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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**

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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[1]) 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, reduced nicotinamide-adenine dinucleotide phosphate (NADPH), and FAD/FMN. 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 Ca^{2+} 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 "nitroxidergic" (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.

[1]Abbreviations: NANC, nonadrenergic noncholinergic; GI, gastrointestinal; IJP, inhibitory junction potential(s); EDRF, endothelium-derived relaxing factor; NO, nitric oxide; NOS, NO synthase; iNOS, inducible NOS; nNOS, neuronal NOS; eNOS, endothelial NOS; L-NMMA, N^G-monomethyl-L-arginine; DMPP, dimethylphenylpiperazinium; L-NA, N^G-nitro-L-arginine; oxyHb, oxyhemoglobin; 5-HT, 5-hydroxytryptamine; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; L-NAME, L-NA methylester; LES, lower esophageal sphincter; DAF, diaminofluorescein/diacetate; VIP, vasoactive intestinal polypeptide; NYP, neuropeptide Y; PACAP, pituitary adenylyl cyclase-activating peptide; CART, cocaine- and amphetamine-regulated transcript; M & B 22948, 2-*o*-propoxyphenyl-8-azapurin-6-one; BH_4, tetrahydrobiopterin; UK 14304, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine; SNI-2011, (±)*cis*-2-methyl-spiro(1,3-okathiolane-5,3′)quinuclidine.

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 treatment of the tissue 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 at 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 nitrergic 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 nitrergic 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 nitrergic 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 Pelckmans (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 N^G-oxidation, only NH_2OH caused relaxations that exhibited a pharmacological profile similar to that induced by nitrergic 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. (2004) demonstrated that using a protocol involving strong homogenization of the rat small intestine, about 50% of the immune reactive nNOS, was particle-bound in a subcellular structure.

Ca^{2+} together with calmodulin is required for nNOS activation (Bredt and Snyder, 1990). In the canine ileocolonic junction, ω-conotoxin GVIA, an N-type Ca^{2+} channel inhibitor (McCleskey et al., 1987; Miller, 1987), or exposure of the tissue to Ca^{2+}-free media reduced the inhibitory response to nitrergic nerve stimulation, whereas L-type Ca^{2+} channel inhibitors nifedipine and verapamil were without effect (Boeckxstaens et al., 1993a). NO induced relaxation of rectal circular muscle with a concomitant decrease in intracellular Ca^{2+} level (Takeuchi et al., 1998a). Therefore, electrically generated nerve action potentials trigger the influx of Ca^{2+} through N-type channels that appear to be prerequisite for the nNOS activation. Evidence for the capacity of recycling citrulline to arginine has been reported in the proximal colon (Shuttleworth et al., 1995) and internal anal sphincter smooth muscle (Rattan and Chakder, 1997). The presence of enzymes, such as argininosuccinate synthetase and argininosuccinate lyase (Hecker et al., 1990), capable of transforming citrulline to arginine, has immunohistochemically been established (Shuttleworth et al., 1995). This seems to be one of the mechanisms of maintaining the function of nitrergic neurons in the GI tract.

Nicotine (Toda et al., 1992; Kojima et al., 1993; McLaren et al., 1993; Mozhorkova et al., 1994), DMPP (Boeckxstaens et al., 1993a; Takeuchi et al., 1996), K^+ (Toda et al., 1992), 5-HT (Boeckxstaens et al., 1990b; Bogers et al., 1991), ATP, and GABA (Boeckxstaens et al., 1991b) reportedly stimulate nitrergic nerves and release NO or a NO analog in the GI tract. Nerve action potentials appear to contribute to the inhibitory response to these chemical stimuli because the induced relaxation is susceptible to tetrodotoxin. The actions of nicotine and DMPP were diminished by hexamethonium. The Ca^{2+} channels involved in the Ca^{2+} influx for nNOS 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 nerve stimulation (Boeckxstaens et al., 1993a). A similar finding as to the Ca^{2+} 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 M_1-muscarinic receptor activation by McN-A-343 also produced NO-mediated relaxation in the intestine (Olgart and Iversen, 1999). The response was abolished by L-NA, L-NMMA, ODQ (1*H*-[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 M_1 receptors are stimulated by the agonist to generate nitrergic 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-sydnoninime, 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 nitrergic 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 nitrergic 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 nitrergic 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 nitrergic 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 nitrergic inhibitory responses in most mammalian GI tracts, including that of humans.

C. Is the Nitrergic 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 nitrergic 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

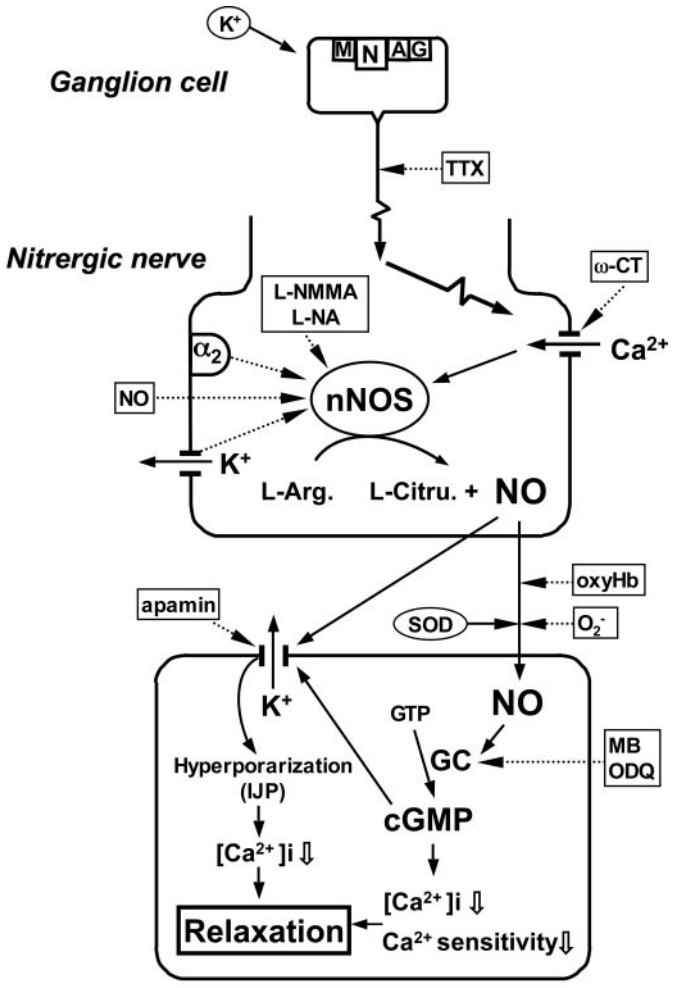

FIG. 1. Schematic presentation of synaptic transmission via NO from nitrergic nerves to GI smooth muscle cells. Solid lines represent stimulation, and dotted lines represent inhibition. M, N, A, and G on ganglionic cell membrane, M_1 muscarinic, nicotinic, purinergic, and GABAergic receptors, respectively. TTX, tetrodotoxin; ω-CT, ω-conotoxin GVIA; L-NMMA, N^G-monomethyl-L-arginine; L-NA, N^G-nitro-L-arginine; α_2, prejunctional α_2-adrenoceptor; oxyHb, oxyhemoglobin; SOD, superoxide dismutase; O_2^-, superoxide anion; GTP, guanosine triphosphate; MB, methylene blue; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3*a*]quinoxalin-1-one; cGMP, cyclic GMP.

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 Cu^{2+}/Zn^{2+} 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 Bayguinov 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). Histochemical 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 P_{2Y}-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 P_{2X} purinoceptors are involved in the ATP-induced relaxation. Recently, De Man et al. (2003) suggested the major involvement of P_{2Y} receptors of the P_{2Y1} subtype located postjunctionally and P_{2X} 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 Y_1 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. Kadowaki 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 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 (Kortezova 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 (Kortezova 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 nNOS 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 antiarrythmic therapy in patients with mexiletine, a class Ib antiarrythmic 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 (Kohjitani et al., 2003b). The intravenous anesthetics, ketamine and midazolam, inhibited the K^+-induced neurogenic relaxation of the rabbit LES (Kohjitani 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 (Kohjitani 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 nutcracker 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 nutcracker 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 Ca^{2+} ATPase appeared to play a role in nitrergic relaxation (Van Geldre and Lefebvre, 2004). From studies using wild-type and M_3 muscarinic receptor knockout mice, Stengel and Cohen (2003) obtained evidence supporting the presence of M_1 receptor-mediated relaxation in the

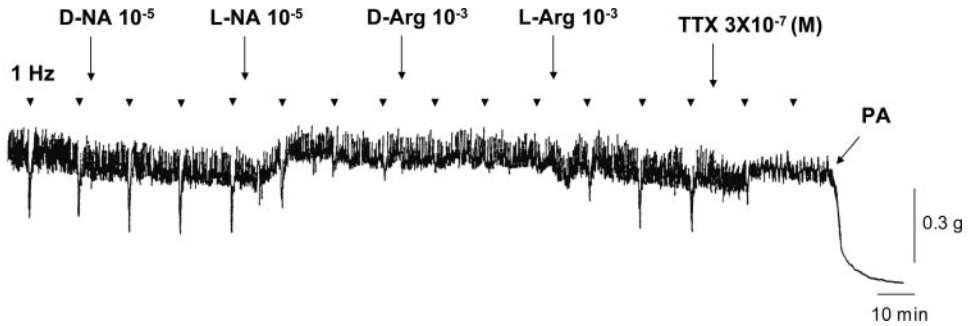

FIG. 2. Modification by D- and L-NA, D-and L-arginine, and tetrodotoxin of the relaxant response to transmural electrical stimulation at 1 Hz for 10 s of a Japanese monkey pyloric sphincter treated with atropine and partially contracted with bradykinin. L-NA abolished and L-arginine reversed the inhibitory response that was abolished by tetrodotoxin. PA represents 10^{-4} M papaverine that produced the maximal relaxation. Arrowheads denote the application of electrical stimulation.

stomach and suggested that when the M_3 receptor responsible for contraction was eliminated or blocked, M_1 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 electronmicroscopic 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 $nNOS_{\alpha\text{-a,-b,-c}}$ and $nNOS_{\beta}$, 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 distension-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 (Morschl 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.

nNOS-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 nNOS 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). nNOS 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 hypocontractility 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), hamster (Matsuyama et al., 1999, 2002), 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., 1998), and pig (Kajimoto et al., 2000) that would contribute to the regulation of the ileocolonic transit of intraluminal contents. Muscarinic M_1 receptors also mediate nitrergic inhibitory responses in the rat intestine (Olgart and Iversen, 1999) and cat duodenum (Shikova and Kortezova, 2000). NO is likely an inhibitory neurotransmitter in the human jejunal longitudinal smooth muscle, acting via a mechanism mediated by guanylyl cyclase; however, other mechanisms are also involved (Zyromski 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., 1998b), 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). Nitrergic innervation also contributes to the regulation of the smooth muscle tone in the rat rectum (Takeuchi et al., 1998a) 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 rectoanal neuronal pathway. Histochemical studies demonstrated nitrergic 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., 1997). Nitrergic nerve stimulation induced relaxation in association with an increase in the tissue cyclic GMP content in the rabbit sphincter of Oddi (Szilvassy 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., 1994), 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 (Kaufman 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 (Szilvassy 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 (Szilvassy 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*-acetylcysteine (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., 1998b). 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 (Kirchgessner 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 (Vaquero 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). Dibutyryl 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 nitrergic, 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 electrogenic 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 electrogenic 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_1 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 enzyme 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 (Kirchgessner 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

efferent nitrergic nerves under the experimental conditions used or to a nitrergic nerve-independent mechanism remains to be determined.

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 (Venkova and Krier, 1994), rabbit (Ciccocioppo 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 comediate 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

months), and old (24–25 months) rats, NANC inhibitory responses sensitive to L-NAME decreased with age, but those susceptible to ATP desensitization were not influenced by age (Smits and Lefebvre, 1996b). Relaxations induced by exogenous NO did not differ in different age groups. The nitrergic contribution to NANC relaxation supposedly decreased with age. In the gastric fundus of rats (3-, 12-, and 24-months-old), cyclic AMP-mediated relaxant responses to VIP, sustained electrical stimulation, and forskolin were reduced with age, but the response to dibutyryl cyclic AMP was not, suggesting that the defect seems to occur at the level of the adenylyl cyclase. On the other hand, cyclic GMP-mediated inhibitory responses to NO and to short train electrical stimulation were similar in the three age groups (Smits and Lefebvre, 1995). The relative number of nitrergic neurons, as demonstrated with NADPH diaphorase staining, was similar in the ileum of 6- and of 26-month-old rats (Belai et al., 1995). Smits and Lefebvre (1996c) also noted that nitrergic relaxation increased during development from 2 to 8 weeks in the rat gastric fundus. In contrast, Takeuchi et al. (1998b) demonstrated that relaxations induced by nitrergic nerve stimulation seen in the jejunum of 2-week-old rats were attenuated in that of 4-week-old rats and were absent in that of 8- and 50-week-old rats. The same tendency was seen in the ileum, proximal and distal colon, and rectum. Sensitivity to exogenous NO was higher in younger rat jejunum and proximal colon. The population of NOS-immunoreactive neurons in the rectum was denser in 4-week-old rats than in 50-week-old rats. Functioning of nitrergic nerves and sensitivity to NO of smooth muscles appear to be reduced with advancing age in the rat intestine.

