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Nitric Oxide and the Gastrointestinal Tract

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Abstract: Nitric oxide is one of the smallest molecules in nature. Many mammalian cells can synthesize nitric oxide. It modulates immune function, blood vessel dilatation and neurotransmission. It is synthesized from arginine in a two-step enzyme reaction by Nitric Oxide Synthase (NOS) via the formation of the intermediate N-hydroxy-L-arginine. Over the last years numerous studies have been carried out and the important role of Nitric Oxide (NO) as endogenous modulator of numerous physiological functions has been shown. Still some areas are ill defined and lacking studies related to the exact role of such intriguing molecule. Gastrointestinal tract is one of the areas where the role of nitric oxide is scarcely studied and results are controversial. In the gastrointestinal tract (GIT) NO participates in the modulation of the smooth musculature tone, such as the regulation of intestinal peristalsis, gastric emptying and antral motor activity. It also regulates acid and gastric mucus secretion, alkaline production and is involved in the maintenance of mucosal blood flow. In physiological conditions, NO acts as an endogenous mediator modulating both, the repairing and integrity of the tissues and demonstrate gastroprotective properties against different types of aggressive agents. However, high concentrations of NO are related to numerous pathological processes of GIT. This review article brief out the findings of the studies demonstrating role of NO in various physiological and pathological conditions of gastrointestinal tract.

Key words: Nitric oxide, gastrointestinal tract, arginine, nitric oxide synthase, GI diseases

INTRODUCTION

Nitric oxide is generated in tissues from arginine by Nitric Oxide Synthase (NOS). Nitric oxide synthase enzyme exists in three isoforms encoded by distinct genes. Neuronal (nNOS or Type 1) and endothelial (eNOS or Type 3) are constitutive, calcium dependent and present in the neural tissue and in the vascular endothelium, respectively. Inducible NOS (iNOS or Type 2) is Ca^{++}-independent and is induced by bacterial endotoxins and cytokines in macrophages, endothelium, smooth muscle, liver, fibroblast and neutrophils. Constitutive NOS (cNOS) is responsible for production of NO in physiological context. In contrast, inducible NOS (iNOS) produce NO in pathophysiological circumstances. NO is implicated in mechanisms maintaining the integrity of the gastric epithelium. Activity of nNOS and eNOS produces low levels of NO for a short period of time. iNOS when induced provides a continuous supply of high levels of NO. Nitric oxide is uncharged and it diffuses freely across cell membranes. In biological systems its half-life is less than 30 sec. NO is less reactive than many free radicals and it cannot react with itself. NO mediates its effects as a physiological messenger via production of cGMP by activating guanylate cyclase. Interactions of NO with thiol groups may also provide a mechanism whereby NO can be transported to the target cell. Nitrosylation of thiol proteins may also be involved in remodeling of axon terminals. Under conditions of oxidative stress, e.g., when high levels of NO are synthesized by iNOS and intracellular levels of superoxides are high, the intracellular thiol pool is depleted. NO can react with superoxide (O_2^-) to produce peroxynitrite ($ONOO^-$) and subsequently the hydroxyl radical, which are more toxic than NO itself (Bult *et al*., 1990; Moncada and Higgs, 1993, 1995).

Neuronal NOS (nNOS) is a predominant isoform of NOS in the enteric nervous system besides the other constitutive and calcium dependent endothelial NOS and the inducible calcium independent iNOS. Nitric oxide. The molecule of the millennium, is an important non-adrenergic non-cholinergic double-edged neurotransmitter having protective as well as cytotoxic effect. The protective effect relates with its retrograde influence over the release of stimulating neurotransmitter and thereby regulates the neural transduction. The derogatory effect of NO relates with its ability to generate cytotoxic free radicals (Bult *et al*., 1990; Meulemans and

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Schuurkes, 1993; Moncada and Higgs, 1993; Sugita *et al*., 2003). The fundamental question regarding the exact roles of NO in various biological events has not been well defined. This is especially true in the gastrointestinal (GI) tract. It was reported to protect against GI mucosal damage and promote ulcer healing; on the other hand, NO promotes or even initiates inflammatory responses when combined with other reactive oxygen species throughout the GI tract. This article reviews the differential actions of NO on various biological disciplines concerning the defensive and the detrimental effects of NO on the gastrointestinal tract.

