Involvement of Nitric Oxide in Neuromuscular Transmission in Canine Proximal Colon (43950C)

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It is well established that discrete populations of neurons throughout the nervous system have the capacity to synthesize and release nitric oxide (NO). Although nitric oxide synthase (NOS) was first isolated from the cerebellum, some of the most comprehensive evidence for a neurotransmitter role for NO comes from studies of smooth muscles controlled by the autonomic nervous system. Since the 1960s, it has been appreciated that stimulation of enteric neurons supplying gastrointestinal smooth muscle releases inhibitory neurotransmitters, which cause smooth muscle hyperpolarization and relaxation (1, 2). The actions of this transmitter (or group of transmitters) are not antagonized by muscarinic or adrenergic receptor antagonists, leading to the term "nonadrenergic, noncholinergic" (NANC) neurotransmission being used to refer to this process. In the 30 years or so that followed the original characterization of these responses, NANC relaxations have been characterized in many peripheral tissues, including most regions of the gastrointestinal tract, urogenital tract, and neurogenic relaxation of many blood vessels. For many years, the identity of transmitter(s) responsible for these responses remained unknown or controversial, principally because potent and specific antagonists could not be found.

In the 1980s, studies of rodent anococcygeus and bovine retractor penis muscles revealed similarities between the actions of an unidentified autonomic transmitter and endothelium-derived relaxing factor (EDRF). Following the discovery that EDRF was synthesized by NOS and its synthesis could be abolished by arginine analogues, selective tools became available to test the hypothesis that NO was released as an autonomic neurotransmitter. Since that time, a large number of studies have reported that arginine analogs that block NO synthesis effectively antagonize nerve-stimulated smooth muscle relaxations and markers for NOS have been localized in autonomic motor neurons in many of these tissues. A number of reviews are available describing the discovery of autonomic nitric oxide–dependent transmission (also termed "nitrergic" transmission, see Ref. 3) and its role in regulation of smooth muscle function (3–9).

The relative simplicity of nerve smooth muscle preparations makes them useful for investigations of nitrergic transmission. In this short review, we discuss nitrergic neurotransmission in canine colonic muscles as a model of peripheral inhibitory neurotransmission. We have also included a discussion of several postjunctional mechanisms that may contribute to the actions of NO as an enteric inhibitory neurotransmitter.

Localization and Activity of Nitric Oxide Synthase

Neuronal Nitric Oxide Synthase. Following the isolation of NOS it was demonstrated that discrete populations of neurons throughout the central and peripheral nervous systems contain NOS-like immunoreactivity (NOS-LI) and that these neurons are often identical to those stained with the NADPH diaphorase histochemical method (10, 11). Using antisera raised against rat cerebellar NOS (12), nNOS-like immunoreactivity (nNOS-LI) was found in a subpopulation of myenteric and submucosal neurons in canine proximal colon. These neurons are also labeled using NADPH diaphorase histochemistry, so that, as in other regions of the nervous system, NADPHd can be a useful marker for NOS-LI–positive neurons. NADPHd staining and nNOS-LI is also found in many nerve fibers supplying the circular and longitudinal muscle layers (13). These findings suggest that NO could be synthesized in response to elevated intracellular calcium concentration in intrinsic neurons supplying the smooth muscle layers.

Evidence for release of NO from canine colonic intrinsic neurons has been obtained using a chemilu-

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minescence detection method. In these experiments, samples from stimulated tissues were subjected to reducing conditions to regenerate NO from its oxidation products (NO_2^- and NO_3^-) and NO measured by chemiluminescence. Release of NOx was antagonized by (i) inhibitors of NOS activity (ii), removal of extracellular calcium ions, and (iii) tetrodotoxin, suggesting that NO release was due to stimulation of a calcium-dependent isoform of nitric oxide synthase (14).

