Role of Nitric Oxide in Physiology and Pathology of the Gastrointestinal Tract

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Abstract: In this paper the physiological role of NO and isoforms of NOS in the gastrointestinal tract and the involvement of NO in pathological processes of digestive tract as well as the perspective of therapeutic use of NO-donating drugs and selective inhibitors of phosphodiesterase in the treatment of gastric diseases were presented.

Key Words: Nitric oxide, constitutive nitric oxide synthase, inducible nitric oxide synthase, gastrointestinal tract, inflammatory process, malignant transformations, NO-donor drugs, phosphodiesterase inhibitors.

INTRODUCTION

Studies of the role of nitric oxide (NO) have been carried out for over twenty years. In 1992, NO was named the molecule of the year by the Science journal. Six years later, the Nobel prize for Medicine was awarded to Robert Furchgott, Louis Ignarro and Ferid Murad for the identification of NO as an important signaling molecule in the cardiovascular system [1]. Nowadays in clinical chemistry many different methods for determining NO or its metabolites are used including: "real time" $NO^{\bullet}$ determination, determination of stable end-products nitric/nitrate, incorporation of stable heavy nitrogen isotopes into nitrite and nitrate, determination of nitric oxide synthase activity as well as spectroscopy: electron paramagnetic resonance and near-infrared [2].

Nitric oxide is a gaseous small molecule generated by nitric oxide synthase (E.C. 1.14.13.39, NOS) which oxidizes the terminal nitrogen atom of L-arginine, leading to production of L-citrulline and NO as presented in Fig. (**1**).

NOS requires the following cofactors to exert its action: flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), calmodulin (CaM), tetrathydrobiopterin (BH_4) and heme [3,4].

There are three isoforms of NOS [3,5,6]:

- two forms constitutively expressed nitric oxide synthase (cNOS): neuronal (nNOS or type I NOS) and endothelial (eNOS or type III NOS) nitric oxide synthase; Ca^{2+}-dependent
- inducible nitric oxide synthase (iNOS or type II NOS); Ca^{2+}-independent

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Both isoforms of cNOS generates NO in an intermittent way over seconds or minutes in small amounts (pmol/l). nNOS is located in the neurons of central and peripheral nervous system (non adrenergic non cholinergic fibres). In the gastrointestinal tract the main role of nNOS-derived NO is the control of the smooth muscles' relaxation. eNOS is mainly localized in platelets and endothelial cells. It is involved in the maintenance of gastrointestinal mucosa integrity *via* modulation of gastric mucosal blood flow, epithelial secretion and barrier function. It also plays a role in inhibition of leukocytes, platelets and mast cells adhesion [1,7-9].

Based on these data, it is thought that both eNOS and nNOS are involved in homeostasis.

Main activator of NOS is change in cellular calcium levels. The constitutive isoforms of NOS: eNOS and nNOS show an increased activity following increase in calcium and therefore calmodulin concentration in the cell as well as following increased activity of phosphorylation. Especially activity of eNOS could be regulated by a number of different kinases and phosphatases with special role of the protein kinase Akt. On the other hand iNOS activity is mostly regulated at the transcriptional level and through its intracellular distribution [9].

The activity of NOS is also regulated by endogenously produced competitive inhibitors present in the plasma and cells, that inhibit (in some cases irreversibly) both the constitutive and the inducible NOS [10]. Some inhibitors of NOS are shown in Fig (**2**).

The iNOS isoform expression may be induced by certain immunological factors such as cytokines, which may lead to continuous (over hours or days) NO production resulting in cellular concentration if this compound of nmols/l. iNOS isoform is localised in endothelial cells, smooth vascular muscle, neutrophils, macrophages and hepatocytes. Large amounts of NO synthesized from the inducible isoform have

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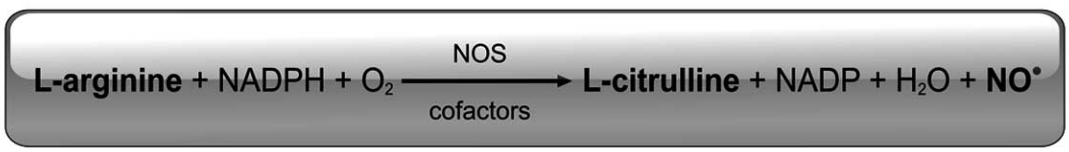
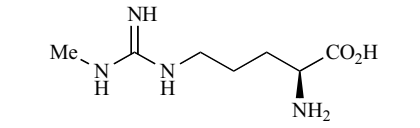


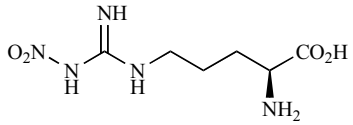
Fig. (1). Synthesis reaction of NO• molecule.

been implicated in pathology (especially inflammatory processes) of the gastrointestinal tract [1,6,7,9,11].

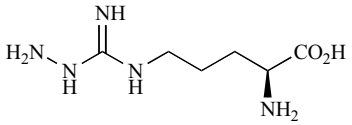
In past years it was confirmed, that an important factor determining biological function of NO is its steady-state concentration. Particular cellular responses are differentially regulated by a specific NO concentration. Five basic distinct concentration levels of NO activity were proposed for regulation of particular cellular processes: cGMP-mediated processes ([NO]<1-30 nmol), Akt phosphorylation ([NO]=30-100 nmol}, stabilization of HIF-1alpha ([NO]=100-300 nmol, phosphorylation of p53 ([NO]>400 nmol), and nitrosative stress (1μmol) [12]. So far, low levels of NO synthesized by both isoforms of cNOS were considered as protective - promoting cell survival and proliferation, whereas higher, excessive quantities of NO generated by iNOS were treated as harmful leading to cytotoxicity [13].



