REVIEW ARTICLE

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Role of Nitric Oxide in Gastrointestinal Tract

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Endogenous nitric oxide (NO) formed from L-arginine plays an important role as non-adrenergic, non-cholinergic inhibitory neurotransmitter in the gastrointestinal (GI) tract. NO plays an important neuromodulator role in relaxation of various smooth muscles of GI tract and is also involved in the secretion of fluids, electrolytes and gastric acid. It is also involved in the various gastrointestinal pathological changes much as inflammatory bowel syndrome. In future, NO donors and specific inhibitors of nitric oxide synthase may gain clinical importance in number of gastric disorders. NO donors can be used in combination with non-steroidal antiinflammatory drugs (NSAIDs) in reducing their gastric side effects. In this article the extensive role of NO in gastrointestinal system has been discussed.

Since the 1960s it has been widely recognised that stimulation of a certain class of nerves within the wall of the gut elicits relaxation, these nerves are non-adrenergic and non-cholinergic nerves (NANC)[1]. The physiological and anatomical evidence for this hypothesis is already quite extensive and it postulated that NANC inhibitory responses are mediated by NO at every level of the GI tract. In the GI tract, NO can bring about changes in secretion, motility, blood flow, electrolyte and water absorption, mucosal protection, inflammation and many of these changes are transduced via activation of intestinal epithelial cells which contains mostly particulate guanylate cyclase[2] and NO is thought to stimulate only the specific soluble guanylate cyclase[3,4].

Evidence for involvement of NO:

Several evidences suggests that NO serves as a NANC inhibitory neurotransmitter in GI tract and is supported by some of the following illustrations[5].

1. Immunoreactivity studies have shown the presence of NO synthase in the guinea pig small intestine [6].
2. Immunohistochemical studies have shown that the enzyme necessary for NO synthesis expressed in enteric neurons[7].
3. It was reported that NO synthase copurifies with NADPH diaphorase in central and peripheral neurons. These findings suddenly accelerated studies of the distribution of NO synthase, because NADPH diaphorase is a relatively cheap and simple histological stain that has been used for several years to stain selected population of neurons[8].
4. *In vitro* studies of muscles from all levels of GI tract have also shown that L-arginine analogues which inhibit NO synthesis, reduce inhibitory effects of NANC neurotransmission[8,9].

All these studies have provided evidence for the existence of nitrergic neurons in which NO serves as a neurotransmitter in various gastrointestinal functions.

Mechanism of release of NO in GI smooth muscle:

The possibility of NO serving as a neurotransmitter has met with some skepticism, because it is an extremely labile, freely diffusible molecule that cannot easily stored in secretory vesicles. This would mean that the classical concept of

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storage and quantum release of transmitter might not be applicable to NO releasing neurons. As inducible NO synthase is involved, therefore influx of Ca^{++} into varicosities during activation could increase synthesis of NO[10]. A mechanism has been suggested that regarding storage of NO in secretory vesicles, but little evidence is available to support this hypothesis (fig.1).

Both forms of NO synthases are dimeric enzymes, each isoform contains iron protoporphyrins IX (haem), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin as bound prosthetic groups. They also contain binding sites for L-arginine, NADPH and Ca^{+2}-calmodulin. Functionally, NOS enzymes are bimodal in that they combine oxygen and reductase domains. The oxygenase domain contains haem while reductase domains binds Ca^{+2}-calmodulin, FMN, FAD and NADPH, it is believed that the flavins accepts electron from NADPH and transfer them to the haem iron which binds O_2 and catalyses stepwise oxidation of L-arginine to NO and L-citrulline. L-Arginine is usually present in excess in endothelial cytoplasm, so the rate of production of NO is determined by the activity of enzyme rather than by substrate availability[11,12].

The activity of constitutive isoform of NOS is controlled by intracellular Ca^{+2}-calmodulin. The most important stimuli controlling endothelial NO Synthesis in resistance vessels under physiological conditions are probably mechanical, pulsative flow and shear stress being important. The occupation of receptors by acetylcholine, substance P, and brady-

