Gastrointestinal Function Regulation by Nitrergic Efferent Nerves

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Abstract—Gastrointestinal (GI) smooth muscle responses to stimulation of the nonadrenergic noncholinergic inhibitory nerves have been suggested to be mediated by polypeptides, ATP, or another unidentified neurotransmitter. The discovery of nitric-oxide (NO) synthase inhibitors greatly contributed to our understanding of mechanisms involved in these responses, leading to the novel hypothesis that NO, an inorganic, gaseous molecule, acts as an inhibitory neurotransmitter. The nerves whose transmitter function depends on the NO release are called “nitrergic”, and such nerves are recognized to play major roles in the control of smooth muscle tone and motility and of fluid secretion in the GI tract. Endothelium-derived relaxing factor, discovered by Furchgott and Zawadzki, has been identified to be NO that is biosynthesized from L-arginine by the constitutive NO synthase in endothelial cells and neurons. NO as a mediator or transmitter activates soluble guanylyl cyclase and produces cyclic GMP in smooth muscle cells, resulting in relaxation of the vasculature. On the other hand, NO-induced GI smooth muscle relaxation is mediated, not only by cyclic GMP directly or indirectly via hyperpolarization, but also by cyclic GMP-independent mechanisms. Numerous cotransmitters and cross talk of autonomic efferent nerves make the neural control of GI functions complicated. However, the findings...
related to the nitrergic innervation may provide us a new way of understanding GI tract physiology and pathophysiology and might result in the development of new therapies of GI diseases. This review article covers the discovery of nitrergic nerves, their functional roles, and pathological implications in the GI tract.

I. Introduction

Nonadrenergic noncholinergic (NANC) inhibitory neurotransmission has long been recognized in the gastrointestinal (GI) tract (Abrahamsson, 1986). Although part of the induced responses to efferent nerve stimulation, such as relaxation and inhibitory junction potentials (IJP), are apparently mediated by peptides (Fahrenkrug, 1993; Shuttleworth and Keef, 1995) and ATP (Burnstock, 1972), the major mechanisms remained unclarified until 1990. In the late 1980s, three groups independently reported that endothelium-derived relaxing factor (EDRF) first discovered by Furchgott and Zawadzki (1980) is nitric oxide (NO) (Ignarro et al., 1987, 1988; Palmer et al., 1987; Furchgott, 1988). Murad et al. (1978) provided evidence that nitroglycerin exerts vasorelaxation due to the release of NO which activates soluble guanylyl cyclase to yield cyclic GMP. Moncada et al. (1989) reported that NO is formed by an enzyme, called NO synthase (NOS) that transforms L-arginine to L-citrulline in the presence of cofactors such as tetrahydrobiopterin (BH4) and FAD/FRN. In contrast to the inducible form of the NOS (iNOS), the two constitutive NO synthases, i.e., the neuronal (nNOS) and endothelial (eNOS), are activated by Ca2+ in the presence of calmodulin (Bredt and Snyder, 1990). Palmer et al. (1988) first introduced the compound N(G)-monomethyl-L-arginine (L-NMMA), which inhibits the enzyme, and helped us to elucidate the physiologically important roles of NO in the whole body. This led to the discovery that the major inhibitory neurotransmitter of the efferent autonomic nervous system at the level of the anococcygeus muscle (Li and Rand, 1989), retractor penis (Gillespie and Sheng, 1990), artery (Toda and Okamura, 1990), gut (Bult et al., 1990; Toda et al., 1990a), and trachea (Tucker et al., 1990) is NO. In the field of GI tract research, detailed analyses established the role of neurons in which NO acts as an inhibitory neurotransmitter. These nerves, called “nitrergic” (Rand, 1992a) or “nitrooxidergic” (Toda and Okamura, 1992), receive electrical information from the central nervous system via parasympathetic preganglionic fibers and ganglia. This review article describes the history of the discovery of nitrergic nerves in the GI tract, the nitrergic innervation in various regions of the GI tract, and the interaction with other autonomic nerves, the blood flow regulation of the GI tract by these nerves, as well as their pathophysiological implications.

II. Nitric Oxide as a Neurotransmitter

A. Discovery of Nitrergic Nerves

Bult et al. (1990), Toda et al. (1990a), and Li and Rand (1990) for the first time provided evidence that NO acts as an inhibitory NANC neurotransmitter in the isolated canine ileocolonic junction, canine duodenal longitudinal muscle, and rat gastric fundus, respectively. According to Bult et al. (1990), superfused ileocolonic preparations liberate vasorelaxant substance(s) in response to transmural electrical stimulation or dimethylphenylpiperazinium (DMPP). The induced relaxation was abolished by abolishment of the response with the NOS inhibitor N(G)-nitro-L-arginine (L-NA), superoxide anions, or oxyhemoglobin (oxyHb), and the effect of L-NA was reversed by L-arginine. Toda et al. (1990a) and Li and Rand (1990) noted that relaxations induced by electrical field stimulation of duodenal and gastric fundus strips were abolished by L-NMMA, but not by D-NMMA, and the addition of L-arginine, but not D-arginine, restored the responses. Release of the neurotransmitter NO from the myenteric plexus by electrical stimulation has been visualized with NO/luminal-induced chemiluminescence in the guinea pig ileum (Wiklund et al., 1996, 1997). In addition, evidence supporting the idea that nNOS activation and NO formation from L-arginine are involved in the relaxation induced by electrical nerve stimulation in the rat gastric fundus has been obtained by measuring L-citrulline in the incubation medium (Curro et al., 1996). Direct evidence for the release of NO from neurons has been obtained by detecting the major metabolites of the NO-citrulline pathway with capillary electrophoresis in the single cell level (Moroz et al., 1999).

NOS immunoreactive and NADPH diaphorase-positive nerve fibers have histologically been shown to be present in the myenteric plexus of the rat intestine (Bredt et al., 1990) and the myenteric and submucous plexuses of the GI tract from other mammals (Barbiers et al., 1993; Nichols et al., 1995; Rodrigo et al., 1998).
Vittoria et al. (2000) studied the distribution of nitricergic neurons in the bovine gut from the esophagus to the rectum and noted the NOS immunoreactivity and NADPH diaphorase to be constantly colocalized in the same neuron.

Burns et al. (1996), Ward et al. (1998), and Ward and Sanders (2001) revealed that nitricergic inhibitory responses are abolished in mutant rats that lack c-Kit-positive interstitial cells of Cajal in the esophagus, stomach, and pyloric sphincter; thus, the interstitial cells are expected to play quite an important role in nitricergic neurotransmission, possibly by transducing electrical and mechanical NO signals. Immunohistochemical distribution of c-Kit-positive cells and NOS-positive neurons has also been demonstrated in the human gut (Nemeth and Puri, 2001) and esophageal sphincter (Watanabe et al., 2002).

Review articles by Sanders and Ward (1992), Rand (1992b), Lefebvre (1993, 2000), Boeckxstaens and Peckmans (1997), and Curro and Preziosi (1998) summarize the evidence supporting the hypothesis that NO or its analog acts as an inhibitory neurotransmitter in the GI tract. However, Correia et al. (2000) noted that among the products that can be formed during NOS catalyzed L-arginine N⁰-oxidation, only NH₂OH caused relaxations that exhibited a pharmacological profile similar to that induced by nitricergic nerve stimulation in the rat duodenum, and these observations suggested that this molecule may be an actual neurotransmitter acting directly or by a release of NO.

NO is synthesized from L-arginine to yield citrulline by the constitutive nNOS (Forstermann et al., 1994). Neuronal NOS in the brain (Bredt and Snyder, 1990) and iNOS (Hevel et al., 1991; Stuehr et al., 1991) are mostly soluble enzymes, whereas eNOS is more than 90% particulate (Forstermann et al., 1991). The nNOS-containing subcellular structure has not morphologically been confirmed. A recent study by Van Geldre et al. (2000) demonstrated that using a protocol involving strongly homogenization of the rat small intestine, about 50% of the immune reactive nNOS, was particle-bound immediately after NOS activation in response to DMPP were suggested to be of a non-L, non-N-type, which was in contrast to the N-type channel involved in the response to electrical NO induced relaxation (Boeckxstaens et al., 1993a). A similar finding as to the Ca²⁺ channel types responsible for the action of nicotine was also obtained in the cerebral artery, although in the arteries, the action of nicotine was resistant to tetrodotoxin, which abolished the response mediated by nerve action potentials (Toda et al., 1995). The M₁-muscarinic receptor activation by McN-A-343 also produced NO-mediated relaxation in the intestine (Olgart and Iversen, 1999). The response was abolished by 1-NA, 1-NMMA, ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), a soluble guanylyl cyclase inhibitor (Garthwaite et al., 1995), and tetrodotoxin, whereas guanethidine or hexamethonium did not affect the response. Susceptibility to tetrodotoxin is consistent with the view that ganglionic M₁ receptors are stimulated by the agonist to generate nitricergic nerve action potentials.