Endothelial Nitric Oxide Synthase. Immunoreactivity for nNOS and NADPHd staining are not detectable in smooth muscle cells in this tissue; however, immunoreactivity for endothelial type NOS (eNOS-LI) can be found in the endothelium of small intramural blood vessels and also in interstitial cells (15). Interstitial cells (ICs) are a morphologically distinct cell type found within gastrointestinal smooth muscles and are recognized by their numerous mitochondria, multiple branching processes, basal lamina, and caveolae. These cells are hypothesized to act as pacemakers for gastrointestinal muscles by initiating phasic depolarizations of the smooth muscle cell syncytium (16, 17).

Evidence for release of NO from ICs was obtained using a bioassay method in which individual smooth muscle cells were used as NO sensors (18). Enzymatic dispersions containing ICs and smooth muscle cells were loaded with the calcium indicator Fluo-3. Brief application of NO to smooth muscle cells produced a prompt decrease in intracellular calcium ion concentration ($[Ca^{2+}]_i$) providing an assay for NO release from nearby ICs in mixed dispersions. Selective exposure of an IC to the calcium channel agonist BAY K 8644 (picospritz) produced an increase in IC $[Ca^{2+}]_i$ and a decrease in $[Ca^{2+}]_i$ in nearby (<100 μm) smooth muscle cells. The NOS inhibitor L-NAME did not prevent the actions of BAY K 8644 on ICs, but prevented subsequent responses in nearby smooth muscle cells (18). These results suggest that increases in $[Ca^{2+}]_i$ can stimulate eNOS in ICs.

Evidence for a Neurotransmitter Role

Inhibitory Junction Potentials. Electrical field stimulation (EFS) of gastrointestinal nerve-muscle preparations often results in a transient hyperpolarization of smooth muscle cells. These inhibitory junction potentials (ijps) are the electrical response of muscle cells to release of inhibitory neurotransmitter(s) from intrinsic neurons. ijps are recorded from the circular muscle of canine proximal colon; however, the amplitude of these events is quite dependent on the position of the intracellular microelectrode. Muscle cells near the myenteric plexus usually show the least negative resting potential (~40 mV) and single pulses of electrical field stimulation (EFS) produce relatively large (~30 mV), tetrodotoxin-sensitive ijps. As the electrode is moved across the thickness of the muscle (towards the submucosa), a progressive increase in resting membrane potential is noted with a corresponding decrease in ijp amplitude (19). These responses are not reduced by adrenoceptor antognists or atropine and are therefore sometimes referred to as NANC ijps. The possible involvement of NO in inhibitory transmission was therefore first tested on evoked ijps recorded from circular muscle cells near the myenteric plexus region.

L-NAME and other inhibitors of NO synthesis (L-NNA, L-NMMA) greatly decrease the amplitude of ijps evoked by single pulses of EFS. Detectable decreases in ijp amplitude were seen within minutes of L-NAME addition and reached a maximum within approximately 15–20 min (20). In many preparations, ijps evoked by single stimuli are completely abolished by NOS inhibitors (used at concentrations of 100 μ*M*). Subsequent addition of L-arginine, but not D-arginine, reverses the actions of L-NAME (20), implying that the actions of L-NAME were due to stereo-specific interaction with the arginine binding site of NOS.

Most studies of ijps are performed in the presence of atropine, since the major excitatory neurotransmitter acetylcholine is also released by single pulses of EFS and its influence on the muscle (an excitatory junction potential [ejp]) may mask a component of the ijp. The underlying cholinergic ejp can be more clearly seen when the ijp is antagonized by L-NNA in the absence of atropine. Interestingly, the same is not true for L-NAME—in the absence of atropine this agent antagonizes the ijp without revealing a cholinergic ejp (Shuttleworth, Sanders, unpublished observations). This difference may be explained by the weak muscarinic antagonist activity of this arginine analog (21).