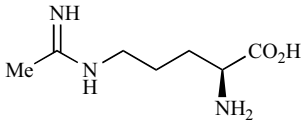
N$^{\omega}$-monomethyl-L-arginine



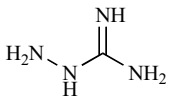
N$^{\omega}$-nitro-L-arginine



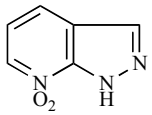
N$^{\omega}$-amino-L-arginine



N-$^{\delta}$iminoethyl-L-ornithine



aminoguanidine



7-nitroindazole

Fig. (2). Chemical structures of inhibitors of nitric oxide synthase.

Recently that view has been questioned. As it was initially reported for nNOS and eNOS, also iNOS activity may be connected with the reduction of leukocyte-endothelium interaction, platelet aggregation and protection of mucosal microcirculation. A low level of iNOS expression may also reflect a positive host-defense response to a challenge [14,15].

The primarily protective effects of NO were described for picomolar and nanomolar levels of NO concentration [16]. Under physiological conditions eNOS generates short bursts of NO (10-30 nmol/l) causing relaxation of smooth muscle cells and stimulating proliferation of endothelial cells [17-19].

It was found e.g. that low NO concentration (<50 nmol) transciently induced extracellular signal-regulated kinase (ERK) phosphorylation, which is prosurvival, while higher concentration (>100 nmol) proangiogenic transcription factor hypoxis inducible factor 1α (HIF1 α), which promotes angiogenesis and potentially carcinogenesis [20]. NO concentration >300 nmol/l produced by iNOS have antitumor and antiangiogenetic properties regarding induction of phosphorylation of p53 resulting in cytostasis and cell death [19,21].

MOLECULAR MECHANISMS OF NO ACTIONS

NO is a free radical, which has an unpaired electron in its outer orbit, and this is the basis of its biological activity, allowing for electrochemical interactions between NO and metals, e.g., the active site of guanylate cyclase (GC) [22]. Through its lipophilic property it is able to diffuse quickly and initiate inter- and intracellular signals.

At low (nanomolar) concentrations the most important effect related to NO signaling pathway is activation of soluble guanylate cyclase (sGC) (E.C. 4.6.1.2, GC) [23]. NO binds to the sGC heme group giving rise to a change in the structure of the enzyme, which after activation increases the production of the cGMP (cyclic guanosine monophosphate) with subsequent activation of protein kinase G, as presented in Fig. (**3**), leading through downstream phosphorylation cascades to effector functions [1,4,22,24].

In mammals there are two different types of cGMP-dependent protein kinases CGH1 and cGK2 which mediate localized and global NO signaling. Activation of the NO/cGMP/cGK1 pathway induces relaxation of smooth muscles by lowering the cytosolic calcium level and/or by calcium desensitization of the contractile elements, which makes an important mechanism of NO effect in gastrointestinal tract [25].

On the other hand cGMP regulates the activity of phosphodiesterases (PDEs), which modulate the duration and amplitude of cyclic nucleotide signaling.

Phosphodiesterases play critical role in hydrolysis and controlling intracellular cAMP and cGMP. PDEs constitute a large superfamily of enzymes grouped into 11 broad families based on their distinct ninetic properties, regulatory mechanisms and sensitivity to selective inhibitors [26,27]. In smooth

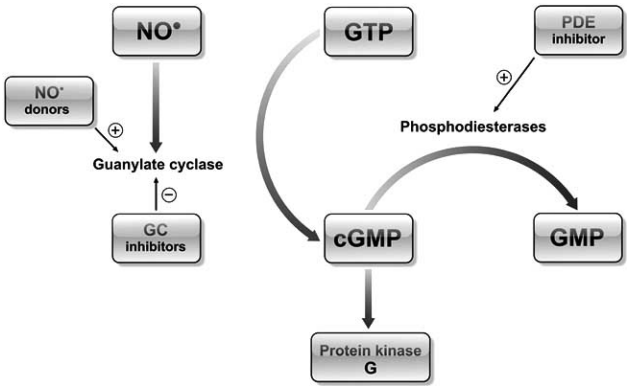


Fig. (3). NO• signaling pathway.

muscle cells four major families of PDEs have been identified, including Ca^{2+}/calmodulin-stimulated PDE1, cGMP-inhibited PDE3, cAMP-specific PDE4 and cGMP-specific PDE5, but so far in gastrointestinal tract only myorelaxant activity of PDE5 was confirmed [27,28].

Recently a novel cGMP-dependent pathway of NO inhibition due to blocking activation of sGC by matrix protein thrombospondin-1 (TSP1) was described [29]. Thrombospondin-1 is a glycoprotein secreted by macrophages and endothelial cells that modulates endothelial and vascular smooth muscle cell adhesion, proliferation, motility and survival *via* several cell surface receptors. It was found in that antiangiogenic potency of TSP1 increases more than 100-fold in the presence of physiological levels of NO [29]. In both *in vitro* and *in vivo* experimental models of human vascular endothelial cells HUVEC and mice, respectively, administration of NO-releasing compound DETA/NO, which releases NO in slower rate for a prolonged period ($t_{1/2}$ ~ 24 h) significantly enhanced cellular outgrowth [18]. This effect was abolished by NOS inhibitor L-NAME. DETA-NO mediated triphasic regulation of TSP1 expression, which depended on extracellular signal-regulated kinase (pERK) phosphorylation. Under experimental conditions particular phases of this triphasic response in the expression of TSP1 were as follows: decreasing at NO level = 0,1 μmol (similar to those generated endogenously by eNOS), rebounding at 100 μmol and decreasing again at 1000 μmol. Conversely exogenous TSP1 inhibited NO-mediated perk activity. This effect was cGMP-dependent. The results of this study suggest that there are some dose-dependent positive and negative feed-back loops between NO and TSP-1. Further studies confirm that at low, physiological level of NO, TSP1 binding to CD47 and CD36 ligands on cell surface inhibits NO signaling by blocking sGC, resulting in the decrease in cGMP synthesis with subsequent reduction of cGMP-dependent protein kinase activity [19,21,30,31]. This antagonism of NO signaling allows TSP1 at circulating in organism level to protect tissues against angiogenic responses to NO as well as to NO-stimulated chemotaxis, endothelial cells motility and adhesion of endothelial cells on type I collagen which results in maintaining homeostasis [29 31].

