). The inhibitory effects of nitrovasodilators have been correlated with activation of guanylate cyclase and increased levels of intracellular cGMP (7). Furthermore, exogenous administration of 8-BrcGMP itself has been shown to produce smooth muscle relaxation including LES relaxation (13) and circular muscle hyperpolarization (1). In the longitudinal muscle, however, 8-BrcGMP produced a biphasic response, showing that cGMP can cause both relaxation as well as contraction of this muscle. This was consistent with the view that cGMP-dependent pathways exist in the longitudinal muscle and that both the inhibitory and excitatory actions of NO donors could be mediated by increases in intracellular cGMP.

The similarity of the response to SNP and 8-BrcGMP provides only indirect evidence for the involvement of cGMP in the effects of NO. Therefore, we further investigated the role of cGMP using the inhibitors of guanylate cyclase, MB and LY-83583. Although the precise mechanism by which these inhibitors work has not been fully elucidated (16), they have been clearly shown to inhibit NO-induced cGMP accumulation (3). In the ELM, a significant relaxation was found to persist in the presence of guanylate cyclase inhibitors. This, however, does not exclude the possibility that NO donors also exert a concomitant cGMP-dependent relaxation in the ELM, which is masked by the prominent cGMP-independent relaxation. In fact, the residual tone at maximal relaxation with SNP or NO was higher in guanylate cyclase inhibitor-treated tissues than in the control tissues. This is consistent with partial inhibition of the relaxation by the guanylate cyclase inhibitors and suggests that a cGMP-dependent relaxation may coincide with the cGMP-independent relaxation of the ELM by NO donors. However, because the guanylate cyclase blockers also increased the resting tone, the contribution of the cGMP-dependent component of ELM relaxation remains uncertain. The mechanism for the increased tone is itself unclear. The guanylate cyclase inhibitors serve as superoxide generators and can directly neutralize NO (3). MB and cystamine have been shown to produce contraction of the LES that is not related to their inhibitory effect on cGMP (11).

SNP and NO produced significant relaxation of the ELM in the presence of MB or LY-83583, showing that the relaxant action of SNP or NO is insensitive to both the superoxide generating and the guanylate cyclase-inhibiting properties of these agents. Such a cGMP-independent mechanism of NO donor-associated relaxation has been reported in the LES (9) and is in contrast to the cGMP-dependent hyperpolarization of esophageal circular muscle (1). cGMP-dependent and cGMP-independent pathways of NO donor-induced relaxations have also been reported in other smooth muscles (12). The mechanism of cGMP-independent smooth muscle relaxation to NO donor is not known. SNP has been shown to cause a decrease in intracellular Ca²⁺, which is cGMP independent (4) and direct interaction with the sulfhydryl groups of cell surface receptors and ion channels (17).

The most important finding of this study was that NO donors cause contraction of the ELM and that this excitatory effect involves both guanylate cyclase and cyclooxygenase pathways. The ability of NO donors to cause smooth muscle contraction is unusual. NO donors did not produce contraction of either the ECM or the LES. Similarly, 8-BrcGMP also produced contraction in the ELM but not in the ECM or LES. Moreover, the excitatory action of NO donors was abolished by the guanylate cyclase inhibitors MB and LY-83583. These observations

**Fig. 3.** A proposed pathway for the mechanism of relaxation-contraction sequence produced by SNP and NO. SNP and NO produce contraction of esophageal longitudinal muscle via cGMP, which in turn acts by stimulating endogenous eicosanoids of the cyclooxygenase pathway. On the other hand, SNP and NO produce relaxation largely via a cGMP-independent pathway. MB, methylene blue; Indo, indomethacin; PG, prostaglandin.
support the involvement of increases in intracellular cGMP in ELM contraction. The observation of cGMP involvement in ELM contraction is of interest. Although the initial studies from the early 1970's suggested the involvement of cGMP in smooth muscle contraction, most of the subsequent studies have supported a role for cGMP in smooth muscle relaxation rather than contraction (see Ref. 12). Indomethacin did not modify the NO donor or 8-BrcGMP induced relaxations but abolished their excitatory effects. This suggests that NO donors may act via cGMP to stimulate the synthesis and release of prostaglandins and thereby cause contraction of ELM. cGMP has been linked to the release of certain leukotrienes (16). The antagonism of the excitatory action of 8-BrcGMP by indomethacin suggests that arachidonic acid metabolites of the cyclooxygenase rather than lipoxygenase pathway are involved in ELM contraction. Prostaglandins, specifically PGE$_2$ and PGF$_2\alpha$, have been demonstrated to be the major metabolites of arachidonic acid metabolism in gastrointestinal smooth muscle (15). It is worth noting that whereas PGF$_2\alpha$ causes contraction and PGE$_2$ causes relaxation of the LES, both agents cause contraction of ELM (19). Further studies are needed to identify the sites of action of cGMP and NO on the eicosanoid pathway. NO donors have recently been reported to be involved in the rebound depolarization that is blocked by indomethacin (20). Studies are also needed to define the roles of cGMP and the eicosanoid pathway in the “rebound” contraction that follows inhibition due to activation of nonadrenergic noncholinergic nerves in gastrointestinal smooth muscle.

We thank Dr. Hamid I. Akbarali for comments and Yanli He for technical assistance.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-31092.

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Received 8 April 1993; accepted in final form 19 May 1993.

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