Further support for the involvement of NO as a neurotransmitter mediating ijps comes from responses to exogenous NO. Addition of NO solutions produced a concentration-dependent hyperpolarization of smooth muscle cells. The time course of these responses was dependent on the rate of delivery and removal of NO from the superfusion medium; however, brief NO exposures produced rapid-onset hyperpolarizations that closely resembled events evoked by nerve-stimulation (22). ijps and responses to exogenous NO were both antagonized by exposure to oxyhemoglobin, an agent that binds extracellular NO with high avidity. These observations strengthen the contention that NO acts as a neurotransmitter, suggesting that the activity of NO requires intercellular movement rather than activity as an intracellular messenger (22, 23).

Inhibition of Slow Waves and Phasic Contractions. It was mentioned above that in the canine proximal colon there is a gradient in resting membrane potential across the thickness of the circular muscle layer. In contrast to cells near the longitudinal muscle

layer, cells near the innermost surface (near the submucosa) have relatively negative resting potentials (~80 mV) and show regular spontaneous depolarizations called slow waves (24). During the plateau phase of each slow wave, resting potential is maintained at approximately −35 mV for a number of seconds, providing a period of opening of voltage-dependent calcium channels sufficient to produce a phasic contraction (16). EFS produces frequency-dependent inhibition of slow wave duration, and these effects are abolished by L-NAME or oxyhemoglobin. Exogenous addition of NO solutions mimics the effects of EFS, reducing slow wave duration (23). These observations are consistent with effects seen in contractile recordings of circular muscle strips, where L-NNA prevents EFS-stimulated inhibition of phasic contractile activity (25).

Co-Transmission

The canine proximal colon is a useful preparation to study mechanisms of nitrergic transmission, since ijps are often abolished by inhibitors of NO synthesis and there is little contribution from other inhibitory neurotransmitters to complicate analysis. However, it is important to note that in some tissues ijps evoked by single stimuli do not appear to involve NO synthesis. For example in intracellular microelectrode recordings from human colonic or jejunal circular muscle, ijps evoked by single pulses (or trains of impulses at low frequency) appear completely unaffected by L-NAME, L-NNA or L-NMMA. However, if a train of pulses is given at higher frequency (e.g., 5 Hz for 30s) the inhibitory event is composed of two components, an initial NOS-inhibitor resistant component and a sustained hyperpolarization that is antagonized by NOS inhibitors or oxyhemoglobin (26, 27). Thus, NO is presumed to act as an inhibitory transmitter to mediate the sustained component of the compound ijp and contribute to nerve-stimulated relaxation in human colonic tissues (28). The NOS inhibitor–resistant ijps in human colonic circular muscle are antagonized by apamin, a peptide toxin that blocks a type of Ca^{2+}-activated K^+ channel in gastrointestinal (GI) smooth muscle. The transmitter(s) responsible for apamin-sensitive transmission remain contraversial, but ATP has long been a favored candidate (29) and recently the peptide pituitary adenylate cyclase activating peptide (PACAP) has been advanced as a possible mediator (30).

ijps evoked by single pulses in canine colon are abolished by NOS-inhibitors and unaffected by apamin, and therefore are not dependent on the fast transmitter discussed above. However, under some conditions, a component of transmission in canine colon is resistant to arginine analogs and oxyhemoglobin, suggesting the involvement of additional inhibitory transmitter(s). This has been demonstrated in tension recordings of smooth muscle strips where a combination of antagonists has been used to block the actions of NO and multiple excitatory transmitters that may mask the actions of other inhibitory transmitters. The circular muscle of the canine proximal colon shows spontaneous periodic phasic contractile activity. In the presence of atropine and adrenoceptor antagonists, EFS produces frequency-dependent, tetrodotoxin-sensitive inhibition of these contractions. These nerve-stimulated relaxations are prevented by arginine analogues (L-NAME, L-NMMA) or by oxyhemoglobin (25). Furthermore, when nitrergic transmission is blocked a noncholinergic contraction is revealed during the stimulation period (31). Subsequent antagonism of the noncholinergic excitation (using an antagonist of tachykinin NK2 receptors) then reveals nerve-stimulated inhibition during the stimulus period (32). This suggests that, in addition to NO, another inhibitory transmitter(s) is released and that its actions are usually masked by the concomitant release of excitatory peptides during nondiscriminatory EFS. Vasoactive intestinal peptide (VIP) is a candidate for the transmitter, since VIP-like immunoreactivity is co-localized with NOS-LI in intrinsic nerve cell bodies and fibres supplying the circular muscle and VIP is an effective inhibitory agonist in this tissue (32).