In pathological conditions, when NO is produced in higher amounts by iNOS, the NO signaling pathway may be independent of GC activation. In this situation NO may directly regulate the function of ion channels, enzymes and other proteins through the nitrosylation of cysteine thiols in proteins leading to, e.g., inhibition of DNA synthesis and enzymes linked to Krebs' cycle, respiratory chain electron's transport and the glucidic metabolism. Another mechanism of NO cytotoxicity is the generation of nitrogen species. NO as a free radical can interact with reactive species such as the superoxide radical, thereby generating peroxynitrite and trioxide of dinitrogen. Peroxynitrite is responsible for oxidation of proteins, lipids and nucleic acids as well as for nitration of protein tyrosine residues and hydroxylation of residues of other amino acids. Peroxynitrous acid is a stronger oxidizing agent than either nitric oxide or superoxide, from which it may be formed, and readily oxidize thiols, ascorbate and other molecules. Trioxide of dinitrogen leading to nitrosylation of different substrates may contribute to a chronic inflammation and carcinogenic processes [1,22,32]. Formation of peroxynitrite in activated macrophages is beneficial, while its formation elsewhere is harmful causing oxidative damage to tissues and giving rise to various pathological conditions [33,34]. Peroxynitrite is not a radical since the unpaired electrons of its precursors are now paired. However, peroxynitrite is a far more cytotoxic species than either of its parents, NO or superoxide [22]. Also alternative route for peroxinitrite formation, involving the reaction between nitroxyl ion and O_2 does occur. Peroxinitrite also reacts with carbon dioxide with production of nitrosoperoxocarbonate $ONO_2CO_2^-$. As the HCO_3^- concentrations in body fluids are relatively high, the formation of adduct may take place more rapidly then other reactions associated with peroxinitrite toxicity [34].

NO is a precursor of a family of reactive compounds, collectively called reactive nitrogen species, in a manner analogous to the fate of superoxide and it's family of reactive oxygen species. One misconception is that NO (NO·) reacts with amines and thiols. *N*-nitrosoamines and *S*-nitrosothiols are typically formed *via* donation of a nitrosonium equivalent (NO^+) from dinitrogen trioxide (N_2O_3) to the nucleophilic residue [18,19]. The function of many proteins and enzymes has been altered by nitrosation *in vitro*. The mechanism through which these modifications may occur in biological systems is a not fully recognized. Many have questioned the relevance of nitrosation *via* NO autoxidation, reasoning that sufficient levels of NO cannot be achieved *in vivo* to satisfy the rate-limiting step for dinitrogen trioxide formation, which is second order in NO. In contrast to NO autoxidation, the reaction between NO and superoxide anion is first order in both reactants and occurs at near diffusion control, so the peroxynitrite may also serve as an intermediary in a pathway leading to formation of NO adducts (e.g. nitrosation products) [35,36]. Some results suggest that N-nitrosoamines and S-nitrosothiols may be produced through both nitrosation and oxidative nitrosylation dependent mechanisms, which are strongly influenced by the relative rates of NO and superoxide formation [35]. It was found in *in vitro* study that reactive nitrogen species-mediated protein modification could be also an important mechanism of NO regulation of matrix metalloproteinase-9 (MMP-9) activity secreted from macrophages both by guanylyl-cyclase-dependent modulation of the MMP-9/TIMP-1 balance and proteolytical regulation of MMP-1 and MMP-13, which can cleave the prodomain of MMP-9 [37].

Other additional cGMP-independent action of NO is nitration of tyrosine residues in proteins. Peroxynitrite is a strong oxidant capable of modifying most biological molecules and compounds, including such amino acids as tyrosine, tryptophan, cysteine, and methionine [38]. Tyrosine nitration is mediated by reactive nitrogen species such as peroxynitrite anion ($ONOO^-$) and nitrogen dioxide ($NO_2^{\bullet}$), formed as secondary products of $NO^{\bullet}$ metabolism in the presence of oxidants including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and transition metal centers. Despite the capacity of peroxynitrite to mediate tyrosine nitration *in vitro*, its role on nitration *in vivo* has been questioned, and alternative pathways, including the nitrite-H_2O_2-heme-peroxidase and transition metal-dependent mechanisms, have been proposed [39]. Although 3-nitrotyrosine content in proteins has been revealed as a relevant biomarker of nitric oxide -dependent oxidative stress *in vivo.*

NO is a pleiotropic molecule which may play a dual role in apoptosis. It allows the formation of $ONNO^-$ by reacting with superoxide anions and triggers the increase in the membrane permeability and in the calcium concentration, stimulating apoptosis this way [40,41]. On the other hand, NO activates *in vitro* Bcl-2/Bcl-X_L resulting in inhibition of the Bax/Bak pathway and thereby blocking the caspases cascade [42-44]. Another mechanism by which NO may regulate apoptosis is by modulating p53. NO-mediated DNA damage can trigger p53 accumulation and induce apoptosis [45].

NO is produced at many different sites in the gastrointestinal tract and has been connected to both physiological and pathological processes as presented in Fig. (**4**).

NITRIC OXIDE PARTICIPATION IN PHYSIOLOGICAL PROCESSES

Gastrointestinal Tract Motility

NO synthetized by nNOS plays an important role in esophageal, gastric and intestinal motility regulation. It appears to be the dominant non-adrenergic, non-cholinergic inhibitory neurotransmitter in the enteric nervous system, which is involved in the vagally-mediated accommodation reflex of the stomach and colon to food ingestion as well as in the coordinated control of contractile function during the peristaltic reflex, resulting in inhibition of esophageal smooth muscle function and small bowel motility and also promotion of gastric accommodation and emptying [46]. Nitric oxide is the major post-ganglionic inhibitory neurotransmitter to the lower esophageal sphincter and recent evidence suggest that there my be a novel nitrergic, preganglionic, vagal pathway involved in this sphincter relaxation [47]. Pharmacological inhibitors of NOS in the esophagus contribute to reducing the latency between swallows and contraction in the distal esophagus, increasing in the lower esophageal sphincter pressure and peristaltic wave pressure as well as decreasing the number of transient lower esophagus relaxations [48]. In the stomach pharmacological inhibitors of NOS cause an increase in the frequency of gastric contractions, a decrease in basal and after-meals fundic volume; whereas in the small intestine action of pharmacological inhibitors of NOS results in an increase in fasting motor activity [9,49-51].