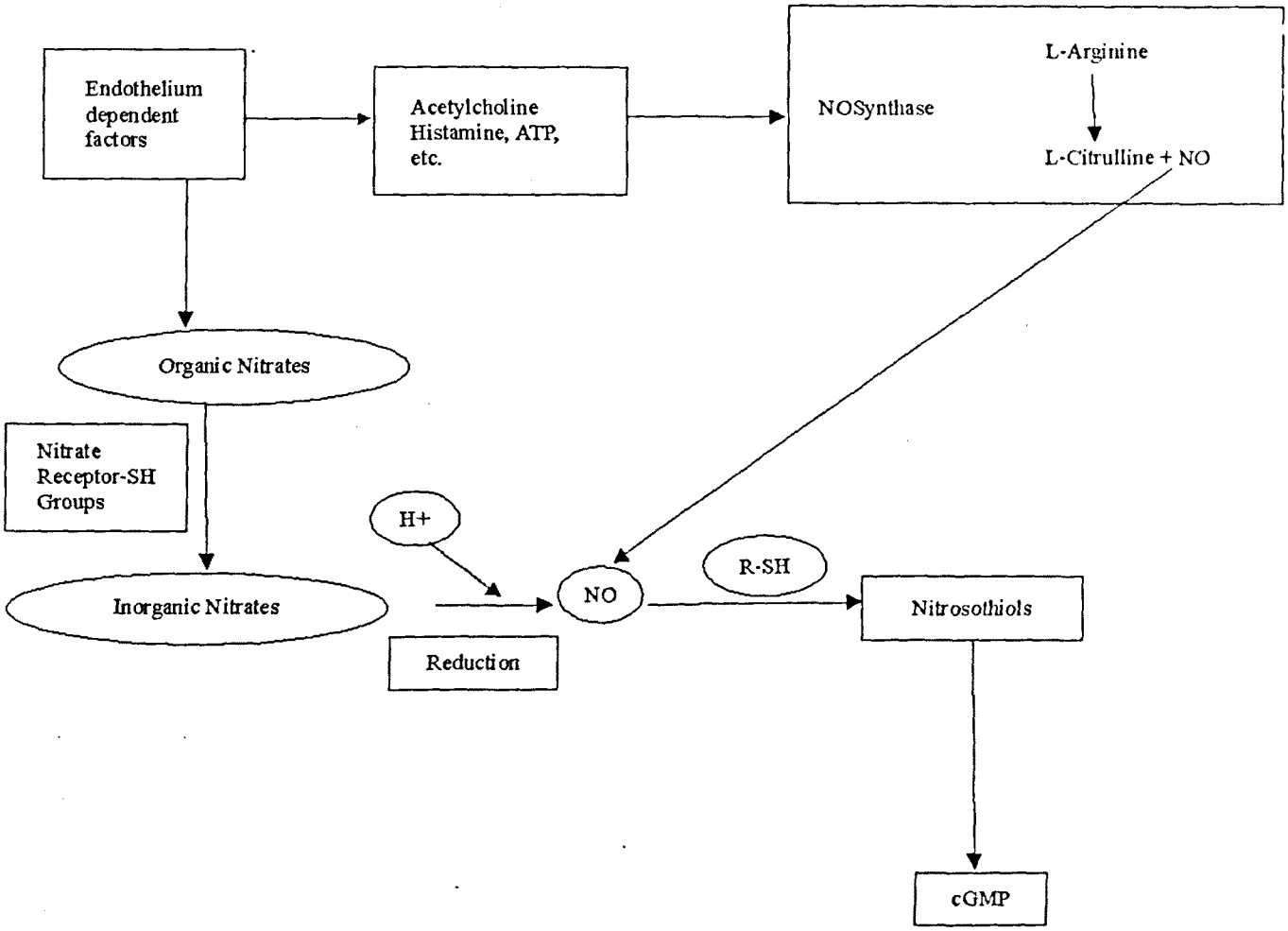

Fig. 1: Mode of release of NO

The neuromodulators convert L-arginine to L-citrulline by activating NO synthase. NO released will conjugate with thiol group of NO receptors and forms nitrosothiols which will activate soluble guanylate cyclase. The organic nitrates also act in the same way after reduction from inorganic nitrate to NO.

kinin, which increases [Ca^{+2}] thereby stimulating NO biosynthesis. The nitrate receptor possesses -SH groups, which reduce nitrate to inorganic nitrate and nitric oxide. The formation of nitrosothiols can be proposed to stimulate guanylate cyclase, which leads to an increase in intracellular cGMP formation[11].

Mechanism of action of NO:

The physiologically most relevant action of NO is the activation of soluble guanylate cyclase by nitrosation of its haem moiety. The subsequent increase in cGMP level alters the activity of three main target proteins, cGMP-regulated ion channels, cGMP-regulated phosphodiesterases and cGMP-dependent proteinkinases[9,13] (fig. 2).