In appendices from patients (newborn period up to 3 years of age) with suspected appendicitis that were histologically normal with conventional examination, the density of NADPH diaphorase-positive myenteric plexus was similar to that in the large bowel. The myenteric plexus of appendix specimens from patients older than 4 years showed smaller ganglia connected by thin nerve bundles compared with the large ganglia and nerve bundles in the large bowel (Nemeth et al., 2003). The differences in the architecture of the myenteric plexus in patients older than 4 years suggest an altered function and motility of the appendix in the early years of life. Wester et al. (1998b) found that the density of NADPH diaphorase-positive ganglion cells in the submucous plexus of human distal colon excised during postmortem examination from patients who died of non-GI diseases markedly decreased with age.

VII. Prejunctional Regulation

De Man et al. (1995a) noted that in the rat gastric fundus, treatment with NO-releasing substances, such as nitroglycerin and 3-morpholino-sydnoninime, reduced transient relaxations induced by short periods of electrical stimulation, mediated by endogenous NO, to a similar extent as treatment with L-NA. 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 $NaNO_2$, 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 ^{3}H-choline, exogenous NO inhibited electrically evoked ^{3}H 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 ^{3}H-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 underlying 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 α_2-adrenoceptor activation did (Leclere and Lefebvre, 2001). In conscious dogs, vagal cooling, atropine, and hexamethonium 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 nitrergic 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 α_2-adrenoceptors; blockade of K^+ channels enhances the release, whereas α_2-adrenoceptor activation reduces the release of the nitrergic transmitter, possibly by activating K^+ channels. An inhibitory prejunctional enkephalinergic mechanism in nitrergically 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 diethylthiocarbamic acid, De Man et al. (2001) suggested that catalase might protect the nitrergic 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 nitrergic nerve stimulation were refractory to the α_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 norepinephine 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 splanchinic ganglionectomy is regulated by α_2-adrenoceptors (Nishizaki et al., 2003).

VIII. Gastrointestinal Blood Flow Regulation by Nitrergic 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,

1995; Elliott and Wallace, 1998). 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 (Laszlo 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), thyrotropin-releasing hormone analog (Kiraly et al., 1993), and cholescytokinin (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 the 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 nonsteroidal 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-induced 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 norepinephrine, 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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REFERENCES

Abrahamsson H (1986) Non-adrenergic non-cholinergic nervous control of gastrointestinal motility patterns. *Arch Int Pharmacodyn Ther* **280 (Suppl):**50–61.
Adeghate E, al-Ramadi B, Saleh AM, Vijayarasathy C, Ponery AS, Arafat K, Howarth FC, and El-Sharkawy T (2003) Increase in neuronal nitric oxide synthase content of the gastroduodenal tract of diabetic rats. *Cell Mol Life Sci* **60:**1172–1179.
Alexander B (1998) The role of nitric oxide in hepatic circulation. *Nutrition* **14:**376–390.
Allescher HD, Lu S, Daniel EE, and Classen M (1993) Nitric oxide as putative nonadrenergic noncholinergic inhibitory transmitter in the opossum sphincter of Oddi. *Can J Physiol Pharmacol* **71:**525–530.
Allescher HD, Tougas G, Vergara P, Lu S, and Daniel EE (1992) Nitric oxide as a putative nonadrenergic noncholinergic inhibitory transmitter in the canine pylorus in vivo. *Am J Physiol* **262:**G695–G702.
Ancha H, Ojeas H, Tedesco D, Ward A, and Harty RF (2003) Somatostatin-induced gastric protection against ethanol: involvement of nitric oxide and effects on gastric mucosal blood flow. *Regul Pept* **110:**107–113.
Ayajiki K, Tanaka T, Okamura T, and Toda N (2000) Evidence for nitroxidergic innervation in monkey ophthalmic arteries in vivo and in vitro. *Am J Physiol* **279:**H2006–H2012.
Baccari MC, Romagnani P, and Calamai F (2000) Impaired nitrergic relaxations in the gastric fundus of dystrophic (mdx) mice. *Neurosci Lett* **282:**105–108.
Bagyanszki M, Roman V, and Fekete E (2000) Quantitative distribution of NADPH-diaphorase-positive myenteric neurons in different segments of the developing chicken small intestine and colon. *Histochem J* **32:**679–684.
Baker RA, Saccone GT, Brookes SJ, and Toouli J (1993) Nitric oxide mediates nonadrenergic, noncholinergic neural relaxation in the Australian possum. *Gastroenterology* **105:**1746–1753.
Balemba OB, Mortensen K, Semuguruka WD, Hay-Schmidt A, Johansen MV, and Dantzer V (2002) Neuronal nitric oxide synthase activity is increased during granulomatous inflammation in the colon and caecum of pigs infected with Schistosoma japonicum. *Auton Neurosci* **99:**1–12.
Balsiger BM, Ohtani N, Anding WJ, Duenes JA, and Sarr MG (2001) Chronic extrinsic denervation after small bowel transplantation in rat jejunum: effects and adaptation in nitrergic and non-nitrergic neuromuscular inhibitory mechanisms. *Surgery* **129:**478–489.
Barahona MV, Sanchez-Fortun S, San Andes MD, Ballesteros E, Contreras J, and San Andres M (1998) Involvement of the L-arginine/nitric oxide neural pathway in non-adrenergic, non-cholinergic relaxation of the bovine oesophageal groove. *J Auton Pharmacol* **18:**65–73.
Barahona MV, Sanchez-Fortun S, San Andres MD, Rodriguez C, and San Andres M (1999) Involvement of cyclic GMP-dependent mechanism in the nitrergic relaxation of the bovine oesophageal groove. *J Auton Pharmacol* **19:**39–47.
Baraona E, Shoichet L, Navder K, and Lieber CS (2002) Mediation by nitric oxide of the stimulatory effects of ethanol on blood flow. *Life Sci* **70:**2987–2995.
Barbier AJ and Lefebvre RA (1993) Involvement of the L-arginine: nitric oxide pathway in nonadrenergic noncholinergic relaxation of the cat gastric fundus. *J Pharmacol Exp Ther* **26:**172–178.
Barbiers M, Timmermans JP, Adriansen D, De Groodt-Lasseel MH, and Scheuermann DW (1995) Projections of neurochemically specified neurons in the porcine colon. *Histochem Cell Biol* **103:**115–126.
Barbiers M, Timmermans JP, Scheuermann DW, Adriansen D, Mayer B, and De Groodt-Lasseel MH (1993) Distribution and morphological features of nitrergic neurons in the porcine large intestine. *Histochemistry* **100:**27–34.
Bartho L, Benko R, Lazar Z, Illenyi L, and Horvath OP (2002) Nitric oxide is involved in the relaxant effect of capsaicin in the human sigmoid colon circular muscle. *Naunyn-Schmiedeberg's Arch Pharmacol* **366:**496–500.
Bayguinov O, Keef KD, Hagen B, and Sanders KM (1999) Parallel pathways mediate inhibitory effects of vasoactive intestinal polypeptide and nitric oxide in canine fundus. *Br J Pharmacol* **126:**1543–1552.
Bealer JF, Natuzzi ES, Bucher C, Ursell PC, Flake AW, Adzick NS, and Harrison MR (1994) Nitric oxide synthase is deficient in the aganglionic colon of patients with Hirschsprung's disease. *Pediatrics* **93:**647–651.
Belai A and Burnstock G (1994) Evidence for coexistence of ATP and nitric oxide in non-adrenergic, non-cholinergic (NANC) inhibitory neurons in the rat ileum, colon and anococcygeus muscle. *Cell Tissue Res* **278:**197–200.
Belai A and Burnstock G (2000) Pattern of distribution and co-localization of NOS and ATP in the myenteric plexus of human fetal stomach and intestine. *Neuroreport* **11:**5–8.
Belai A, Cooper S, and Burnstock G (1995) Effect of age on NADPH-diaphorase-containing myenteric neurons of rat ileum and proximal colon. *Cell Tissue Res* **279:**379–383.
Berrino L, Hey C, Rossi F, and Racke K (1995) Stimulation-induced ^{3}H-L-citrulline accumulation in isolated longitudinal muscle myenteric plexus preparations of rat small intestine: a measure to characterize nitrergic neurons. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:**506–511.
Berthoud HR (1995) Anatomical demonstration of vagal input to nicotinamide acetamide dinucleotide phosphate diaphorase-positive (nitrergic) neurons in rat fundic stomach. *J Comp Neurol* **358:**428–439.
Beyak MJ, Xue S, Collman PI, Valdez DT, and Diamant NE (2000) Central nervous system nitric oxide induces oropharyngeal swallowing and esophageal peristalsis in the cat. *Gastroenterology* **119:**377–385.
Boeckxstaens GE, De Man JG, De Winter BY, Herman AG, and Pelckmans PA (1994) Pharmacological similarity between nitric oxide and the nitrergic neurotransmitter in the canine ileocolonic junction. *Eur J Pharmacol* **264:**85–89.
Boeckxstaens GE, De Man JG, De Winter BY, Moreels TG, Herman AG, and Pelckmans PA (1995) Bioassay and pharmacological characterization of the nitrergic neurotransmitter. *Arch Int Pharmacodyn Ther* **329:**11–26.
Boeckxstaens GE, De Man JG, Pelckmans PA, Cromheeke KM, Herman AG, and Van Maercke YM (1993a) Ca^{2+} dependency of the release of nitric oxide from non-adrenergic non-cholinergic nerves. *Br J Pharmacol* **110:**1329–1334.
Boeckxstaens GE, De Man JG, Pelckmans PA, Herman AG, and Van Maercke YM (1993b) Alpha 2-adrenoceptor-mediated modulation of the nitrergic innervation of the canine isolated ileocolonic junction. *Br J Pharmacol* **109:**1079–1084.
Boeckxstaens GE and Pelckmans PA (1997) Nitric oxide and the non-adrenergic non-cholinergic neurotransmission. *Comp Biochem Physiol A Physiol* **118:**925–937.
Boeckxstaens GE, Pelckmans PA, Bogers JJ, Bult H, De Man JG, Oosterbosch L, Herman AG, and Van Maercke YM (1991a) Release of nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. *J Pharmacol Exp Ther* **256:**441–447.
Boeckxstaens GE, Pelckmans PA, Bult H, De Man JG, Herman AG, and Van Maercke YM (1990a) Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. *Eur J Pharmacol* **190:**239–246.
Boeckxstaens GE, Pelckmans PA, Bult H, De Man JG, Herman AG, and Van Maercke YM (1991b) Evidence for nitric oxide as mediator of non-adrenergic, non-cholinergic relaxations induced by ATP and GABA in the canine gut. *Br J Pharmacol* **102:**434–438.
Boeckxstaens GE, Pelckmans PA, De Man JG, Bult H, Herman AG, and Van Maercke YM (1992) Evidence for a differential release of nitric oxide and vasoactive intestinal polypeptide by nonadrenergic noncholinergic nerves in the rat gastric fundus. *Arch Int Pharmacodyn Ther* **318:**107–115.
Boeckxstaens GE, Pelckmans PA, Herman AG, and Van Mearcke YM (1993c) Involvement of nitric oxide in the inhibitory innervation of the human isolated colon. *Gastroenterology* **104:**690–697.
Boeckxstaens GE, Pelckmans PA, Rampart M, Bogers JJ, Verbeuren TJ, Herman AG, and Van Maercke YM (1990b) Pharmacological characterization of 5-hydroxy-

