

Amplification of nitric oxide signaling by interstitial cells isolated from canine colon

(nonadrenergic, noncholinergic inhibitory nerves/enteric nervous system/nitric oxide synthase/neurotransmission/cell-to-cell communication)

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ABSTRACT The effects of nitric oxide (NO) on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were studied in enzymatically dispersed interstitial cells (ICs) and smooth muscle cells (SMCs) isolated from canine colon. $[Ca^{2+}]_i$ was monitored by using fluo-3 and video fluorescence imaging techniques. Exogenous NO caused an increase in $[Ca^{2+}]_i$ in ICs and a decrease in $[Ca^{2+}]_i$ in SMCs. Effects of NO on ICs were not blocked by removal of extracellular Ca^{2+} but were blocked by ryanodine, suggesting that NO caused release of Ca^{2+} from intracellular stores. When $[Ca^{2+}]_i$ was elevated in an IC by micropressure ejection of Bay K 8644, $[Ca^{2+}]_i$ decreased in nearby SMCs, suggesting release of a diffusible substance. The diffusible substance may be NO or an NO-related substance based on blockade of transmission by N^G -nitro-L-arginine methyl ester, N^G -monomethyl-L-arginine, or oxyhemoglobin. The elevation of $[Ca^{2+}]_i$ in ICs by NO, which, in turn, might cause further release of NO and elevation of $[Ca^{2+}]_i$, suggests a positive feedback and amplification mechanism in these cells. Elevation of $[Ca^{2+}]_i$ in SMCs had no effect on adjacent SMCs. Our data suggest that ICs may play a central role in amplification of NO signaling and propagation of inhibitory wave fronts.

Several recent studies have led to the concept that nitric oxide (NO) serves as a neurotransmitter in inhibitory regulation of visceral smooth muscles (see ref. 1 for review). The involvement of NO in nonadrenergic, noncholinergic inhibition is supported by morphological studies that have demonstrated the presence of NO synthase-like immunoreactivity in cell bodies and varicose processes of enteric neurons (2-5). Physiological and biochemical experiments have also supported the role of NO in inhibitory neurotransmission: (i) electrical field stimulation releases a substance identified as NO by bioassay (6), (ii) drugs that block NO synthesis reduce responses to enteric inhibitory nerve stimulation (7-10), (iii) hyperpolarizing responses to enteric inhibitory nerve stimulation can be mimicked by exogenous NO or NO carriers (11-13), (iv) drugs that affect cGMP (presumed to be the mediator of the effects of NO) similarly affect inhibitory neurotransmission (14), and (v) levels of L-[3H]citrulline (used as an indicator of NO synthesis) are increased by agonist stimulation of isolated myenteric ganglia (15). Taken together, these data suggest that enteric inhibitory neurons possess the enzymatic apparatus necessary to synthesize NO from L-arginine and NO can be released during nerve stimulation to inhibit smooth muscle activity.

In the gastrointestinal (GI) tract, a cell type referred to as interstitial cells (ICs) is often closely associated with enteric neurons and electrically coupled to smooth muscle cells (SMCs) (e.g., see refs. 16-19). At present it is not known whether ICs or SMCs are the "effectors" in enteric inhibitory

transmission, but for several years it has been suggested that ICs facilitate communication between the enteric nervous system and SMCs. Physiological studies have suggested that inhibitory neurotransmission is concentrated in pacemaker regions in the canine colon (20) where there is a particularly high density of ICs (17, 19). Since ICs are physically close to enteric varicosities, these cells would be expected to be exposed to NO released from nerves. Therefore, it is important to determine whether ICs respond to NO and whether effects are transmitted to SMCs. Specific responses of ICs are difficult to ascertain from intact preparations because of electrical coupling to SMCs. We have developed an isolated preparation of ICs from the canine colon (21) and have characterized ionic currents (22) and Ca^{2+} oscillations in these cells (23). In the present study, we monitored changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) elicited by NO in ICs to determine the ability of ICs to transmit NO responses to nearby SMCs. Results suggest that ICs respond to NO and may amplify and propagate inhibitory signals by producing more NO via a positive feedback mechanism.