In experimental model in which mouse gastric fundus and small intestine muscle preparations mounted in organ baths were exposed to electric field stimulation and to NO and NO donors relaxations of longitudinal muscle strips was abolished by specific inhibitors of soluble guanylate cyclase (ODQ and ns2028), which indicates that relaxations to endogenous NO in gastric fundus and small intestine are completely dependent on cGMP [52].

In an animal model, RhoA/Rho-kinase independent Ca2+ desensitization pathway contributed to the relaxation by NO in circular smooth muscle strips of the distal colon [53]. NO also mediates nonadrenergic, noncholinergic relaxation in the murine internal anal sphincter [54].

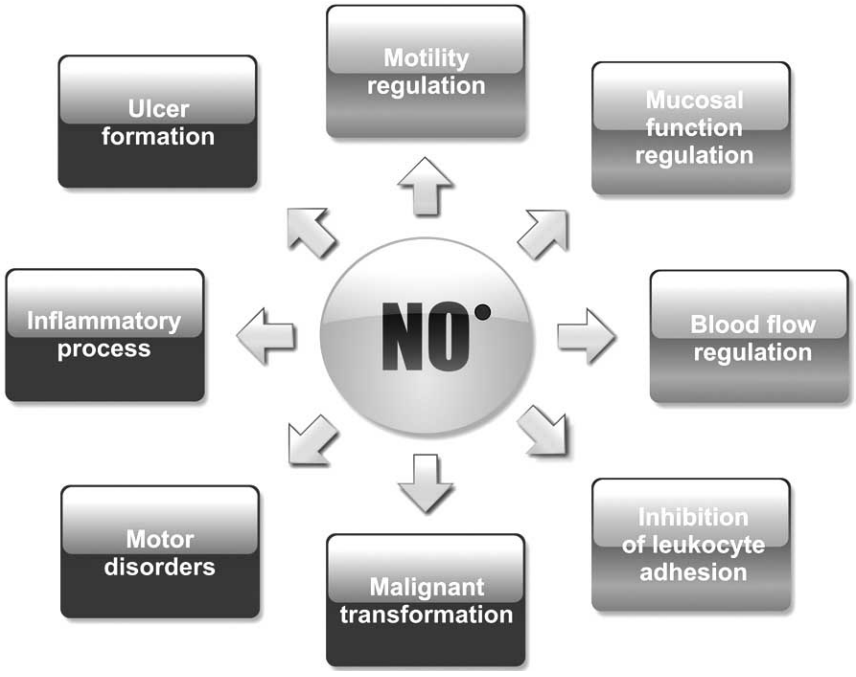


Fig. (4). Role of $NO^{\bullet}$ in physiological and pathological processes in the gastrointestinal tract – summary.

Endogenous NO may play a role in the regulation of small intestinal nutrient transit by regulating small intestine's motility as in healthy subjects after infusion of L-NAME duodenal transit was delayed and the frequency and amplitude of duodenal pressure waves increased significantly [55]. NO is also an inhibitory neurotransmitter in the Oddi's sphincter. Inhibition of endogenous NO production enhances contractility while exogenous NO decreases sphincter contractility and electrical activity, both in experimental pig model and *in vitro* [56,57].

In an experimental model of mice exposed to intestinal surgery deficiency or pharmacological inhibition of iNOS protected intestinal pacemaker cells from postoperative damage resulting in reduction of pacemaking activity in the distance up to 5 cm from the anastomosis site with subsequent postsurgical dysmotility and ileus, which was observed in wild-type mice with unchanged iNOS function [58].

Gastrointestinal Mucosa Function and Blood Flow Regulation

NO synthesized by eNOS participates in regulation of alkaline production, secretion of acid and gastric mucosa as well as in vascular perfusion, and tissue regeneration in gastrointestinal tract. Furthermore, NO has also a cytoprotective effect on gastric mucosa *via* protection against different aggressive agents [1,9,11]. The continuous NO release, through eNOS and nNOS expression, contributes to the physiologic gastrointestinal mobility, tonus, permeability and blood flow to the vessels of gastric wall.

Results of *in vitro* study confirmed that NO increases HCO_{3-} secretion in mouse duodenum and that two types of phosphodiesterase: PDE1 and PDE3 are involved in the regulation of duodenal HCO_{3-} secretion, as inhibitors of both enzymes increased the basal secretion at high doses, as well as potentiated HCO_{3-} response to PGE(2) and in case of PDE1 also to NO donor at lower doses that had no effect by themselves on the basal secretion [59].

The influence of NO and NOS isoforms activity on protection of gastric lesions due to ichaemia/reperfusion-induced mucosal injury shows unequivocal character, as in mice with reperfusion after occlusion of celiac artery, prior admistristaration of L-NAME (a non-selective NOS inhibitor) significantly aggravated visible hemorrhagic lesions of gastric mucosa, whereas selective iNOS inhibitor (1400W) prevented the occurrence of these lesions [60].

The activity of iNOS can also be associated with a reduction in the platelet aggregation and leukocyte/endothelium interaction, as well as microcirculatory protection of the gastric mucous membrane [24].

