Inducible nitric oxide synthase in intestinal inflammation

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Targeting Nitric Oxide in the Gastrointestinal Tract

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INTRODUCTION

The overall function of the gastrointestinal (GI) tract is to take up nutrients and remove waste. The major physiologic processes that occur in the GI tract are motility, secretion, digestion, absorption and elimination. Nitric oxide (NO) plays a critical role in several of these physiological processes (figure 1). In this review some important aspects of nitric oxide (NO) in the pathophysiology of diseases of the GI tract (table 1) are discussed.

Figure 1. Diagram of the effects of NO in the gastrointestinal tract.

SOURCES OF NITRIC OXIDE IN THE GASTROINTESTINAL TRACT

In the human gut there is enzymatic, non-enzymatic and bacterial production of NO. Two constitutively expressed calcium dependent isoforms of nitric oxide synthase (NOS) are responsible for the enzymatic production of small amounts (nanomolar) of NO: neuronal NOS (nNOS, type I), and endothelial NOS (eNOS, type III). These isoforms are primarily regulated by intracellular calcium, via Ca2+ activated calmodulin. The third, inducible isoform (iNOS, type II) is not regulated by calcium because
activated calmodulin is inserted at the time of synthesis. This isoform produces large amounts of NO (micromolar) for a limited period of time (figure 2). The induction of iNOS usually occurs in states of inflammation and immune activation. In the gut, inflammatory ¹, epithelial ¹, endothelial ² and neuronal cells ³ can express iNOS ⁴. 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 (NO₃⁻) and nitrite (NO₂⁻). NO can also be formed from dietary nitrate which in the oral cavity is reduced by bacterial reductases to nitrite ⁵. This nitrite can be acidified in the gastric lumen yielding NO gas ⁶,⁷. NO

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Table 1. Involvement of nitric oxide (NO) in the pathophysiology of diseases of the GI tract.

<table>
<thead>
<tr>
<th>Action</th>
<th>Main function</th>
<th>Involved Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-adrenergic non-cholinergic (NANC) neurotransmitter</td>
<td>peristalsis, relaxation of smooth muscles</td>
<td>oesophageal spasms, gastroparesis, chronic intestinal pseudo-obstruction, bacterial overgrowth, ileus, toxic megacolon.</td>
</tr>
<tr>
<td>smooth muscle relaxant</td>
<td>sphincter relaxation</td>
<td>gastro-oesophageal reflux disease, achalasia, infantile hypertrophic pyloric stenosis, sphincter of Oddi dysfunction, Hirschsprung’s disease, healing of anal fissure</td>
</tr>
<tr>
<td>inhibition of platelet aggregation and leukocyte adhesion, mast cell stabilisation, increasing mucus production, inhibition of Th1 cytokine production</td>
<td>mucosal protection against injury and inflammation</td>
<td>NSAID gastropathy, celiac disease, inflammatory bowel disease, diverticulitis</td>
</tr>
<tr>
<td>oxygen radical; reaction with superoxide, iron and thiol groups</td>
<td>bactericidal</td>
<td>infectious diarrhoea, Helicobacter pylori infection, increased permeability in sepsis, actions of laxatives, bile acid induced diarrhoea, microscopic colitis</td>
</tr>
<tr>
<td></td>
<td>induction or inhibition of chloride secretion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>induction or inhibition of apoptosis carcinogen (nitrosamines)</td>
<td>intestinal metaplasia, colonic polyp and tumour development, carcinogenesis in the GI tract</td>
</tr>
</tbody>
</table>

Table 1. Involvement of nitric oxide (NO) in the pathophysiology of diseases of the GI tract.
production from the reaction of hydrogen peroxide with arginine is another example of non-enzymatic NO production. Finally anaerobic bacteria in the colon produce NO, using nitrite and nitrate as substrates.