The molecular basis of the sensory systems of visualization and olfaction is related to the action of cation channels, which are regulated by cellular cGMP levels. These proteins have one binding site for cGMP. Binding of cGMP critically determines the function of these sensors. Activation of the vertebrate rod photoreceptor cells by light results in hydrolysis of cGMP. The drop in cellular cGMP concentration leads to closure of a cGMP-regulated cation channel with subsequent hyperpolarization of the cell; this is the primary neuronal response in visualization[14]. Activation of odorant receptors in olfactory cilia is likely to be connected to an inositol-trisphosphate-mediated rise in intracellular Ca^{2+} concentration and activation of a constitutive NOS. NO increases cGMP levels in adjacent neurons, which in turn mediates further activation of the odorant signaling pathway by opening Ca^{2+} channels[15].

Phosphodiesterases play a key role in controlling the actions of the secondary messengers cAMP and cGMP of the six family members of phosphodiesterases. Binding of cGMP to conserved non-catalytic cGMP binding domains directly regulates the enzyme activity of type II and type III proteins. Type II phosphodiesterases are stimulated by cGMP binding, whereas the type III enzymes are inhibited by cGMP. Therefore, cGMP can increase cAMP levels via inhibition of type III phosphodiesterase. In contrast, activation of type II enzymes increase cAMP hydrolysis and accordingly decrease cAMP concentrations[16].

Two major classes of cGMP-dependent protein kinases have been identified, the soluble type I (GKI) and the membrane-bound type II (GKII) enzymes. GKI is further subdivided in a and b isoforms. GKI exists as a dimer, whereas, GKII is a monomer. Protein analysis of GKI revealed a dimerization domain, which includes an autophosphorylation and an autoinhibitory site, two cGMP binding domains and a catalytic domain. GKII is predominantly expressed in the intestinal epithelial brush border and is involved in intestinal Cl^- absorption and secretion. GKI is expressed in various tissues and its role in physiology is much better characterized. In myocytes, activation of GKI modulates contractility by inhibiting an inward Ca^{++} current. In the kidney, GKI is expressed in different cell types such as messangial cells, smooth muscle cells and myofibroblasts. Functionally, it has been demonstrated that GKI regulates an amiloride-sensitive Na^+ channel in the inner medullary collecting duct. In the cerebellum, GKI is highly expressed in Purkinje cells. However, its role in these cells is not yet clear. Furthermore, in smooth muscle cells and platelets, GKI inhibits agonist-induced elevations in Ca^{2+} concentration and thus modulates smooth muscle contraction and platelet activation[13,16].

PHYSIOLOGICAL ACTIONS OF NO

Regulation of gut blood flow:

NO plays an important role in vascular tone within the GI circulation. Different physiological stimuli may participate in NO release, such as shear stress[17], changes in Ca^{+2} concentration[18] or changes in oxygen tension[19]. The stress produced on the endothelial luminal surface in response to increase in blood flow is suggested to be the most important physiological stimulus for endothelial NO release[20]. NO has been suggested to involve in resting gastric mucosal microcirculation[21] and also blood flow in the jejunum[22] and large intestine[23].

In the stomach, an increase in gastric mucosal blood flow caused by secretagogues, such as pentagastrin can be affected without any influence on acid secretion by administration of N^G-nitro-L-arginine-methylester (L-NAME) and this effect can be reversed by co-administration of L-arginine[24]. Back diffusion of H^+ leads to an increase in gastric blood flow, and this increase can be inhibited by L-NAME treatment, indicating the involvement of endogenous prostanoids in cirrhotic animals[25]. The gastric blood flow, in response to a nitrovasodilator that acts through NO release is impaired in these animals[26]. These findings suggest that NO and PG's can interact in the regulation of gastric blood flow in healthy and cirrhotic animals.

In the intestine, sympathetic vasoconstriction of intestinal arterioles is increased during NOS inhibition and this can be reversed by administration of L-arginine. NO is also involved in intestinal hyperemia in response to luminal application of certain foodstuffs. For example, glucose admin-

istration into rat ileal loops resulted in an increase in blood flow, which was blocked by L-NAME. The most likely source of this NO was considered to be the micro vascular endothelium[27]. Recently it has been suggested that constitutive NO synthase effectively counteracts the damaging actions on microvascular integrity of mediators, including thromboxanes, PAF, leukotrienes and vasopressin, released during surgical intervention[28].