tryptamine receptors in the canine terminal ileum and ileocolonic junction. *J Pharmacol Exp Ther* **254:**652–658.
Boeckxstaens GE, Pelckmans PA, Ruyjens IF, Bult H, De Man JG, Herman AG, and Van Maercke YM (1991c) Bioassay of nitric oxide released upon stimulation of non-adrenergic non-cholinergic nerves in the canine ileocolonic junction. *Br J Pharmacol* **103:**1085–1091.
Bogers JJ, Pelckmans PA, Boeckxstaens GE, De Man JG, Herman AG, and Van Maercke YM (1991) The role of nitric oxide in serotonin-induced relaxations in the canine terminal ileum and ileocolonic junction. *Naunyn-Schmiedeberg's Arch Pharmacol* **344:**716–719.
Borjesson L, Nordgren S, and Delbro DS (1997) DMPP causes relaxation of rat distal colon by a purinergic and a nitrergic mechanism. *Eur J Pharmacol* **334:**223–231.
Bortolotti M, Mari C, Giovannini M, Pinna S, Miglioli M, and Pandolfo N (2001a) Effects of sildenafil on esophageal motility of normal subjects. *Dig Dis Sci* **46:** 2301–2306.
Bortolotti M, Mari C, Lopilato C, La Rovere L, and Miglioli M (2001b) Sildenafil inhibits gastroduodenal motility. *Aliment Pharmacol Ther* **15:**157–161.
Bortolotti M, Mari C, Lopilato C, Porrazzo G, and Miglioli M (2000) Effects of sildenafil on esophageal motility of patients with idiopathic achalasia. *Gastroenterology* **118:**253–257.
Bortolotti M, Pandolfo N, Giovannini M, Mari C, and Miglioli M (2002) Effect of sildenafil on hypertensive lower oesophageal sphincter. *Eur J Clin Investig* **32:** 682–685.
Boyle FC, Craig JW, and Muir TC (1991) Nitric oxide in inhibitory transmission in the guinea-pig lower oesophageal sphincter. *Br J Pharmacol* **104:**5P.
Brandt CT, Tam PK, and Gould SJ (1996) Nitrergic innervation of the human gut during early fetal development. *J Pediatr Surg* **31:**661–664.
Bredt DS, Hwang PM, and Snyder SH (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature (Lond)* **347:**768–770.
Bredt DS and Snyder SH (1990) Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* **87:**682–685.
Brown JF, Keates AC, Hanson PJ, and Whittle BJR (1993) Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells. *Am J Physiol* **265:**G418–G422.
Brown JF, Tepperman BL, Hanson PJ, Whittle BJR, and Moncada S (1992) Differential distribution of nitric oxide synthase between cell fractions isolated from the rat gastric mucosa. *Biochem Biophy Res Commun* **184:**680–685.
Browse DJ, Mathie RT, Benjamin IS, and Alexander B (1994) The transhepatic action of ATP on the hepatic arterial and portal venous vascular beds of the rabbit: the role of nitric oxide. *Br J Pharmacol* **113:**987–993.
Brzozowski T, Konturek SJ, Sliwowski Z, Drozdowicz D, Zaczek M, and Kedra D (1997) Role of L-arginine, a substrate for nitric oxide-synthase, in gastroprotection and ulcer healing. *J Gastroenterol* **32:**442–452.
Brzozowski T, Ptak A, Kwiecien S, Pajdo R, Drozdowicz D, Pawlik M, Konturek SJ, and Pawlik WW (2003) Importance of nitric oxide (NO) and adenosine in the mechanism of gastric preconditioning induced by short ischemia. *Rocz Akad Med Bialymst* **48:**42–47.
Bucher B, Ouedraogo S, Tschopl M, Paya D and Stoclet JC (1992) Role of the L-arginine-NO pathway and of cyclic GMP in the electrical field-induced noradrenaline release and vasoconstriction in the rat tail artery. *Br J Pharmacol* **107:**976–982.
Bult H, Boeckxcstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, and Herman AG (1990) Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature (Lond)* **345:**346–347.
Burns AJ, Lomax AEJ, Torihashi S, Sanders KM, and Ward SM (1996) Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc Natl Acad Sci USA* **93:**12008–12013.
Burnstock G (1972) Purinergic nerves. *Pharmacol Rev* **24:**509–581.
Calignano A, Whittle BJR, Di Rosa M, and Moncada S (1992) Involvement of endogenous nitric oxide in the regulation of rat intestinal motility in vivo. *Eur J Pharmacol* **229:**273–276.
Campbell RW (1987) Mexiletine. *N Engl J Med* **316:**29–34.
Cellek S, Fowell NA, and Moncada S (2003) Two phases of nitrergic neuropathy in streptozotocin-induced diabetic rats. *Diabetes* **52:**2353–2362.
Chakder S and Rattan S (1998) Involvement of pituitary adenylate cyclase-activating peptide in opossum internal anal sphincter relaxation. *Am J Physiol* **275:**G769–G777.
Chang KC, Koo EB, Lee GW, Kang YJ, and Lee HY (1998) Comparison of relaxations evoked by photoactivation of NO-containing compounds and nitrergic nerve stimulation in 5-hydrosytryptamine- and potassium-contracted rat gastric fundus. *Gen Pharmacol* **30:**585–591.
Chen RYZ, Ross G, Chyu K-Y, and Guth PH (1993) Role of L-arginine-derived nitric oxide in cholinergic dilation of gastric arterioles. *Am J Physiol* **265:**H2110–H2116.
Chiu JH, Lui WY, Chen YL, and Hong CY (1998) Local somatothermal stimulation inhibits the motility of sphincter of Oddi in cats, rabbits and humans through nitrergic neural release of nitric oxide. *Life Sci* **63:**413–428.
Christinck F, Jury J, Cayabyab F, and Daniel EE (1991) Nitric oxide may be final mediator of nonadrenergic, noncholinergic inhibitory junction potential in the gut. *Can J Physiol Pharmacol* **69:**1448–1458.
Ciccocioppo R, Onori L, Messori E, Messori E, Candura SM, Coccini T, and Tonini M (1994) Role of nitric oxide-dependent and -independent mechanisms in peristalsis and accommodation in the rabbit distal colon. *J Pharmacol Exp Ther* **270:**929–937.
Colpaert EE and Lefebvre RA (2000) Influence of bilirubin and other antioxidants on nitrergic relaxation in the pig gastric fundus. *Br J Pharmacol* **129:**1201–1211.
Colpaert EE, Timmermans JP, and Lefebvre RA (2002) Influence of antioxidant depletion on nitrergic relaxation in the pig gastric fundus. *Br J Pharmacol* **135:** 917–926.
Conklin JL, Du C, Murray JA, and Bates JN (1993) Characterization and mediation of inhibitory junction potentials from opossum lower esophageal sphincter. *Gastroenterology* **104:**1439–1444.
Correia NA, Cavalcanti PM, Oliveira RB, and Ballejo G (1999) Effect of hydroquinone, hydrocobalamin and carboxy-PTIO on non-adrenergic non-cholinergic nerve mediated relaxations of the rat duodenum. *J Auton Pharmacol* **19:**233–240.
Correia NA, Oliveira RB, and Ballejo G (2000) Pharmacological profile of nitrergic nerve-, nitric oxide-, nitrosoglutathione- and hydroxylamine-induced relaxations of the rat duodenum. *Life Sci* **68:**709–717.
Cortesini C, Cianchi F, Infantino A, and Lise M (1995) Nitric oxide synthase and VIP distribution in enteric nervous system in idiopathic chronic constipation, *Dig Dis Sci* **40:**2450–2455.
Costa M, Brookes SJ, Steels PA, Gibbins I, Burcher E, and Kandiah CJ (1996) Neurochemical classification of myenteric neurons in the guinea-pig ileum. *Neuroscience* **75:**949–967.
Cottrell DF, McGorum BC, and Pearson GT (1999) The neurology and enterology of equine grass sickness: a review of basic mechanisms. *Neurogastroenterol Motil* **11:**79–92.
Couceyro P, Paquet M, Koylu E, Kuhar MJ, and Smith Y (1998) Cocaine- and amphetamine-regulated transcript (CART) peptide immunoreactivity in myenteric plexus neurons of the rat ileum and co-localization with choline acetyltransferase. *Synapse* **30:**1–8.
Cuffari C, Bass J, Rubin S, and Krantis A (1997) Dysplastic nitrergic neurons in the rectum of a patient with rectal ectasia. *J Pediatr Surg* **32:**1237–1240.
Cullen JJ, Conklin JL, Murray J, Ledlow A, and Rosenthal G (1996) Effects of recombinant human hemoglobin on opossum sphincter of Oddi motor function in vivo and in vitro. *Dig Dis Sci* **41:**289–294.
Curro D and Preziosi P (1997) Involvement of vasoactive intestinal polypeptide in nicotine-induced relaxation of the rat gastric fundus. *Br J Pharmacol* **121:**1105–1112.
Curro D and Preziosi P (1998) Non-adrenergic non-cholinergic relaxation of the rat stomach. *Gen Pharmacol* **31:**697–703.
Curro D, Volpe AR, and Preziosi P (1996) Nitric oxide synthase activity and non-adrenergic non-cholinergic relaxation in the rat gastric fundus. *Br J Pharmacol* **117:**717–723.
Dalziel HH, Thornbury KD, Ward SM, and Sanders KM (1991) Involvement of nitric oxide synthetic pathway in inhibitory junction potentials in canine proximal colon. *Am J Physiol* **260:**G789–G792.
D'Amato M, Curro D, and Montuschi P (1992) Evidence for dual components in the non-adrenergic non-cholinergic relaxation in the rat gastric fundus: role of endogenous nitric oxide and vasoactive intestinal polypeptide. *J Auton Nerv Syst* **37:** 175–186.
Daniel EE, Bowes TJ, and Jury J (2002) Roles of guanylate cyclase in responses to myogenic and neural nitric oxide in canine lower esophageal sphincter. *J Pharmacol Exp Ther* **301:**1111–1118.
De Giorgio R, Parodi JE, Brecha NC, Brunicardi FC, Becker JM, Go VL, and Sternini C (1994) Nitric oxide producing neurons in the monkey and human digestive system. *J Comp Neurol* **342:**619–627.
De Man JG, Boeckxstaens GE, De Winter BY, Moreels TG, Herman AG, and Pelckmans PA (1995a) Inhibition of non-adrenergic non-cholinergic relaxations by nitric oxide donors. *Eur J Pharmacol* **285:**269–274.
De Man JG, Boeckxstaens GE, De Winter BY, Moreels TG, Misset ME, Herman AG, and Peckmans PA (1995b) Comparison of the pharmacological profile of S-nitrosothiols, nitric oxide and the nitrergic neurotransmitter in the canine ileocolonic junction. *Br J Pharmacol* **114:**1179–1184.
De Man JG, Boeckxstaens GE, Pelckmans PP, De Winter BY, Herman AG, and Van Maercke YM (1993) Prejunctional modulation of the nitrergic innervation of the canine ileocolonic junction via potassium channels. *Br J Pharmacol* **110:**559–564.
De Man JG, De Winter BY, Boeckxstaens GE, Herman AG, and Pelckmans PA (1996a) Effect of Cu^{2+} on relaxations to the nitrergic neurotransmitter, NO and S-nitrosothiols in the rat gastric fundus. *Br J Pharmacol* **119:**990–996.
De Man JG, De Winter BY, Boeckxstaens GE, Herman AG, and Pelckmans PA (1996b) Effect of thiol modulators and Cu/Zn superoxide dismutase inhibition on nitrergic relaxation in the rat gastric fundus. *Br J Pharmacol* **119:**1022–1028.
De Man JG, De Winter BY, Moreels TG, Herman AG, and Pelckmans PA (1998) S-Nitrosothiols and the nitrergic neurotransmitter in the rat gastric fundus: effect of antioxidants and metal chelation. *Br J Pharmacol* **123:**1039–1046.
De Man JG, De Winter BY, Seerden TC, De Schepper HU, Herman AG, and Pelckmans PA (2003) Functional evidence that ATP or a related purine is an inhibitory NANC neurotransmitter in the mouse jejunum: study on the identity of P2X and P2Y purinoceptors involved. *Br J Pharmacol* **140:**1108–1116.
De Man JG, Moreels TG, De Winter BY, Herman AG, and Pelckmans PA (1999) Neocuproine potentiates the activity of the nitrergic neurotransmitter but inhibits that of S-nitrosothiols. *Eur J Pharmacol* **381:**151–159.
De Man JG, Moreels TG, De Winter BY, Herman AG, and Pelckmans PA (2001) Pre- and postjunctional protective effect of neocuproine on the nitrergic neurotransmitter in the mouse gastric fundus. *Br J Pharmacol* **132:**277–285.
De Man JG, Pelckmans PA, Boeckxstaens GE, Bult H, Oosterbosch L, Herman AG, and Van Maercke YM (1991) The role of nitric oxide in inhibitory non-adrenergic non-cholinergic neurotransmission in the canine lower oesophageal sphincter. *Br J Pharmacol* **103:**1092–1096.
De Meyer GR, Kockx M, Knaapen MW, Martinet W, De Cleen DM, Bult H, and Herman AG (2003) Nitric oxide donor molsidomine favors features of atherosclerotic plaque stability during cholesterol lowering in rabbits. *J Cardiovasc Pharmacol* **41:**970–978.
Depoortere I, Thijs T, and Peeters TL (2002) Generalized loss of inhibitory innervation reverses serotonergic inhibition into excitation in a rabbit model of TNBS-colitis. *Br J Pharmacol* **135:**2011–2019.
Desai KM, Sessa WC, and Vane JR (1991) Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. *Nature (Lond)* **351:**477–479.
De Winter BY, Boeckxstaens GE, De Man JG, Moreels TG, Herman AG, and Pelckmans PA (1997) Effect of adrenergic and nitrergic blockade on experimental ileus in rats. *B J Pharmacol* **120:**464–468.
De Winter BY, Bredenoord AJ, De Man JG, Moreels TG, Herman AG, and Pelck-