**B. Mechanism of Nitric Oxide Actions**

EDRF is identical to NO in blood vessels (Ignarro et al., 1987; Palmer et al., 1987; Furchgott, 1988), and NO exerts its actions via activation of soluble guanylyl cyclase in smooth muscle cells that produce cyclic GMP as an intracellular messenger (Waldman and Murad, 1987). These hypotheses have widely been accepted in vascular smooth muscle. However, in GI smooth muscles, the NO-induced relaxation is mediated by the guanylyl cyclase-cyclic GMP pathway and also by membrane hyperpolarization. In GI smooth muscles from animals (Desai et al., 1991; McLaren et al., 1993; Olgart and Iversen, 1999) or humans (Bartho et al., 2002), involvement of cyclic GMP in NO-induced relaxation has been derived from studies using the soluble guanylyl cyclase inhibitors methylene blue and ODQ or the cyclic...
GMP phosphodiesterase inhibitor zaprinast (Ward et al., 1992) or measuring directly the cyclic GMP contents (Toda et al., 1992). Ward et al. (1992) suggested that inhibitory junction potentials mediated by neurogenic NO are presumably coupled with the enhanced production of cyclic GMP. Ny et al. (2000) noted that electrical field stimulation caused a biphasic relaxation, an initial rapid one followed by a slowly developing phase, in stomach fundus strips from wild-type mice, whereas only a slow relaxation was observed in the strips from mice lacking cyclic GMP-dependent protein kinase I. These relaxations were mediated by NO and a non-NO inhibitory molecule. The responses to 3-morpholino-sydnonimine, a NO donor, and 8-bromo-cyclic GMP were markedly impaired in the strips from the knockout mice. It was suggested that cyclic GMP-dependent protein kinase I plays a central role in the NO/cyclic GMP signaling cascade. In contrast, the relaxation induced by neurogenic NO was not inhibited by treatment with methylene blue (Allescher et al., 1993; Takakura and Muramatsu, 1999). Cyclic GMP-independent, apamin-sensitive relaxation induced by NO was also reported by Martins et al. (1995) and Borjesson et al. (1997).

NO and NO donors enhance the open state of Ca\(^{2+}\)-activated K\(^+\) channels and mimic hyperpolarization by NANC inhibitory nerve stimulation (Thornbury et al., 1991). The apamin-sensitive and -insensitive IJP is abolished by L-NAME, suggesting that nerve-derived NO opens Ca\(^{2+}\)-activated K\(^+\) channels to induce hyperpolarization (Christinck et al., 1991). Transmural field stimulation at high frequencies elicited fast and slow phases of relaxation and hyperpolarization, and L-NA depressed the slow phase and partially reduced the fast phase, suggesting that the NO-induced hyperpolarization is involved in the relaxation (Shimamura et al., 1993; Suzuki et al., 2003). Cl\(^-\) channels may also be involved in the NO-evoked IJP (Suzuki et al., 2003). From studies on wild mice and mutant mice that lacked the inositol triphosphate type 1 receptor, Suzuki et al. (2000) noted that the lack of this receptor attenuated IJP evoked by nitric-ergic nerve stimulation but did not alter those associated with norepinephrine. It was suggested that basal release of NO caused an oscillatory pattern of electrical and mechanical activity. Suthamnatpong et al. (1994) suggested that IJP is not involved in relaxation in the rat proximal colon. On the other hand, there are a number of reports showing that NO and other transmitters are independently involved in the responses to nitricergic nerve stimulation. In the lower esophageal sphincter (LES), relaxations induced by NANC inhibitory nerve stimulation were reduced by NOS inhibitors and oxyHb, and the remaining response was abolished by apamin (Boyle et al., 1991). In the human colon, NO- and ATP-induced relaxations mimicked relaxations evoked by NANC neurogenic stimulation, and the L-NA-resistant response was abolished by apamin, suggesting that NO and possibly ATP are involved in the inhibitory neurotransmission (Boeckxstaens et al., 1993c). Zagorodnyuk and Maggi (1994) have suggested that apamin-sensitive and apamin-resistant components of the evoked IJP utilize different NANC transmitters, i.e., ATP and NO, respectively. However, according to Imaeda et al. (1998), both the nitricergic and adrenergic (ATP-mediated) inhibitory nerves contribute equally to the IJP generation via opening of Ca\(^{2+}\)-activated K\(^+\) channels, and the relaxation is mainly produced by NO in a membrane potential-independent manner. Okamura et al. (1998b) have reported that L-NA-resistant relaxation induced by NANC inhibitory nerve stimulation in the canine colon is suppressed by ouabain and lowering of external K\(^+\), suggesting the involvement of the electrogenic Na\(^+\) pump. In this preparation, K\(^+\) channel inhibitors, such as apamin, charybdotoxin, tetraethylammonium, and glibenclamide, are without effect. The relaxing factor responsible for the Na\(^+\) pump activation has not yet been identified.

Possible mechanisms involved in NO-induced relaxation and hyperpolarization are 1) cyclic GMP-dependent reduction of cellular free Ca\(^{2+}\) without changing the membrane potential, 2) cyclic GMP-dependent opening of apamin-sensitive K\(^+\) channels or other types of ion channels to produce hyperpolarization and relaxation, and 3) cyclic GMP-independent mechanisms, such as actions of NO on ion channels involved in muscle contractility, either directly or via membrane hyperpolarization. Figure 1 summarizes the mechanisms of action of neurogenic NO in the GI tract. Proposed mechanisms of action of nitricergic nerve stimulation differ in animal species and regions of the GI tract studied. Despite the evidence for a cyclic GMP-independent mechanism in some rodents, cyclic GMP seems to be a key substance for nitric-ergic inhibitory responses in most mammalian GI tracts, including that of humans.

C. Is the Nitricergic Neurotransmitter Free Radical Nitric Oxide or a Stable Analog of Nitric Oxide?

This question has been raised soon after EDRF was identified to be NO (Ignarro et al., 1987; Palmer et al., 1987; Furchgott, 1988). The reason for the question was the observation that responses to EDRF or nitricergic nerve stimulation in some smooth muscle preparations, including the GI tract, were not reduced by superoxide anion generators or a NO scavenger (La et al., 1996) in concentrations sufficient to suppress the effect of exogenous NO or NO donors. In addition, the neurogenic response was not augmented by superoxide dismutase. Therefore, stable analogs of NO like S-nitrosothiols or unidentified substance(s) were postulated to be the NANC neurotransmitters in the canine colon (Thornbury et al., 1991), rat gastric fundus (Jenkinson et al., 1995), and rat duodenum (Correia et al., 1999). Using the bioassay method, it was reported that NOS products, considered to be NO or S-nitrosothiols liberated from neurons in the donor tissue, relaxed the assay tissue and
that this response was potentiated by superoxide dismutase (Boeckxstaens et al., 1991a,c), suggesting that the released substance was susceptible to superoxide anions. If the substance had been released in a stable form, superoxide anions would not have destroyed its activity. Furthermore, the fluorescent method using DAF-2 (4,5-diaminofluorescein/diacetate) and 4-amino-5-methylamino-2',7'-difluorofluorescein for detection of NO demonstrated the release of NO in rat brain slices (Kojima et al., 1998, 2001). There is no visible fluorescence of these indicators interacting with S-nitrosothiols (T. Nagano, personal communication), suggesting that DAF only binds to NO. If the thiol compounds were stable enough to be protected from degradation in extracellular fluids, the data obtained from the bioassay method and with DAF would not support the hypothesis that the neurotransmitter is a stable analog of NO.

Evidence against the idea that S-nitrosothiols act as intermediate compounds has been presented elsewhere (De Man et al., 1995b, 1998, 1999), and many other investigators, who sought a way to solve this problem, were also led to the conclusion that free radical NO most likely acts as the nitrergic neurotransmitter by excluding the possible involvement of S-nitrosothiols (Boeckxstaens et al., 1994; De Man et al., 1996a,b; Lefebvre, 1996; Colpaert and Lefebvre, 2000). Immunohistochemical analysis of porcine gastric fundus revealed a 100% colocalization of Cu²⁺/Zn²⁺ superoxide dismutase and nNOS in the intrinsic neurons of the myenteric plexus (Colpaert et al., 2002). Therefore, it is hypothesized that there are endogenous antioxidant defense mechanisms, enzymatic and nonenzymatic, for the neurotransmission process to be mediated by free NO. Similar conclusions were also obtained from studies using an irreversible inhibitor of superoxide dismutase (Martin et al., 1994; Lilley and Gibson, 1995; Paisley and Martin, 1996; Okamura et al., 1998a; Tanaka et al., 1999).

Goyal and He (1998) noted that nitrergic slow IJP and hyperpolarization evoked by the NO redox donor, diethylenetriamine/NO adduct, were suppressed by guanylyl cyclase inhibitors but not by apamin in the guinea pig ileal circular muscle, whereas hyperpolarization caused by sodium nitroprusside and solubilized NO gas was antagonized by apamin but not by guanylyl cyclase inhibitors. Thus, they speculated that NO redox is the nitrergic neurotransmitter.

D. Cotransmitters Responsible for Nonadrenergic Noncholinergic Inhibitory Responses

Coexistence of choline acetyltransferase/cholinesterase with nNOS, VIP, and neuropeptide Y (NPY) in the neurons of the myenteric plexus (Fang et al., 1993; Ekblad et al., 1994; Barbiers et al., 1995) would indicate that nerve fibers containing these molecules are of vagal origin. It has been demonstrated that nitrergic as well as cholinergic nerves innervating the cerebral and ophthalmic arteries arise from the superior salivatory nucleus in the brain stem sending off parasympathetic efferent neurons (Spencer et al., 1990) via the greater petrosal nerve and pterygopalatine ganglion (Ayajiki et al., 2000; Toda et al., 2000a,b; Toda and Okamura, 2003). A histological study has demonstrated that vagal preganglionic efferents terminate on NOS-containing neurons in the rat gastric fundus (Berthoud, 1995).