MATERIALS AND METHODS

Cells were isolated and identified as ICs or SMCs as described (see ref. 21). Freshly isolated ICs and SMCs were allowed to attach to the bottom of 35-mm culture dishes and were placed on an inverted microscope. Cells were loaded with the AM form of the Ca^{2+} -sensitive dye fluo-3 (12 μM ; 30 min) as described (21, 23). ICs and SMCs were illuminated at 470 ± 20 nm and fluorescent signals > 510 nm were recorded as a relative measure of $[Ca^{2+}]_i$. Fluorescent signals were collected by using a video-based imaging system that has been described (23). The locations of cells of interest were specified by using a computer mouse and custom-designed software. Signals were recorded as the fluorescence within a rectangular region over each cell. Fluorescent responses were normalized by dividing by the maximum response to the bath perfusion of a solution containing 1 μM Bay K 8644. Thus, the ratio associated with the ordinate of each trace represents a fraction of the increase in $[Ca^{2+}]_i$ relative to the response to 1 μM Bay K 8644. By continuously frame-grabbing images, the computer was able to track the activity of a number of individual cells within a field of view simultaneously. NO was introduced by bolus application of saturated solution (1 mM) to the bath. Bay K 8644 was applied to individual cells by micropressure ejection (2 bars; 15 msec) using a Picospritzer apparatus (General Valve, Fairfield, NJ). Other agents were added or removed from solutions by rapidly exchanging bath medium.

Hanks' perfusion buffer, NO stock solutions, oxyhemoglobin, and methemoglobin were prepared as described (11,

23). Drugs used were ryanodine (Agri Systems International, Wind Gap, PA) and Bay K 8644 (Sigma). *N*^G-nitro-L-arginine methyl ester (L-NAME) and *N*^G-monomethyl-L-arginine were obtained from Sigma and fluo-3/AM was obtained from Molecular Probes. Data are expressed as means \pm SE.

RESULTS

Bolus application of NO to the bath caused an increase in fluo-3 fluorescence of ICs, indicating an increase in $[Ca^{2+}]_i$ (Fig. 1, upper trace). When boluses were applied that raised $[NO]$ to 1 μ M (3 μ l of saturated solution), the increase in fluo-3 fluorescence had an average $t_{1/2}$ of 15 ± 4 sec and recovered to a resting level of fluorescence after an average of 4.2 ± 0.8 min ($n = 16$). The average amplitude of the peak response was 0.52 ± 0.13 of the response to the perfusion of 1 μ M Bay K 8644. When smaller boluses of NO were applied, the increase in $[Ca^{2+}]_i$ was transient (lasting <10 sec). Larger concentrations of NO (maximal bath $[NO]$, 10 μ M) did not significantly alter responses compared to bolus applications that raised the bath $[NO]$ to 1 μ M. Increases in $[Ca^{2+}]_i$ were observed in both freshly dispersed ICs ($n = 16$) and in ICs that were cultured (see ref. 23) for up to 2 weeks ($n = 30$). Repeated applications of NO over a period of several hours elicited fluorescent responses that were similar in amplitude, shape, and duration compared to initial responses.

SMCs responded differently to NO. Bolus addition of 1 μ M NO caused an abrupt decrease in fluo-3 fluorescence, indicating a decrease in $[Ca^{2+}]_i$. The decrease in fluorescence averaged -1.03 ± 0.10 ($n = 29$) of the response to Bay K 8644 (1 μ M). As illustrated in Fig. 1 (lower trace), in some preparations (9 of 29) the decrease in $[Ca^{2+}]_i$ occurred in two phases: the abrupt decrease in fluorescence was followed by a transient recovery and then a slow decline in $[Ca^{2+}]_i$. In the majority of SMC responses to NO (20 of 29), the transient recovery phase was not apparent. Similar responses were obtained in SMCs whether they were in close proximity to ICs ($n = 13$) or in dishes that contained no identifiable ICs ($n = 16$).