Recycling of Citrulline

NOS produces citrulline stoichiometrically with NO and recent studies have demonstrated that some NO-releasing cells (e.g., macrophages, endothelial, and induced vascular smooth muscle cells) have the capacity to regenerate arginine from citrulline. Citrulline can be converted to arginine via the production of argininosuccinate by the sequential activities of argininosuccinate synthetase (AS) and argininosuccinate lyase (AL). It has been proposed that under some conditions this pathway maintains adequate levels of arginine required for NO production (Fig. 1) (33–36).

In the canine proximal colon, AS-like immunoreactivity (AS-LI) and AL-like immunoreactivity (AL-LI) are found in many nerve-fibres supplying the circular and longitudinal muscle layers. Immunoreactivity for both markers was also found in a subpopulation of myenteric and submucosal nerve cell bodies, suggesting that immunoreactive fibres within the muscle are of intrinsic origin. Sequential processing revealed that all AS-LI positive neurons were NADPHd positive and the converse was also true (i.e., all NADPHd positive neurons contained AS-LI). There was also virtually complete co-localization between AL-LI and NADPHd. Thus, although direct demonstration was not possible, it can be deduced that these three markers (NADPHd, AS-LI, AL-LI) are all found in the same neuronal population, suggesting that NO-

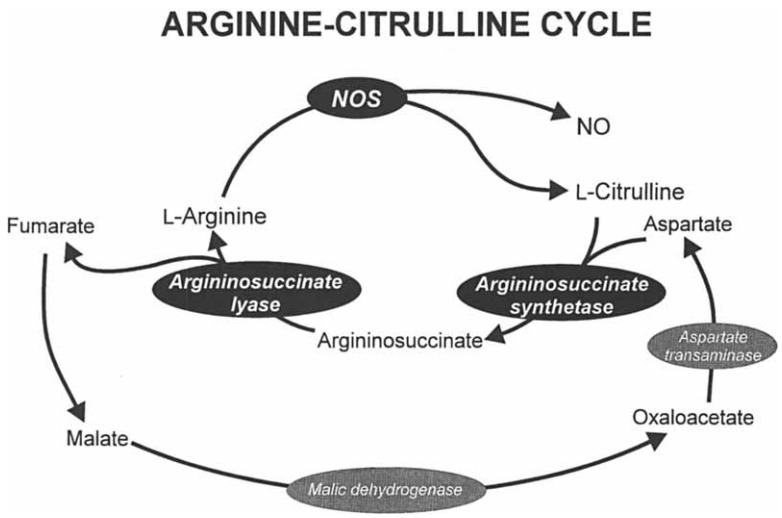

Figure 1. Arginine-citrulline cycle. This figure is adapted from Ref. 36 to explain how citrulline could be recycled to provide arginine for NO synthesis. In the canine proximal colon, NOS, argininosuccinate synthetase, and argininosuccinate lyase appear to be co-localized within neurons supplying the muscularis. Functional evidence suggests that citrulline is efficiently converted to arginine to sustain neuronal NO synthesis in this tissue (see text for details).

releasing inhibitory motor neurons contain the enzymatic apparatus to recycle citrulline to arginine (37).