NO decreases histamine-stimulated acid secretion in gastric glands. The inhibition of gastric secretion by NO is connected with increasing cGMP levels in the parietal cells in biopsy material from healthy subjects involving the activation of guanylate cyclase [61]. Furthermore, NO is responsible for the healing process of chronic gastric ulcers. It enhances blood flow to the ulcer margin and increases the number of capillaries in the granulation tissue at the ulcer bed. The inhibition of NOS results in a delay in ulcer healing [62]. NO inhibits the leukocytes, platelets and mast cells adhesion to endothelial cells and through this mechanism it protects tissue from ischaemia-reperfusion injury [63-65].

The inhibitory action of No on leukocyte adhesion is mediated by P-selectin, beta integrins, phospholipase A2, PAF (platelet activating factor) and leukotriene B4 [66]. The inhibitory influence of NO on platelet function occurs largely controlled by cGMP-phosphoinositide 3-kinase–dependent mechanisms, although other mechanisms involving pathways independent of cGMP and peroxynitrate-dependent protein nitration have been postulated [67,68].

NITRIC OXIDE PARTICIPATION IN PATHOLOGICAL PROCESSES

Ulcer Formation, Inflammatory Process and Motor Disorders

Studies in both animal models and humans indicate that NO is involved in gastrointestinal ulcer formation and inflammation.

Expression of iNOS shows topographical variations in Helicobacter pylori (Hp) positive patients in the following diseases: gastritis and duodenal and gastric ulcers. There is a significantly higher expression of iNOs mRNA in the duodenum when there is duodenal ulcer, gastritis in the antrum and corpus or gastric ulcer in the antrum. Eradication of Hp decreases iNOS mRNA expression in the duodenum in the case of duodenal ulcer or gastric ulcer in the antrum. Diverse topographical patterns of iNOS expression induced by Hp infection may explain the mechanisms by which this bacterium elicits different clinical disorders [69]. Hp infection upregulates also eNOS activity and induces angiogenesis resulting in inflammatory lesions of gastric mucosa [70]. In Hp-positive dyspeptic patients expression of eNOS in the mucosa of the stomach body and antrum was significantly higher than in Hp-negative patients. Moreover Hp-positive patients showed higher expression of CD34-positive blood vesels in the mucosa of antrum, which was correlated with gastric inflammation and activity.

NO plays an important role in pathogenesis of inflammatory bowel diseases (IBD). A significant cellular source of NO during intestinal inflammation is the colonic epithelial cell, but also other cells as i.e. granulocytes could release NO in this process [46]. In IBD patients increased mucosal iNOS expression and activity, increase in release of NO into the rectal lumen as well as increased nitrotirosine levels were observed [71-74].

An increase in iNOS expression is correlated with exacerbation of IBD due to tissue-associated larger contribution to the recruitment of inflammatory cells. In experimental model of mice with dextran sodium sulfate (DSS)-induced colonic inflammation in wild-type mice and bone marrow chimeras with normal iNOS function an inflammatory process was characterized by bloody diarrhea and a high disease activity index as well as elevated colonic myeloperoxidase concentration, while chimeras both with iNOS-deficient blood cells and i-NOS-deficient tissue function exhibited attenuated disease activity index as well as significantly reduced colonic myeloperoxidase activity [75].

Elevated mucosal NO in IBD contributes to secretory dysfunction *via* inhibition of the epithelial responsiveness to cAMP (cyclic adenosine-monophosphate)-dependent secretagogues [76]. Increased NO production in IBD may contribute to reduced colonic motility and toxic megacolon [77,78].

It was also confirmed both *in vitro* and *in vivo* that necrotising enterocolitis is associated with mucosal release of NO and that exogenous NO inhibits enterocyte migration in a dose-dependent manner *via* aSHP-2-mediated activation of the Rho-GTP-ase-FAK signaling pathway, while phosphorylation of focal adhesion kinase (FAK) activity in enterocytes occurs in an NO-dependent manner [79].

NO is also involved in regulation of colonic mucosa blood flow in the course of colitis. In 2 different experimental models of colitis colonic blood flow in visualized mucosa of exteriorized rat distal colon was reduced by administration of NOS inhibitor (l-NNA), but not by iNOS inhibitor (l–NIL). In both colitis models vascular resistance increased compared to control indicating a higher level of vasodilating NO. Moreover in rats with experimental colitis iNOS and eNOS mRNA level increased, whereas nNOS mRNA level remained at the control level, which suggests that colitis results in increased colonic mucosal blood flow most probably due to increased eNOS activity [80].

NO together with other free radicals is also involved in the ulcerogenic effect of passive smoking on colitis. The mechanism is probably connected to the interaction with superoxide to produce peroxynitrite, which initiates lipid peroxidation [11].

In ulcerative colitis (UC) and Crohn′s disease (CD) differences in the expression and distribution of NOS isoforms in immune and endothelial cells were observed. iNOS-IR (immunoreactive) cells were much more numerous in inflamed mucosa of UC than CD. Inducible NOS-IR/CD15-IR cells are significantly elevated in inflamed compared to uninflammed UC mucosa. In CD, the percentage of iNOS-IR/CD68-IR cells was lower in inflamed sites. Activity of iNOS was higher in inflamed sites in UC, whereas in CD no changes were observed. The ratio of eNOS/CD-31-IR was higher in patients with UC as compared to patients with CD [81].

In UC the mucosa releases interleukin 1-β (IL-1β), hydrogen peroxide (H_20_2) and NO, which may contribute to the impaired Ca^{+2} release and decrease sigmoid smooth muscle contractility [82]. In chronic IBD microvascular endothelial dysfunction characterized by loss of NO-dependent dilatation may contribute to reduced perfusion, poor wound healing, and maintenance of chronic inflammation [83].