Li and Rand (1990) have reported that NANC-mediated neurogenic relaxation of the rat gastric fundus is reduced and VIP-induced relaxation is abolished by treatment with a VIP antibody. The residual relaxation in the presence of VIP antibody is further reduced by L-NMMA. Therefore, these authors suggested that NO as well as VIP is involved in the NANC-mediated relaxation. There is also evidence for a differential release of
NO and VIP by NANC nerves in the rat gastric fundus (Boeckxstaens et al., 1992; D'Amato et al., 1992). In studies on isolated feline and porcine gastric fundus, Barbier and Lefebvre (1993) and Lefebvre et al. (1995) have concluded that short-lasting and sustained relaxations induced by electrical nerve stimulation are mediated by NO and that a peptide, possibly VIP, is involved during sustained, low-frequency stimulation. NO-independent inhibitory responses to NANC nerve stimulation were reduced by α-chymotrypsin in the canine proximal colon (Keef et al., 1994). Jin et al. (1996) and Murthy et al. (1996) suggested the dual origin of NO from nerves and muscle and its interplay with VIP in regulating relaxation of the rabbit and rat stomach. Recent studies by Dick and Lefebvre (2000) and Dick et al. (2001, 2002) on pig, guinea pig, and mouse gastric fundus smooth muscle cells have provided evidence that inducible NOS probably induced by the isolation procedure may be involved in the relaxant effect of VIP, thereby underscoring the importance of the experimental method used. From findings that nicotine-induced NANC relaxation in the gastric fundus was reduced by a specific anti-VIP serum and that nicotine increased the outflow of VIP- and histidine isoleucine-like immunoreactivity from the strips, involvement of VIP and possibly histidine isoleucine in the mechanical response was suggested (Curro and Preziosi, 1997). According to Baygijnov et al. (1999), the NANC inhibitory neurotransmitters in the gastric fundus are NO and VIP mediating responses via cyclic GMP and cyclic AMP, and there is no evidence supporting a serial cascade in which VIP is coupled to NO-dependent responses. In the proximal colon, the VIP-induced inhibitory response was reportedly induced by stimulation at the high frequency of 20 Hz only when nitrergic and purinergic NANC responses were depressed (Matsuyama et al., 2003). Histochromic studies have demonstrated the colocalization of NOS immunoreactivity or NADPH-diaphorase and VIP-like immunoreactivity in myenteric plexus and enteric neurons (Keef et al., 1994; Barbiers et al., 1995; Lefebvre et al., 1995; Guo et al., 1997).

Contribution of NO and ATP to NANC inhibitory neurotransmission has been noted in the human colon (Boeckxstaens et al., 1993c), rat pyloric sphincter (Soediono and Burnstock, 1994), rat ileum (Smits and Lefebvre, 1996a), and mouse jejunum (De Man et al., 2003). Soediono and Burnstock (1994) have concluded that ATP seems to act through P2Y-purinoceptors in the rat pyloric sphincter. Studies on the rat pylorus by Ishiguchi et al. (2000) have also shown that NO and ATP act in concert to mediate NANC relaxation. However, these authors suggested that P2X purinoceptors are involved in the ATP-induced relaxation. Recently, De Man et al. (2003) suggested the major involvement of P2Y receptors of the P2Y1 subtype located postjunctionally and P2X receptors located pre- and/or postjunctionally in the mouse jejunum. Coexistence of ATP and NO was reported in myenteric neurons in the rat ileum (Belai and Burnstock, 1994). NOS-related activity and NPY immunoreactivity are extensively colocalized in layers of the human (Nichols et al., 1994) and porcine gut wall (Wu et al., 2003). NOS-positive neurons of the myenteric plexus display Y1 NPY receptors in the human colon (Peaire et al., 1997), suggesting possible modulation of nitrergic nerve function by NPY receptors. It has been reported that NANC inhibitory responses to nerve stimulation are mediated by NO and by neurotensin via apamin-sensitive K+ channels in the rat ileum (Yamaji et al., 2002).

Pituitary adenylyl cyclase-activating peptide (PACAP) is postulated to be an inhibitory neurotransmitter in the rat colon (Grider et al., 1994; Kishi et al., 1996) and guinea pig taenia coli (Jin et al., 1994). McConalogue et al. (1995) noted that in the guinea pig taenia caecum, nitrergic IJP were reduced in tissues desensitized to PACAP and that the IJP and hyperpolarization induced by exogenous PACAP were blocked by apamin. Cocaine- and amphetamine-regulated transcript (CART) peptide, originally isolated from the rat striatum (Douglass et al., 1995), is also expressed in the peripheral nervous system (Dun et al., 2000) and enteric neurons (Couceyro et al., 1998). Ekblad et al. (2003) found that CART peptide is present in numerous myenteric neurons throughout the rat GI tract, and the addition of CART attenuated the NO donor-induced relaxations in rat colonic strips, suggesting CART may exert a modulatory action on NO neurotransmission. Kadowniki et al. (1999) demonstrated that about 30% of the total NOS-immunoreactive neurons had contact with 5-HT-positive nerve fibers in the guinea pig distal colon and suggested that NO may participate in the 5-HT-evoked Cl− secretion.

E. In Vivo Studies

Nitrergic inhibitory responses of the GI tract were also observed in vivo studies. Electrical stimulation of the peripherally cut end of the right vagus nerve resulted in relaxations of the opossum LES and caused peristaltic and nonperistaltic contractions in the esophageal body (Tottrup et al., 1991b). L-NA abolished the sphincter relaxation evoked by nerve stimulation, this effect being fully reversed by L-arginine infusion. The L-arginine-NO pathway seems to play an important role in the relaxation, but not for esophageal body peristalsis. In conscious dogs, L-NAME delayed gastric emptying of solid meals, and L-arginine given with L-NAME shortened the delay of gastric emptying. This delay was due to stimulation of pyloric and proximal duodenal contractions (Orihata and Sarna, 1994). They speculated that NO facilitated gastric emptying by partially inhibiting pyloric and proximal duodenal contractions. Pyloric sphincter relaxations may also be involved. De Winter et al. (2002) showed that L-NAME delayed the gastric emptying of a semiliquid meal in mice without any effect on the intestinal transit. In conscious dogs
with enterically isolated ileocolonic loops, L-NA increased the phasic pressure in ileum and ileocolonic sphincter and the sphincter tone, but did not abolish colonic relaxation during ileal distension, suggesting that non-nitrergic nerve pathways mediate the reflex colonic relaxation (Leelakusolvong et al., 2002). In the anesthetized rat, L-NAME increased jejunal intraluminal pressure and initiated phasic intestinal contractions. These responses were inhibited by concurrent administration of L-arginine, but not D-arginine (Calignano et al., 1992). Endogenous NO is suggested to play a role in the modulation of intestinal motility. From findings obtained in anesthetized rats that an initial increase in intragastric pressure by vagal nerve stimulation was enhanced while the induced decrease in intragastric pressure was abolished by L-NAME, Lefebvre et al. (1991) suggested that NO is involved in gastric relaxation. In anesthetized dogs, pyloric activity induced by duodenal field stimulation was inhibited by antral field stimulation and electrical vagal stimulation, and intra-arterial L-NAME reduced the inhibition. It has been reported that the functional significance of NO release from NANC nerves in the isolated guinea pig stomach is to bring about adaptive relaxation through a reflex response to increases in gastric pressure (Desai et al., 1991). In conscious rats exposed to cold at 4°C for 2 to 3 h, nitrergic and cholinergic neurons were activated in the gastric myenteric ganglia through vagal nicotinic pathways (Yuan et al., 2001).

III. Nitrergic Innervation in Various Regions

A. Esophagus

It has been reported that NANC inhibitory responses are mainly mediated by nitrergic nerves in esophagus from the opossum (Christinck et al., 1991; Murray et al., 1991), cat (Ny et al., 1995), cow (Barahona et al., 1998), and human (Richards et al., 1995) and also in the LES from the dog (De Man et al., 1991; Daniel et al., 2002), opossum (Tottrup et al., 1991a,b; Conklin et al., 1993; Uc et al., 1999), guinea pig (Imaeda et al., 1998), cat (Korteza et al., 1996), mouse (Kim et al., 1999; Sivarao et al., 2001), and human (McKirdy et al., 1992; Preiksaitis et al., 1994; Tomita et al., 1997). Biphasic, rapid and sustained, relaxations of the feline LES are suggested to be mediated by NO and VIP or VIP-like peptides (Korteza et al., 1996), and those of the opossum sphincter appear to be mediated by NO and an unidentified substance (Uc et al., 1999). In LES strips from humans, inhibitory motorneurons were efficiently stimulated both by electrical field stimulation and stimulation of nicotinic receptors located in nerve terminals, releasing NO and an apamin-sensitive neurotransmitter (Gonzalez et al., 2004). In studies on the LES from wild-type and genetically engineered eNOS and nNOS mice, Kim et al. (1999) noted that relaxations to electrical nerve stimulation were abolished by L-NA in wild-type mice, and the responses were not affected by a lack of the eNOS gene, but were absent in nNOS knockout mice. There was no difference in the sensitivity to a NO donor in these three groups. Thus, the authors concluded that nNOS rather than eNOS is the enzymatic source of NO. Cyclic GMP is involved in the NO-mediated relaxation in canine (Daniel et al., 2002) and bovine LES (Barahona et al., 1999). Histological studies have demonstrated the presence of neurons containing nNOS or NADPH diaphorase and costaining for VIP and galanin in the human (Singaram et al., 1994), cat (Ny et al., 1995), monkey (Rodrigo et al., 1998), guinea pig (Morikawa and Komuro, 1998), bovine (Vittoria et al., 2000), and porcine esophagus (Wu et al., 2003). Nitrergic innervation was also observed in striated muscles of the guinea pig (Morikawa and Komuro, 1998) and rat esophagus (Neuhuber et al., 1994; Kuramoto et al., 1999).