The increase in $[Ca^{2+}]_i$ in ICs could be due to a transmembrane flux of Ca^{2+} or to release of Ca^{2+} from intracellular stores. A mechanism involving transmembrane influx of Ca^{2+} was tested for by removing Ca^{2+} from the extracellular medium. Fig. 2A (upper trace) shows a typical increase in $[Ca^{2+}]_i$ in response to a bolus application of 1 μ M NO with 1.8 mM Ca^{2+} in the external medium (control response). The medium was then switched to one with low Ca^{2+} (i.e., no Ca^{2+} added and 5 mM EGTA). The lower trace shows that an

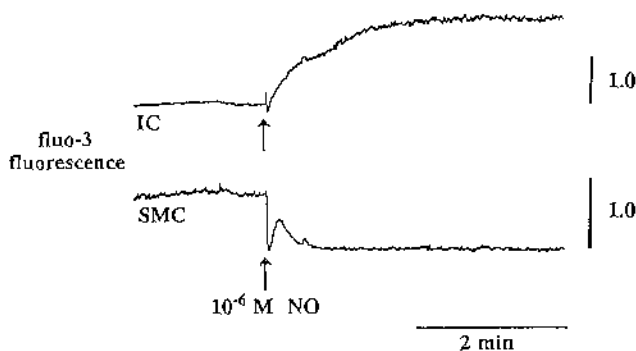


FIG. 1. Responses of freshly dispersed ICs and SMCs to exogenous NO. Cells were isolated from a muscle strip and located in the same dish. Changes in $[Ca^{2+}]_i$ were monitored by fluo-3 fluorescence. Fluorescent changes were normalized to the responses to the perfusion of Bay K 8644 (1 μ M). A bolus of NO was added to the bath at the time indicated (arrows) (maximum $[NO]$, 1 μ M). In response to NO, $[Ca^{2+}]_i$ increased in the IC (upper trace) and decreased in the SMC (lower trace).

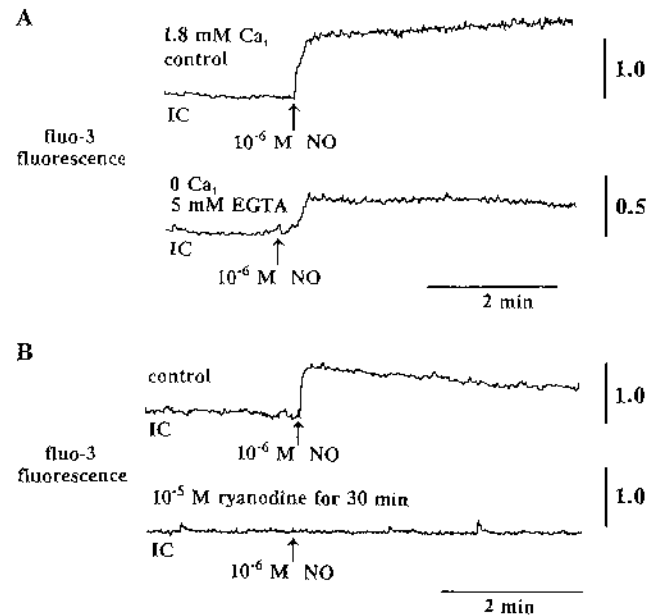


FIG. 2. Removal of intracellular and extracellular sources of Ca^{2+} . (A) Upper trace shows a control response of an IC to NO (maximum, 1 μ M in bath) in normal (1.8 mM) $[Ca^{2+}]_o$. External medium was then switched to one containing no added Ca^{2+} and 5 mM EGTA. Addition of NO continued to produce an increase in $[Ca^{2+}]_i$ (lower trace). (B) In a separate experiment, a control response to 1 μ M NO was obtained before addition of 10 μ M ryanodine (upper trace). After a 30-min incubation in ryanodine, the response to NO was abolished (lower trace).

increase in $[Ca^{2+}]_i$ could be obtained in the absence of external Ca^{2+} . NO caused an increase in $[Ca^{2+}]_i$ in all ICs tested ($n = 4$) within 5 min after removal of Ca^{2+} from the external medium. These results suggest that external Ca^{2+} is not the primary source for the NO-induced increase in $[Ca^{2+}]_i$ in ICs.