**NITRIC OXIDE IN INTESTINAL 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 occurs by a food bolus. This distension is detected by local enteric afferent neurons. These neurons project to interneurons, which send their stimulus either upstream (orally) or downstream (aborally). Upstream interneurons are stimulatory and induce contractions via the neurotransmitters acetylcholine and substance P. Downstream interneurons are inhibitory and act via the neurotransmitters somatostatin, γ-aminobutyric acid (GABA). These inhibitory interneurons regulate other interneurons that use endogenous opiates, vasoactive intestinal peptide (VIP) and NO to inhibit muscular contraction. 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 (NANC) inhibitory neurotransmitter in the gut. Inhibitory motor neurons mediate receptive and accommodative relaxations and control the opening of sphincters. Indeed nNOS-/- mice showed increased lower oesophageal sphincter (LES) relaxations and gastroparesis. Impaired NO release is observed in diseases with non-relaxing sphincters or bowel segments like achalasia, infantile hypertrophic pyloric stenosis and Hirschprung's disease. 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 cholangiopancreatography (ERCP) to relax the sphincter of Oddi and inhibit duodenal motility 16. Isosorbide dinitrate (ISDN) ointment is locally applied to relax the anal sphincter in order to heal anal fissures 17. Furthermore, ISDN tablets are used to treat oesophageal spasms 18. Transient LES relaxation (TLESR) are important in gastroesophageal reflux disease (GERD). Intravenous infusion of the NOS inhibitor N-monomethyl-Arginine (L-NMMA) 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 GERD 19.

The contractile activity of the gut has an intriguing pattern, which is known as the migrating motor complex (MMC). This MMC has three phases of activity with minimal contractile activity in the fast state (phase I), mixing contractility in the fed state (phase II) and high amplitude peristaltic contractions (phase III). Several studies have shown that inhibitors of NOS initiating premature phase III contractions whereas NO donors disrupt the MMC 20-22. 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 highly induced iNOS in the colonic smooth muscles. Selective iNOS inhibition could be a treatment strategy in this life threatening condition 23. Several motility disorders like chronic intestinal pseudo-obstruction and even constipation might relate to the enteric NO system, thereby suggesting new pharmacological treatment options.

WITRIC OXIDE IN INTESTINAL SECRETION AND ABSORPTION

In the gut lumen, NO has a half life of less than 6 seconds, 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 bloodflow or indirectly by stimulating neuronal reflexes, and releases of, or interactions, with other agents. For example, NO activates soluble guanylate cyclase and this results in cGMP generation, a potent activator of intestinal secretion 24. NO can also induce vasoactive intestinal polypeptide (VIP) an important neurotransmitter in secretomotor neurons 25. Furthermore, NO causes an increase of prostaglandin (PG) E2 production, a known secretory molecule 26. Apart from indirect effects on secretory molecules, NO may also exert direct secretory effects by opening of chloride channels 27. NO is one of the mediators of the intestinal secretion and laxative induced diarrhoea induced by castor oil 28, magnesium sulfate 29, and anthraquinone containing laxatives such as senna and cascara 30, as well as the
diphenylmethanes: phenolphthalein and bisacodyl. Bile acid infusion in the left colon induces NO generation suggesting that NO is also involved in bile salt induced diarrhoea. Patients with collagenous or lymphocytic colitis produce watery diarrhoea 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 diarrhoea. Topical administration of the NOS inhibitor N\(^6\)-monomethyl-L-arginine (L-NMMA) reduced fluid secretion in patients with collagenous colitis. In contrast NO can also reduce fluid secretion as demonstrated in Clostridium difficile and cholera toxin induced diarrhoea. Early studies with NOS inhibitors already showed that NO promotes absorption under basal conditions (for review see). Interestingly the secretory effect of the NOS inhibitor N\(^6\)-nitro-L-arginine (L-NAME) could be reversed by loperamide a known antidiarrhoeal opioid. The mechanisms of the proabsorptive actions of NO are not fully understood but may involve the opening of basolaterally located potassium channels in enterocytes. 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.