In the anesthetized opossum and cat, L-NAME or L-NA inhibited LES relaxation induced by vagal stimulation, swallowing, and balloon distension (Paterson et al., 1992) and decreased the amplitude of peristaltic contraction in the very distal esophagus (Xue et al., 1996), suggesting that NO is an important mediator for the timing of peristalsis in the distal esophagus for LES relaxation and for swallowing. In cats under ketamine sedation, it was suggested that tonic contractile activity in the esophageal body is mainly caused by a continuous cholinergic excitatory input, and a NO inhibitory mechanism may have a complementary role in the tonic regulation (Zhang et al., 2004). According to Beyak et al. (2000), intravenous injections of L-NA reduced the number of oropharyngeal swallows and the induction of primary peristalsis in the smooth muscle portion but not in the striated muscle portion, whereas administration of L-NA into the fourth ventricle in the brain reduced the swallows and peristalsis in both smooth and striated muscles. Therefore, NO released in the central nervous system appears to be involved in esophageal peristalsis and swallowing, and the neural substrates mediating striated and smooth muscle peristalsis may be anatomically and neurochemically distinct. In humans, intravenous infusions of L-NMMA resulted in a reduction of the latency period between swallows and the onset of contractions in the distal esophagus (Konturek et al., 1997b) or increased the amplitude of peristaltic pressure waves in the distal esophagus and inhibited the increase in LES relaxation during gastric distension (Hirsch et al., 1998). Endogenous NO is likely to be involved in the timing of esophageal peristalsis and sphincter relaxation in humans.

On the basis of histological studies, diminished nitrergic inhibitory neurotransmission to the LES (Mearin et al., 1993) or to interstitial cells of Cajal in the LES was speculated to be the pathophysiological mechanism of esophageal achalasia (Watanabe et al., 2002). Sivarao et al. (2001) noted that mice with NOS gene disruption had LES hypertension with impaired relaxation resem-
bling achalasia. On the other hand, wild-type mice lacking intramuscular interstitial cells of Cajal had a hypotensive sphincter with unimpaired relaxation, suggesting that the interstitial cells of Cajal do not play a role in nitrergic neurotransmission. NADPH diaphorase histochemistry showed a reduction of myenteric nitrergic neurons and fibers in the circular muscle of congenital esophageal stenosis, but the peptidergic neurons (VIP, substance P, and galanin) were unaltered (Singaram et al., 1995). The specific lack of NO inhibitory innervation may be an important mechanism for the pathogenesis of stenosis and aperistalsis of the esophagus in this disorder. In contrast, nitrergic relaxation of LES obtained from patients with reflux esophagitis was increased compared with that in normal sphincter specimens (Tomita et al., 2003). Portal hypertension activates NOS genes in the esophageal mucosa in rats, and this phenomenon may facilitate the development and rupture of esophageal varices (Tanoue et al., 1996).

Cardiac antiarrhythmic therapy in patients with mexiletine, a class Ib antiarrhythmic drug, is accompanied by a high incidence of gastrointestinal side effects (Campbell, 1987). Mexiletine attenuated NANC relaxation of the rabbit LES, possibly by inhibiting myenteric nitrergic neurotransmission (Kohjitan et al., 2003b). The intravenous anesthetics, ketamine and midazolam, inhibited the K⁺-induced neurogenic relaxation of the rabbit LES (Kohjitan et al., 2001). The authors suggest that ketamine inhibits the response by the extracellular production of superoxide anions and that midazolam inhibits it by inhibiting NOS activity (Kohjitan et al., 2003a).

Intragastric infusion of sildenafil, a phosphodiesterase-5 inhibitor, induced a decrease in LES tone and resting pressure and in the amplitude and propagation velocity of esophageal contractions in normal subjects (Bortolotti et al., 2001a; Rhee et al., 2001). Reduced degradation of intracellular messenger cyclic GMP may participate in the enhanced nitrergic inhibition. Intravenous administration of sildenafil also reduced the amplitude of esophageal contractions and LES pressure in anesthetized cats (Zhang et al., 2001). The authors suggested that the effect may benefit patients with achalasia. In patients with idiopathic achalasia (Bortolotti et al., 2000) and symptomatic hypertensive LES (Bortolotti et al., 2002), sildenafil infused into the stomach in a double-blind manner decreased the LES tone, residual pressure, and wave amplitude. In an open study on 11 patients with nutracker esophagus, hypertensive LES, or achalasia and in a randomized double-blind study on six volunteers, sildenafil lowered LES pressure and propulsive forces in the esophageal body in both the healthy subjects and patients (Eherer et al., 2002). A subset of patients with hypertensive LES or nutracker esophagus may benefit from sildenafil, but the side effects might be a limiting factor.

B. Stomach

Neurogenic relaxation and IJP were reportedly mediated by the neurotransmitter NO in the gastric fundus and pyloric sphincters from the rat (Boeckxstaens et al., 1991a; McLaren et al., 1993; Jenkinson et al., 1995; Curro et al., 1996; Curro and Preziosi, 1998; De Man et al., 1998; Lefebvre, 1998), guinea pig (Kojima et al., 1993), dog (Bayguinov et al., 1999), pig (Colpaert and Lefebvre, 2000), mouse (Selemidis and Cocks, 2000; Suzuki et al., 2000; Ergun and Ogulener, 2001), Japanese monkey (N. Toda, K. Ayajiki, and T. Okamura, unpublished data), and human (Tomita et al., 1999; Tonini et al., 2000). Typical responses to nitrergic nerve stimulation of the isolated monkey pyloric sphincter are shown in Fig. 2. L-NA abolished the relaxation that was reversed by L-arginine, but not D-arginine, suggesting that the response is associated exclusively with nerve-derived NO. It was reported that the ability of the nitrergic neurotransmitter to induce relaxation of the rat gastric fundus was influenced by the mechanism used to induce tone, and sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase appeared to play a role in nitrergic relaxation (Van Geldre and Lefebvre, 2004). From studies using wild-type and M₁ muscarinic receptor knockout mice, Stengel and Cohen (2003) obtained evidence supporting the presence of M₁ receptor-mediated relaxation in the

![Fig. 2](https://example.com/fig2.png)
stomach and suggested that when the M3 receptor responsible for contraction was eliminated or blocked, M1 receptor-mediated nitrergic relaxation was enhanced, possibly leading to alterations in gastric emptying. Melatonin had no effect on the basal tonus of the isolated rat gastric fundus but reduced the inhibitory response to nitrergic nerve stimulation (Storr et al., 2002b). Together with findings obtained from the electrophysiological study on mouse colonic preparations and studies on NOS activity in enteric synaptosomes and melatonin receptor transcripts in rat GI tracts, these authors concluded that a direct inhibition of NOS activity might be involved in the reduction by melatonin of inhibitory neurogenic responses.

Histochemical studies of the human stomach (Belai and Burnstock, 2000; Smith et al., 2001) and electron-microscopic analyses of the cat pylorus (Feher et al., 2001) showed the localization of neurons containing nNOS or NADPH diaphorase. Site-specific gene expression of nNOS variants, including nNOSα,-b,-c and nNOSβ, was revealed in distinct functional regions (e.g., gastric fundus versus pylorus) of human (Saur et al., 2000) and rat gastrointestinal tract (Saur et al., 2002). They suggested that the different expression of these proteins is implicated in different biological functions. Miller et al. (2001) demonstrated colocalization of NOS-immunoreactive cell bodies of myenteric ganglia and those containing heme oxygenase-2 in the human antrum and jejunum, and they postulated a possible role for CO as a neurotransmitter and a possible interaction between heme oxygenase and NOS pathways in inhibitory neurotransmission.

A cyclic GMP-dependent mechanism appears to be involved in the nitrergic relaxation of the rat and mouse gastric fundus (Williams and Parsons, 1995; Chang et al., 1998; Bayguinov et al., 1999; Selemidis and Cocks, 2000). Cyclic GMP-dependent protein kinase I apparently plays a key role in the NO/cyclic GMP signaling pathway in the mouse gastric fundus (Ny et al., 2000). However, okadaic acid, a protein phosphatase inhibitor, also interfered with the NO-mediated inhibitory neurotransmission (Storr et al., 2002a), suggesting that attenuated dephosphorylation of proteins impairs the inhibitory process. Whether this is due to antagonism of the nitrergic relaxation or enhanced contraction by phosphorylated myosin light chain in association with phosphatase inhibition remains to be determined.

NO released from NANC nerves in the guinea pig stomach appears to be involved in adaptive relaxation through a reflex response to increases in intragastric pressure (Desai et al., 1991). It has been reported that metabolites of L-arginine mediate neuronal inhibition of canine pyloric motor activity in vivo (Allescher et al., 1992) and facilitate gastric emptying by inhibiting pyloric contractions (Orihata and Sarna, 1994). Gastric distention-induced pyloric relaxation appears to be mediated via a vago-vagal reflex and NO release in the rat (Ishiguchi et al., 2001). The primary site of action of nitrergic mechanisms on the gastric fundic tone in conscious dogs is likely to be at a presynaptic level on the vagal cholinergic efferent nerves (Paterson et al., 2000). In healthy volunteers, it is suggested that NO is involved in maintaining the basal fundic tone and in the meal-induced fundic relaxation (Kuiken et al., 2002b), and the gastric accommodation reflex involves activation of nitrergic neurons (Tack et al., 2002).

In the stomach of ovariectomized female rats, NOS activity and mucus secretion were increased, and the severity of ulcer lesions was decreased compared with controls, suggesting that estrogen deficiency protects the gastric mucosa by NO-mediated mucus hypersecretion (Morschel et al., 2000). In contrast, Shah et al. (2000, 2001) noted that nitrergic relaxation of isolated gastric fundus and nNOS protein were increased in rats treated with estrogen, but not by progesterone, or in pregnant rats. The nNOS protein was also increased in ovariectomized rats treated with estrogen when compared with ovariectomized rats receiving vehicle. Results from experiments with L-NAME indicated the involvement of neurogenic NO in the estrogen-induced and pregnancy-related changes. Therefore, the possibility is suggested that estrogen, rather than progesterone, may be responsible for the delay in gastric emptying and increase in colonic transit time observed during pregnancy that is mediated by nitrergic inhibitory neurotransmission.