To test whether release of Ca^{2+} from internal stores was important in the IC response to NO, cells were exposed to NO before and after bathing in ryanodine (10 μ M) for 30 min. A typical IC response to the application of 1 μ M NO is shown in Fig. 2B (upper trace). After ryanodine treatment, no measurable change in $[Ca^{2+}]_i$ was observed when NO was reapplied (lower trace). Inhibition of the NO effect by ryanodine did not appear to be due to a run-down phenomenon during the 30-min incubation in ryanodine, because control experiments showed that NO responses were retained during incubation in normal solution (data not shown). No detectable changes in fluo-3 fluorescence were elicited by NO after incubation in 10 μ M ryanodine ($n = 3$), suggesting that the increase in $[Ca^{2+}]_i$ in response to NO was from intracellular sources.

To test the effects of elevating $[Ca^{2+}]_i$ by using a known Ca^{2+} channel agonist, Bay K 8644 was micropressure ejected onto ICs and SMCs. Previous experiments have demonstrated dihydropyridine-sensitive Ca^{2+} currents in both types of cells (21, 22). The concentration of Bay K 8644 in the micropipette was 10 μ M and the tip (diameter, 40 μ m) was lowered to within 50 μ m of cells. As illustrated in Fig. 3, Bay K 8644 caused an elevation in fluo-3 fluorescence, indicating an increase in $[Ca^{2+}]_i$ in both ICs ($n = 13$) and SMCs ($n = 25$). However, when Bay K 8644 was specifically applied to ICs (Fig. 3A), $[Ca^{2+}]_i$ decreased in nearby SMCs. The average minimum distance (closest points between pairs of cells) separating ICs and SMCs in these experiments was 146 ± 35 μ m, with a range from 40 to 270 μ m. The mean distance between the centers of pairs of cells was 211 ± 38 μ m, with a range from 100 to 350 μ m. Micropressure ejection of Bay K 8644 onto SMCs had no effect on adjacent ICs (Fig. 3B),

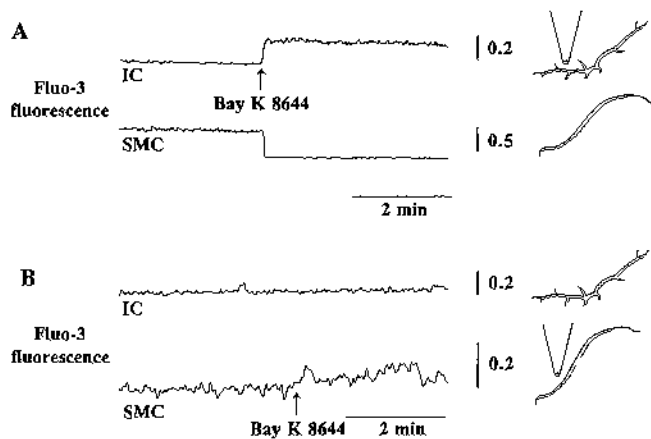


FIG. 3. A two-cell system demonstrating transmission by a diffusible substance. A freshly dispersed IC was identified in close proximity to a SMC (minimum distance separating the two cells, 225 μm ; arrangement shown schematically at right). (A) Bay K 8644 (concentration in pipette, 10 μM) was micropressure ejected toward the IC at the time indicated (arrow). This caused an increase in $[\text{Ca}^{2+}]_i$ in the IC (upper trace) and a decrease in $[\text{Ca}^{2+}]_i$ in the nearby SMC (lower trace). (B) When Bay K 8644 was pressure ejected toward the SMC, $[\text{Ca}^{2+}]_i$ increased in the SMC (lower trace) and no response was observed in the IC (upper trace).

suggesting that (i) it was possible to direct the application of Bay K 8644 to specific cells and (ii) the decrease in $[\text{Ca}^{2+}]_i$ in SMCs caused by application of Bay K 8644 to an IC was due to release of a diffusible substance from the IC when $[\text{Ca}^{2+}]_i$ was elevated.