Intestinal secretion:

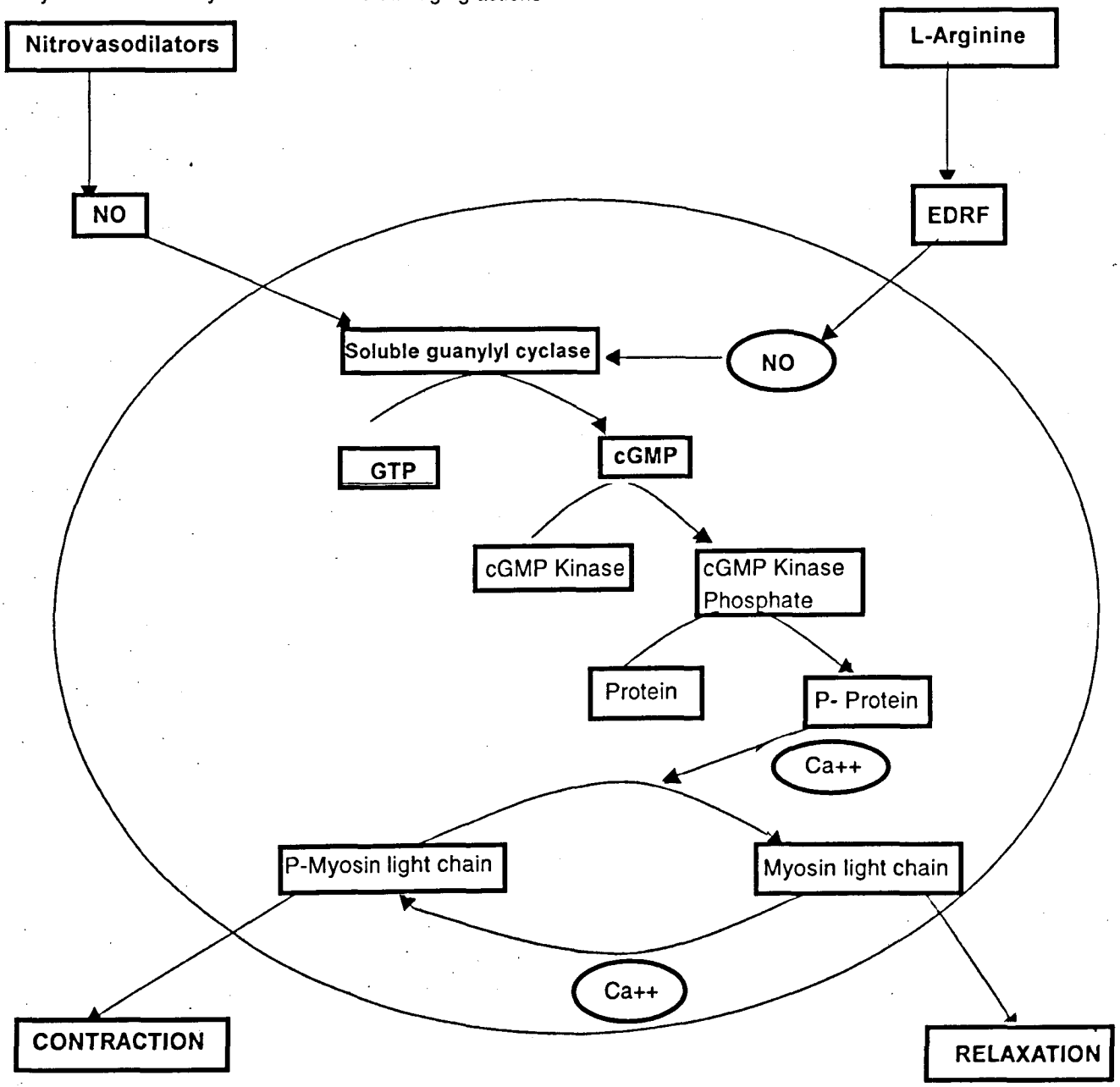

Fig. 2. Mechanism of action of NO.

The NO released in smooth muscle activates soluble guanylyl cyclase and converts guanosine triphosphate into cyclic guanosine monophosphate, which regulates phosphorylation and dephosphorylation of proteins. This controls the process of contraction and relaxation through myosin light chain mechanism.

In recent years, investigators have demonstrated that L-arginine as well as NOS inhibitors are able to influence intestinal secretion[29], but results using these agents are contradictory and depend on the segment of intestine studied, the animal species used and other experimental conditions, including *in vivo* or *in vitro*, intact or stripped tissues, and the type of challenge used. The possible role of NO in 5-HT-induced changes in electrolyte and fluid transport has been studied *in vivo*[30]. It has been demonstrated that L-NNA has inhibited 5-HT-induced fluid and electrolyte transport in jejunum and colon in anaesthetised rats.