The inhibitory effect of corticosteroids on the NO production in the intestinal inflammation might occur *via* the inhibition of mononuclear cell-produced mediators responsible for NO generation colonic epithelial cells [84]. In this *in vitro* study in cultured human colonic epithelial cells collected from patients with UC basal concentration of NO determined by measuring in culture supernatants the stable-end product nitrite using a fluorometric assay was significantly reduced by incubation of paired biopsies with prednisolone and budesonide (1046±170 pmol/100 g protein *vs*. 481±88 and 424±96 pmol/100 μg protein, respectively; P<0.01). Corticosteroids may affect NO production if administered at the early stage of inflammation, whereas they are not effective when the inflammation is already set up [85,86]. Low NO level predicts a poor clinical response to steroid treatment [87]. In this study NO level in rectal mucosa measured by immunochemical method with use of chemiluminescence analyzer in patients with active UC and CD (10950±7610 and 5040±1280 parts per billion (ppb), respectively) was significantly higher as compared to controls (154±71 ppb, P<0.001). In patients with both diseases without clinical response to steroid therapy NO level was only slightly increased (620±270 and 1260±550 ppb, respectively) compared to those with positive therapeutic response (18860± 530 ppb, P<0,001 and 10060±3200 ppb, P<0.05 respectively)

NO level is correlated with IBD and collagenous colitis activity [60]. Rectal NO level measured with chemiluminescence technique by using tonometric balloon method in IBD and collagenous colitis patients was greatly increased (median 3475, 25^{th}-75^{th} percentile, 575-8850 ppb and 9950, 4475-19750 ppb, respectively; P<0.001) while in irritable bowel syndrome patients it was only slightly increased (150, 53-200 ppb; P<001) as compared to healthy control subjects (45, 34-64 ppb). NO level decreases in IBD patients responding to anti-inflammatory treatment. So it may be a rapid tool for distinguishing between bowel inflammation and functional bowel disorders (with a sensitivity of 95% and specificity of 91% in discrimination between these diseases) and also for monitoring patients with IBD [88,89]. The activity of nNOS and iNOS isoforms in colonic mucosa may predict progression of ulcerative colitis, also in patients with ulcerative colitis with morphologically unchanged mucosa. The up-regulation of iNOS and down-regulation of nNOS as well as an increase in the ratio of iNOS /nNOS activity over 0,29 may be a useful tool in the detection of patients with anticipated progression of disease [90].

Increased NO levels can also be found in disorders characterized by minimal mucosal damage, e.g., collagenous and lymphocytic colitis [15,86].

Overproduction of nitric oxide in the liver has been implicated as an important event in endotoxin shock and in other models of hepatic inflammation and injury [91]. Nitric oxide has been reported to downregulate cytochrome P450, to suppress liver protein and DNA synthesis, and to induce apoptosis and necrosis, and these activities seem to be involved in hepatotoxicity [92,93]. NO also inhibits catalase (E.C.1.11.1.6, CAT) activity, suggesting that it may alter the detoxification of cytotoxic oxygen radicals [94].

Moreover, recent studies suggest that impaired nitrergic innervation of the smooth muscles may play a crucial role in several disorders of gastrointestinal motility as achalasia, functional dyspepsia, diabetic gastroparesis, delyed gastric emptying during pregnancy or after vagotomy, infantile hypertrophic pyloric stenosis, Hirschsprung's disease Chagas' disease and diarrhea [15,46,86].

Malignant Transformation

There are several studies on the role of NO in carcinogenic process. NO shows a dual behaviour. It may have both genotoxic and angiogenic properties. At high levels of NOS expression generated by activated macrophages it may be cytostatic and cytotoxic for tumor cells. On the other hand, at low level, NO can have the opposite effect and promote tumor growth. The mechanisms by which NO is capable of killing of tumor cells include direct damage of DNA, inhibition of DNA synthesis and inhibition of the ribonucleoide reductase (E.C. 1.17.4.1, RNR), reduction of activity of cis-aconitase and loosing of large fraction of the iron pool. Other mechanisms which account for this process are connected to: reduction of O_2 consumption, damage of complexes I and II in the mitochondrial electron transport chain, inhibition of complex IV inhibition as well as induction of apoptosis *via* p53 accumulation [95,96]. NO may also modulate tumor DNA repair mechanisms by upregulating p53 [97], poly (ADP-ribose) polimerase (PARP) [98], the DNA-dependent protein kinase (DNA-PK) [99] and block apoptosis. NO can inhibit *in vitro* apoptosis *via* different mechanisms including disruption of transcription factor-dependent Fas ligand expression [100], nitrosylation of caspases 3, 8, 9 [101-103] as well as inhibition of TNF-α-induced apoptosis [104], c-GMP-dependent signaling cascades [105] and modulation of mitochondrial function [106]. These cellular events contribute to accumulation of DNA damage, which causes the numerous mutations necessary for development of invasive cancer. Increased NO-generation in cells may select mutant p53 cell and contribute to tumor angiogenesis by upregulating VEGF (vascular endothelial growth factor). The neovascularization process leads to enhancement of tumor growth, increase in its invasiveness and metastatic ability in gastric cancer patients [107]. It was found that proangiogenic effect of NO in tumors is inhibited in a c-GMP manner by thrombospondin-1 [19]. TSP-1 primarily regulates also NO-induced, long term vascular responses in tumors e.g. tumor blood flow, but only in part because the tumor vasculature has a limited capacity to acutely respond to vasoactive agents [108].

NO induces p53 accumulation and phosphorylation, particularly at P-ser-15, *via* ATM and ATR kinases, which then contributes to cell cycle arrest at G(2)/M. In patients with UC an increase in p53 mutant frequencies of G:C→A:T transitions AT the CpG site of codon 248 and C:G→T:A transitions at codon 247 were found [109].

It was also found *in vitro* that β-catenin can increase the resistance of colonic cancer cells to nitric oxide-induced apoptotic cell death independently of nitric oxide-induced accumulation of p53 [110]. This occurs through inhibition of nitric oxide-induced release of cytochrome *c* from mitochondria and by blocking both the nitricoxide-induced suppression of the antiapoptotic protein Bcl-xL and the phosphorylation of Akt protein kinase B.