mans PA (2002) Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice. *Shock* **18:**125–131.
Dick JM and Lefebvre RA (2000) Interplay between nitric oxide and vasoactive intestinal polypeptide in the pig gastric fundus smooth muscle. *Eur J Pharmacol* **397:**389–397.
Dick JM, Van Molle W, Brouckaert P, and Lefebvre RA (2002) Relaxation by vasoactive intestinal polypeptide in the gastric fundus of nitric oxide synthase-deficient mice. *J Physiol* **538:**133–143.
Dick JM, Van Molle W, Libert C, and Lefebvre RA (2001) Antisense knockdown of inducible nitric oxide synthase inhibits the relaxant effect of VIP in isolated smooth muscle cells of the mouse gastric fundus. *Br J Pharmacol* **134:**425–433.
Donat ME, Wong K, Staines WA, and Krantis A (1999) Heme oxygenase immunoreactive neurons in the rat intestine and their relationship to nitrergic neurons. *J Auton Nerv Syst* **77:**4–12.
Douglass J, McKinzie AA, and Couceyro P (1995) PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J Neurosci* **15:**2471–2481.
Dun NJ, Dun SL, Kwok EH, Yang J, and Chang JK (2000) Cocaine- and amphetamine-regulated transcript-immunoreactivity in the rat sympatho-adrenal axis. *Neurosci Lett* **283:**97–100.
Eherer AJ, Schwetz I, Hammer HF, Petnehazy T, Scheidl SJ, Weber K, and Krejs GJ (2002) Effect of sildenafil on oesophageal motor function in healthy subjects and patients with oesophageal motor disorders. *Gut* **50:**758–764.
Ekblad E, Alm P, and Sundler F (1994) Distribution, origin and projections of nitric oxide synthase-containing neurons in gut and pancreas. *Neuroscience* **63:**233–248.
Ekblad E, Kuhar M, Wierup N, and Sundler F (2003) Cocaine-and amphetamine-regulated transcript: distribution and function in rat gastrointestinal tract. *Neurogastroenterol Motil* **15:**545–557.
Elizalde JI, Mendez A, Gomez J, del Rivero M, Gironella M, Closa D, Quintero E, and Pique JM (2003) Gastric mucosal blood flow changes in Helicobacter pylori infection and NSAID-induced gastric injury. *Helicobacter* **8:**124–131.
Elliott SN and Wallace JL (1998) Nitric oxide: a regulator of mucosal defense and injury. *J Gastroenterol* **33:**792–803.
Ember Z, Reti A, and Feher E (2000) Enzyme- and immunohistochemical localization of nitric oxide synthase in nerves of the porcine pancreas. *Neurosci Lett* **292:**163–166.
Ember Z, Yago M, and Singh J (2001) Distribution of nitric oxide synthase and secretory role of exogenous nitric oxide in the isolated rat pancreas. *Int J Gastrointest Cancer* **29:**77–84.
Ergun Y and Ogulener N (2001) Evidence for the interaction between nitric oxide and vasoactive intestinal polypeptide in the mouse gastric fundus. *J Pharmacol Exp Ther* **299:**945–950.
Esteban FJ, Jimenez A, Barroso JB, Pedrosa JA, del Moral ML, Rodrigo J, and Peinado MA (1998a) The innervation of rainbow trout (Oncorhynchus mykiss) liver: protein gene product 9.5 and neuronal nitric oxide synthase immunoreactivities. *J Anat* **193:**241–249.
Esteban FJ, Jimenez A, Fernandez AP, del Moral ML, Sanchez-Lopez AM, Hemandez R, Garrosa M, Pedrosa JA, Rodrigo J, and Peinado MA (2001) Neuronal nitric oxide synthase immunoreactivity in the guinea-pig liver: distribution and colocalization with neuropeptide Y and calcitonin gene-related peptide. *Liver* **21:**374–379.
Esteban FJ, Pedrosa JA, Jimenez A, del Moral ML, Rodrigo J, and Peinado MA (1998b) Nitrergic innervation of the liver. *Neurosci Lett* **243:**45–48.
Esteban FJ, Pedrosa JA, Jimenez A, Fernandez AP, Ventura ML, Martinez-Murillo R, Rodrigo J, and Peinado MA (1997) Distribution of neuronal nitric oxide synthase in the rat liver. *Neurosci Lett* **226:**99–102.
Fahrenkrug J (1993) Transmitter role of vasoactive intestinal peptide. *Pharmacol Toxicol* **72:**354–363.
Fang S, Wu R, and Christensen J (1993) Intramucosal nerve cells in human small intestine. *J Auton Nerv Syst* **44:**129–136.
Feher E, Altdorfer K, Donath T, and Feher J (2001) Electron-microscopical localization of nitric oxide synthase-containing nerve elements in cat pylorus. *Eur J Morphol* **39:**121–126.
Fisher A, Brandeis R, Karton I, Pittel Z, Gurwitz D, Haring R, Sapoir M, Levy A, and Heldman E (1991) (±)-Cis-2-methyl-spiro(1,3-oxathiolane-5,3′) quinuclidine, an M_1 selective cholinergic agonist, attenuates cognitive dysfunctions in an anima model of Alzheimer's disease. *J Pharmacol Exp Ther* **257:**392–403.
Forstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, and Kleinert H (1994) Nitric oxide synthase isozymes. Characterization, purification, molecular cloning and functions. *Hypertension* **23:**1121–1131.
Forstermann U, Pollock JS, Schmidt HH, Heller M, and Murad F (1991) Calmodulin-dependent endothelium-derived relaxing factor/nitric oxide synthase activity is present in the particulate and cytosolic fractions of bovine aortic endothelial cells. *Proc Natl Acad Sci USA* **88:**1788–1792.
Fritz E, Hammer J, Schmidt B, Eherer AJ, and Hammer HF (2003) Stimulation of the nitric oxide-guanosine 3′,5′-cyclic monophosphate pathway by sildenafil: effect on rectal muscle tone, distensibility and perception in health and in irritable bowel syndrome. *Am J Gastroenterol* **98:**2253–2260.
Furchgott RF (1988) Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid-activatable inhibitory factor from retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide, in *Vasodilatation* (Vanhoutte PM ed) pp 401–414, Raven Press, Ltd., New York.
Furchgott RF and Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond)* **288:**373–376.
Garthwaite J, Southam E, Boulton CL, Nielsen EB, Schmidt K, and Mayer B (1995) Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol Pharmacol* **48:**184–188.
Gillespie JS and Sheng H (1990) The effects of pyrogallol and hydroquinone on the response to NANC nerve stimulation in the rat anococcygeus and the bovine retractor penis. *Br J Pharmacol* **99:**194–196.
Gonzalez AA, Farre R, and Clave P (2004) Different responsiveness of excitatory and inhibitory enteric motor neurons in the human esophagus to electrical field stimulation and to nicotine. *Am J Physiol* **287:**G299–G306.
Goyal RK and He XD (1998) Evidence for NO. redox form of nitric oxide as nitrergic inhibitory neurotransmitter in gut. *Am J Physiol* **275:**G1185–G1192.
Greenberg S, Diecke FPJ, Cantor E, Peevy K, and Tanaka TP (1990) Inhibition of sympathetic neurotransmitter release by modulators of cyclic GMP in canine vascular smooth muscle. *Eur J Pharmacol* **187:**409–423.
Greenblatt EP, Loeb AL, and Longnecker DE (1993) Marked regional heterogeneity in the magnitude of EDRF/NO-mediated vascular tone in awake rats. *J Cardiovasc Pharmacol* **21:**235–240.
Grider JR, Katsoulis S, Schmidt WE, and Jin JG (1994) Regulation of the descending relaxation phase of intestinal peristalsis by PACAP. *J Auton Nerv Syst* **50:**151–159.
Grozdanovic Z, Mayer B, Baumgarten HG, and Bruning GN (1994) Nitric oxide synthase-containing nerve fibres and neurons in the gall bladder and biliary pathways of the guinea-pig. *Neuroreport* **5:**837–840.
Guo R, Nada O, Suita S, Taguchi T, and Masumoto K (1997) The distribution and co-localization of nitric oxide synthase and vasoactive intestinal polypeptide in nerves of the colons with Hirschsprung's disease. *Virchows Arch* **430:**53–61.
Hebeiss K and Kilbinger H (1999) Cholinergic and GABAergic regulation of nitric oxide synthesis in the guinea pig ileum. *Am J Physiol* **276:**G862–G866.
Hecker M, Sessa WC, Harris HJ, Anggard EE, and Vane JR (1990) The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: culture endothelial cells recycle L-citrulline to L-arginine. *Proc Natl Acad Sci USA* **87:**8612–8616.
Hens J, Gajda M, Scheuermann DW, Adriaensen D, and Timmermans JP (2002) The longitudinal smooth muscle layer of the pig small intestine is innervated by both myenteric and submucous neurons. *Histochem Cell Biol* **117:**481–492.
Hevel JM, White KA, and Marletta MA (1991) Purification of the inducible murine macrophage nitric oxide synthase: identification as a flavoprotein. *J Biol Chem* **266:**22789–22791.
Hirakawa H, Kobayashi H, O'Briain DS, and Puri P (1995) Absence of NADPH-diaphorase activity in internal anal sphincter (IAS) achalasia. *J Pediatr Gastroenterol Nutr* **20:**54–58.
Hirano I (1999) Pathophysiology of achalasia. *Curr Gastroenterol Rep* **1:**198–202.
Hirsch DP, Holloway RH, Tytgat GN, and Boeckxstaens GE (1998) Involvement of nitric oxide in human transient lower esophageal sphincter relaxations and esophageal primary peristalsis. *Gastroenterology* **115:**1374–1380.
Holst JJ, Rasmussen TN, and Schmidt P (1994) Role of nitric oxide in neurally induced pancreatic exocrine secretion in pigs. *Am J Physiol* **266:**G206–G213.
Holzer P (1997) Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. *Neuroreport* **8:**2857–2860.
Hryhorenko LM, Woskowska Z, and Fox-Threlkeld JET (1994) Nitric oxide (NO) inhibits release of acetylcholine from nerves of isolated circular muscle of the canine ileum: relationship to motility and release of nitric oxide. *J Pharmacol Exp Ther* **271:**918–926.
Huang PL, Dawson TM, Bredt DS, Snyder SH, and Fishman MC (1993) Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* **75:**1273–1286.
Ignarro LJ, Buga GM, Wood KS, Byrns RE, and Chaudhuri G (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* **84:**9265–9269.
Ignarro LJ, Byrns RE, and Wood KS (1988) Biochemical and pharmacological properties of endothelium-derived relaxing factor and its similarity to nitric oxide radical, in *Vasodilatation* (Vanhoutte PM ed) pp 427–435, Raven Press, New York.
Imaeda K, Joh T, Yamamoto Y, Itoh M, and Suzuki H (1998) Properties of inhibitory junctional transmission in smooth muscle of the guinea pig lower esophageal sphincter. *Jpn J Physiol* **48:**457–465.
Ishiguchi T, Nakajima M, Sone H, Tada H, Kumagai AK, and Takahashi T (2001) Gastric distension-induced pyloric relaxation: central nervous system regulation and effects of acute hyperglycemia in the rat. *J Physiol* **533:**801–813.
Ishiguchi T, Takahashi T, Itoh H, and Owyang C (2000) Nitrergic and purinergic regulation of the rat pylorus. *Am J Physiol* **279:**G740–G747.
Ivancheva C and Radomirov R (1996) Met-enkephalin-dependent nitrergically mediated relaxation in the guinea pig ileum. *Methods Find Exp Clin Pharmacol* **18:**521–525.
Iversen HH, Gusrafsson LE, Leone AM, and Wiklund NP (1994) Smooth muscle relaxing effects of NO, nitrosothiols and a nerve-induced relaxing factor released in guinea-pig colon. *Br J Pharmacol* **113:**1088–1092.
Jenkinson KM, Reid JJ, and Rand MJ (1995) Hydroxocobalamin and haemoglobin differentiate between exogenous and neuronal nitric oxide in the rat gastric fundus. *Eur J Pharmacol* **275:**145–152.
Jin JG, Katsoulis S, Schmidt WE, and Grider JR (1994) Inhibitory transmission in taenia coli mediated by distinct vasoactive intestinal peptide and apamin-sensitive pituitary adenylate cyclase activating peptide receptors. *J Pharmacol Exp Ther* **270:**433–439.
Jin JG, Murthy KS, Grider JR, and Makhlouf GM (1996) Stoichiometry of neurally induced VIP release, NO formation and relaxation in rabbit and rat gastric muscle. *Am J Physiol* **271:**G357–G369.
Kadowaki M, Kuramoto H, and Kuwahara A (1999) Morphological relationship between serotonergic neurons and nitrergic neurons for electrolyte secretion in the submucous plexus of the guinea pig distal colon. *Brain Res* **831:**288–291.
Kajimoto T, Dinning PG, Gibb DB, de Carle DJ, and Cook IJ (2000) Neurogenic pathways mediating ascending and descending reflexes at the porcine ileocolonic junction. *Neurogastroenterol Motil* **12:**125–134.
Kamata K, Kohzuki M, Misawa M, and Kasuya Y (1993) Involvement of nitric oxide pathway in non-adrenergic non-cholinergic (NANC) relaxation in the rat stomach: differential innervation of NANC nerves in the longitudinal and circular muscle of the fundus. *Gen Pharmacol* **24:**1403–1410.
Kaputlu I, Ozdem S, Sadan G, and Gokalp O (1999) Effects of diabetes on non-