Amino acids are actively transported in the small intestine and in general enhances water and electrolyte absorption[31], however the dibasic amino acids L-arginine has been shown to induce water secretion in human jejunum[32]. Electrogenic anion, chloride secretion and inhibition of neutral Na^+ absorption induced by sodium nitroprusside (SNP) appear to be mediated by enteric nerves and prostanoid producing cells of the sub-epithelium, the inhibition of neutral Cl^- absorption appears to be due to direct effect of SNP on the colonic epithelium, the increased release of NO from resident macrophages and mast cells, invading neutrophils, enteric nerves and other cells of the mucosa may play an important role in the production of diarrhea in variety of disease states via the stimulation of intestinal electrolyte transport[33].

In vitro studies have unveiled a role for NO as a secretagogue in the ileum[34] and colon[35]. *In vivo* studies have suggested a possible role for NO in the laxative actions and in the pathophysiology of secretion induced by *E. coli* heat stable enterotoxin[36]. The intestinal epithelium may be exposed to NO release from macrophages and mast cells of the lamina propria, enteric nerves, smooth muscles and endothelium. Therefore, NO may be an important mediator of processes affect intestinal epithelial function. This may be due to the ability of NO to stimulate soluble guanylate cyclase and increase the production of cGMP, a second messenger that acts as a potent secretory agent[36].

Gastric acid secretion:

Parenteral administration of NO donors has been shown to reduce vagally mediated acid secretion[37], as well as histamine-stimulated acid secretion[38]. It has also been noted that FK 409, a NO donor, inhibited pentagastrin stimulated acid secretion in doses that did not affect blood flow and that the effect was probably mediated to some extent, via suppression of histamine release from entero chromaffin cells[39]. It has also been demonstrated that high concentrations of NO donors can inhibit parietal cell activity *in vitro*, suggesting a direct action of NO in acid secretory activity[40]. These effects of endogenous and exogenous NO in acid secretion is complex and appears to depend on the prevailing physiological or pathological conditions.

Gastroduodenal secretion:

Gastric mucus forms a continuous visco-elastic layer over the mucosa. This layer is an important factor in mucosal protection against topical irritation by noxious agents in the lumen. NO donors such as isosorbide dinitrate and S-nitroso-N-acetyl penicillamine (SNAP) have been shown to increase mucus get thickness in rat stomach[41]. NOS inhibitors have been shown to reduce the ability of mucosal cells to secrete and synthesize mucous[42]. It has also been reported that L-arginine treatment could reverse the reduction in gastric cellular mucous secretion in response to hypoxia- reoxygenation *in vitro*[43]. NO has been demonstrated to mediate mucus secretion in response to a number of stimulatory agents[44]. Inhibition of NO synthesis reduced mucus secretion *in vivo* by approximately 20%. Inhibition of NOS activity has been shown to increase resting duodenal alkaline secretion to various degrees in experimental animals, suggesting that endogenous NO may modulate the basal secretion of HCO^-_3[45]. Using a technique that could simultaneously determine NO output and HCO_3^- secretion. It has been observed that luminal acid formation, increased NO output and this was inhibited by L-NMMA treatment[46]. Further more, L-NMMA also reduced the H^+-stimulated increase in alkaline secretion. Luminal administration of NO donors has been shown to increase mucosal HCO_3^- secretion in the dog[47].

Intestinal fluid and electrolyte transport:

The role of NO in intestinal fluid and electrolyte transport appears ambiguous when taking into consideration the studies concerning this matter. *In vitro* studies, applying Ussing chamber technique, have shown that serosal administration of NO or NO donating compounds to stripped intestinal tissue increases short circuit current. Thus indicating a prosecretory and antiabsorptive role of NO[33]. Recently it has been postulated an antisecretory effect of L-NNA on 5-HT-induced intestinal fluid and electrolyte transport in both the parts of jejunum and colon[30]

Conversely, inhibition of NOS *in vivo* resulted in gastric and duodenal HCO_3^-[48] and intestinal fluid secretion and in the enhancement of intestinal epithelial permeability[49], pointing towards an antisecretory and/or proabsorptive role of NO in the intestine. The mechanism of action underlying any

effect of NO on intestinal fluid and electrolyte transport remains unclear. Activation of guanylate cyclase and formation of cGMP is a commonly accepted transduction mechanism of NO[50], and some authors suggest that this mechanism is responsible also for the observed intestinal effect[33, 51]. Moreover, the participation of enteric nerves and arachidonic acid metabolites in the mediation of NO effects is proposed[52].

Gastric emptying:

Studies have shown that NO suppresses duodenal, antral, pyloric and fundic contractions[53]. In a study of the abnormality of GI motility associated with diabetes in experimental animals it was found that normal antral motility could be restored by the NO donors[54]. In humans, intraduodenal triglyceride administration has been shown to inhibit pyloric motility[55]. Administration of L-arginine and glyceryltrinitrate showed decrease in rate of gastric emptying[56] and decreased antral motor activity[57].