NOS-deficient knockout mice showed gastric dilation or enlargement (Huang et al., 1993) and delayed gastric emptying of solids and liquids compared with wild-type and eNOS-deficient mice (Mashimo et al., 2000). Wild-type mice treated with L-NA showed delay in emptying of solids but not liquids. Thus, these authors concluded that chronic depletion of NO from NOS resulted in delayed gastric emptying. Similar defects in gastric emptying were also observed in genetic and streptozotocin-elicited models of diabetes in mice; they also showed impaired nitrergic inhibitory responses of the pyloric muscle and reduced nNOS protein and mRNA in the pylorus (Watkins et al., 2000). NOS expression and pyloric function were restored to normal levels by insulin treatment, and delayed gastric emptying was reversed with sildenafil. These findings implicate novel therapeutic approaches to diabetic gastropathy. Cellek et al. (2003) demonstrated that NOS-containing neurons innervating the gastric pylorus of streptozotocin-induced diabetic rats lost some of nNOS content and function in the first phase and led to complete loss of nitrergic function in the second phase; the changes in the first phase were reversible with insulin therapy, but the neurodegeneration in the second phase was irreversible. In normal subjects, antral and duodenal wave frequency and amplitude were lower during and after sildenafil administration in the gut than during and after placebo (Bortolotti et al., 2001b). NO-mediated brisk relaxant responses were impaired in the gastric fundus of mdx...
dystrophic mice, an animal model of Duchenne muscular dystrophy compared with normal mice (Baccari et al., 2000). Quintana et al. (2004) demonstrated synthesis of NO in postganglionic myenteric neurons during early endotoxemia that seemed to mediate gastric hypococontractility in rats.

C. Duodenum, Jejunum, Ileum, and Ileocolonic Junction

There are numerous data supporting the hypothesis that NO plays a pivotal role in NANC relaxation or IJP associated with electrical field stimulation or nicotinic agonists in the duodenum, jejunum, and ileum from a variety of mammals, including the dog (Toda et al., 1990a, 1991; Bogers et al., 1991), human (Maggi et al., 1991; Zyromski et al., 2001), rat (Berrino et al., 1995; Sotirov et al., 1998; Donat et al., 1999; Correia et al., 2000; Okishio et al., 2000b; Tanovic et al., 2001; Yamaji et al., 2002), guinea pig (Toma et al., 1999), and mouse (Ogulener et al., 2002). The ileocolonic junction also has intense nitrergic innervation in the dog (Boeckxstaens et al., 2001). The ileocolonic junction also has intense nitrergic innervation in the dog (Boeckxstaens et al., 1990a, 1993b; Bogers et al., 1991; Leelakusolvong et al., 2002), cat (Mozhorkova et al., 1994), opossum (Matsuda et al., 1999, 2002), guinea pig (Toma et al., 1999), and mouse (Ogulener et al., 2001). The ileocolonic junction also has intense nitrergic innervation in the dog (Boeckxstaens et al., 1990a, 1993b; Bogers et al., 1991; Leelakusolvong et al., 2002), cat (Mozhorkova et al., 1994), opossum (Matsuda et al., 1999, 2002), guinea pig (Toma et al., 1999), and mouse (Ogulener et al., 2001).

The presence of neurons containing NADPH diaphorase or nNOS has histologically been established in the small intestine from humans (Fang et al., 1993; Timmermans et al., 1994) and animals (Wilhelm et al., 1998; Hens et al., 2002). There is an anatomical heterogeneity of the nitrergic system in the ileum of guinea pig, rat, rabbit, and cat (Wilhelm et al., 1998).

Involvement of endogenous NO in the regulation of intestinal motility was noted in the rat (Calignano et al., 1992) and guinea pig (Holzer, 1997). Kuiken et al. (2002a) have shown that inhibition of NO biosynthesis triggers the onset of a rapidly propagating phase III-like activity and shortens the postprandial period, and they have indicated that NO is involved in the modulation of fasting and postprandial small intestinal motility in humans. In jejunal longitudinal muscle strips obtained from rats subjected to chronic extrinsic denervation for 1 to 8 weeks by small bowel transplantation, contractile responsiveness was inhibited possibly by increased functioning of NANC inhibitory nerves, in which NO was partially involved (Balsiger et al., 2001). In anesthetized rats, laparotomy and manipulation of the small intestine inhibited the intestinal transit of Evans blue, and this inhibition was partially reversed by pretreating the rats with reserpine and completely reversed by additional treatment with L-NA, suggesting the involvement of adrenergic and nitrergic pathways in the pathogenesis of experimental ileus (De Winter et al., 1997). Duodenal strips isolated from rats with experimental diabetes induced by streptozotocin administration responded to nicotine, DMPP, or electrical stimulation with L-NA-sensitive relaxations that were substantially smaller than those in control rats (Martinez-Cuesta et al., 1995; Kaputlu et al., 1999). Therefore, impairment of nitrergic innervation may contribute to the abnormalities of intestinal motility associated with diabetes.

D. Colon, Rectum, and Internal Anal Sphincter

A functional role of nitrergic neurons in the colon has been determined in humans (Bartho et al., 2002) and in dogs (Dalziel et al., 1991; Okamura et al., 1999b), rabbits (Smith and Muir, 1991), rats (Middleton et al., 1993; Borjesson et al., 1997), guinea pigs (Zagorodnyuk and Maggi, 1994), and hamsters (Matsuyama et al., 2003). In healthy subjects and patients with irritable bowel syndrome, stimulation of the NO-cyclic GMP pathway by administration of sildenafil decreased the rectal tone but did not influence rectal distensibility, and relaxation of the rectum was accompanied by an increase in rectal volumes to reach perception thresholds (Fritz et al., 2003). Giant migrating contractions of the rat colon, possibly mediated by neuronal release of acetylcholine, appear to be partially suppressed by constitutive release of NO (Li et al., 2002). Nitricergic innervation also contributes to the regulation of the smooth muscle tone in the rat rectum (Takeuchi et al., 1999a) and in the guinea pig (Stebbing et al., 1996) and opossum internal anal sphincter (Rattan and Chakder, 1997). It was reported that NANC nerve stimulation, CO, and a CO-releasing molecule produced internal anal sphincter relaxation via guanylyl cyclase/cyclic GMP-dependent protein kinase activation (Rattan et al., 2004). Sun et al. (2004) noted that bile acids increased colonic permeability in rats via a mechanism that was inhibited by NO in the distal colon and suggested a possible role of NO in maintaining the epithelial barrier function. Stebbing et al. (1996) provided direct anatomical evidence for a descending nitrergic rectoanul neuronal pathway. Histochmical studies demonstrated nitricergic innervation in the colon of humans (Nichols et al., 1994, 1995; Peaire et al., 1997), pigs (Barbiers et al., 1993), and guinea pigs (Wang et al., 1996).

In colonic strips isolated from rabbits with trinitrobenzene sulfonic acid-induced colitis, relaxations elicited by electrical field stimulation were abolished, and nNOS expression was decreased (Depoortere et al., 2002). Similar findings were also observed in the severely inflamed colon and caecum from pigs infected with *Schistosoma japonicum* (Balemba et al., 2002). These alterations may contribute to impaired intestinal...
motility and absorption. Nitrergic innervation disappeared in the colon of patients with Hirschsprung's disease (Guo et al., 1997) and in the internal anal sphincter of patients with achalasia of this sphincter (Hirakawa et al., 1995).

E. Sphincter of Oddi and Gall Bladder

NO is a major transmitter of NANC neurogenic relaxation of the sphincter of Oddi in the guinea pig (Mourelle et al., 1993; Pauletzki et al., 1993), opossum (Allescher et al., 1993), Australian possum (Baker et al., 1993), rabbit (Lonovics et al., 1994), dog (Tanobe et al., 1995), pig, and human (Sand et al., 1995). Nitrergic nerve stimulation induced relaxation in association with an increase in the tissue cyclic GMP content in the rabbit sphincter of Oddi (Szilvasy et al., 1998). Recombinant human hemoglobin may alter the motor function of the sphincter of Oddi in vitro and in vivo by binding endogenous NO (Cullen et al., 1996). Neurons containing nNOS immunoreactivity or NADPH diaphorase were histochemically detected in the sphincter of Oddi of the human, monkey (De Giorgio et al., 1994), dog (Tanobe et al., 1995), guinea pig (Wells et al., 1995), and possum (Baker et al., 1993; Simula et al., 2001). The presence of NOS has also been demonstrated in the gall bladder and biliary pathways of the guinea pig (Grozdanovic et al., 1995), monkey, and human (De Giorgio et al., 1994; Uemura et al., 1997). In humans, NOS-containing nerve fibers are more abundant in the sphincter of Oddi than in the gall bladder (De Giorgio et al., 1994). In anesthetized cats, L-NA increased the flow resistance exerted by the sphincter of Oddi, but did not alter gall bladder motility, suggesting that nitrergic tonic inhibition is, if any, minimal in the gall bladder (Thune et al., 1995). Cholinergic nerve stimulation or agonists like cholecystokinin and acetylcholine caused contraction of gall bladder smooth muscle, and parasympathetic, nitrergic/VIPergic nerve activation evoked relaxation of the sphincter of Oddi. These events are likely coordinated with motility and secretory events in the upper GI tract, delivering bile into the duodenum (Shaffer, 2000).

In anesthetized dogs, systemic infusion of L-NAME produced hypertension and increased the sphincter of Oddi and duodenal motilities (Kauffman et al., 1993). L-Arginine, but not D-arginine, blocked these increases, suggesting that regulation of the baseline sphincter of Oddi and duodenal motility involves the generation of NO from L-arginine. In the anesthetized Australian possum, high doses of a highly selective iNOS inhibitor influenced the sphincter's motility by inhibiting nNOS activity; thus, the effects need to be considered in relation to therapeutic doses of this agent (Sandstrom et al., 2004). Local somatothermal stimulation applied to anesthetized cats and rabbits relaxed the sphincter of Oddi. Pretreatment with L-NAME blocked the relaxation, which was reversed by L-arginine (Chiu et al., 1998). Local heat-induced sphincter of Oddi relaxation was also seen in patients undergoing endoscopic retrograde cholangiopancreatography. The authors concluded that local thermal stimulation inhibits the sphincter Oddi motility by activating the heat-sensitive neuronal release of NO.