adrenergic, non-cholinergic relaxation induced by GABA and electrical stimulation in the rat isolated duodenum. *Clin Exp Pharmacol Physiol* **26:**724–728.
Kato S, Kitamura M, Korolkiewicz RP, and Takeuchi K (1998) Role of nitric oxide in regulation of gastric acid secretion in rats: effects of NO donors and NO synthase inhibitor. *Br J Pharmacol* **123:**839–846.
Kaufman HS, Shermak MA, May CA, Pitt HA, and Lillemoe KD (1993) Nitric oxide inhibits resting sphincter of Oddi activity. *Am J Surg* **165:**74–80.
Keef KD, Shuttleworth CW, Xue C, Bayguinov O, Publicover NG, and Sanders KM (1994) Relationship between nitric oxide and vasoactive intestinal polypeptide in enteric inhibitory neurotransmission. *Neuropharmacology* **33:**1303–1314.
Kilbinger H, Ginap T, and Erbelding D (1999) GABAergic inhibition of nitric oxide-mediated relaxation of guinea-pig ileum. *Naunyn-Schmiedeberg's Arch Pharmacol* **359:**500–504.
Kim CD, Goyal RK, and Mashimo H (1999) Neuronal NOS provides nitrergic inhibitory neurotransmitter in mouse lower esophageal sphincter. *Am J Physiol* **277:** G280–G284.
King BN, Stoner MC, Haque SM, and Kellum JM (2004) Nitrergic secretomotor neurotransmitter in the chloride secretory response to serotonin. *Dig Dis Sci* **49:**196–201.
Kiraly A, Suto G, and Tache Y (1993) Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. *Eur J Pharmacol* **240:**299–301.
Kirchgessner AL, Liu MT, and Gershon MD (1994) NADPH diaphorase (nitric oxide synthase)-containing nerves in the enteropancreatic innervation: sources, co-stored neuropeptides and pancreatic function. *J Comp Neurol* **342:**115–130.
Kishi M, Takeuchi T, Suthamnatpong N, Ishii T, Nishio H, Hata F, and Takewaki T (1996) VIP and PACAP mediate nonadrenergic, noncholinergic inhibition in longitudinal muscle of rat distal colon: involvement of activation of charybdotoxin- and apamin-sensitive K+ channels. *Br J Pharmacol* **119:**623–630.
Kohjitani A, Miyawaki T, Funahashi M, Higuchi H, Matsuo R, and Shimada M (2003a) Ketamine and midazolam differentially inhibit nonadrenergic noncholinergic lower esophageal sphincter relaxation in rabbits. *Anesthesiology* **98:**449–458.
Kohjitani A, Miyawaki T, Funahashi M, Mitoh Y, Matsuo R, and Shimada M (2001) Intravenous anesthetics inhibit nonadrenergic noncholinergic lower esophageal sphincter relaxation via nitric oxide-cyclic guanosine monophosphate pathway modulation in rabbit. *Anesthesiology* **95:**176–183.
Kohjitani A, Miyawaki T, Funahashi M, Mitoh Y, Matsuo R, and Shimada M (2003b) Mexiletine inhibits nonadrenergic noncholinergic lower oesophageal sphincter relaxation in rabbits. *Eur J Pharmacol* **465:**145–151.
Kojima H, Hirata M, Kodo Y, Kikuchi K, and Nagano T (2001) Visualization of oxygen-concentration-dependent production of nitric oxide in rat hippocampal slices during aglycemia. *J Neurochem* **76:**1404–1410.
Kojima H, Nakatsubo N, Kikuchi K, Urano Y, Higuchi T, Tanaka J, Kudo Y, and Nagano T (1998) Direct evidence of NO production in rat hippocampus and cortex using a new fluorescent indicator DAF-2 DA. *Neuroreport* **9:**3345–3348.
Kojima S, Ishizaki R, and Shimo Y (1993) Investigation of nicotine-induced relaxation of circular smooth muscle of the guinea-pig gastric fundus. *Eur J Pharmacol* **241:**171–175.
Konturek JW, Hengst K, Kulesza E, Gabryelewicz A, Konturek SJ, and Domschke W (1997a) Role of endogenous nitric oxide in the control of exocrine and endocrine pancreatic secretion in humans. *Gut* **40:**86–91.
Konturek JW, Thor P, Lukaszyk A, Gabryelewicz A, Konturek SJ, and Domschke W (1997b) Endogenous nitric oxide in the control of esophageal motility in humans. *J Physiol Pharmacol* **48:**201–209.
Konturek SJ, Bilski J, Konturek PK, Cieszkowski M, and Pawlik W (1993a) Role of endogenous nitric oxide in the control of canine pancreatic secretion and blood flow. *Gastroenterology* **104:**896–902.
Konturek SJ, Brzozowski T, Majka J, Pytko-Polonczyk J, and Stachura J (1993b) Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur J Pharmacol* **239:**215–217.
Kortezova N, Mizhorkova Z, Milusheva E, Verga G, Vizi ES, and Papasova M (1996) Non-adrenergic non-cholinergic neuron stimulation in the cat lower esophageal sphincter. *Eur J Pharmacol* **304:**109–115.
Kortezova NI, Shikova LI, Milusheva EA, Itzev DE, Bagaev VA, and Mizhorkova ZN (2004) Muscarinic modulation of nitrergic neurotransmission in guinea pig gastric fundus. *Neurogastroenterol Motil* **16:**155–165.
Kuiken SD, Tytgat GN, and Boeckxstaens GE (2002a) Role of endogenous nitric oxide in regulating antropyloroduodenal motility in humans. *Am J Gastroenterol* **97:**1661–1667.
Kuiken SD, Vergeer M, Heisterkamp SH, Tytgat GN, and Boeckxstaens GE (2002b) Role of nitric oxide in gastric motor and sensory functions in healthy subjects. *Gut* **51:**212–218.
Kuramoto H, Kawano H, Sakamoto H, and Furness JB (1999) Motor innervation by enteric nerve fibers containing both nitric oxide synthase and galanin immunoreactivities in the striated muscle of the rat esophagus. *Cell Tissue Res* **295:**241–245.
Kwiecien S, Brzozowski T, Konturek PC, and Konturek SJ (2002) The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions. *J Physiol Pharmacol* **53:**761–773.
Kwon NS, Nathan CF, and Stuehr DJ (1989) Reduced biopterin as cofactors in the generation of nitrogen oxides by murine macrophages. *J Biol Chem* **264:**20496–20501.
La M, Li CG, and Rand MJ (1996) Comparison of the effects of hydrocobalamin and oxyhaemoglobin on responses to NO, EDRF and the nitrergic transmitter. *Br J Pharmacol* **117:**805–810.
Laszlo F and Whittle BJR (1994) Constitutive nitric oxide modulates the injurious actions of vasopressin on rat intestinal microcirculation in acute endotoxaemia. *Eur J Pharmacol* **260:**265–268.
Le QL, Zhang J, Xu QZ, and Gao HY (2001) Role of nitric oxide in omeprazole protection of the gastric mucosa in rats. *Di Yi Jun Yi Da Xue Xue Bao* **21:**926–928.
Leclere PG and Lefebvre RA (2001) Influence of nitric oxide donors and of the alpha-2 agonist UK-14,304 on acetylcholine release in the pig gastric fundus. *Neuropharmacology* **40:**270–278.
Leelakusolvong S, Sarr MG, Miller SM, Phillips SF, and Bharucha AE (2002) Role of extrinsic innervation in modulating nitrergic transmission in the canine ileocolonic region. *Am J Physiol* **283:**G230–G239.
Lefebvre RA (1993) Non-adrenergic non-cholinergic neurotransmission in the proximal stomach. *Gen Pharmacol* **24:**257–266.
Lefebvre RA (1996) Influence of superoxide dismutase inhibition on the discrimination between NO and the nitrergic neurotransmitter in the rat gastric fundus. *Br J Pharmacol* **118:**2171–2177.
Lefebvre RA (1998) Influence of a selective guanylate cyclase inhibitor and of the contraction level, on nitrergic relaxations in the gastric fundus. *Br J Pharmacol* **124:**1439–1448.
Lefebvre RA (2000) Pharmacological characterization of the nitrergic innervation of the stomach. *Verh K Acad Geneeskd Belg* **64:**151–166.
Lefebvre E, Baert E, and Barbier AJ (1992) Influence of N^G-nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus. *Br J Pharmacol* **106:**173–179.
Lefebvre RA, Hasrat J, and Gobert A (1991) Influence of N^G-nitro-L-arginine methyl ester on vagally-induced gastric relaxation. *Br J Pharmacol* **104:**4P.
Lefebvre RA, Smits GJ, and Timmermans JP (1995) Study of NO and VIP as non-adrenergic non-cholinergic neurotransmitters in the pig gastric fundus. *Br J Pharmacol* **116:**2017–2026.
Lefebvre RA and Vandekerckhove K (1998) Effect of nitroglycerin and long-term electrical stimulation on nitrergic relaxation in the pig gastric fundus. *Br J Pharmacol* **123:**143–149.
Li CG and Rand MJ (1989) Evidence for a role of nitric oxide in the neurotransmitter system mediating relaxation of the rat anococcygeus muscle. *Clin Exp Pharmacol Physiol* **16:**933–938.
Li CG and Rand MJ (1990) Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur J Pharmacol* **191:**303–309.
Li M, Johnson CP, Adams MB, and Sama SK (2002) Cholinergic and nitrergic regulation of in vivo giant migrating contractions in rat colon. *Am J Physiol* **283:**G544–G552.
Lilley E and Gibson A (1995) Inhibition of relaxations to nitrergic stimulation of the mouse anococcygeus by duroquinone. *Br J Pharmacol* **116:**3231–3236.
Liu HP, Tay SS, and Leong SK (1996) Nitrergic neurons in the pancreas of newborn guinea pig: their distribution and colocalization with various neuropeptides and dopamine-beta-hydroxylase. *J Auton Nerv Syst* **61:**248–256.
Lonovics J, Jakab I, Szilvassy J, and Szilvassy Z (1994) Regional differences in nitric oxide-mediated relaxation of the rabbit sphincter of Oddi. *Eur J Pharmacol* **255:** 117–122.
Lorente S, Doiz O, Trinidad Serrano M, Castillo J, and Lanas A (2002) Helicobacter pylori stimulates pepsinogen secretion from isolated human peptic cells. *Gut* **50:**13–18.
Luman W, Pryde A, Heading RC, and Palmer KR (1997) Topical glyceryl trinitrate relaxes the sphincter of Oddi. *Gut* **40:**541–543.
Macedo MP and Lautt WW (1997) Potentiation to vasodilators by nitric oxide synthase blockade in superior mesenteric but not hepatic artery. *Am J Physiol* **272:**G507–G514.
Maggi CA, Barbanti G, Turini D, and Giuliani S (1991) Effect of N^G-monomethyl L-arginine (L-NMMA) and N^G-nitro L-arginine (L-NOARG) on non-adrenergic non-cholinergic relaxation in the circular muscle of the human ileum. *Br J Pharmacol* **103:**1970–1972.
Martin W, McAllister KHM, and Paisley K (1994) NANC neurotransmission in the bovine retractor penis muscle is blocked by superoxide anion following inhibition of superoxide dismutase with diethyldithiocarbamate. *Neuropharmacology* **33:** 1293–1301.
Martinez-Cuesta MA, Massuda H, Whittle BJR, and Moncada S (1995) Impairment of nitrergic-mediated relaxation of rat isolated duodenum by experimental diabetes. *Br J Pharmacol* **114:**919–924.
Martins SLR, De Oliveira RB, and Ballejo G (1995) Rat duodenum nitrergic-induced relaxations are cGMP-independent and apamin-sensitive. *Eur J Pharmacol* **284:** 265–270.
Mascolo N, Izzo AA, Barbato F, and Capasso F (1993) Inhibitors of nitric oxide synthetase prevent caster-oil-induced diarrhoea in the rat. *Br J Pharmacol* **108:** 861–864.
Mashimo H, Kjellin A, and Goyal RK (2000) Gastric stasis in neuronal nitric oxide synthase-deficient knockout mice. *Gastroenterology* **119:**766–773.
Masunaga H, Ogawa H, Uematsu Y, Tomizuka T, Yasuda H, and Takeshita Y (1997) Long-acting salivation induced by a novel muscarinic receptor agonist SNI-2011 in rats and dogs. *Eur J Pharmacol* **339:**1–9.
Matsuda NM, Feitosa RL Jr, Lemos MC, De Oliveira RB, and Ballejo G (1998) In vitro and in vivo effects of nitric oxide synthase inhibitors and nitric oxide inactivators on the South American opossum ileocolonic junction. *Neurogastroenterol Motil* **10:**245–252.
Matsukado Y, Tsuru H, Sasa M, Kambe M, and Yamakido M (1997) Nonadrenergic, noncholinergic inhibitory innervation in the longitudinal muscle of the rabbit portal vein. *Gen Pharmacol* **29:**815–821.
Matsumoto K, Suita S, Nada O, Taguchi T, and Guo R (1999) Abnormalities of enteric neurons, intestinal pacemaker cells and smooth muscle in human intestinal atresia. *J Pediatr Surg* **34:**1463–1468.
Matsuyama H, El-Mahmoudy A, Shimizu Y, and Takewaki T (2003) Nitrergic prejunctional inhibition of purinergic neuromuscular transmission in the hamster proximal colon. *J Neurophysiol* **89:**2346–2353.
Matsuyama H, Thapaliya S, and Takewaki T (1999) Cyclic GMP-associated apamin-sensitive nitrergic slow inhibitory junction potential in the hamster ileum. *Br J Pharmacol* **128:**830–836.
Matsuyama H, Unno T, El-Mahmoudy AM, Komori S, Kobayashi H, Thapaliya S, and Takewaki T (2002) Peptidergic and nitrergic inhibitory neurotransmissions in