The digestive system is one of the major sources of nitric oxide. Gastrointestinal functions are regulated by autonomic (extrinsic) and enteric (intrinsic) nerves and local hormones. Nitric oxide plays a critical role in several of major physiologic processes of gastrointestinal tract like motility, secretion, digestion, absorption and elimination (Stark and Szurszewski, 1992). In addition nitric oxide takes part in the control of pancreatic secretion and liver functions.

SOURCES OF NITRIC OXIDE IN GASTROINTESTINAL TRACT

Nitric oxide is produced in the gastrointestinal tract either by enzymatic, non-enzymatic or by bacterial production mechanisms. The constitutively expressed and inducible isoform are responsible for the enzymatic production of NO. There are several NOS-independent mechanisms of NO formation. For example, xanthine oxidoreductase is an enzyme that under hypoxic conditions can produce NO by reduction of nitrate (NO_3^-) and nitrite (NO_2^-). Nitric oxide can also be formed from dietary nitrate which in the oral cavity is reduced by bacterial reductases to nitrite (Duncan *et al*., 1995) yielding NO gas after acidification in the gastric lumen (McKnight *et al*., 1997). Nitric oxide production from the reaction of hydrogen peroxide with arginine is another example of non-enzymatic NO production (Nagase *et al*., 1997). Anaerobic bacteria in the colon produces NO using nitrite and nitrate as substrates (Brittain *et al*., 1992; Goretski *et al*., 1990).

NITRIC OXIDE IN GUT MOTILITY

Motility of the GI tract is directly controlled by enteric inhibitory and excitatory motor neurons that innervate the smooth muscle layers. Distension of the gut by a food bolus is detected by local enteric afferent neurons. About 50% of the nerves in the enteric nervous system contain nNOS. These nerves are located in the myenteric plexus and muscle fibers. Bult and colleagues were the first to demonstrate that NO is the most important non-adrenergic, non-cholinergic inhibitory neurotransmitter in the gut (Bult *et al*., 1990). Inhibitory motor neurons mediate receptive and accommodative relaxations and control the opening of sphincters. It has been shown that nNOS deficient mice increased lower oesophageal sphincter relaxations and gastroparesis (Mashimo and Goyal, 1999). Diabetic patients often exhibit GI dysfunctions which continue even after antidiabetic drug therapy. It has been shown that L-arginine, a nitric oxide substrate, inhibited haemoglobin glycation and oxidative stress generation in gastrointestinal tissues in chronic diabetes (Kochar and Umathe, 2009). Non-cholinergic nonadrenergic neural mechanisms involving nerves containing NO have been shown to modulate smooth muscle in the gastrointestinal tract and suggested that release from tonic NO inhibition may be important in the regulation of cyclical fasting small intestinal motility. Russo et al have shown that NO mechanisms play a role in the regulation of fasting small intestinal motor activity in humans (Russo *et al*., 1999).