NO contributes to carcinogenesis during chronic inflammation. The level of NO and NOS in Hp positive patients was higher than that in the negative ones. It was higher in pre-neoplastic diseases such as athrophy, intestinal metaplasia and dysplasia than in active gastritis. These data may suggest that besides Hp, other factors may stimulate the producing of NO and that this molecule plays an important role in the development of pre-neoplastic diseases in humans [111]. The mechanisms implicated in gastric malignancy transformation associated with Hp infection include an increased expression of iNOS and nitrotyrosine, as well as an increment in DNA fragmentation due to stimulation of the apoptotic process [112,113]. The expression of iNOS is significantly more frequent in gastric tubular adenocarcinoma compared to gastric diffuse or polymorphous adenocarcinomas, but process of DNA oxidation and protein nitration occurs in all these subtypes of gastric adenocarcinomas [114].

Increased expression of iNOS in colon adenomas and colon cancer as well as in metastases was also observed [114,115]. Increased NO levels generated by iNOS may also contribute to the progression of colon adenoma to carcinoma by DNA damage, increased expression of COX-2 (cyclooxygenase-2) gene or synthesis of postranslation modifications of proteins [9].

In another study it was shown that tumor extensions and the intensity of metastasis formation is related to the activity of iNOS [116]. It has been also suggested that increased cancer incidence in patients with chronic ulcerative colitis may be connected with excess NO synthesis [117]. In rats with colon cancer induced by using azoxymethane treated with the preferential iNOS inhibitors, SC-51 and aminoguanidine a reduction in the development of aberrant crypts was observed. Similar results were obtained in animals with colon cancer treated with sulindac and celecoxib [118].

Therapeutic Perspective for Drugs Containing NO and Inhibitors of Phosphodiesterase PDE5 in the Treatment of Diseases of Gastrointestinal Tract (Upcoming Drugs)

Non-steroidal anti-inflammatory drugs (NSAIDs) often cause adverse effects in the gastrointestinal tract including dyspeptic symptoms, ulcers and even severe complications like bleeding and perforation [24,119].

NO releasing NSAIDs are a new class of anti-inflammatory drugs obtained by co-administration of NO donor agents with NSAIDs (NO-NSAIDs). They are also called cyclooxygenase (E.C. 1.14.99.1, COX) inhibiting NO-donating drugs (CINODs). They cause fewer adverse effects on gastrointestinal tract in comparison to conventional NSAIDs and COXIBs (cyclooxygenase (COX)-2 selective inhibitors) without impairing their anti-inflammatory and antithrombotic effects [24,120-123].

In the experimental acute gastritis developed by water immersion restraint rat mucosa, pretreatment with NO-donors resulted in reduction in the number of gastric lesions, increment of gastric blood flow, attenuation of lipid peroxidation (decrease in malondialdehyde and 4-hydroxynonenal concentrations) and enhancement of antioxidative properties (increase in superoxide dismutase activity) [124]. The gastroprotection of NO-acetylsalicylic acid (NO-ASA) was also shown under similar experimental conditions [125]. Gastric lesions provoked by water-immersion and restraint stress, ischaemia-reperfusion and ethanol administrations were markedly reduced by NO-ASA as compared with ASA. NO-