As mentioned above, L-NAME blocks ijps in canine colon and this action is reversed by addition of L-arginine to the bathing medium. Thus, the use of an inhibitor that competes for arginine binding can be used to demonstrate substrate dependence of NOS activity. If enteric neurons have the capacity to convert citrulline to arginine, then addition of exogenous citrulline might be expected to overcome L-NAME block of inhibitory transmission. Indeed, citrulline exposure almost completely restored ijp amplitude, inhibition of slow wave duration and inhibition of phasic contractions observed during EFS in the continued presence of L-NAME. Citrulline alone had no effect on muscle activity or control nitrergic transmission, and other neutral amino acids (leucine, serine) had no activity in these experiments (37). This suggests that citrulline can provide a source of arginine for NO synthesis via the production of argininosuccinate. It is noteworthy that Gibson and co-workers reported partial reversal of L-NNA effects by citrulline in murine anococcygeus (38). Thus, although the distributions of AS and AL have not yet been investigated in other peripheral tissues, it is possible that citrulline recycling may be a common feature of autonomic neurons that utilize NO as a neurotransmitter. This pathway may be a primary mechanism by which arginine levels are maintained for NO synthesis in these NO-releasing neurons.

Amplification of NO signaling by Interstitial Cells

It was mentioned above that interstitial cells are a specialized cell type found within gastrointestinal smooth muscles. ICs often found interposed between enteric nerve terminals and smooth muscle cells, and have been postulated to act as transducers of neural inputs to smooth muscle cells. In canine proximal colon, ICs are in close proximity to terminals of VIP/NOS-LI containing neurons (32, 39). To determine whether these cells are in fact influenced by neuronally released NO, targets of NO action were probed with antiserum raised against formaldehyde-like cGMP. Electrical stimulation of intrinsic neurons leads to cGMP-LI accumulation in ICs, and this response is mimicked by addition of exogenous NO solutions (40). In addition to increases in cGMP, it has been found that isolated ICs respond to NO by increasing intracellular free calcium concentration ($[Ca^{2+}]_i$) (18). This raises the possibility that interstitial cells respond to NO released from inhibitory neurons by synthesizing more NO, amplifying the inhibitory signal to surrounding smooth muscle cells (18).

Postjunctional Mechanisms of NO Action

NO or NO donors are inhibitory to tone and phasic contractions in gastrointestinal muscles. Studies on canine colonic muscles have shown that several postjunctional mechanisms may contribute to the inhibitory effects. Two general mechanisms appear to couple NO stimulation to inhibition of mechanical activity: (i) reduction in intracellular $[Ca^{2+}]_i$ and (ii) reduction in the sensitivity of the contractile apparatus to Ca^{2+}. There are many factors that might contribute to the reduction in $[Ca^{2+}]_i$, including hyperpolarization of membrane potential, inhibition of excitable events, inhibition of Ca^{2+} influx or release, and regulation of Ca^{2+} uptake and/or extrusion (Fig. 2). The second messenger pathways that couple NO to these responses are not fully understood, but the extensive literature on vascular smooth muscle suggests the pathway including enhancement in production of cGMP, activation of cGMP-dependent protein kinase, and phosphorylation of cellular proteins may be a major means of transducing NO signals (41). The following discusses second messenger signaling and coupling of cellular effectors in canine colonic muscles.

Stimulation of guanylate cyclase

cGMP levels in canine proximal colon have been measured using enzyme-linked immunosorbent assay (ELISA) methods, and found to increase in response