NITRIC OXIDE IN SECRETION AND ABSORPTION

In the gut lumen, NO has a half-life of less than 6 sec and is rapidly converted into nitrite and nitrate in the presence of oxygen and water. It is highly diffusible in water, lipids and air and it freely traverses cell membranes and passes into adjacent target cells. NO is involved in the intestinal water transport by acting directly on the epithelium and blood flow or indirectly by stimulating neuronal reflexes and releases of, or interactions, with other agents. For example, NO activates soluble guanylate cyclase and this result in cGMP generation, a potent activator of intestinal secretion (Brasitus *et al*., 1976). Nitric oxide 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*., 1992). Dibutyryl cyclic GMP and the cyclic GMP phosphodiesterase inhibitor M and B 22948 also increased the mucus release. These findings, together with the presence of NOS in the gastric mucus cells (Brown *et al*., 1993), 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). Tsuchiya *et al*. (2002) have shown that centrally injected NO donors stimulate gastric acid secretion in both conscious and anesthetized rats through vagus activation. On the other hand, Berg *et al*. (2004) have demonstrated that NO inhibits gastric acid secretion in isolated human gastric glands and that there is endogenous formation of NO within the glandular epithelium in the vicinity of the parietal cells. NO can also induce vasoactive intestinal polypeptide-an important neurotransmitter, in secretomotor neurons (Allescher *et al*., 1996). Furthermore, NO causes an increase of prostaglandin E_2 production, a known secretory molecule (Wilson *et al*., 1996). Apart from indirect effects on secretory molecules, NO may also exert direct secretory effects by opening of chloride channels (Tamai and Gaginella, 1993). It is one of the mediators of the intestinal secretion and laxative-induced diarrhea induced by castor oil (Mascolo *et al*., 1993), magnesium sulfate (Izzo *et al*., 1994) and anthraquinone containing laxatives such as senna and cascara (Izzo *et al*., 1997), as well as the diphenylmethanes: phenolphthalein and bisacodyl (Gaginella *et al*., 1994). Bile acid infusion in the left colon induces NO generation suggesting that NO is also involved in bile salt induced diarrhea (Casellas *et al*., 1996). Patients with collagenous or lymphocytic colitis produce watery diarrhea in the absence of epithelial cell damage. High levels of NO gas in the gut lumen of these patients suggest a role of NO in inflammation-induced diarrhea (Lundberg *et al*., 1997). Topical administration of the NOS inhibitor N-monomethyl-L-arginine reduced fluid secretion in patients with collagenous colitis (Perner *et al*., 2001). In contrast NO can also reduce fluid secretion (Qiu *et al*., 1996) and cholera toxin-induced diarrhoea (Beubler and Schirgi-Degen, 1997). Early studies with NOS inhibitors showed that NO promote absorption under basal conditions. Interestingly, the secretory effect of the NOS inhibitor L-NAME could be reversed by loperamide a known antidiarrhoeal opiod. The mechanisms of the proabsorptive actions of NO are not fully understood but may involve the opening of basolaterally located potassium channels in enterocytes (Izzo *et al*., 1998). In summary it seems that NO can act both as a secretagogue and an absorbagogue depending on the concentrations, local circumstances and on the site of delivery.

NO IN INTESTINAL INFLAMMATION, CARCINOGENESIS AND APOPTOTIC PROCESSES

Nitric oxide is important in maintaining mucosal integrity of GI tract by several mechanisms. Many researchers have shown that NO synthesized via cNOS plays a pivotal role in protecting the GI mucosa from a variety of noxious stimuli through maintenance of mucosal perfusion (Elliott and Wallace, 1998; Salzman 1995). Endogenous NO responsible for the regulation of the vascular tone is derived from nitrergic nerves and vascular endothelial cells. The NO synthase inhibitors decreased gastrointestinal mucosal blood flow and increased vascular resistance, despite an increase in systemic blood pressure in anesthetized rats (Pawlik *et al*., 1995), cats (Macedo and Lautt, 1997) and in awake rats (Greenblatt *et al*., 1993). 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 (DeMeyer *et al*., 2003), also prevented the ischemia/reperfusion injury of the rat small intestine (Ozturk *et al*., 2003). A continuous supply of blood to the gastrointestinal mucosa is vital during periods of injury. Nitric oxide has been suggested to have cytoprotective effects, mainly via the regulation of mucosal blood flow, in endotoxin and ethanol-induced intestinal injury (Baraona *et al*., 2002; Sugita *et al*., 2003) and the gastroprotective effects of somatostatin (Ancha *et al*., 2003), adrenomedullin (Salomone *et al*., 2003), thyrotropin-releasing hormone analog (Kiraly *et al*., 1993) and cholecytokinin (West *et al*., 2002) are partly mediated by the endogenous release of NO. Nitric oxide from cNOS plays a critical role in modulating the defensive mechanisms in the GI tract which was reported to be largely due to its anti-inflammatory action and improvement of the integrity of the mucosa. Nitric oxide has been shown to inhibit the adhesion molecule on neutrophils and the expression of P-selectin on the vascular endothelium (Bamick *et al*., 1997; Davenpeck *et al*., 1994). This would greatly improve the inflammatory response in tissues. NO was also reported to down regulate the release of some inflammatory mediators from mast cells (Hogaboam *et al*., 1993; Mashini *et al*., 1991; Salvemini *et al*., 1990). Likewise, NO could also modulate the actions of macrophage-derived