Another method to stimulate a selected IC while avoiding the possibility of diffusion of drugs throughout the bath is to mechanically agitate an IC using an unfilled, glass micropipette (like those typically used for intracellular recordings). When a pipette was carefully inserted into an IC, there was generally a small, transient increase in $[\text{Ca}^{2+}]_i$ (likely dependent on the degree of damage to the cell during microelectrode entry) and no response or only a small transient decrease in $[\text{Ca}^{2+}]_i$ in adjacent SMCs. Upon withdrawal of the pipette, there was a sustained increase in $[\text{Ca}^{2+}]_i$ in the impaled IC and a decrease in $[\text{Ca}^{2+}]_i$ in nearby SMCs (data not shown). These results also suggest that a diffusible substance might be released from ICs in response to the elevation in $[\text{Ca}^{2+}]_i$.

It is possible that NO could be the diffusible substance since NO synthesis is Ca^{2+} dependent in many cells (see ref. 24). If NO is the substance that mediates responses in SMCs when $[\text{Ca}^{2+}]_i$ increases in ICs, then transmission between ICs and SMCs should be blocked by inhibitors of NO synthesis. Bay K 8644 was micropressure ejected onto ICs and changes in $[\text{Ca}^{2+}]_i$ were monitored in the selected IC and adjacent SMCs. After recording control responses (Fig. 4A), cells were exposed to L-NAME or *N*^G-monomethyl-L-arginine ($n = 4$) for 3 min. Then the IC was reexposed to Bay K 8644 by micropressure ejection in the presence of the arginine analogues. The amplitude of the increase in fluorescence was in the range 1–6% of the initial control responses. Arginine analogues also blocked the decrease in $[\text{Ca}^{2+}]_i$ in nearby SMCs in response to selective elevation of $[\text{Ca}^{2+}]_i$ by Bay K 8644 in ICs. An example of the block by L-NAME is shown in Fig. 4B.

We also tested the effects of oxyhemoglobin (known to sequester NO; ref. 25) on the transmission between ICs and SMCs. Bay K 8644 (10 μM) micropressure ejected onto ICs in normal buffer produced typical responses in ICs and nearby SMCs (see Fig. 3A). The red color of oxyhemoglobin reduced the signal/noise ratio of the Ca^{2+} -sensitive dye

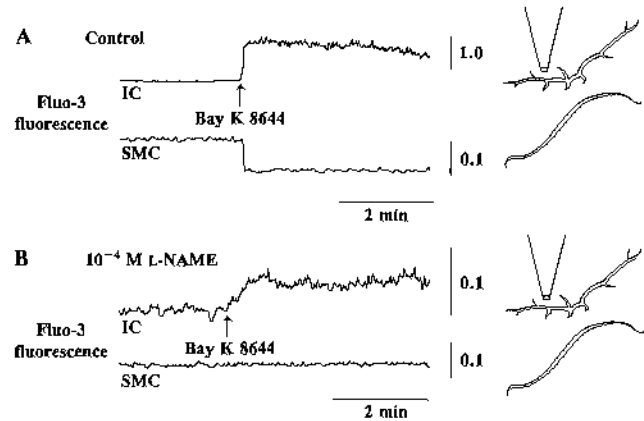


FIG. 4. Transmission from IC to SMC was blocked by L-NAME. (A) Decrease in $[\text{Ca}^{2+}]_i$ is shown in a SMC when Bay K 8644 was applied to a nearby IC (minimum distance separating the two cells, 205 μm). (B) Response in the IC was substantially reduced and response in the SMC was blocked when 100 μM L-NAME was added to the bath.