**NITRIC OXIDE IN INTESTINAL INJURY, REPAIR, CARCINOGENESIS AND APOPTOSIS**

The GI tract is remarkably protected against damage by ingested irritants (food components, alcohol, drugs); endogenous secretions (acid, bile, proteolytic enzymes) and potentially harmful actions of enteric microbes. The mucosal barrier is composed of a rapidly replaced monolayer of intestinal epithelial cells (IEC) and non-specific agents such as lysozyme, acid and mucus. NO is important in maintaining mucosal integrity by several mechanisms. A continuous supply of blood to the gastrointestinal mucosa is vital during periods of injury. Especially eNOS derived NO plays an important role in the calcitonin gene-related peptide (CGRP) mediated, reactive hyperaemic response to mucosal injury. Studies with irritants like ethanol, hydrochloric acid or the vasoconstrictive agent endothelin showed reduced gastric mucosal damage when NO donors were administered while gastric mucosal damage was increased when NO scavengers or NOS inhibitors were given. These findings spurred the development of adding NO donating groups (nitroxybutyl) to known ulcerogenic non steroidal anti-inflammatory drugs (NSAIDs). Indeed, NO -NSAIDs did not cause the gastric mucosal injury normally associated with NSAIDs and could even protect against gastric mucosal injury associated with endotoxin or hemorrhagic shock. Apart from increasing mucosal blood flow, the protective actions of NO may also involve reduction of leucocyte adherence, inhibition of mast cell activation, inhibition of Th1 type cytokines by inactivation of cytokine processing, and stimulation of gastric mucus secretion. In addition to its protective actions in response to injury, NO is also a modulator of mucosal repair. Konturek et al
demonstrated retarded gastric ulcer healing by NOS inhibitors and accelerated healing by the NO donor glyceryl trinitrate. Delayed ulcer healing was also found in iNOS knockout mice with acetic-acid induced colitis. Studies with cutaneous wounds demonstrated that NOS enhances collagen production by fibroblasts. Transfection with the gene for iNOS into the wound of the skin had beneficial effects. Angiogenesis is important in both wound repair and carcinogenesis. NO derived from eNOS expressed in mammary tumor cells promoted tumor growth and metastasis by stimulation of tumor cell migration, invasiveness and angiogenesis. However, in another study with colon carcinoma cells the presence of eNOS inversely correlated with their metastatic potential. In this study the absence of NO production in metastatic cells induced platelets aggregation that increased the adhesion of metastatic cells to the vascular endothelium and promoted their successful implantation. Precancerous lesions such as gastric intestinal metaplasia and colonic polyps express iNOS. 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. 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. NO may also potentiate DNA damage by inhibition of DNA repair mechanisms.

The capacity of NO to induce apoptosis was first appreciated by Albina, who showed that NO caused apoptosis in macrophages. Accumulation of the tumor suppressor protein p53 has been described as an essential and early indicator of NO-induced apoptosis. Although high concentration of NO is a well-established cytotoxic and pro-apoptotic effector molecule, there is growing evidence that low concentrations of NO can also inhibit apoptosis. First, NO can oxidize intracellular reduced glutathione and thereby change the antioxidant levels within the cell, resulting in oxidative or nitrosative stress. This stimulates the induction of heat shock proteins HSP32 and 70, which protects cells from apoptotic cell death. Second, caspase proteases contain a single cysteine at the catalytic site that is essential for activity. Suppression of the effector caspase-3 activity by S-nitrosylation of the essential cysteine cysteine cysteine can block apoptosis. Finally, NO may inhibit cytochrome C release from mitochondria by inhibiting bcl-2 cleavage. 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.

NITRIC OXIDE IN INTESTINAL INFECTION AND INFLAMMATION

Despite intestinal barriers there is a constant interaction between antigens in the gut lumen and the mucosal immune system. The presence of inflammatory cells in the gut mucosa reflects this "tolerant" mucosal immune response against the
normal gut content. NO plays an important role in both barrier and immune function. Inhibition of NO with N\textsuperscript{a}-nitro-L-arginine (L-NAME) resulted in a rapid increase of trans-epithelial movement of the small molecule \textsuperscript{51}Cr-EDTA (MW 330)\textsuperscript{68} indicating increased permeability. The increased permeability for small molecules occurred without overt injury and could be restored by exogenous NO suggesting that NO maintains an intact mucosal barrier. However, intestinal epithelial cells (IEC) exposed to high amounts of NO showed increased permeability\textsuperscript{69}. Induction of iNOS can yield micromolar amounts of NO for sustained periods. The induction of iNOS is mediated by the nuclear transcription factor \textsuperscript{κ}B (NF-\textsuperscript{κ}B)\textsuperscript{70}. Various stimuli like viruses, microbial products, pro-inflammatory cytokines (TNF-\textsubscript{α}, IL-1, IL-6), T- and B-cell mitogens, physical and chemical stress can activate NF-\textsuperscript{κ}B\textsuperscript{71}. Because a combination of cytokines (IL-1, IFN-\textgammaTNF-\textsubscript{α}) and endotoxin (LPS) are needed for the in vitro induction of iNOS in native colon cells and in intestinal tumour cell lines these cells are believed to be relatively resistant to cytokine induced NF-\textsuperscript{κ}B activation. A decreased \textit{IκB} kinase (IKK) activity and consequent resistance to \textit{IκBα} degradation is postulated as a protective response of IEC’s to remain quiescent in the hostile colon environment\textsuperscript{72}. Indeed, many immunohistochemical studies\textsuperscript{73,74,75} even combined with Western blotting\textsuperscript{1,76} could not demonstrate epithelial iNOS expression in normal gut mucosa. However, Roberts et al.\textsuperscript{77} found iNOS mRNA and Perner et al.\textsuperscript{78} demonstrated iNOS protein expression in the normal colonic mucosa suggesting a low grade physiological induction of iNOS in the normal colon.