the hamster jejunum: regulation of vasoactive intestinal peptide release by nitric oxide. *Neuroscience* **110:**779–788.
McCleskey EW, Fox AP, Feldman DH, Cruz LJ, Olivera BM, Tsien RW, and Yoshikami D (1987) ω-Conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc Natl Acad Sci USA* **84:**4327–4331.
McConalogue K, Lyser DJK, and Furness JB (1995) Electrophysiological analysis of the actions of pituitary adenylyl cyclase activating peptide in the taenia of the guinea-pig caecum. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:**538–544.
McKirdy HC, McKirdy ML, Lewis MJ, and Marshall RW (1992) Evidence for involvement of nitric oxide in the non-adrenergic non-cholinergic (NANC) relaxation of human lower oesophageal sphincter muscle strips. *Exp Physiol* **77:**509–511.
McKirdy HC, Richardson CE, Green JT, Rhodes J, Williams GT, and Marshall RW (2004) Differential effect of nitric oxide synthase inhibition on sigmoid colon longitudinal and circular muscle responses to nicotine and nerve stimulation. *Br J Surg* **91:**229–234.
McLaren A, Li CG, and Rand MJ (1993) Mediators of nicotine-induced relaxations of the rat gastric fundus. *Clin Exp Pharmacol Physiol* **20:**451–457.
Mearin F, Mourelle M, Guarner F, Salas A, Riveros-Moreno V, Moncada S, and Malagelada JR (1993) Patients with achalasia lack nitric oxide synthase in the gastro-oesophageal junction. *Eur J Clin Investig* **23:**724–728.
Middleton SJ, Cuthbert AW, Shorthouse M, and Hunter JO (1993) Nitric oxide affects mammalian distal colonic smooth muscle by tonic neural inhibition. *Br J Pharmacol* **108:**974–979.
Milbourne EA and Bygrave FL (1995) Does nitric oxide play a role in liver function? *Cell Signal* **7:**313–318.
Miller SM, Reed D, Sarr MG, Farrugia G, and Szurszewski JH (2001) Haem oxygenase in enteric nervous system of human stomach and jejunum and co-localization with nitric oxide synthase. *Neurogastroenterol Motil* **13:**121–131.
Miller RJ (1987) Multiple calcium channels and neuronal function. *Science (Wash DC)* **235:**46–52.
Molero X, Guarner F, Salas A, Mourelle M, Puig V, and Malagelada JR (1995) Nitric oxide modulates pancreatic basal secretion and response to cerulein in the rat: effects in acute pancreatitis. *Gastroenterology* **108:**1855–1862.
Moncada S, Palmer RM, and Higgs EA (1989) Biosynthesis of nitric oxide from L-arginine: a pathway for the regulation of cell function and communication. *Biochem Pharmacol* **38:**1709–1715.
Morikawa S and Komuro T (1998) Distribution of myenteric NO neurons along the guinea-pig esophagus. *J Auton Nerv Syst* **74:**91–99.
Moroz LL, Gillette R, and Sweedler JV (1999) Single-cell analyses of nitrergic neurons in simple nervous systems. *J Exp Biol* **202:**333–341.
Morschl E, Bretus I, Pavo I, Topa L, Weiszhar Z, and Laszlo F (2000) Nitric oxide-mediated muscle hypersecretion protects the stomach of ovariectomized rats. *Eur J Pharmacol* **392:**R5–R7.
Mourad FH, Barada KA, Abdel-Malak N, Bou Rached NA, Khoury CI, Saade NE, and Nassar CF (2003) Interplay between nitric oxide and vasoactive intestinal polypeptide in inducing fluid secretion in rat jejunum. *J Physiol* **550:**863–871.
Mourad FH, O'Donnell LJ, Andre EA, Bearcroft CP, Owen RA, Clark MI, and Farthing MJ (1996) L-Arginine, nitric oxide and intestinal secretion: studies in rat jejunum in vivo. *Gut* **39:**539–544.
Mourelle M, Guarner F, Moncada S, and Malagelada JR (1993) The arginine/nitric oxide pathway modulates sphincter of Oddi motor activity in guinea pigs and rabbits. *Gastroenterology* **105:**1299–1305.
Mozhorkova Z, Kortezova N, Bredy-Dobreva G, and Papasova M (1994) Role of nitric oxide in mediating non-adrenergic non-cholinergic relaxation of the cat ileocecal sphincter. *Eur J Pharmacol* **265:**77–82.
Murad F, Mittal CK, Arnold WP, Katsuki S, and Kimura H (1978) Guanylate cyclase activation by azide, nitro compounds, nitric oxide and hydrosyl radical and inhibition by hemoglobin and myoglobin. *Adv Cyclic Nucleotide Protein Phosphorylation Res* **9:**145–158.
Murray J, Du C, Ledlow A, Bates JN, and Conklin JL (1991) Nitric oxide: mediator of nonadrenergic noncholinergic responses of opossum esophageal muscle. *Am J Physiol* **261:**G401–G406.
Murthy KS, Grider JR, Jin JG, and Makhlouf GM (1996) Interplay of VIP and nitric oxide in the regulation of neuromuscular function in the gut. *Ann NY Acad Sci* **805:**355–362.
Nankervis CA, Reber KM, and Nowicki PT (2001) Age-dependent changes in the postnatal intestinal microcirculation. *Microcirculation* **8:**377–387.
Nemeth L and Puri P (2001) Three-dimensional morphology of c-Kit-positive cellular network and nitrergic innervation in the human gut. *Arch Pathol Lab Med* **125:**899–904.
Nemeth L, Rolle U, Reen DJ, and Puri P (2003) Nitrergic hyperinnervation in appendicitis and in appendices histologically classified as normal. *Arch Pathol Lab Med* **127:**573–578.
Neuhuber WL, Worl J, Berthoud HR, and Conte B (1994) NADPH-diaphorase-positive nerve fibers associated with motor endplates in the rat esophagus: new evidence for co-innervation of striated muscle by enteric neurons. *Cell Tissue Res* **276:**23–30.
Nichols K, Staines W, and Krantis A (1994) Neural sites of the human colon colocalize nitric oxide synthase-related NADPH diaphorase activity and neuropeptide Y. *Gastroenterology* **107:**968–975.
Nichols K, Staines W, Wu JY, and Krantis A (1995) Immunopositive GABAergic neural sites display nitric oxide synthase-related NADPH diaphorase activity in the human colon. *J Auton Nerv Syst* **50:**253–262.
Nishizaki K, Nakao K, Ishii H, Yamanaka H, Tokunaga A, Nakagawa K, Yamamura T, and Noguchi K (2003) Induction of neuronal nitric oxide synthase by sympathetic denervation is mediated via alpha 2-adrenoceptors in the jejunal myenteric plexus. *Brain Res* **965:**121–129.
Ny L, Alm P, Larsson B, Ekstrom P, and Andersson KE (1995) Nitric oxide pathway in cat esophagus: localization of nitric oxide synthase and functional effects. *Am J Physiol* **268:**G59–G70.
Ny L, Pfeifer A, Aszodi A, Ahmad M, Alm P, Hedlund P, Fassler R, and Andersson KE (2000) Impaired relaxation of stomach smooth muscle in mice lacking cyclic GMP-dependent protein kinase I. *Br J Pharmacol* **129:**395–401.
Ogulener N, Ergun Y, Dondas N, and Dikmen A (2001) The influence of nitric oxide donors on the responses to nitrergic nerve stimulation in the mouse duodenum. *Eur J Pharmacol* **421:**121–131.
Ohno N, Xue L, Yamamoto Y, and Suzuki H (1996) Properties of inhibitory junction potential in smooth muscle of the guinea-pig gastric fundus. *Br J Pharmacol* **117:**974–978.
Okamura T, Fujioka H, Ayajiki K, and Toda N (1998a) Modifications by superoxide-generating agent, neurotransmitters and neuromodulators of nitroxidergic nerve function in monkey cerebral arteries. *J Pharmacol Exp Ther* **286:**1321–1325.
Okamura T, Tanobe Y, Fujioka H, Ayajiki K, and Toda N (1998b) Mechanism of neurogenic relaxation and modification of the response by enteric substances in isolated dog colon. *Eur J Pharmacol* **358:**245–252.
Okishio Y, Niioka S, Takeuchi T, Nishio H, Hata F, and Takatsuji K (2000a) Differences in mediator of nonadrenergic, noncholinergic relaxation of the distal colon between Wistar-ST and Sprague-Dawley strains of rats. *Eur J Pharmacol* **388:**97–105.
Okishio Y, Niioka S, Yamaji M, Yamazaki Y, Nishio H, Takeuchi T, and Hata F (2000b) Mediators of nonadrenergic, noncholinergic relaxation in Sprague Dawley rat intestine. *J Vet Med Sci* **62:**821–828.
Olgart C and Iversen HH (1999) Nitric oxide-dependent relaxation induced by M_1 muscarinic receptor activation in the rat small intestine. *Br J Pharmacol* **127:**309–313.
Orihata M and Sarna SK (1994) Inhibition of nitric oxide synthase delays gastric emptying of solid meals. *J Pharmacol Exp Ther* **271:**660–670.
Ozturk H, Aldemir M, Dokucu AI, Yagmur Y, Kilinc N, and Sahin AH (2003) The nitric oxide donor molsidomine prevents ischemia/reperfusion injury of the adult rat small intestine. *Pediatr Surg Int* **19:**305–308.
Pa V, Reid JJ, and Rand MJ (1992) Attenuation of vasoconstriction by endogenous nitric oxide in rat caudal artery. *Br J Pharmacol* **107:**1121–1128.
Paisley K and Martin W (1996) Blockade of nitrergic transmission by hydroquinone, hydrocobalamin and carboxy-PTIO in bovine retractor penis: role of superoxide anion. *Br J Pharmacol* **117:**1633–1638.
Palmer RMJ, Ferrige AG, and Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond)* **327:**524–526.
Palmer RMJ, Rees DD, Ashton DS, and Moncada S (1988) L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun* **153:**1251–1256.
Patel AG, Toyama MT, Nguyen TN, Cohen GA, Ignarro LJ, Reber HA, and Ashley SW (1995) Role of nitric oxide in the relationship of pancreatic blood flow and exocrine secretion in cats. *Gastroenterology* **108:**1215–1220.
Paterson CA, Anvari M, Tougas G, and Huizinga JD (2000) Nitrergic and cholinergic vagal pathways involved in the regulation of canine proximal gastric tone: an in vivo study. *Neurogastroenterol Motil* **12:**301–306.
Paterson WG, Anderson MA, and Anand N (1992) Pharmacological characterization of lower esophageal sphincter relaxation induced by swallowing, vagal efferent nerve stimulation and esophageal distension. *Can J Physiol Pharmacol* **70:**1011–1015.
Pauletzki JG, Sharkey KA, Davison JS, Bomzon A, and Shaffer EA (1993) Involvement of L-arginine-nitric oxide pathways in neural relaxation of the sphincter of Oddi. *Eur J Pharmacol* **232:**263–270.
Pawlik WW, Gustaw P, Jacobson ED, Sendur R, and Czarnobilski K (1995) Nitric oxide mediates intestinal hyperaemic responses to intraluminal bile-oleate. *Pflugers Arch* **429:**301–305.
Peaire AE, Krantis A, and Staines WA (1997) Distribution of the NPY receptor subtype Y_1 within human colon: evidence for NPY targeting a subpopulation of nitrergic neurons. *J Auton Nerv Syst* **67:**168–175.
Pique JM, Whittle BJR, and Esplugues JV (1989) The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur J Pharmacol* **174:**293–296.
Pompolo S and Furness JB (1993) Sources of inputs to longitudinal muscle motor neurons and ascending interneurons in the guinea-pig small intestine. *Cell Tissue Res* **280:**549–560.
Pou S, Pou WS, Bredt DS, Snyder SH, and Rosen GM (1992) Generation of superoxide by purified brain nitric oxide synthase. *J Biol Chem* **267:**24173–24176.
Preiksaitis HG, Tremblay L, and Diamant NE (1994) Nitric oxide mediates inhibitory nerve effects in human esophagus and lower esophageal sphincter. *Dig Dis Sci* **39:**770–775.
Premaratne S, Xue C, McCarty JM, Zai M, McCuen RW, Johns RA, Schepp W, Neu B, Lippman R, Melone PD, and Schubert ML (2001) Neuronal nitric oxide synthase: expression in rat parietal cells. *Am J Physiol* **280:**G308–G313.
Quintana E, Hernandez C, Alvarez-Barrientos A, Esplugues JV, and Barrachina MD (2004) Synthesis of nitric oxide in postganglionic myenteric neurons during endotoxaemia: implications for gastric motor function in rats. *FASEB J* **18:**531–533.
Rand MJ (1992a) Nitrergic neurotransmission: nitric oxide as a mediator of non-adrenergic, non-cholinergic neuroeffector transmission. *Clin Exp Pharmacol Physiol* **19:**147–169.
Rand MJ (1992b) New perspectives in neurotransmission: nitric oxide, the mediator of nitrergic transmission. *Proc Aust Physiol Pharmacol Soc* **23:**1–6.
Rattan S, Al Haj R, and De Godoy MA (2004) Mechanism of internal anal sphincter relaxation by CORM-1, authentic CO and NANC nerve stimulation. *Am J Physiol* **287:**G605–G611.
Rattan S and Chakder S (1997) L-Citrulline recycling in opossum internal anal sphincter relaxation by nonadrenergic, noncholinergic nerve stimulation. *Gastroenterology* **112:**1250–1259.
Rhee PL, Hyun JG, Lee JH, Kim YH, Son HJ, Kim JJ, Paik SW, Rhee JC, and Choi KW (2001) The effect of sildenafil on lower esophageal sphincter and body motility in normal male adults. *Am J Gastroenterol* **96:**3251–3257.
Richards WG, Stamler JS, Kobzik L, and Sugarbaker DJ (1995) Role of nitric oxide