Mucosal protection:

NO donors have been shown to diminish thermal injury associated venule constriction within the GI tract[58]. Prophylactic administration of L-arginine improved intestinal barrier function after mesenteric ischemia in the rat[59]. In the intestine, administration of NO donors provides significant protection against the mucosal and microvascular dysfunction associated with ischemia reperfussion[60]. It has also been shown to be effective in reducing gastric lesion in response to water immersion stress[61] and ischemia followed by reperfusion[62]. It has also been noted that NO donors are also useful in overcoming gastric injury provoked by NSAIDs[63].

Intestinal motility:

The *in vitro* studies have shown that electrical stimulation of NANC nerves relaxes gastro intestinal smooth muscle, which was supported by *in vivo* study. The intravenous administration of NO synthase inhibitor, L-NAME produced intraheminal pressure in the jejunum and by direct observation in this acute preparation. The actions of L-NAME were inhibited by L-arginine but not by D-arginine[64].

The carbachol-induced increased intestinal transit was reduced by L-NAME but not by D-NAME[65]. L-arginine counteracted the effects of L-NAME, supports the involvement of NO. Bisacodyl and phenolphthalein increased transit through the gastrointestinal tract was inhibited by L-NAME in a stereospecific manner[66]. In case of castor oil treated rats, the increased transit was inhibited dose dependently by L-NAME and this was inhibited by L-arginine[67]. Sodium choleate increased the intestinal transit of charcoal meal was inhibited by L-NAME and was reversed by L-arginine and isosorbide-5-mononitrate (IMN)[68].

Endogenous nitric oxide can exert a physiological relaxant action that opposes the action of contractile mediators. *In vitro* studies demonstrated that this could stimulate a descending inhibitory reflex involving NO[69]. NO interacts with the part of neuronal mechanisms affecting the gastro intestinal musculature and hence may be involved in the physiological modulation of peristalsis and intestinal transit[70].

Recently the characteristics of the nitrergic neuronal network and its relationship with the smooth muscle fibres were studied, which provided a morphological basis for investigating intestinal motility disorders[71]. The role of NO in 5-HT-induced intestinal motility has also been demonstrated in rats[72]. The role of NO mechanisms in the regulation of fasting small intestinal activity in humans using a specific NO synthase inhibitor, L-NMMA has been studied. The duration of phase I activity was reduced after the intravenous infusion of L-NMMA than with saline[73].

The induction of diarrhea involves an increase in intestinal transit and/or an increased luminal fluid secretion. In pathophysiological states such as inflammatory bowel syndrome, colitis, ileitis, NO may be produced at a higher concentration, capable of evoking net secretion. An increase in intestinal transit is believed to be due to reduced resistance to flow, because of relaxation of the circular smooth muscle of the intestine[74]. This may soften the stool and promotes greater ease of fluid flow from proximal to distal gut segments[75] and also seems to be involved in relaxation of the gut during the peristaltic reflex[76].

Relaxation of lower esophageal sphincter:

A study has been done by using lower esophageal sphincter and found complete inhibition of NANC-induced relaxations with N^G-nitro-L-arginine (L-NNA)[77], the mechanism of NO-induced relaxation of GI smooth muscles is a subject of much investigation. A body of evidence suggests that NO may have some association with vasoactive intestinal peptide (VIP), a signaling peptide that has also been identified as a NANC mediator. However, other studies have also demonstrated that, although vagal activation evokes both VIP and NO release; there is no evidence for any interaction, and the relaxation induced by VIP does not involve NO[78].

Relaxation of ileocecal sphincter:

Field stimulation of the muscles caused release of a substance with chemical characteristic similar to NO, this substance was inactivated by haemoglobin and its release was inhibited by arginine analogues. Endogenous NO caused concentration dependent relaxation of muscles from the ileocolonic junction. L-NMMA also increased basal tension and this effect was partially reversed by L-Arginine but not by D-Arginine[79]. The canine ileocolonic sphincter was one of the first and has been one of the most thoroughly characterized preparation in terms of the role of NO as the NANC neurotransmitter.

Relaxation of intestinal, colon and anal sphincters:

In a study of intertaenial longitudinal muscle from the guinea pig colon, it has been noted that, L-NNA and haemoglobin reduced the amplitude of NANC-mediated relaxation[80]. L-NAME reduced NANC nerve induced relaxation in distal colon and small bowel by 30-40%[81]. Local distension of the small intestine and colon results in a neural reflex that causes the bowel to contract oral to the site of distension and relax distal to the distension.