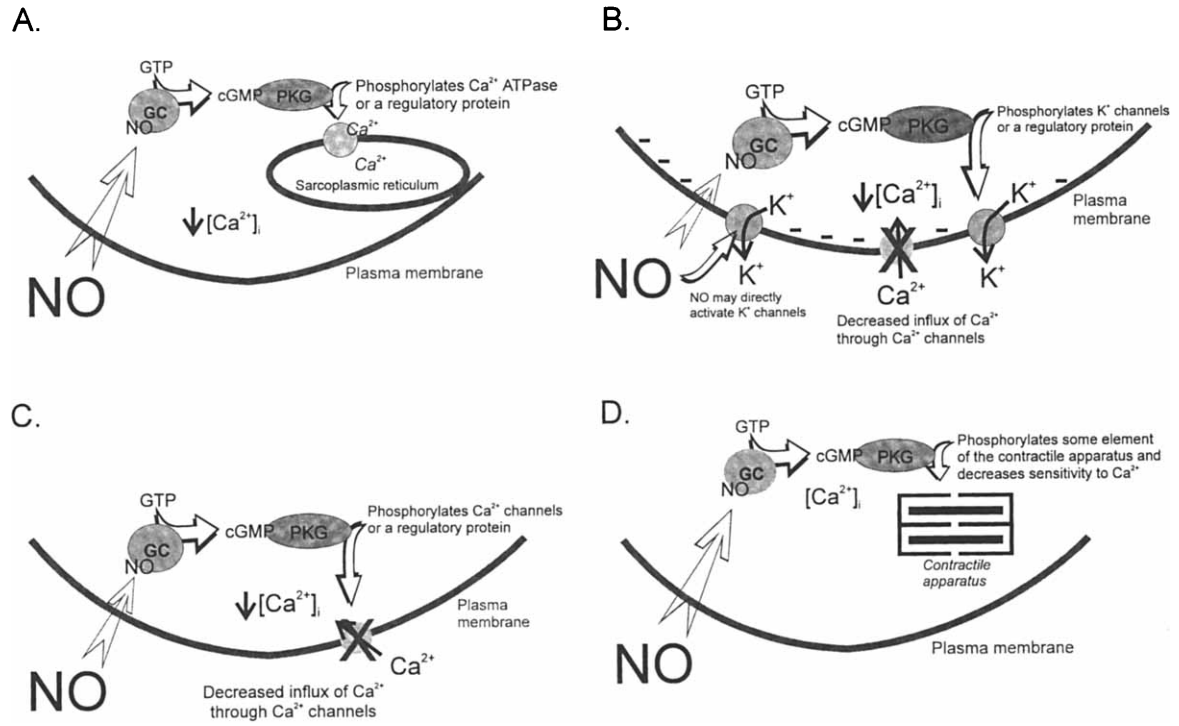

Figure 2. One of the major mechanisms by which NO inhibits smooth muscles is to reduce $[Ca^{2+}]_i$. Several mechanisms might contribute to this response. (A) Reduced $[Ca^{2+}]_i$ could result from an increase in uptake of Ca^{2+} into the sarcoplasmic reticulum (SR). In this scheme, NO activates guanylate cyclase (GC); enhanced cGMP activates protein kinase G (PKG); PKG phosphorylates a transport protein or regulatory protein, facilitating uptake of Ca^{2+}. (B) Reduced $[Ca^{2+}]_i$ could occur as a result of reduced entry of Ca^{2+} through L-type Ca^{2+} channels. In this scheme NO regulates the open probability of K^+ channels either via a direct mechanism or by activation of PKG and phosphorylation of channel or regulatory proteins. Increased K^+ conductance would tend to hyperpolarize membrane potential and reduce excitability. These changes would reduce the open probability of Ca^{2+} channels. (C) Reduced $[Ca^{2+}]_i$ could occur as a result of reduced entry of Ca^{2+} through L-type Ca^{2+} channels by a direct regulation of these channels. In this scheme PKG would phosphorylate one of the proteins making up Ca^{2+} channels and reduce the open probability at any given potential. (D) The reduction in force caused by NO may be due to a reduction in the sensitivity of the contractile apparatus to Ca^{2+}. In this scheme, PKG phosphorylates some element of the contractile apparatus.

to nerve stimulation or exposure to NO (42). The cellular targets of NO and sites of cGMP accumulation were also investigated using antisera raised against formaldehyde-linked cGMP. NO or nerve-stimulation caused an increase in cGMP-LI in smooth muscle cells, interstitial cells of Cajal, and some enteric neurons (40). Membrane-permeable analogs of cGMP (8-Br-cGMP, dibutyryl cGMP) mimicked the effects of NO, causing hyperpolarization of membrane potential, inhibition of excitability, and reduction in the amplitude of phasic contractions (42). These data suggest that NO is coupled to enhanced production of cGMP in smooth muscle cells and enhanced cGMP levels mediate responses similar to NO.