in human esophageal circular smooth muscle in vitro. *J Thorac Cardiovasc Surg* **110:**157–164.
Rodrigo J, Uttenthal LO, Peinado MA, Esteban FJ, Fernandez AP, Serrano J, Martinez de Velasco J, Santacana M, Bentura ML, Martinez de Murillo R, and Pedrosa JA (1998) Distribution of nitric oxide synthase in the esophagus of the cat and monkey. *J Auton Nerv Syst* **70:**164–179.
Rolfe VE, Brand MP, Heales SJ, Lindley KJ, and Milla PJ (1997) Tetrahydrobiopterin regulates cyclic GMP-dependent electrogenic Cl− secretion in mouse ileum in vitro. *J Physiol* **503:**347–352.
Salomone S, Caruso A, Cutuli VM, Mangano NG, Prato A, Amico-Roxas M, Bianchi A, and Clementi G (2003) Effects of adrenomedullin on the contraction of gastric arteries during reserpine-induced gastric ulcer. *Pediatrics* **24:**117–122.
Salzman AL (1995) Nitric oxide in the gut. *New Horiz* **3:**352–364.
Sand J, Arvola P, Jantti V, Oja S, Singaram C, Baer G, Pasricha PJ, and Nordback I (1997) The inhibitory role of nitric oxide in the control of porcine and human sphincter of Oddi activity. *Gut* **41:**375–380.
Sanders KM and Ward SM (1992) Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. *Am J Physiol* **262:**G379–G392.
Sandstrom P, Woods CM, Brooke-Smith M, Saccone GT, Toouli J, and Svanvik J (2004) Highly selective iNOS inhibition and sphincter of Oddi motility in the Australian possum. *Acta Physiol Scand* **181:**321–331.
Sari R, Nemeth J, Rorszasz R, Horvath P, Blasig IE, Ferdinandy P, Nag I, Lonovics J, and Szilvassy Z (2001) Impaired by lovastatin of neural relaxation of the rabbit sphincter of Oddi. *Eur J Pharmacol* **432:**91–97.
Satoh Y, Takeuchi T, Yamazaki Y, Okishio Y, Nishio H, Takatsuji K, and Hata F (1999) Mediators of nonadrenergic, noncholinergic relaxation in longitudinal muscle of the intestine of ICR mice. *J Smooth Muscle Res* **35:**65–75.
Saur D, Neuhuber WL, Gengenbach B, Huber A, Schusziarra V, and Allescher HD (2002) Site-specific gene expression of nNOS variants in distinct functional regions of rat gastrointestinal tract. *Am J Physiol* **282:**G349–G358.
Saur D, Paehge H, Schusdziarra V, and Allescher HD (2000) Distinct expression of splice variants of neuronal nitric oxide synthase in the human gastrointestinal tract. *Gastroenterology* **118:**849–858.
Sayardoust S and Ekstrom J (2003) Nitric oxide-dependent in vitro secretion of amylase from innervated or chronically denervated parotid glands of the rat in response to isoprenaline and vasoactive intestinal peptide. *Exp Physiol* **88:**381–387.
Schicho R, Schemann M, Holzer P, and Lippe IT (2001) Mucosal acid challenge activates nitrergic neurons in myenteric plexus of rat stomach. *Am J Physiol* **281:**G1316–1321.
Selemidis S and Cocks TM (2000) Nitrergic relaxation of the mouse gastric fundus is mediated by cyclic GMP-dependent and ryanodine-sensitive mechanisms. *Br J Pharmacol* **129:**1315–1322.
Shaffer EA (2000) Review article: control of gall-bladder motor function. *Aliment Pharmacol Ther* **14 (Suppl 2):**2–8.
Shah S, Hobbs A, Singh R, Cuevas J, Ignarro LJ, and Chaudhuri G (2000) Gastrointestinal motility during pregnancy: role of nitrergic component of NANC nerves. *Am J Physiol* **279:**R1478–R1485.
Shah S, Nathan L, Singh R, Fu YS, and Chaudhuri G (2001) E2 and not P4 increases NO release from NANC nerves of the gastrointestinal tract: implications in pregnancy. *Am J Physiol* **280:**R1546–R1554.
Shikova LI and Kortezova NI (2000) Participation of muscarinic acetylcholine receptors in nitric oxide pathway in cat duodenum. *Acta Physiol Pharmacol Bulg* **25:**57–61.
Shimamura K, Fujisawa A, Toda N, and Sunano S (1993) Effects of N^G-nitro-L-arginine on electrical and mechanical responses to stimulation of non-adrenergic, non-cholinergic inhibitory nerves in circular muscle of the rat gastric fundus. *Eur J Pharmacol* **231:**103–109.
Shinozaki K, Kashiwagi A, Nishio Y, Okamura T, Yoshida Y, Masada M, Toda N, and Kikkawa Y (1999) Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent relaxation through nitric oxide/O_2^- imbalance in insulin-resistant rat aorta. *Diabetes* **48:**2437–2445.
Shiraishi S, Okamura T, Kodama M, and Toda N (1998) Mechanisms underlying the neurogenic relaxation in dog isolated hepatic arteries. *J Cardiovasc Pharmacol* **31:**372–376.
Shuttleworth CW, Burns AJ, Ward SM, O'Brien WE, and Sanders KM (1995) Recycling of L-citrulline to sustain nitric oxide-dependent enteric neurotransmission. *Neuroscience* **68:**1295–1304.
Shuttleworth CW and Keef KD (1995) Roles of peptides in enteric neuromuscular transmission. *Regul Pept* **56:**101–120.
Simula ME, Brookes SJ, Meedeniya AC, Toouli J, and Saccone GT (2001) Distribution of nitric oxide synthase and vasoactive intestinal polypeptide immunoreactivity in the sphincter of Oddi and duodenum of the possum. *Cell Tissue Res* **304:**31–41.
Singaram C, Sengupta A, Sweet MA, Sugarbaker DJ, and Goyal RK (1994) Nitrergic and peptidergic innervation of the human oesophagus. *Gut* **35:**1690–1696.
Singaram C, Sweet MA, Gaumnitz EA, Cameron AJ, and Camilleri M (1995) Peptidergic and nitrergic denervation in congenital esophageal stenosis. *Gastroenterology* **109:**275–281.
Sivarao DV, Mashimo HL, Thatte HS, and Goyal RK (2001) Lower esophageal sphincter is achalasiac in nNOS(−/−) and hypotensive in W/W(v) mutant mice. *Gastroenterology* **121:**34–42.
Slivka A, Chttani R, Carr-Locke DL, Kobzik L, Bredt DS, Loscalzo J, and Stamler JS (1994) Inhibition of sphincter of Oddi function by the nitric oxide carrier S-nitroso-N-acetylcysteine in rabbits and humans. *J Clin Investig* **94:**1792–1798.
Smith AD and Muir TC (1991) The involvement of nitric oxide in neuronally mediated relaxation in the rabbit distal colon. *Br J Pharmacol* **104:**201P.
Smith TK and McCarron SL (1998) Nitric oxide modulates cholinergic reflex pathways to the longitudinal and circular muscle in the isolated guinea-pig distal colon. *J Physiol* **512:**893–906.
Smith VC, Dhatt N, and Buchan AM (2001) The innervation of the human antropyloric region: organization and composition. *Can J Physiol Pharmacol* **79:**905–918.
Smits GJ and Lefebvre RA (1995) Influence of age on the signal transduction pathway of non-adrenergic non-cholinergic neurotransmitters in the rat gastric fundus. *Br J Pharmacol* **114:**640–647.
Smits GJ and Lefebvre RA (1996a) ATP and nitric oxide: inhibitory NANC neurotransmitters in the longitudinal muscle-myenteric plexus preparation of the rat ileum. *Br J Pharmacol* **118:**695–703.
Smits GJM and Lefebvre RA (1996b) Influence of age on cholinergic and inhibitory nonadrenergic noncholinergic responses in the rat ileum. *Eur J Pharmacol* **303:**79–86.
Smits GJM and Lefebvre RA (1996c) Development of cholinergic and inhibitory non-adrenergic non-cholinergic responses in the rat gastric fundus. *Br J Pharmacol* **118:**1987–1994.
Soediono P and Burnstock G (1994) Contribution of ATP and nitric oxide to NANC inhibitory transmission in rat pyloric sphincter. *Br J Pharmacol* **113:**681–686.
Sotirov E and Papasova M (2000) Nitric oxide modulates release of noradrenaline in guinea-pig gastric fundus. *Brain Res Bull* **51:**401–405.
Sotirov ES, Itzev DE, and Papasova MP (1998) Modulation of electrically evoked responses in rat duodenum by activation of nicotinic cholinoceptors. *Clin Exp Pharmacol Physiol* **25:**331–335.
Spencer SE, Sawyer WB, Wada H, Platt KB, and Loewy AD (1990) CNS projections to the pterygopalatine parasympathetic preganglionic neurons in the rat: a retrograde transneuronal viral cell body labeling study. *Brain Res* **534:**149–169.
Stebbing JF, Brading AF, and Mortensen NJ (1996) Nitric oxide and the rectoanal inhibitory reflex: retrograde neuronal tracing reveals a descending nitrergic rectoanal pathway in a guinea-pig model. *Br J Surg* **83:**493–498.
Stengel PW and Cohen ML (2003) M_1 receptor-mediated nitric oxide-dependent relaxation unmasked in stomach fundus from M_3 receptor knockout mice. *J Pharmacol Exp Ther* **304:**675–682.
Storr M, Folmer R, Kurjak M, Schusdziarra V, and Allescher HD (2002a) Okadaic acid inhibits relaxant neur4al transmission in rat gastric fundus in vitro. *Acta Physiol Scand* **175:**29–36.
Storr M, Koppitz P, Sibaev A, Kurjak M, Franck H, Schusdziam V, and Allescher HD (2002b) Melatonin reduces non-adrenergic, non-cholinergic relaxant neurotransmission by inhibition of nitric oxide synthase activity in the gastrointestinal tract of rodents in vitro. *J Pineal Res* **33:**101–108.
Stuehr DJ, Cho HJ, Kwon NS, Weise MF, and Nathan CF (1991) Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD and FMN-containing flavoprotein. *Proc Natl Acad Sci USA* **88:**7773–7777.
Sugita H, Ueno T, Shimosegawa T, and Yoshimura T (2003) Direct detection of nitric oxide and its roles in maintaining gastric mucosal integrity following ethanol-induced injury in rats. *Free Radic Res* **37:**159–169.
Sun Y, Fihn BM, Jodal M, and Sjovall H (2004) Inhibition of nitric oxide synthesis potentiates the colonic permeability increase triggered by luminal bile acids. *Acta Physiol Scand* **180:**167–175.
Suthamnatpong N, Hosokawa M, Takeuchi T, Hata F, and Takewaki T (1994) Nitric oxide-mediated inhibitory response of rat proximal colon: independence from changes in membrane potential. *Br J Pharmacol* **112:**676–682.
Suzuki H, Takano H, Yamamoto Y, Komuro T, Saito M, Kato K, and Mikoshiba K (2000) Properties of gastric smooth muscle obtained from mice which lack inositol trisphosphate receptor. *J Physiol* **525:**105–111.
Suzuki H, Ward SM, Bayguinov YR, Edwards FR, and Hirst GD (2003) Involvement of intramuscular interstitial cells in nitrergic inhibition in the mouse gastric antrum. *J Physiol* **546:**751–763.
Szilvassy Z, Nagy I, Szilvassy J, Jakab I, Csati S, and Lonovics J (1996) Impaired nitrergic relaxation of the sphincter of Oddi of hyperlipidaemic rabbits. *Eur J Pharmacol* **301:**R17–R18.
Szilvassy Z, Sari R, Nemeth J, Nagy I, Csati S, and Lonovics J (1998) Improvement of nitrergic relaxation by farnesol of the sphincter of Oddi from hypercholesterolaemic rabbits. *Eur J Pharmacol* **353:**75–78.
Tack J, Demedts I, Meulemans A, Schuurkes J, and Janssens J (2002) Role of nitric oxide in the gastric accommodation reflex and in meal induced satiety in humans. *Gut* **51:**219–224.
Takahashi T (2003) Pathophysiological significance of neuronal nitric oxide synthaxes in the gastrointestinal tract. *J Gastroenterol* **38:**421–430.
Takahashi T and Owyang C (1998) Regional differences in the nitrergic innervation between the proximal and the distal colon in rats. *Gastroenterology* **115:**1504–1512.
Takakura K and Muramatsu I (1999) Pharmacological comparison between the nitrergic responses produced by intramural nerve stimulation and exogenous NO-donors in rat gastric fundus. *Jpn J Pharmacol* **80:**155–161.
Takeuchi K, Ohuch T, Kato S, and Okabe S (1993) Cytoprotective action of L-arginine against HCl-induced gastric injury in rats: involvement of nitric oxide. *Jpn J Pharmacol* **61:**13–21.
Takeuchi T, Kishi M, Ishii T, Nishio H, and Hata F (1996) Nitric oxide-mediated relaxation without concomitant changes in cyclic GMP content of rat proximal colon. *Br J Pharmacol* **117:**1204–1208.
Takeuchi T, Niioka S, Kishi M, Ishii T, Nishio H, Hata F, Takewaki T, and Takatsuji K (1998a) Nonadrenergic, noncholinergic relaxation mediated by nitric oxide with concomitant change in Ca^{2+} level in rectal circular muscle of rats. *Eur J Pharmacol* **353:**67–74.
Takeuchi T, Niioka S, Yamaji M, Okishio Y, Ishii T, Nishio H, Takatsuji K, and Hata F (1998b) Decrease in participation of nitric oxide in nonadrenergic noncholinergic relaxation of rat intestine with age. *Jpn J Pharmacol* **78:**293–302.
Tanaka T, Okamura T, Handa J, and Toda N (1999) Neurogenic vasodilation mediated by nitric oxide in porcine cerebral arteries. *J Cardiovasc Pharmacol* **33:**56–64.
Tanobe Y, Okamura T, Fujimura M, and Toda N (1995) Functional role and histo-