cytokines on mucosal cells, which improved the side effects on the GI tract (Fiorucci *et al*., 1999). Endogenous NO on the healing process of gastric ulcers has been investigated extensively by the use of L-arginine, a substrate for NOS and N_G-monomethyl-L-arginine, an inhibitor of NOS (Brzozowski *et al*., 1997; Konturek *et al*., 1993). Cigarette smoke exposure delayed ulcer healing and decreased gastric blood flow and angiogenesis at the ulcer margin. These changes were accompanied by a significant reduction of cNOS activity but not prostaglandin E_2 and vascular endothelial growth factor levels (Ma *et al*., 1999). In contrast, heparin, an anti-coagulant, was reported to increase mucosal regeneration, proliferation and angiogenesis, which are likely to be stimulated by growth factors as well as cNOS activity (Li *et al*., 1999, 2000). NO also implicates in mechanisms maintaining the integrity of the gastric epithelium by regulating mucosal blood flow (Whittle *et al*., 1990, 1994). NO can directly affect mucus secretion by activating soluble guanylate cyclase and raising intracellular cyclic GMP. Indeed NO donors stimulate mucus secretion by intact rat stomach and isolated mucosal cells (Brown *et al*., 1993). Endogenous NO also contributes to the inhibition of gastric acid secretion (Esplugues *et al*., 1994). It was noted that prior treatment with inhibitors of NOS abolished the reactive response to topical mild irritants and greatly increased the susceptibility of stomach damage (Lippe and Holzer, 1992).

These findings spurred the development of adding NO donating groups to known ulcerogenic non-steroidal anti-inflammatory drugs. Delayed ulcer healing was also found in iNOS knockout mice with acetic-acid induced colitis (McCafferty *et al*., 1997). Studies with cutaneous wounds demonstrated that NO enhances collagen production by fibroblasts (Schaffer *et al*., 1997). Angiogenesis is important in both wound repair and carcinogenesis. Nitric oxide derived from eNOS expressed in mammary tumor cells promoted tumor growth and metastasis by stimulation of tumor cell migration, invasiveness and angiogenesis (Jadeski *et al*., 2000). However, in another study with colon carcinoma cells the presence of NOS inversely correlated with their metastatic potential (Radomski *et al*., 1991). Selective inhibition of iNOS showed a reduced development of aberrant crypt foci indicating that specific iNOS inhibition could be a chemopreventive strategy for colon cancer (Rao *et al*., 1999). Early studies concerning the role of NO in carcinogenesis were focused on its potential to nitrosate (addition of NO^+) amines, including those in DNA, forming nitrosamines. Nitrosamines can lead to direct mutations or to the generation of carcinogens (Moncada and Higgs, 1995). NO may also potentiate DNA damage by inhibition of DNA repair mechanisms (Jaiswal *et al*., 2001).

Thus, NO functions as double edged sword which depending on its local concentration, either protects cells against apoptosis, or on the contrary, induces apoptosis. Low concentrations protect B lymphocytes against viral infections, whereas high concentrations induce apoptosis in macrophages, hepatocytes, glial cells, neurons and other cells of the immune system. NO promotes apoptosis in macrophages, CD4+/CD8+ thymocytes, condriocytes and pancreatic B cells (Albina *et al*., 1997; Clancy *et al*., 1997). Caspases can be activated by the release of cytochrome C and possibly by other factors diffusing from the mitochondrial intermembrane space into the cytoplasm (Kluck *et al*., 1997; Kroemer *et al*., 1997). Some antiapoptotic proteins like Bcl-2 and Bcl-xl hinder this release, whereas it can be activated by proapoptotic Bax proteins (Jurgensmeier *et al*., 1998). NO plays a dual role in the regulation of the process. On one hand, it allows the formation of ONNO by reacting with superoxide anions and it triggers the increase in the membrane permeability and also in the calcium concentration, stimulating apoptosis by means of these mechanisms (Hortelano *et al*., 1997; Packer *et al*., 1997). On the other hand, NO activates Bcl-2/Bcl-xl resulting in the inhibition of the Bax/Bak pathway and thereby blocking the caspases cascade (Hortelano *et al*., 1997; Jurgensmeier *et al*., 1998; Levine, 1997). Cytochrome C intervenes in the cellular destruction, but it also promotes the release of some anti-apoptotic substances which block the caspases (Grossmann *et al*., 1998). Another mechanism by which NO prevents the apoptotic process is by modulating transcriptional factors AP-1, NF-κB and extra cellular signals similar to TNF (Clem *et al*., 1998; Hsu *et al*., 1997). Thus, NO may act as a bifunctional regulator of apoptosis with inhibition of apoptosis in case of oxidative stress as present in mucosal injury and induction of apoptosis in carcinogenesis.