Another way to test the involvement of cGMP is to block or delay the breakdown of these messengers. Inhibitors of phosphodiesterase type V activity (e.g., M&B22948-zaprinast) increase the duration of ijps, suggesting that cGMP-dependent mechanisms may activate the currents responsible for these events. Other data tends to argue against this hypothesis, however, since methylene blue, an inhibitor of soluble guanylate cyclase in many smooth muscle preparations, had little or no effect on ijps (42). Others reported that methylene blue reduced the inhibitory effects of nerve stimulation on contractions (25). Thus, it is possible that the electrical effects of NO may be mediated by both cGMP-dependent and cGMP-independent mechanisms.

Activation of K^+ current

The hyperpolarization response to NO is likely to be due to the activation of K^+ channels. This mechanism could also contribute to a reduction in $[Ca^{2+}]_i$ because a significant amount of Ca^{2+} necessary for activation of GI muscles is due to influx via voltage-dependent Ca^{2+} channels. Hyperpolarization and/or reduction in action potentials or slow waves would tend to reduce the open probability of voltage-dependent Ca^{2+} channels.

Patch clamp recordings from single smooth muscle cells isolated from canine proximal colon have shown that large-conductance, Ca^{2+}-activated K^+ channels (IK_{Ca}) are activated by NO, NO donors and cGMP analogs (22, 43). This response is likely to be mediated via activation of cGMP-dependent protein

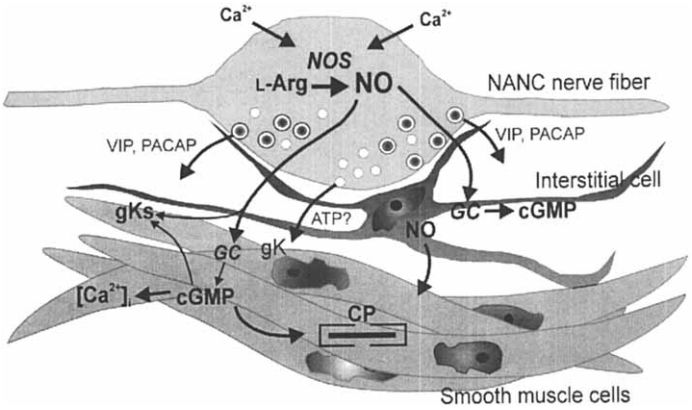

Figure 3. Summary of structures and mechanisms involved in mediating NO-dependent neurotransmission. NO is synthesized in varicosities of nonadrenergic, noncholinergic (NANC) neurons when Ca^{2+} rises as a result of activation. These neurons may also release other inhibitory transmitters, including ATP and the peptides VIP and PACAP. NANC varicosities in the GI tract are often in close association with interstitial cells of Cajal. Some classes of interstitial cells have the capacity of responding to NO by activation of guanylate cyclase (GC), production of cGMP, and production of more NO. In smooth muscles, NO from smooth muscle cells and interstitial cells activates GC. cGMP formed is coupled to a reduction in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and a reduction in the sensitivity of the contractile apparatus (CP) to Ca^{2+}. A significant part of the reduction in $[Ca^{2+}]_i$ appears to be due to activation of K^+ channels (gKs), either directly or via cGMP-dependent mechanisms. Increase K^+ conductance leads to hyperpolarization and reduction in excitability. Both of these phenomena reduce the open probability of L-type Ca^{2+} channels and reduce influx of Ca^{2+}.

kinase (44), but direct (i.e., non–cGMP-dependent) activation of IK_{Ca} has also been observed in smooth muscle cells isolated from rabbit coronary artery (45). The direct mechanism does not appear to be relevant in canine colonic cells, however, because NO donors had no effect on the open probability of IK_{Ca} in inside-out patches (43). Activation of IK_{Ca} does not appear to contribute significantly to inhibitory transmission under basal conditions because ijps are unaffected by blockers of IK_{Ca} (charybdotoxin or tetraethylammonium ions).