It was noted that nitrergic relaxation of the sphincter of Oddi was impaired in hyperlipidemic rabbits (Szilvasy et al., 1996). Neurogenic relaxation and an increase in the tissue cyclic GMP content were reversed in preparations from hyperlipidemic rabbits that underwent a treatment with farnesol (Szilvasy et al., 1998). In the sphincter of Oddi isolated from rabbits pretreated with lovastatin, nitrergic relaxation was impaired, and the relaxation was recovered by combined treatment with lovastatin and farnesol (Sari et al., 2001). Topical applications of the NO donor S-nitroso-N-acetylcyesteine (Slivka et al., 1994) or nitroglycerin (Luman et al., 1997) to patients undergoing routine sphincter Oddi manometry reduced or abolished the phasic and tonic contractions of the sphincter of Oddi. A novel clinical approach using local NO donors may be utilized to control GI motility and regulate sphincteric function.

F. Liver and Pancreas

1. Liver. At the hepatic hilus in the cat, a rich plexus of nNOS-immunoreactive nerve fibers and ganglia was detected around the interlobular branches of the bile duct, and the fibers were observed running along the length of a few vessels and in the ducts of the deep parenchyma (Esteban et al., 1999b). It is suggested that nNOS is involved in the autonomic control of hepatic blood flow and the regulation of hepatobiliary excretory activity and hepatocellular metabolic function. Similar results were also observed in the rat, rainbow trout, and guinea pig liver (Esteban et al., 1997, 1998a, 2001).

Canalicular bile secretion was stimulated by NO, possibly released from the intraparenchymal nitrergic nerves in isolated hepatocyte couplets (Trauner et al., 1998). Evidence supporting the idea that neurogenic NO is involved in vasodilatation has been obtained in isolated canine hepatic arteries (Shiraishi et al., 1998), rabbit portal vein (Matsukado et al., 1997), and isolated perfused rabbit liver (Browse et al., 1994). Intracellular roles of NO in the liver may involve drug metabolism and blood storage (Milbourne and Bygrave, 1995). Endogenous NO is important in the regulation of intra- and intercellular liver functions (Alexander, 1998).

2. Pancreas. The innervation of the pancreas may be considered as an extension of the enteric nervous system (Kirchgesner et al., 1994). Histological studies demonstrated nerve fibers and ganglia containing NADPH-diaphorase or nNOS-immunoreactivity in the dog (Umehara et al., 1997), pig (Ember et al., 2000), guinea pig (Liu et al., 1996), and rat pancreas (Wang et al., 1999; Ember et al., 2001). In the dog pancreas, the positive fibers were numerous around pancreatic ducts and moderate around the arteries and the acini but few in the
islets. In contrast, in the rat pancreas, few fibers were present around the pancreatic ducts and acini and more abundant fibers were localized in the islets (Umehara et al., 1997). In the porcine pancreas, positive nerve fibers appeared within nerve bundles of the interlobular spaces and as varicose fibers in the vicinity of pancreatic blood vessels; most of the islet cells were NOS-immunoreactive (Ember et al., 2000). These results indicate evident species variations of nitrergic innervation. On the basis of histological data, NO of both neuronal and extraneuronal origin may regulate blood flow and secretion of the pancreas.

Inhibition by NOS inhibitors of basal and stimulated pancreatic secretion in vivo was reported in the dog (Konturek et al., 1993a), pig (Holst et al., 1994), cat (Patel et al., 1995), and rat (Molero et al., 1995). Konturek et al. (1997a) provided evidence that the suppression of NOS reduced pancreatic enzyme secretion, plasma insulin, and pancreatic polypeptide levels, suggesting that endogenous NO affects both exocrine and endocrine pancreatic secretion in humans. Nitrergic neurotransmission appears to play an important role in the pancreatic secretion in the rat and may induce effects opposite to those of eNOS activity (Vaquerio et al., 1998). In the isolated perfused porcine pancreas with intact vagus nerve supply, there was evidence for an essential role of NO in vagus nerve-induced pancreatic secretion and basal vascular tone, but not in vagally induced vasodilatation (Holst et al., 1994).

IV. Secretion and Neurogenic Nitric Oxide

NO donors, such as sodium nitroprusside, S-nitroso-N-acetylpenicillamine, and isosorbide dinitrate, stimulated mucus secretion from a suspension of isolated gastric cells (Brown et al., 1993). Dibutyril cyclic GMP and the cyclic GMP phosphodiesterase inhibitor M & B 22948 also increased the mucus release. These findings, together with the presence of NOS in the gastric mucus cells (Brown et al., 1992), suggest a role for NO in mediating gastric mucus release. On the basis of studies on chloride secretion and changes in short-circuit current in the isolated rat distal colon, King et al. (2004) suggested that NO is a secretomotor neurotransmitter in response to serotonin. Expression of nNOS in parietal cells suggests a participation of endogenous NO in the regulation of gastric acid secretion (Premaratne et al., 2001). Helicobacter pylori increased pepsinogen secretion from dispersed human peptic cells through a Ca^{2+}- and NO-mediated intracellular pathway (Lorente et al., 2002). Intragastric administration of HCl stimulated a subpopulation of nitricergic, but not cholinergic, myenteric plexus neurons, which might play a role in secretion, vasodilatation, and muscle relaxation (Schicho et al., 2001).

In a perfused segment of rat jejunum in situ, l-arginine in the perfusate induced fluid secretion. L-NAME had no effect on basal fluid movement, but reversed the l-arginine-induced secretion. Therefore, l-arginine was suggested to induce intestinal fluid secretion through production of NO (Mourad et al., 1996, 2003). In the isolated ileum of wild-type mice, l-arginine increased NO release, cyclic GMP production, and electrolytic Cl^- secretion. However, these actions were reduced in tetrahydrobiopterin (BH_4)-deficient hph-1 mice, suggesting that BH_4 might be a target for the modulation of electrolytic ion transport; this highlights the complexity of the interactions between NO and cyclic GMP in the gut (Rolfe et al., 1997). It has been reported that BH_4 is a critical factor in producing NO from l-arginine (Kwon et al., 1989), and BH_4 deficiency not only reduces the production of NO but also enhances superoxide generation (Pou et al., 1992; Shinozaki et al., 1999). SNI-2011, a novel muscarinic M_3 agonist (Fisher et al., 1991) that possesses a long-lasting sialogogic action (Masunaga et al., 1997), increased amylase secretion from parotid tissues in rats (Yuan et al., 2003). This effect was inhibited by a cell-permeable Ca^{2+} chelator or inhibitors of calmodulin kinase II, nNOS, soluble guanylyl cyclase, cyclic GMP-dependent protein kinase, and myosin light chain kinase. SNI-2011 induced an increase in DAF-2 fluorescence from parotid acinar cells loaded with DAF-2, corresponding to an increase in the NO production. In nNOS knockout mice, SNI-2011 did not release amylase. The authors suggested involvement of the NOS-cyclic GMP pathway in the SNI-2011-induced amylase secretion from parotid acinar cells. Activation of β-adrenoceptors and VIP receptors likely causes amylase secretion from the rat parotid glands partly through a NO/cyclic GMP-dependent intracellular pathway involving the activity of nNOS, possibly of acinar origin (Sayardoust and Ekstrom, 2003).

In humans, the addition of L-NMMA reduced pancreatic enzymatic secretion stimulated by secretin as well as caerulein, plasma insulin, and pancreatic polypeptide levels; this inhibitory action was reversed by l-arginine, suggesting that endogenous NO affects both exocrine and endocrine pancreatic secretion (Konturek et al., 1997a). Amylase secretion evoked by l-arginine from isolated rat pancreatic lobules was prevented by L-NA, and NADPH diaphorase-containing nerve fibers were histologically determined in the pancreas (Kirchgesner et al., 1994). In anesthetized rats in which the stomach was mounted in an ex vivo chamber, intragastric administration of NO donors inhibited the increase in acid secretion in response to pentagastrin. L-NAME did not affect basal acid secretion but potentiated the stimulated acid secretion. Pentagastrin increased the release of nitrite and nitrate in the gastric lumen, and the effect was abolished by L-NAME (Kato et al., 1998). It was suggested that exogenous or endogenous NO has an inhibitory action on gastric acid secretion. Whether the reason for the inability of L-NAME to affect the basal acid secretion is due to a lack of tonic impulses along the
V. Species and Regional Differences in the Nitrergic Regulation

A lot of data are available describing the diversity of nitrergic nerve function and histology in different regions of the GI tract from a variety of mammals. However, it is often quite difficult to compare the data obtained by different investigators under various experimental conditions. Therefore, we only summarize those results obtained from authors who specifically mentioned similarities or differences of nitrergic innervation in GI regions or in animal species.

Lefebvre et al. (1992) reported that in circular muscle strips of the guinea pig gastric fundus, relaxations induced by short-lasting and sustained electrical stimulation of NANC inhibitory nerves were abolished by L-NA, the inhibition being reversed by L-arginine. They noted that the contribution of nitrergic nerves to sustained NANC relaxation was much more important in the guinea pig gastric fundus compared with the rat. Clear species variations in the functioning of nitrergic nerves were also seen in the distal colon; neurogenic NO significantly contributed to the inhibitory response in the cat (Venkov and Krier, 1994), rabbit (Ciccióppo et al., 1994), guinea pig (Iversen et al., 1994), and mouse (Satoh et al., 1999), whereas there was no contribution in the Wistar-ST rat (Okishio et al., 2000a).