Despite relative resistance of IEC’s to NF-\textsuperscript{κ}B activation several pathogenic organisms such as Salmonella, Shigella, Listeria and Helicobacter species can activate NF-\textsuperscript{κ}B\textsuperscript{79} and induce iNOS\textsuperscript{80} in IEC’s. NO in itself may not be toxic to bacteria, in fact certain enteric bacteria contain nitrate reductase and produce NO by their own. However, NO becomes cytotoxic when generated together with superoxide (O\textsubscript{2}\textsuperscript{•}⁻) forming peroxynitrite (OONO\textsuperscript{−}) a powerful antimicrobial agent\textsuperscript{81}. Epithelial iNOS induction has clearly been demonstrated in inflammatory conditions such as; diverticulitis\textsuperscript{1}, inflammatory bowel disease (IBD)\textsuperscript{1,2,23,75,76} and celiac disease\textsuperscript{82}. Epithelial iNOS induction and NO production may cause increased intestinal permeability as observed in septic patients\textsuperscript{83}. Indeed selective inhibition of iNOS in endotoxemic rats ameliorated mucosal permeability for dextran (MW 4000)\textsuperscript{84} and reduced bacterial translocation\textsuperscript{85}. The absence of bacterial translocation in endotoxemic iNOS knockout mice further supports a pathogenic role of epithelial derived NO in sepsis.

The role of NO in IBD is less obvious. NO can react with superoxide (O\textsubscript{2}\textsuperscript{•}⁻) anions yielding the toxic reactive nitrogen intermediate peroxynitrite (OONO\textsuperscript{−}). In addition to oxidation reactions, peroxynitrite can nitrate tyrosine to produce 3-nitrotyrosine. Evidence for peroxynitrite mediated damage of epithelial cells in IBD was found in one\textsuperscript{1} but not in two other studies\textsuperscript{75,76}. Studies using inhibitors of NOS in experimental colitis showed little improvement\textsuperscript{87,88} no effects\textsuperscript{89,90}, or even worse effects\textsuperscript{91} on colitis probably due to the lack of iNOS specificity of the inhibitors.
used. The first report of experimental colitis in iNOS knockout mice showed delayed healing and persistent inflammation in acetic-acid induced colitis 53. Studies with trinitrobenzene sulphonic acid (TNBS) induced colitis in iNOS KO mice showed resistance to TNBS colitis in one 92 and increased inflammation in two other studies 93,94. In the dextran sulphate sodium (DSS) colitis model in iNOS KO mice showed resistance to DSS in three studies 95,96,97. Finally, a chronic colitis which develops spontaneously in interleukin 10 (IL-10) deficient mice, developed at the same rate and intensity in mice deficient in both the IL-10 and iNOS genes 98. Considering the absence of macroscopic ulcerations in the presence of large amounts of NO in patients suffering from microscopic colitis a protective role of NO is suggested 99. Indeed a NO donating mesalazine derivative had an additional beneficial effect on TNBS induced colitis 100. The reduced gastro-intestinal toxicity of NO donating non steroidal anti-inflammatory drugs (NSAID) 44 and aspirin 101 are in agreement with a protective effect of NO on intestinal epithelial cells. A part from the above-mentioned beneficial effects of NO in mucosal injury, NO can also inhibit NF-κB activation 102. Therefore high amounts of NO may serve in a negative feedback loop to block prolonged activation of NF-κB thereby limiting chronic inflammation.

CONCLUSIONS

It is clear that nanomolar amounts of NO produced by calcium dependent nNOS has a physiological role in peristalsis and sphincter function. Absence of locally produced NO can result in aperistalsis and obstructive sphincters. NO produced by eNOS is essential in maintaining mucosal blood flow. Absence of NO results in an increased susceptibility of the GI tract to injury.

Selective NO delivery by gene therapy or by NO donating compounds might offer new therapeutic options in motility disorders of the gut or to prevent mucosal injury. The effects of micromolar amounts of NO as produced by iNOS are less well understood. On the one hand large amounts of NO can increase gut permeability, induce apoptosis and stimulate intestinal secretion. On the other hand NO can block apoptosis and reduce inflammation by inhibiting NF-κB activation. Further studies with selective iNOS inhibition by specific iNOS blockers or anti-sense therapy are needed to elucidate the role of NO in the gastrointestinal tract.

References