Two additional classes of K^+ channel were activated by NO and NO donors in colonic myocytes (43). These channels have conductances of approximately 80 ps (K_{NO1}) and <4 ps (K_{NO2}), In symmetrical K^+ gradients NO-stimulated openings of K_{NO2} were observed at a constant holding potential of −60 mV, raising the possibility that these channels could contribute to hyperpolarization caused by NO during ijps (43). Interestingly, both K_{NO1} and K_{NO2} could be directly activated by NO donors in inside out patches, presumably by a mechanism independent of cGMP. Direct modulation of ion channels by NO is reported to be due to change in redox state and prevented by exposure to the reducing agent dithiotreitol (DTT) or the alkylating agent N-ethylmaleimide (NEM) (45, 46). These agents both prevented activation of K_{NO1} and K_{NO2} in canine colonic myocytes, suggesting that direct regulation of K^+ channels may occur by a similar mechanism (43). One or both of these currents are attractive candidates for NO-mediated ijps and direct activation by redox modification might explain the observed insensitivity of ijps to methylene blue. Unfortunately, selective blockers of these channels have not been identified, so it is difficult to test this hypothesis directly.

Regulation of Ca^{2+} influx

Another means of inhibiting excitable events in GI muscles might be to inhibit L-type Ca^{2+} current since action potentials and, in some cases, slow waves depend upon these channels (16). Others have observed NO-dependent regulation of L-type Ca^{2+} currents in vascular and visceral smooth muscles (47, 48). Studies of canine colonic muscles have also demonstrated inhibition of L-type current by membrane permeable analogues of cGMP (Koh SD, Sanders KM, unpublished observations) and NO-donors (Campbell JD, Sanders KM, unpublished observations). But it appears that relatively high concentrations of NO may be required to achieve these effects.

Regulation of the sensitivity of the contractile apparatus to Ca^{2+}

Cyclic nucleotide–dependent mechanisms have been shown to decrease the sensitivity of the contractile apparatus to Ca^{2+} (49). This causes a rightward shift in the Ca^{2+}-force relationship, and even if Ca^{2+} levels remain the same, the amount of force produced decreases. Such a mechanism may be important in the mechanical effects of NO in GI muscles. cGMP causes a reduction of force in Ca^{2+} clamped skinned fibers (50), and we have found that addition of NO to colonic muscles depolarized with high external K^+ causes re-

duction in force without a change in $[Ca^{2+}]_i$ as measured by fura-2 (Sato K, Sanders KM, unpublished observations). At present, however, it is unclear what degree of importance should be attributed to this mechanism during enteric inhibitory neurotransmission.

Conclusions

The evidence is very strong that NO serves as a neurotransmitter in some autonomic neurons. In the canine proximal colon, NOS is localized in fibers and varicosities of inhibitory motor neurons that course through the muscle layers. Excitation of inhibitory neurons enhances Ca^{2+} entry into varicosities and activates NOS. In the GI tract enteric inhibitory neurons not only possess the ability to synthesize NO, they may also recycle the by-product, citrulline, back to arginine, thus sustaining inhibitory neurotransmission. NO appears to diffuse freely from nerve terminals and into nearby postjunctional cells. Interstitial cells appear to be innervated by nerves that release NO, and postjunctional effects in these cells include production of cGMP and synthesis of additional NO. In smooth muscle cells NO signals are transduced by guanylate cyclase, the production of cGMP, and activation of protein kinase G, but direct stimulation by NO of some cellular effectors, such as K^+ channels also appears to play a role. Responses of smooth muscle cells include activation of K^+ channels, inhibition of Ca^{2+} channels, and a reduction in the sensitivity of the contractile apparatus to Ca^{2+}. All of these factors may contribute to the reduction in mechanical activity produced by stimulation of enteric inhibitory neurons. NO-dependent neurotransmission is critical for many of the physiological processes of the GI tract, such as relaxation of sphincters, gastric accommodation, and receptive relaxation during feeding, and the descending inhibition arc of the peristaltic reflex.

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