fluo-3. However, when 1% oxyhemoglobin was added to the external medium, an increase in $[\text{Ca}^{2+}]_i$ could still clearly be observed in ICs after micropressure ejection of Bay K 8644. Oxyhemoglobin blocked responses in nearby SMCs ($n = 3$). In control experiments using a similar protocol with methemoglobin (which caused a similar reduction in the signal/noise ratio of fluorescent responses), decreases in fluorescence in nearby SMCs were not blocked.

The fluorescent changes observed in SMCs in response to NO provide a convenient assay for transmission by the diffusible substance released from ICs. We used such a technique to address the question of whether SMCs themselves can be induced to release NO. Pairs of SMCs were located in close proximity to each other. When Bay K 8644 (10 μM) was applied to one of the SMCs in the pair, an increase in fluorescence was observed in the cell near the pipette and a response was not observed in an adjacent SMC (Fig. 5A). When Bay K 8644 was micropressure ejected onto the other cell, an increase in fluorescence was observed, indicating that the cell was responsive to agonists (Fig. 5B). Similar experiments were repeated in 17 pairs of SMCs separated by minimum distances ranging from 2 to 240 μm (average, $112 \pm 19 \mu\text{m}$). Bay K 8644 consistently produced an increase in $[\text{Ca}^{2+}]_i$ in the cell near the pipette tip, but this never caused a decrease in fluorescence in an adjacent cell. Results of these experiments suggest that, in normal buffer, SMCs do not release NO in response to an increase in $[\text{Ca}^{2+}]_i$.

DISCUSSION

Recent studies have characterized differences in the ionic currents of SMCs and ICs (22). ICs express a low-threshold Ca^{2+} current not found in SMCs and the non- Ca^{2+} -dependent, voltage-dependent K current of ICs inactivates at more negative potentials than the equivalent current in SMCs. These differences may facilitate the pacemaker function proposed for these cells (21, 26). This study demonstrates another physiological difference between ICs and SMCs. In response to NO, $[\text{Ca}^{2+}]_i$ increased in ICs and decreased in SMCs. Removal of extracellular Ca^{2+} did not abolish the increase in $[\text{Ca}^{2+}]_i$ caused by NO, whereas exposure to ryanodine completely blocked the response. These results suggest that intracellular Ca^{2+} stores are necessary for the NO-induced increase in $[\text{Ca}^{2+}]_i$ in ICs. We did not investigate the mechanism by which $[\text{Ca}^{2+}]_i$ decreased in SMCs in response to NO. Lincoln and Cornwell (27) have suggested that Ca^{2+} sequestration into stores can be en-

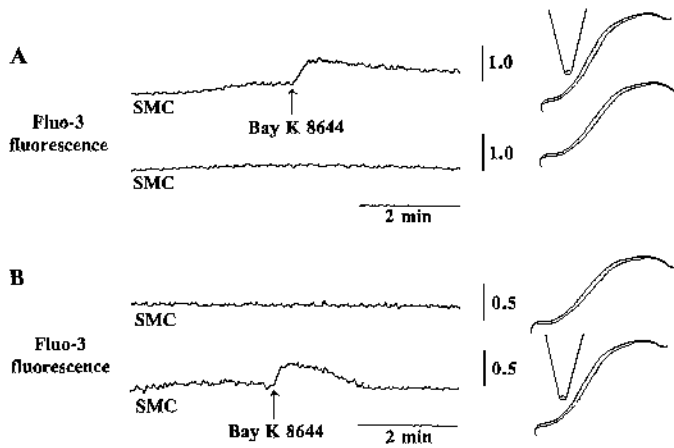


FIG. 5. NO is not released from SMCs by elevated $[Ca^{2+}]_i$. Two SMCs separated by a minimum distance of $120 \mu m$ were identified. (A) When Bay K 8644 was micropressure ejected near one of the SMCs, a transient increase in $[Ca^{2+}]_i$ was observed, but no response was observed in the second SMC. (B) When Bay K 8644 was micropressure ejected near the second SMC, a transient increase in $[Ca^{2+}]_i$ was observed in that cell, but no response was observed in the other SMC.

hanced via a cGMP-dependent mechanism, and Garg and Hassid (28) have shown a decrease in $[Ca^{2+}]_i$ in fibroblasts via a cGMP-independent mechanism.