NO IN GI DISEASES

Impaired NO release is observed in diseases with non-relaxing sphincters or bowel segments like achalasia (Mearin *et al*., 1993), infantile hypertrophic pyloric stenosis (Vanderwinden *et al*., 1992) and Hirschprung's disease (Larsson *et al*., 1995). nNOS gene therapy may

perhaps in the future become a new treatment options. Topical NO donors have already been used in patients undergoing endoscopic retrograde cholangiopancreaticography to relax the sphincter of Oddi and inhibit duodenal motility (Slivka *et al*., 1994). Isosorbide dinitrate (ISDN) ointment is locally applied to relax the anal sphincter in order to heal anal fissures (Schouten *et al*., 1996). Furthermore, ISDN tablets are used to treat oesophageal spasms (Parker and MacKinnon, 1981). Transient LES relaxation is important in gastroesophageal reflux disease. Intravenous infusion of the NOS inhibitor N-monomethyl-L-arginine in healthy volunteers caused a decrease in the gastric distension-triggered TLESR's and an increase in oesophageal peristaltic amplitude and velocity. This indicates that inhibition of NO might be of benefit for patients with gastroesophageal reflux disease (Hirsch *et al*., 1998). The contractile activity of the gut has an intriguing pattern, which is known as the migrating motor complex. Several studies have shown that inhibitors of NOS initiate premature phase III contractions whereas NO donors disrupt the migrating motor complex (Russo *et al*., 1999; Stark and Szurszewski, 1992; Wink *et al*., 1997). Therefore, selective inhibition of nitric oxide synthase could be a treatment option in patients with bacterial overgrowth due to an impaired phase III activity. Also a toxic megacolon in patients with ulcerative colitis is probably to a certain extent caused by overproduction of NO by iNOS in the colonic smooth muscles. Selective iNOS inhibition could be a treatment strategy in this life threatening condition (Mourelle *et al*., 1995). It has been shown that gastrointestinal dysfunction in diabetic rats relates with a decline in tissue L-arginine content and consequent low levels of nitric oxide. Daily supplementation of L-arginine (100 mg kg^{-1}, p.o.) for eight weeks to diabetic and NOS inhibitor treated non-diabetic group was found to restore the gastric emptying and intestinal transit and improved the levels of NO in GI tissues. The findings indicate that diabetes-induced L-arginine deficiency and consequent low levels of NO in GI tissues could be possible cause for the GI dysfunction and L-arginine supplementation can prevent the same (Umathe *et al*., 2009). Several motility disorders like chronic intestinal pseudo-obstruction and even constipation might relate to the enteric NO system, thereby suggesting new pharmacological treatment options.

It is clear that in gastrointestinal tract nanomolar amounts of NO produced by calcium dependent nNOS and eNOS has a physiological role while absence of NO results in an increased susceptibility of the GI tract to injury. The lack of GI side effects, together with the potential for an increase therapeutic benefit, due to its additional pharmacological activities, thereby significantly improving the risk/benefit ratio, open the possibility for a wide range of application for those promising compounds, that should be explored in depth. Thus, findings related to the nitrergic innervations may provide us a new way of understanding GI tract physiology and pathophysiology.

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