PATHOPHYSIOLOGICAL ROLE OF NO

Diarrhea:

In some pathological conditions such as ulcerative colitis[82], trinitrobenzene sulfonic acid (TNBS)-induced colitis[83] and chronic ileitis, NO can be produced at higher concentrations capable of evoking net secretion. Such NO appears to contribute to the diarrheal response in TNBS-induced ileitis and the spontaneous colitis seen in human leukocyte antigen (HLA)-B27 transgenic rats, and in chronic colitis model in the rhesus monkey[84-86]. Sepsis is known to be associated with profound alterations in the synthesis of NO. The activity of the constitutive form of NO synthesis is increased within hours of endotoxemia; this is followed by an increase in the expression of the inducible form of NO synthase in various key organs including lung, spleen, liver, heart, and vascular endothelium[87]. Elevated activity of NO synthase is associated with the laxative action of several intestinal secretagogues, including castor oil, phenolphathalein, bisacodyl and magnesium sulphate. Studies using isoform selective and nonselective inhabitatos ameliorate intestinal secretion, depaending on the model evalluated. L-NAME or L-NMMA effectively reduce the diarrheal response to these laxatives listed above, but inhibitors of iNOS are only effective against bile salt- and cascara-mediated secretion[88]. The mechanism involved in diarrhea induced by laxative agents may involve the release of NO from epithelial cells, myenteric neurons and smooth muscles. The liberated NO may act on the epithelial cells to stimulate anion secretion and luminal fluid accumulation. Relaxation of the intestinal smooth muscles in concert with intraluminal fluid accumulation may lead to diarrhea (fig. 3)

The diarrhea may be associated with pathogenic factors with overproduction of NO. Activation of a calcium-dependent mechanism is responsible for the enhanced activity of NO synthase. Pathogenic factors increased calcium flux or mucosal permeability[89, 90] across intestinal brush border cells. Since the constitutive form of NO synthase is calcium calmodulin-dependent, these agents may increase the permeability of the epithelial layer to calcium ions, leading to an increase in intracellular calcium and thus enhance calmodulin stimulation of NO synthase activity[91].

L-NAME has inhibited the sodium choleate-induced diarrhea in rats[70]. The laxative action of magnesium sulphate had been reversed by the administration of L-NAME[92]. Intraperitoneal administration of L-NAME and L-NMMA has inhibited or prevented castor oil- induced diarrhea in the rats[93]. Bisacodyl and phenolphthalein produced diarrhea, that was delayed in onset by intraperitoneal administration of NO synthase inhibitor, L-NAME and the effect was reversed by the NO donors, isosorbide-5-mononitrate and NO precurssor L-arginine[68]. Recently the involvement of NO in 5-HT-induced diarrhea has been demonstrated in fasted mice[80].

Intestinal inflammatory disease:

Induction of Ca^{+2}-dependent colonic NOS has been observed in a model of inflammatory bowel disease induced by TNBS in the rat with in the initial 3 d after challenge[94]. In other studies, levels of nitrite in the luminal lavage of the inflammed guinea pig ileum were elevated when measured 7 d following TNBS administration[84]. Administration of L-NAME in the drinking water reduced the inflammatory response as determined by myeloperoxidase levels and lowered both protein and nitrite levels in the lavage fluid[84], supporting a role for NO in the inflammatory process. Elevated plasma of nitrate and nitrite were also observed in the chronic but not the acute phases of colonic inflammation induced by bacterial wall polymers in the rat[95].

Nitrite levels in rectal dialysates were elevated in patients with active ulcerative colitis[97]and augmented levels of citrulline have been detected in biopsies of inflamed human colon[98]. In more direct studies on colonic NOS in inflammatory bowel diseases, a six-fold increase in enzyme activity

that was calcium independent was found in colonic mucosal biopsies from patients with ulcerative colitis[81]. The cellular location of the iNOS in inflammed colonic mucosa is not yet defined, but may reflect activity of infiltrating or resident inflammatory cells or the induction of NOS in the other mucosal cells, including epithelial cells. Such NO may contribute to the vascular injury and other inflammatory signs associated with ulcerative colitis.