Some authors have suggested that ICs may serve as intermediates in neurotransmission from enteric nerves to the syncytium of SMCs (29, 30). This hypothesis is based primarily on morphological studies showing that ICs are often in close proximity to the varicosities of nerve fibers and appear to be interposed between nerves and SMCs. It is possible that receptors for neurotransmitters are expressed by ICs. Then neurotransmitters could elicit electrical responses in ICs that could be transmitted to SMCs via gap junctions occurring between these cells (17, 19). The present study demonstrates that ICs possess a receptor mechanism for NO, one of the major inhibitory transmitters released from enteric inhibitory nerves (see ref. 1). These data also provide functional support for the concept that ICs serve as intermediates in enteric inhibitory neurotransmission.

This study also shows that ICs respond to NO with an elevation of $[Ca^{2+}]_i$. In response to the increase in $[Ca^{2+}]_i$, ICs appear to release a diffusible substance capable of reducing $[Ca^{2+}]_i$ in nearby SMCs. The diffusible substance may be NO because (i) its effects on SMCs were mimicked by exogenous NO, (ii) release of the diffusible substance was blocked by arginine analogues, which are known to block NO synthase (see ref. 24), and (iii) transmission between ICs and SMCs was blocked by oxyhemoglobin, which is known to bind to and sequester extracellular NO (25). If the diffusible substance is NO, or a carrier of NO, then the following concept is possible: (i) release of NO increases $[Ca^{2+}]_i$ in ICs, (ii) this stimulus increases synthesis and release of NO, and (iii) together these mechanisms create a positive-feedback loop within ICs, which amplify NO signals. The reduced $[Ca^{2+}]_i$ response caused by Bay K 8644 in the presence of arginine analogues (see Fig. 2B) supports the concept of positive feedback. After inhibition of NO synthesis, Bay K 8644 increases $[Ca^{2+}]_i$, but the response may not be reinforced by positive-feedback amplification.

At present, the enzyme responsible for producing NO in ICs is unknown. Apparent activation of NO release by Bay K 8644 suggests that a Ca^{2+} -dependent NO synthase is involved. Recently, Daniel *et al.* (31) have reported NO synthase-like immunoreactivity in ICs of the canine intestine.

In addition to neurons, there are other cell types present within the wall of the GI tract that could release NO and

trigger the amplification mechanism: (i) endothelial cells in blood vessels release NO, and small blood vessels can be found close to ICs *in situ* (S. M. Ward and K.M.S., unpublished observations), and (ii) macrophages and other cells express a Ca^{2+} -independent form of NO synthase that can produce relatively large amounts of NO when the enzyme is induced by cytokines (see ref. 24). In addition, ICs generate spontaneous oscillations in $[Ca^{2+}]_i$ (23), and it is possible that these events are accompanied by rhythmic bursts of NO synthesis. NO from any of these sources could be amplified by the IC network.

Furthermore, amplification of NO signals could result in the active propagation of inhibitory transmission through a network of ICs. The NO released by one IC should increase $[Ca^{2+}]_i$ in nearby ICs and propagate the positive feedback mechanisms. This extracellular chemical propagation pathway might be augmented by intracellular electrochemical transmission. The increase in $[Ca^{2+}]_i$ would create gradients in Ca^{2+} (and possibly other second messengers) between cells. Gap junctions, which are plentiful between ICs *in situ* (17), allow the exchange of ions and small molecules. The flow of ions and second messengers from cell to cell could facilitate propagation of an inhibitory wave front.