logical demonstration of nitric-oxide-mediated inhibitory nerves in dog sphincter of Oddi. *Neurogastroenterol Mot* **7:**219–227.
Tanoue K, Ohta M, Tarnawski AS, Wahlstrom KJ, Sugimachi K, and Sarfeh IJ (1996) Portal hypertension activates the nitric oxide synthase genes in the esophageal mucosa of rats. *Gastroenterology* **110:**549–557.
Tanovic A, Jimenez M, and Fernandez E (2001) Actions of NO donors and endogenous nitrergic transmitter on the longitudinal muscle of rat ileum in vitro: mechanisms involved. *Life Sci* **69:**1143–1154.
Tepperman BL and Whittle BJR (1992) Endogenous nitric oxide and sensory neuropeptides interact in the modulation of the rat gastric microcirculation. *Br J Pharmacol* **105:**171–175.
Thatikunta P, Chakder S, and Rattan S (1993) Nitric oxide synthase inhibitor inhibits catecholamines release caused by hypogastric sympathetic nerve stimulation. *J Pharmacol Exp Ther* **267:**1363–1368.
Thornbury KD, Ward SM, Dalziel HH, Carl A, Westfall DP, and Sanders KM (1991) Nitric oxide and nitrosocysteine mimic nonadrenergic, noncholinergic hyperpolarization in canine proximal colon. *Am J Physiol* **261:**G553–G557.
Thune A, Delbro DS, Nilsson B, Friman S, and Szanvik J (1995) Role of nitric oxide in motility and secretion of the feline hepatobiliary tract. *Scand J Gastroenterol* **30:**715–720.
Timmermans JP, Adriaensen D, and Lefebvre R (1999) Postnatal development of nitrergic neurons in the myenteric plexus of rat stomach. *Histochem Cell Biol* **111:**429–434.
Timmermans JP, Barbiers M, Scheuermann DW, Bogers JJ, Adriaensen D, Fekete E, Mayer B, Van Marck EA, and De Groodt-Lasseel MH (1994) Nitric oxide synthase immunoreactivity in the enteric nervous system of the developing human digestive tract. *Cell Tissue Res* **275:**235–245.
Toda N, Ayajiki K, Tanaka T, and Okamura T (2000a) Preganglionic and postganglionic neurons responsible for cerebral vasodilatation mediated by nitric oxide in anesthetized dogs. *J Cerebral Blood Flow Metab* **20:**700–708.
Toda N, Baba H, and Okamura T (1990a) Role of nitric oxide in non-adrenergic, non-cholinergic nerve-mediated relaxation in dog duodenal longitudinal muscle strips. *Jpn J Pharmacol* **53:**281–284.
Toda N, Baba H, Tanobe Y, and Okamura T (1992) Mechanism of relaxation induced by K^+ and nicotine in dog duodenal longitudinal muscle. *J Pharmacol Exp Ther* **260:**697–701.
Toda N, Inoue S, Okunishi H, and Okamura T (1990b) Intra- and extraluminally-applied acetylcholine on the vascular tone or the response to transmural stimulation in dog isolated mesenteric arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* **341:**30–36.
Toda N and Okamura T (1990) Possible role of nitric oxide in transmitting information from vasodilator nerve to cerebroarterial muscle. *Biochem Biophy Res Commun* **170:**308–313.
Toda N and Okamura T (1992) Regulation by nitroxidergic nerve of arterial tone. *News Physiol Sec* **7:**148–152.
Toda N and Okamura T (2003) The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. *Pharmacol Rev* **55:**271–324.
Toda N, Tanaka T, Ayajiki K, and Okamura T (2000b) Cerebral vasodilatation induced by stimulation of the pterygopalatine ganglion and greater petrosal nerve in anesthetized monkeys. *Neuroscience* **96:**393–398.
Toda N, Tanobe Y, and Baba H (1991) Suppression by N^G-nitro-L-arginine of relaxations induced by non-adrenergic, non-cholinergic nerve stimulation in dog duodenal longitudinal muscle. *Jpn J Pharmacol* **57:**527–534.
Toda N, Uchiyama M, and Okamura T (1995) Prejunctional modulation of nitroxidergic nerve function in canine cerebral arteries. *Brain Res* **700:**213–218.
Todorov S, Pozzoli C, Zamfirova R, and Poli E (2003) Prejunctional modulation of non-adrenergic non-cholinergic (NANC) inhibitory responses in the isolated guinea-pig gastric fundus. *Neurogastroenterol Motil* **15:**299–306.
Toma H, Nakamura K, Emson PC, and Kawabuchi M (1999) Immunohistochemical distribution of c-Kit-positive cells and nitric oxide synthase-positive nerves in the guinea-pig small intestine. *J Auton Nerv Syst* **75:**93–99.
Tomita R, Kurosu Y, and Munakata K (1997) Relationship between nitric oxide and non-adrenergic non-cholinergic inhibitory nerves in human lower esophageal sphincter. *J Gastroenterol* **32:**1–5.
Tomita R, Tanjoh K, Fujisaki S, and Fukuzawa M (1999) The role of nitric oxide (NO) in the human pyloric sphincter. *Hepatogastroenterology* **46:**2999–3003.
Tomita R, Tanjoh K, Fujisaki S, and Fukuzawa M (2003) Physiological studies on nitric oxide in the lower esophageal sphincter of patients with reflux esophagitis. *Hepatogastroenterology* **50:**110–114.
Tonini M, De Giorgio R, De Ponti F, Sternini C, Spelta V, Dionigi P, Barbara G, Stanghellini V, and Corinaldesi R (2000) Role of nitric oxide- and vasoactive intestinal polypeptide-containing neurones in human gastric fundus strip relaxations. *Br J Pharmacol* **129:**12–20.
Tottrup A, Knudsen MA, and Gregersen H (1991a) The role of the L-arginine-nitric oxide pathway in relaxation of the opossum lower oesophageal sphincter. *Br J Pharmacol* **104:**113–116.
Tottrup A, Svane D, and Forman A (1991b) Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. *Am J Physiol* **260:**G385–G389.
Trauner M, Mennone A, Gigliozzi A, Fraioli F, and Boyer JL (1998) Nitric oxide and guanosine 3′,5′-cyclic monophosphate stimulate bile secretion in isolated rat hepatocyte couplets, but not in isolated bile duct units. *Hepatology* **28:**1621–1628.
Tucker JF, Brave SR, Charalambopus L, Hobbs AJ, and Bibson A (1990) L-N^G-nitro arginine inhibits non-adrenergic, non-cholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *Br J Pharmacol* **100:**663–664.
Uc A, Oh ST, Murray JA, Clark E, and Conklin JL (1999) Biphasic relaxation of the opossum lower esophageal sphincter: roles of NO, VIP and CGRP. *Am J Physiol* **277:**G548–G554.
Uemura S, Pompolo S, Furness JB, and Hardy KJ (1997) Nitric oxide synthase in neurons of the human gall-bladder and its colocalization with neuropeptides. *J Gastroenterol Hepatol* **12:**257–265.
Umehara K, Kataoka K, Ogura T, Esumi H, Kashima K, Ibata Y, and Okamura H (1997) Comparative distribution of nitric oxide synthase (NOS) in pancreas of the dog and rat: immunocytochemistry of neuronal type NOS and histochemistry of NADPH-diaphorase. *Brain Res Bull* **42:**469–478.
Van Crombruggen K and Lefebvre RA (2004) Nitrergic-purinergic interactions in rat distal colon motility. *Neurogastroenterol Motil* **16:**81–98.
Vanderwinden JM, De Laet MH, Schiffmann SN, Mailleux P, Lowenstein CJ, and Snyder SH (1993) Nitric oxide synthase distribution in the enteric nervous system of Hirschsprung's disease. *Gastroenterology* **105:**969–973.
Vanderwinden JM, Mailleux P, Schiffmann SN, Vanderhaeghen JJ, and De Laet MH (1992) Nitric oxide synthase activity in infantile hypertrophic pyloric stenosis. *N Engl J Med* **327:**511–515.
Van Geldre LA, Fraeyman NH, Peeters TL, Timmermans JP, and Lefebvre RA (2004) Further characterization of particulate neuronal nitric oxide synthase in rat small intestine. *Auton Neurosci* **110:**8–18.
Van Geldre LA and Lefebvre RA (2004) Nitrergic relaxation in rat gastric fundus: influence of mechanism of induced tone and possible role of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase. *Life Sci* **74:**3259–3274.
Vaquero E, Molero X, Puig-Divi V, and Malagelada JR (1998) Contrasting effects of circulating nitric oxide and nitrergic transmission on exocrine pancreatic secretion in rats. *Gut* **43:**684–691.
Venkova K and Krier J (1994) A nitric oxide- and prostaglandin-dependent component of NANC off-contractions in cat colon. *Am J Physiol* **266:**G40–G47.
Vittoria A, Costagliola A, Carrese E, Mayer B, and Cecio A (2000) Nitric oxide-containing neurons in the bovine gut, with special reference to their relationship with VIP and galanin. *Arch Histol Cytol* **63:**357–368.
Waldman SA and Murad F (1987) Cyclic GMP synthesis and function. *Pharmacol Rev* **39:**163–196.
Wang J, Zheng H, and Berthoud HR (1999) Functional vagal input to chemically identified neurons in pancreatic ganglia as revealed by Fos expression. *Am J Physiol* **277:**E958–E964.
Wang XY, Paterson C, and Huizinga JD (2003) Cholinergic and nitrergic innervation of ICC-DMP and ICC-IM in the human small intestine. *Neurogastroenterol Motil* **15:**531–543.
Wang XY, Wong WC, and Ling EA (1996) Localization of choline acetyltransferase and NADPH diaphorase activities in the submucous ganglia of the guinea-pig colon. *Brain Res* **712:**107–116.
Ward SM, McKeen ES, and Sanders KM (1992) Role of nitric oxide in non-adrenergic, non-cholinergic inhibitory junction potentials in canine ileocolonic sphincter. *Br J Pharmacol* **105:**776–782.
Ward SM, Morris G, Reese L, Wang XY, and Sanders KM (1998) Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterology* **115:**314–329.
Ward SM and Sanders KM (2001) Physiology and pathophysiology of the interstitial cell of Cajal: from bench to bedside. I. Functional development and plasticity of interstitial cells of Cajal networks. *Am J Physiol* **281:**G602–G611.
Watanabe Y, Ando H, Seo T, Katsuno S, Marui Y, Ono Y, and Torihashi S (2002) Attenuated nitrergic inhibitory neurotransmission to interstitial cells of Cajal in the lower esophageal sphincter with esophageal achalasia in children. *Pediatr Int* **44:**145–148.
Watkins CC, Sawa A, Jaffrey S, Blackshaw S, Barrow RK, Snyder SH, and Ferris CD (2000) Insulin restores neuronal nitric oxide synthase expression and function that is lost in diabetic gastropathy. *J Clin Investig* **106:**373–384.
Wells DG, Talmage EK, and Mawe GM (1995) Immunohistochemical identification of neurons in ganglia of the guinea pig sphincter of Oddi. *J Comp Neurol* **352:**106–116.
West SD, Helmer KS, Chang LK, Cui Y, Greeley GH, and Mercer DW (2002) Cholecystokinin secretagogue-induced gastroprotection: role of nitric oxide and blood flow. *Am J Physiol* **284:**G399–G410.
Wester T, O'Briain S, and Puri P (1998a) NADPH diaphorase-containing nerve fibers and neurons in the myenteric plexus are resistant to postmortem changes: studies in Hirschsprung's disease and normal autopsy material. *Arch Pathol Lab Med* **122:**461–466.
Wester T, O'Briain S, and Puri P (1998b) Morphometric aspect of the submucous plexus in whole-mount preparations of normal distal colon. *J Pediatr Surg* **33:**619–622.
Weusten BL and Smout AJ (1999) Primary oesophageal motility disorders: how primary are they? *Eur J Gastroenterol Hepatol* **11:**1345–1347.
Wiklund CU, Olgart C, Wiklund NP, and Gustafsson LE (1993) Modulation of cholinergic and substance P-like neurotransmission by nitric oxide in the guinea-pig ileum. *Br J Pharmacol* **110:**833–839.
Wiklund NP, Cellek S, Leone AM, Iversen HH, Gustafsson LE, Brundin I, Furst VW, Flock A, and Moncada S (1997) Visualisation of nitric oxide released by nerve stimulation. *J Neurosci Res* **47:**224–232.
Wiklund NP, Iversen HH, Leone AM, Gustafsson LE, Furst V, Cellek S, Flock A, and Moncada S (1996) Real-time imaging of nerve-induced nitric oxide release, in *The Biology of Nitric Oxide* (Moncada S, Stamler J, Gross S, and Higgs EA eds) part 5, p 228, Portland Press Ltd, London.
Wilhelm M, Batori Z, Pasztor I, and Gabriel R (1998) NADPH-diaphorase positive myenteric neurons in the ileum of guinea-pig, rat, rabbit and cat: a comparative study. *Eur J Morphol* **36:**143–152.
Williams SJ and Parsons ME (1995) Nitric oxide, an enteric nonadrenergic-noncholinergic relaxant transmitter: evidence using phosphodiesterase V and nitric oxide synthase inhibition. *Br J Pharmacol* **116:**1789–1796.
Wu M, Majewski M, Wojtkiewicz J, Vanderwinden JM, Adriaensen D, and Timmermans JP (2003) Anatomical and neurochemical features of the extrinsic and intrinsic innervation of the striated muscle in the porcine esophagus: evidence for regional and species differences. *Cell Tissue Res* **311:**289–297.
Xue S, Valdez D, Collman PI, and Diamant NE (1996) Effects of nitric oxide synthase blockade on esophageal peristalsis and the lower esophageal sphincter in the cat. *Can J Physiol Pharmacol* **74:**1249–1257.

Yamaji M, Ohta M, Yamazaki Y, Fujinami K, Fujita A, Takeuchi T, Hata F, and Takewaki T (2002) A possible role of neurotensin in NANC relaxation of longitudinal muscle of the jejunum and ileum of Wistar rats. *Br J Pharmacol* **137:**629–636.

Yoneda S and Suzuki H (2001) Nitric oxide inhibits smooth muscle responses evoked by cholinergic nerve stimulation in the guinea pig gastric fundus. *Jpn J Physiol* **51:**693–702.

Yuan PQ, Tache Y, Miampamba M, and Yang H (2001) Acute cold exposure induces vagally mediated Fos expression in gastric myenteric neurons in conscious rats. *Am J Physiol* **281:**G560–G568.

Yuan Z, Iida H, Inoue N, Ishikawa Y, and Ishida H (2003) Effect of SNI-2011 on amylase secretion from parotid tissue in rats and in neuronal nitric oxide synthase knockout mice. *Eur J Pharmacol* **464:**197–206.

Zagorodnyuk V and Maggi CA (1994) Electrophysiological evidence for different release mechanism of ATP and NO as inhibitory NANC transmitters in guinea-pig colon. *Br J Pharmacol* **112:**1077–1082.

Zhang X, Tack J, Janssens J, and Sifrim DA (2001) Effect of sildenafil, a phosphodiesterase-5 inhibitor, on oesophageal peristalsis and lower oesophageal sphincter function in cats. *Neurogastroenterol Motil* **13:**325–331.

Zhang X, Tack J, Janssens J, and Sifrim DA (2004) Neural regulation of tone in the oesophageal body: in vivo barostat assessment of volume-pressure relationship in the feline oesophagus. *Neurogastroenterol Motil* **16:**13–21.

Zizzo MG, Mule F, and Serio R (2004) Interplay between PACAP and NO in mouse ileum. *Neuropharmacology* **46:**449–455.

Zyromski NJ, Duenes JA, Kendrick ML, Balsiger BM, Farrugia G, and Sarr MG (2001) Mechanism mediating nitric oxide-induced inhibition in human jejunal longitudinal smooth muscle. *Surgery* **130:**489–496.