In the rat gastric fundus, relaxant responses to electrical field stimulation sensitive to L-NA were larger in circular muscle strips than in longitudinal ones (Kamata et al., 1993), suggesting that the density of nitrergic nerves in the stomach fundus is richer in circular muscle than in longitudinal muscle. In strips of the sigmoid colon obtained from patients with colorectal cancer, responses to NO released by neural stimulation were also greater in circular than in longitudinal muscle (McKirdy et al., 2004). These observations are supported by histological studies on NOS- and NADPH-positive neurons in the porcine intestine (Barbiers et al., 1993). In the rat colon, NO-mediated NANC relaxations were greater in the proximal portion than in the distal one. The average number of NOS-immunoreactive cells and the density of the NOS mRNA were higher in the proximal colon than in the distal colon (Takahashi and Owyang, 1998). On the basis of these data, they postulated the physiological role of the proximal colon as an organ for fecal storage and absorption of excess fluid. Satoh et al. (1999) pharmacologically characterized the transmitters or mediators responsible for NANC relaxation in the mouse intestine. Their findings indicate that NO solely mediates relaxation of the longitudinal muscle of the ileum, whereas NO and VIP comediately it in the jejunum; NO, VIP, and PACAP in the proximal colon; and NO and PACAP in the distal colon. There were clear differences in the magnitude of the L-NA-sensitive relaxant response of the Wistar rat intestine; the responsiveness was marked in the proximal colon, moderate in the ileum and distal colon, and small or none in the jejunum and rectum (Okishio et al., 2000a). In the isolated canine sphincter of Oddi (Tanobe et al., 1995) and longitudinal muscles of the canine duodenum (Toda et al., 1991), neurogenic relaxations sensitive to L-NA did not differ under treatment with atropine, whereas the NO-mediated relaxations in the canine colon (Okamura et al., 1998b) was approximately half of those in the sphincter of Oddi and duodenum. Contractions induced by cholinergic nerve stimulation were substantially larger in the duodenum compared with the colon and were absent in the sphincter of Oddi.

VI. Development and Aging

From histochemical studies on the distribution of neurons containing NOS immunoreactivity or NADPH diaphorase in the GI tract of human fetuses and newborns, Timmermans et al. (1994) reported that in the stomach, NOS immunoreactivity was confined to the myenteric plexus and nerve fibers in the outer smooth musculature. In the pyloric region, a few nitrergic perikarya were seen in the inner submucous plexus, and in the small intestine, nitrergic neurons clustered in primary nerve strands of the myenteric plexus up to the 26th week of gestation. The marked morphological differences observed between nitrergic neurons within the developing human GI tract support the existence of distinct subpopulations of NOS-containing enteric neurons acting as interneurons or inhibitory motor neurons. According to Brandt et al. (1996), NADPH diaphorase-positive nerve fibers had appeared by 12 weeks in the myenteric ganglia at all levels of the human gut, and nitrergic innervation in the submucous plexus became evident after 14 weeks. As gestational age increased, nitrergic innervation became richer and more organized. By 23 weeks’ gestation, nitrergic innervation had matured to the pattern observed in the postnatal gut. The onset and pace of development of nitrergic innervation are similar to adrenergic and cholinergic innervation and occur before peptidergic innervation. In the rat stomach, the percentage of NADPH diaphorase-positive neurons in the proximal part was higher than in the antral part (Timmermans et al., 1999). This difference persisted in groups of 1 day, 1 week, 2 weeks, 1 month, and 2 months of age. In the developing small intestine and colon in the chicken (incubation days 12 and 19), quantitative analysis revealed a decrease with age in the NADPH diaphorase-positive nerve cell density, but not the total number of myenteric nitrergic cells, with age (Bagyanszki et al., 2000).

In longitudinal muscle-myenteric plexus preparations of the ileum of young (3–4 months), adult (12–13 years), and old (26 months) rats, the number of NOS-immunoreactive myenteric neurons was approximately constant. These observations are supported by quantitative data obtained by different investigators under various conditions and used or to a nitrergic nerve-independent mechanism remains to be determined.
duced transient relaxations induced by short periods of electrical stimulation, mediated by endogenous NO, to a similar extent as treatment with L-NAME. Prolonged periods of stimulation induced a sustained relaxation mediated by NO and VIP; the NO-releasing substances did not affect this response but slowed down its onset. The authors suggest the presence of an autoregulatory mechanism for the nitrergic innervation. It was reported that in the mouse duodenum, relaxations mediated by nerve-derived NO, but not those induced by acidified NaNO₂, were inhibited by the NO donors nitroglycerin and sodium nitroprusside (Ogulener et al., 2001). NO scavengers or superoxide generators did not influence the neurogenic relaxation but attenuated the inhibitory effect of NO donors. In addition, nitrergic nerve-mediated relaxations were attenuated by 8-bromo-cyclic GMP but not by 8-bromo-cyclic AMP. They suggested that exogenous NO donors had a prejunctional inhibitory effect on the nitrergic nerve-mediated relaxation, the inhibition being mediated at least in part by the cyclic GMP pathway. However, they did not investigate whether nerve-derived NO has any autoregulatory role. In contrast, Lefebvre and Vandekerckhove (1998) reported evidence against this hypothesis using the pig gastric fundus. Their conclusion was that nitroglycerin could induce a postjunctional tolerance to nitrergic nerve stimuli, but there was no evidence for a prejunctional inhibition of nNOS by NO. However, to solve the problems regarding the prejunctional inhibition of and postjunctional tolerance to neurogenic and exogenous NO in various animal species and different GI regions await future studies.

It has been suggested that NO may not only function as a neurotransmitter released from neurons to relax smooth muscle but may also be involved in communication between interneurons (Pompolo and Furness, 1993; Costa et al., 1996). The histological study by Wang et al. (2003) showed that interstitial cells of Cajal associated with the deep muscular plexus in the human distal ileum formed synapse-like junction with cholinergic and nitrergic nerves and gap junctions with smooth muscle cells. Wiklund et al. (1993) demonstrated that in the guinea pig ileum previously exposed to ³H-choline, exogenous NO inhibited electrically evoked ³H overflow as well as responses to exogenous cholinergic agonists, indicating that NO has the ability of pre- and postsynaptic neuromodulation. NOS inhibitors enhanced contractile responses to cholinergic nerve stimulation, but did not influence the responses to exogenous acetylcholine. Therefore, they concluded that cholinergic neurotransmission might be reduced by endogenous NO, acting prejunctionally. Inhibition by neurogenic NO of the release of acetylcholine from stimulated neurons was also determined in the canine ileum previously incubated with ³H-choline (Hryhorenko et al., 1994). In the guinea pig gastric fundus, NO released from inhibitory nerves reduced the cholinergic neurotransmission (Ohno et al., 1996). According to Yoneda and Suzuki (2001), it appears that the suppression of excitatory junctional po-
tentials is mainly a prejunctional event and that the depression of mechanical responses is mainly induced postjunctionally. In studies on neuronal pathways underlyng reflex responses of muscle layers activated by mucosal stimulation in the isolated guinea pig colon segment, Smith and McCarron (1998) obtained evidence supporting the idea that endogenous NO facilitated or depressed the release of acetylcholine from interneurons in the ascending or descending nervous pathways, respectively; the NO action appeared to be mediated through soluble guanylyl cyclase. NO donors did not influence the basal release and electrically evoked release of $^3$H-choline in the pig gastric fundus, whereas $\alpha_2$-adrenoceptor activation did (Leclere and Lefebvre, 2001). In conscious dogs, vagal cooling, atropine, and hexamethionium increased intragastric volume, indicating a contribution of vagal and enteric cholinergic pathways to the maintenance of fundic tone. Intravenous L-NA increased the fundic tone, and the effects of L-NA were prevented by vagal cooling or atropine. The relaxing effects of nerve-derived NO appear to be related to an inhibition of ongoing vagal cholinergic activity, NO acting at a prejunctional site on cholinergic efferent nerves (Paterson et al., 2000). Administration of muscarinic agonists increased the optical density of NADPH-positive neurons in guinea pig gastric fundus and inhibited NO-mediated relaxation to electrical field stimulation via activation of prejunctional M_1 receptor subtypes (Kortezova et al., 2004).

NANC inhibitory responses mediated by NO were inhibited by the nonselective K$^+$ channel blockers 4-aminopyridine and tetramethylammonium and by the large conductance Ca$^{2+}$-activated K$^+$ channel blocker charybdotoxin, but not by the blocker of small conductance Ca$^{2+}$-activated K$^+$ channels apamin. Prejunctional charybdotoxin-sensitive K$^+$ channel opening seems to participate in the inhibition of NO release from the nitricergic nerves (De Man et al., 1993). An extended study by the same group (Boeckxstaens et al., 1993b, 1995) led them to conclude that the NO release is prejunctionally regulated by K$^+$ channels and $\alpha_2$-adrenoceptors; blockade of K$^+$ channels enhances the release, whereas $\alpha_2$-adrenoceptor activation reduces the release of the nitricergic transmitter, possibly by activating K$^+$ channels. An inhibitory prejunctional enkephalinergic mechanism in nitricergically mediated relaxations was noted in the longitudinal muscle of guinea pig ileum (Ivancheva and Radomirov, 1996). GABAergic inhibition of NO release was also noted in the guinea pig ileum; GABA_A (Hebeiss and Kilbinger, 1999) and also GABA_B receptors (Kilbinger et al., 1999) may be involved in this prejunctional inhibition. On the basis of studies with the copper chelator neocuproine and the Cu$^{2+}$/Zn$^{2+}$ superoxide dismutase inhibitor diethylenetriaminepentaacetic acid, De Man et al. (2001) suggested that catalase might protect the nitricergic neurotransmitter mainly at the prejunctional site, whereas Cu$^{2+}$/Zn$^{2+}$ superoxide dismutase protects it at the postjunctional site. However, in contrast to the data in favor of a prejunctional modulation by endogenous substances so far presented, a recent study on the isolated guinea pig gastric fundus by Todorov et al. (2003) indicated that relaxations induced by nitricergic nerve stimulation were refractory to the $\alpha_2$-adrenoceptor agonist UK 14304 and to a variety of receptor agonists and antagonists, acting at GABA, serotonin, opioid, muscarinic, histamine, and cannabinoid receptors.