Positive-feedback mechanisms require a means of inactivation. Some possibilities include the following: (i) Release of Ca^{2+} from intracellular stores appears to be a mechanism to activate NO synthase in ICs. NO may hyperpolarize ICs, reduce Ca^{2+} entry, and eventually reduce the Ca^{2+} within intracellular stores. (ii) The increase in $[Ca^{2+}]_i$ caused by NO may increase Ca^{2+} -activated K conductances (see ref. 11), cause hyperpolarization, and reduce Ca^{2+} entry. (iii) NO or Ca^{2+} may affect second messenger systems in ICs that may reduce the Ca^{2+} dependence of NO synthase or inhibit the second messenger systems that cause Ca^{2+} release.

Recent studies have suggested that NO may also be produced by SMCs from the rat gastric fundus and rabbit intestine (32, 33), and this may serve to amplify neurotransmission due to vasoactive intestinal peptide released from enteric nerves (32). By using responses of SMCs as a bioassay for NO (see Fig. 5), we found that Bay K 8644 increased $[Ca^{2+}]_i$ in SMCs, but these effects were not transmitted to other SMCs in close proximity. In other words, the diffusible substance produced by ICs in response to an increase in

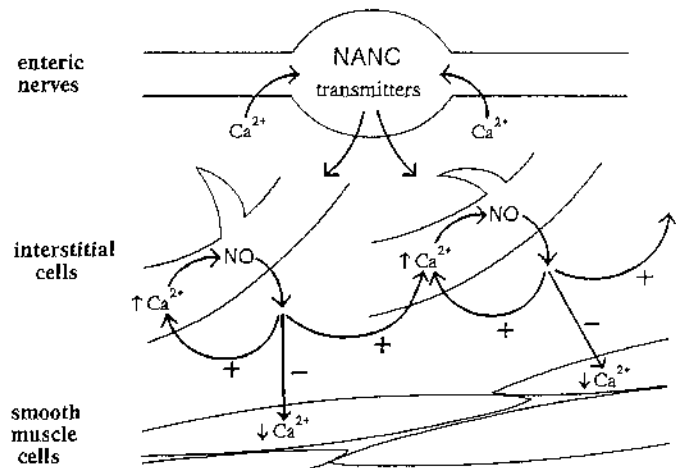


FIG. 6. Schematic illustration of mechanisms involved in a propagating wave of relaxation. In this example, positive feedback mechanisms are initiated by release of NO from enteric inhibitory nerves. This causes an increase in $[Ca^{2+}]_i$ in ICs, which induces further synthesis of NO. Release of NO causes an increase in $[Ca^{2+}]_i$ in nearby ICs, perpetuating the positive-feedback mechanism for NO release. Effect of NO release on nearby SMCs is to decrease $[Ca^{2+}]_i$, resulting in an actively propagated wave of relaxation. NANC, nonadrenergic noncholinergic.

$[Ca^{2+}]_i$ was not produced by SMCs. Vasoactive intestinal peptide (10–100 μ M) stimulation of SMCs also produced no effects in nearby SMCs ($n = 5$; data not shown). Explanations for the apparent contradiction with recent studies (32) include possible species differences, different properties of SMCs in different regions of the GI tract, or insufficient production of NO to be sensed by adjacent cells.

In summary, ICs appear to possess a means to amplify NO signaling in GI muscles. Fig. 6 illustrates this hypothesis. In this example, release of NO from enteric inhibitory nerves triggers an increased $[Ca^{2+}]_i$ in nearby ICs. This increases synthesis and release of NO from the ICs, which further increases $[Ca^{2+}]_i$. NO can diffuse to nearby ICs to trigger and perpetuate the feedback loop within the network of ICs. NO may also diffuse to nearby SMCs, causing inhibition. This system provides a mechanism for active propagation of inhibitory signals and represents a novel concept in cell-to-cell communication within smooth muscles.

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