CONCLUSION

The role of NO may depend on the nature of the tissue, the environment involved, the local response and interac-

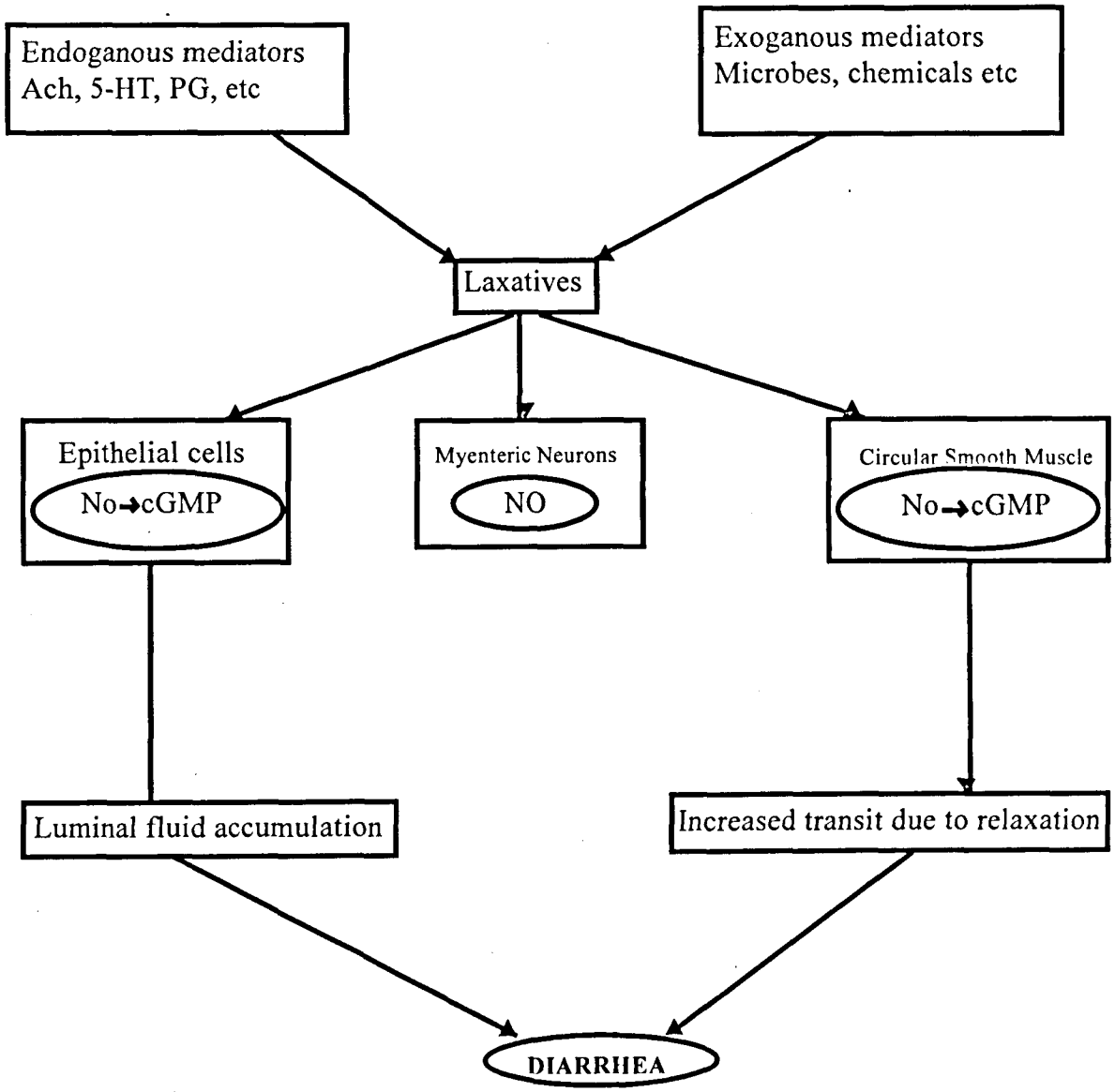

Fig. 3: Mechanism of involvement of NO in diarrhea.

The nitric oxide released from endogenous and exogenous factors either by increasing luminal fluid accumulation, which soften the stool, or by relaxation of circular smooth muscle leads to an increased intestinal transit.

tion with the other mediators. The products of iNOS may have a greater role in the early phase of inflammation, such as inflammatory bowel disease, but lower level of iNOS activity could have a beneficial action in the process of resolution of the inflammatory response. It appears that NO pathway may offer a number of diverse therapeutic opportunities for diseases of the gut. The use of NO production inhibitors has been evaluated for antiinflammatory properties in the gut. The use of NO donors in a range of motility problems is under investigation. The therapeutic value of NSAIDs containing NO is under experimental and clinical evaluation. A large number of studies that have been aimed at evaluating the mechanism of relaxation of NO in GI smooth muscles have only indicated that still more studies are needed before completely understanding its role. NO can surely play an important role in future in clinical gastroenterology in the treatment of a number of GI disorders.

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