Nerve-derived NO also inhibits purinergic neurotransmission in the hamster proximal colon (Matsuyama et al., 2003). Van Crombruggen and Lefebvre (2004) suggest that NO increases the sensitivity of rat distal colonic muscle strips to NO and ATP, and ATP induces neuronal release of NO. The release of PACAP and VIP from NANC nerves is enhanced by exogenous and possibly neurogenic NO (Chakder and Rattan, 1998). Zizzo et al. (2004) have also provided evidence indicating that NO stimulates the release of PACAP from inhibitory neurons in the isolated mouse ileum.

In the anesthetized opossum, hypogastric nerve stimulation caused a rise in the intraluminal pressure of the internal anal sphincter accompanied by an increase in the norepinephrine, epinephrine, and dopamine levels in plasma (Thatikunta et al., 1993). Administration of L-NA decreased the response to nerve stimulation, and this effect was reversed by L-arginine. Therefore, NO seems to have a facilitatory role in the release of sympathetic neurotransmitters. In the isolated guinea pig gastric fundus previously incubated with $^3$H-norepinephrine, the nicotinic agonist DMPP or electrical field stimulation caused an increase in the $^3$H overflow, and this effect was suppressed by L-NA, suggesting that endogenous NO increases the release of norepinephrine from adrenergic nerves (Sotirov and Papasova, 2000). In contrast, NO inhibits (Greenberg et al., 1990) or does not affect (Toda et al., 1990b; Bucher et al., 1992; Pa et al., 1992) the release of norepinephrine from adrenergic nerves in blood vessels. In rats subjected to splanchinic ganglionectomy, nNOS expression in the jejunal myenteric plexus increased compared with sham-operated rats. Treatment with clonidine reversed the changes induced by ganglionectomy, suggesting that nNOS expression in the myenteric plexus after splanchic ganglionectomy is regulated by $\alpha_2$-adrenoceptors (Nishizaki et al., 2003).

VIII. Gastrointestinal Blood Flow Regulation by Nitricergic Nerves

Many data are available supporting the idea that NO synthesized via constitutive NOS plays a pivotal role in protecting the GI mucosa from a variety of noxious stimuli, including caustic ingestion, chemical irritants, ischemia, ischemia/reperfusion injury, and endotoxin shock, through maintenance of mucosal perfusion (Salzman,
Endogenous NO responsible for the regulation of the vascular tone is derived from nitrergic nerves and vascular endothelial cells. Nitrergic nerves innervating GI blood vessels are present in the human, monkey, dog, cow, and guinea pig (reviewed by Toda and Okamura, 1992, 2003).

In anesthetized rats (Pique et al., 1989; Tepperman and Whittle, 1992; Chen et al., 1993; Pawlik et al., 1995) and cats (Macedo and Lautt, 1997) and in awake rats (Greenblatt et al., 1993), L-NA, L-NAME, or L-NMMA decreased gastrointestinal mucosal blood flow and increased vascular resistance, despite an increase in systemic blood pressure. In rats with stress-induced gastric injury, pretreatment with NO donors resulted in reduction of gastric lesions, increase in gastric blood flow, and increase in superoxide dismutase activity, suggesting that suppression of reactive oxygen species plays an important role in the action of NO donors (Kwiecien et al., 2002). The NO donor molsidomine, known to increase the expression of superoxide dismutase (De Meyer et al., 2003), also prevented the ischemia/reperfusion injury of the rat small intestine (Ozturk et al., 2003).

NO has been suggested to have cytoprotective effects, mainly via the regulation of mucosal blood flow, in endotoxin and ethanol-induced intestinal injury (Laslo and Whittle, 1994; Baraona et al., 2002; Sugita et al., 2003), and the gastroprotective effects of somatostatin (Ancha et al., 2003), adrenomedullin (Salomone et al., 2003), omeprazol (Le et al., 2001), thyrotrpin-releasing hormone analog (Kiraly et al., 1993), and cholecystokinin (West et al., 2002) are partly mediated by the endogenous release of NO. Gastric preconditioning in rats, by exposing the mucosa to a brief period of ischemia which increases its resistance to a subsequent ischemic insult, raised the gastric flood flow and luminal NO content, suggesting that preconditioning is one of the major protective mechanisms in the stomach that involves the vasodilatory mediator NO (Brzozowski et al., 2003). During the early phase of a H. pylori infection in mice, gastric hyperemia associated with increased NO production may exert some protective role against non-steroidal anti-inflammatory drug-induced gastric injury (Elizalde et al., 2003). On the other hand, NO-releasing acetylsalicylic acid seems to exhibit mucosal protective and healing effects on stress-induced gastric lesions.

Intestinal hemodynamics significantly changes during the transition from fetal to newborn life. Basal vascular resistance decreased following birth, then decreased further, and began to increase between postnatal days 12 and 30 (Nankervis et al., 2001). These changes appear to be largely mediated by an increase in the production of NO from the endothelium and possibly from the perivascular nerves. The unique hemodynamic conditions that characterize the perinatal and newborn intestine may be part of the overall physiological transition that occurs as the fetus, once born, replaces the placenta with its GI tract to obtain nutrition (Nankervis et al., 2001).

IX. Pathological Implications

Numerous reports in the literature have suggested that endogenous NO may participate in the ethiopathogenesis of digestive tract diseases. Deficiency of nitrergic innervation, as evidenced by a paucity of NADPH diaphorase-positive neurons, has been shown in GI tissues from patients with various diseases (Takahashi, 2003), such as infantile hypertrophic pyloric stenosis (Vanderwinden et al., 1992), Hirschsprung’s disease (Vanderwinden et al., 1993; Bealer et al., 1994; Wester et al., 1998a), internal anal sphincter achalasia (Hirakawa et al., 1995), lower esophageal achalasia (Hirano, 1999; Weusten and Smout, 1999; Watanabe et al., 2002), rectal ectasias (Cuffari et al., 1997), and intestinal atresia (Matsumoto et al., 1999). In contrast, excessive production of NO may cause the persistent inhibition of intestinal motility in human idiopathic chronic constipation (Cortesini et al., 1995). In the duodenum isolated from rats with experimental diabetes, evidence for a reduction in intestinal NOS activity associated with an impairment of the NANC relaxation was noted (Martinez-Cuesta et al., 1995). It was thus suggested that a defect of the intestinal nitrergic activity could contribute to the motility dysfunction observed in diabetes. However, the expression of nNOS in neurons and fibers of the myenteric plexus of the gastroduodenal tract was increased in streptozotocin-induced diabetic rats compared with control rats, suggesting that the increase in the nNOS level may explain why impaired gastric emptying is common in patients with diabetes (Adeghate et al., 2003). Increased nitrergic neuronal activity can account for many of the clinical signs of equine grass sickness, namely, dysphagia, generalized ileus, gastric dilation, peripheral vasodilatation, and salivary hypersecretion (Cottrell et al., 1999). In a rat model of experimental ileus induced by laparotomy followed by evisceration and manipulation of the small intestine and caecum, L-arginine enhanced the inhibition of the intestinal transit of Evans blue; L-NA partially reversed the inhibition, suggesting that an enhanced release of NO, possibly from nitrergic nerves, is partly involved in the inhibitory effect of surgical manipulation (De Winter et al., 1997).

The inhibition of NOS by L-NMMA delayed the healing of chronic gastric ulcer and reduced the blood flow at the ulcer margin in rats, suggesting that endogenous NO plays an important role in the maintenance of blood flow around the ulcer and thus in its healing (Konturek et al., 1993b). The protective action of L-arginine against chronic gastric ulcer in rats appears to involve gastric hyperemia mediated by NO, and the ulcer healing properties of L-arginine may depend upon its hyperemic and angiogenic actions, possibly involving NO (Brzozowski et al., 1997). On the other hand, the cytoprotective effect
of L-arginine against HCl-induced gastric injury in rats invokes endogenous prostaglandins rather than NO (Takeuchi et al., 1993). Copious diarrhea produced following the oral administration of castor oil to rats was inhibited or prevented by L-NMMA or L-NAME by decreasing the intestinal fluid accumulation and Na⁺ secretion, suggesting that endogenous NO participates in castor oil-induced diarrhea (Mascolo et al., 1993).

X. Summary

The hypothesis that NO synthesized from L-arginine via nNOS acts as a neurotransmitter of NANC inhibitory nerves innervating GI smooth muscles is now widely accepted. The nerve whose transmitter function depends on the release of NO is called “nitrergic”. Neurogenic NO plays a crucial role in the control of smooth muscle tone and motility, particularly in sphincters, and also of fluid secretion in the GI tract. In addition to GI smooth muscle relaxation, NO released from the nitrergic nerve and from the vascular endothelium elicits vasodilatation and subsequently increases mucosal blood flow, which might help to protect the mucosa against noxious stimuli. In the GI smooth muscles, NO-mediated relaxation is mediated not only by cyclic GMP but also by cyclic GMP-independent mechanisms. Release of NO from nitrergic nerves is facilitated or inhibited by neurotransmitters from coinnervating autonomic nerves, such as acetylcholine, ATP, peptides, and noradrenaline, that act on prejunctional receptors. Various pathological conditions or substances that inhibit the synthesis of NO, enhance its degradation, or inhibit the guanylyl cyclase are accompanied by an increase in GI muscle contractility and an impairment of the GI function. The findings related to the nitrergic innervation may provide us a new way of understanding GI tract physiology and pathophysiology.

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