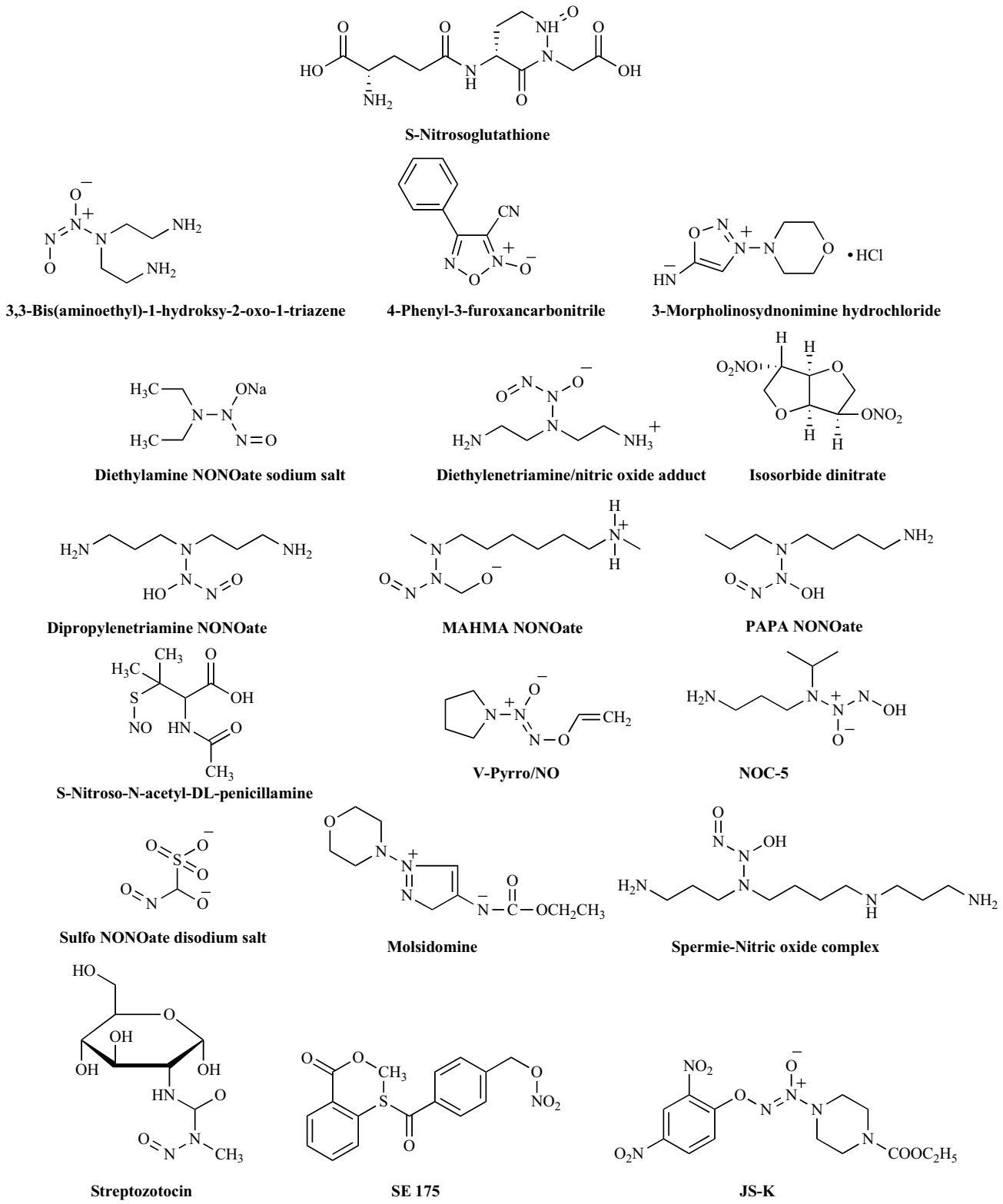


Fig. (5). Chemical structures of some NO donors.

ASA therapy was associated with a decrease in proinflammatory cytokines such as TNF-α and IL-1, and also with a decrease in reactive oxygen species (ROS) generation, an increase in prostaglandin E2 level as well as a decrease in suppression of superoxide dismutase (E.C. 1.15.1.1, SOD) and glutathione peroxidase (E.C. 1.11.1.9, GPX) activity.

So far the efficacy and safety of NO-NSAIDs and CI-NODs have been established in phase I and II studies. Modulation of the iNOS generating excessive NO that can lead to subsequent cytotoxic moieties, such as peroxynitrite, may have therapeutic possibilities in a range of inflammatory diseases of the gut. Likewise, agents that promote the decomposition of peroxynitrite or removal of its other component, superoxide, may also prove to be of use [126].

The alternative for NO-releasing drugs would be to transfer NOS-encoding cDNA sequences into cancer cells for gene therapy purpose. It was demonstrated that transfection of K-1735 melanoma cells with an iNOS cDNA expression cassette suppressed tumorogenicity and abrogated metastasis. In order to protect NO-generating cells from the host immune response they may be encapsulated within a semipermeable alginate-poly-L-lysine membrane. Following delivery to tumor site, high levels of NO and nitrogen species can be generated by administration of appropriate inducer [127].

As impaired generation of NO by nitrergic nerves liberating it as neuromodulator can lead i.e. to achalasia and malfunctions of sphincters in gastrointestinal tract. In such cases NO donors may mimic nitrergic nerve-mediated responses and could be effective in the treatment of these pathologies [128]. Some NO donors are shown in Fig (**5**).

Taking into account a crucial role of phosphodiesterases in NO signaling pathways it seems that phosphodiesterase inhibitors especially of PDE5 could play in future an important role in the treatment of gastrointestinal tract disorders.

So far only efficacy of Sildenafil presented in Fig. (**6**), as a selective inhibitor of cGMP-specific phosphodiesterase PDE5 acting *via* NO-cGMP/K(ATP) pathway, was estimated in clinical trials.

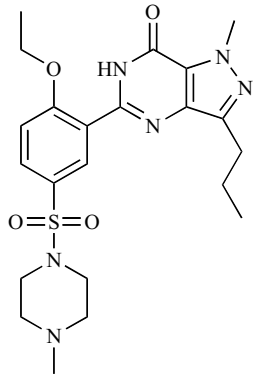


Fig. (6). Chemical structure of selective PDE5 inhibitor sildenafil.

In animal model sildenafil significantly reduced ethanol-induced gastric damage measured in samples of rat stomach with a planimetry programme. L-NAME an inhibitor of NOS alone, without L-arginine significantly reversed the protective effect of this drug. Inhibition of guanylate cyclase by ODQ completely abolished the gastric protective effect of sildenafil. Moreover glibenclamide (K(ATP) channel stimulator) alone reversed, while administered simultaneously with diazoxide did not alter this effect [129]. In other experimental *in vitro* study sildenafil inhibited in a concentration-dependent manner the spontaneous contractions of isolated rat duodenal strips. The syldenafil-induced myorelaxation was significantly decreased by NOS inhibitor (L-NAME) while NO donor (sodium nitroprusside), as well as blockers of K(+) channels (4-aminopiridine and glybenclamide) enhanced this effect [27]. The above results suggest potential possibility of application of sildenafil in the treatment of duodenal contractility disorders *via* activation of the NO-K+ channel pathway.

It was also found that sildenafil which has gastrointestinal myorelaxant properties alters the intragastric ditribution of food without causing gastric stasis [130]. In this study sildenafil (50 mg) along with radio-opaque markers shortened the time taken for the initial radioactivity to fall by 50%, decreased highest activity value in the proximal stomach, and significantly increased highest activity value in the distal stomach, whereas gastric emptying or gastric clearance of radio markers remained unchanged. In other randomized trial administration of sildenafil (50 mg) increased postprandial gastric volume, induced prolonged and higher gastric relaxation and slowed liquid emptying rate in healthy subject, confirming involvement of a nitrergic pathway in the meal-induced accommodation in humans [131]. The observed effect of sildenafil on gastric fundus function suggests a therapeutic potential for selective phosphodiesterase inhibitors in patients with impaired gastric accommodation.

Further targets for pharmaceutical exploitation are likely to come from both genomic and molecular insight into the processes that regulate the NO system.

The results obtained in many studies encourage the research on the role of NO in the gastrointestinal tract, and on the mechanisms of stimulation and inhibition of NOS. Better knowledge of these mechanisms will probably permit the creation of new and safe drugs for treatment of many gastrointestinal disorders.

CONCLUSIONS

The present review article attempts to highlight the important role of NO both in physiological and pathological processes of the gastrointestinal tract. However, despite of important advances in understanding NO effects, more research is still necessary to improve the knowledge of mechanisms of its action. Thus, better understanding the role of NO in gastrointestinal tract function at the molecular level will probably have new therapeutic implications for the treatment of many disorders of digestive system.

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