Role of nitric oxide in intestinal water and electrolyte transport

Since Palmer et al and Ignarro et al showed that vascular endothelial cells could synthesise nitric oxide (NO), this soluble gas has emerged as an important mediator, messenger and regulator of cell function in a number of physiological systems and pathophysiological states. The effect of NO on the intestinal epithelium, the local microcirculation, the enteric nervous system, and inflammatory cascades has implicated it as a potential mediator of intestinal water and electrolyte transport. Data produced by different groups over the past few years have been contradictory, some showing NO as an absorbatagogue and others as a secretagogue.

**Biology of nitric oxide and its source in the intestine**

In biological systems, NO has a half life of less than 5 seconds, rapidly degrading to nitrite and nitrate in the presence of oxygen and water. Being soluble in both water and lipid, it freely traverses cell membranes and passes into adjacent target cells. The potential sources of NO in the gut are the endothelial cells, the intrinsic intestinal tissue (mast cells, epithelium, smooth muscle, neurones), residing and infiltrating leucocytes (neutrophils and monocytes), reduction of luminal gastric nitrates, and to a lesser extent denitrification by commensal intestinal bacteria. Nitric oxide is formed from l-arginine by the action of a stereospecific group of enzymes called nitric oxide synthases (NOS). In the gut, NOS have been localised in the myenteric and submucosal neurones in the subepithelial compartment and lamina propria including submucosal arteries and villous, and in the apical epithelial cells. Nitric oxide can be produced by enteroctyes through both the constitutive and the inducible NOS.

Nitric oxide has been considered as a regulator of basal intestinal water transport, as a mediator of pathological conditions such as ischemia-reperfusion, or indirect through the enteric nervous system or the regulation of intestinal blood flow is still not completely clear (fig 1, 1–4). In vivo studies are more controversial. Even before the discovery of NO, Hegarty et al and Hellier et al showed that l-arginine, unlike other amino acids, induced water secretion in ileum, implying that NO has a proabsorptive effect. Li et al found that a saturated NO solution had no effect on Isc in rat ileum whereas other investigators demonstrated an increase in Isc after serosal addition of the NO donors sodium nitroprusside (SNP), isosorbide dinitrate (ISDN), S-nitroso acetyl penicillamine (SNAP), or saturated NO solutions to guinea pig ileum, rat ileum, and human colon, suggesting that at high doses NO has a secretory effect (fig 1, A–C).

**Effect of nitric oxide donors on intestinal water and electrolyte transport**

The effect of NO donors on small and large intestinal water and electrolyte transport depends on the route of administration and the method used to study the effect. In vitro studies showed that addition of sodium nitrite to the serosal side of mouse ileum resulted in a decrease in Isc, implying that NO has a proabsorptive effect. Li et al showed that a saturated NO solution had no effect on Isc in rat ileum whereas other investigators demonstrated an increase in Isc after serosal addition of the NO donors sodium nitroprusside (SNP), isosorbide dinitrate (ISDN), S-nitroso acetyl penicillamine (SNAP), or saturated NO solutions to guinea pig ileum, rat ileum, and human colon, suggesting that at high doses NO has a secretory effect (fig 1, A–C).

In vivo studies are more controversial. Even before the discovery of NO, Hegarty et al and Hellier et al showed that l-arginine, unlike other amino acids, induced water secretion when perfused in human jejunum. In addition, Thomas et al showed, 15 years ago, that SNP given subcutaneously induced fluid accumulation in mouse intestine. Similarly, we found that intraluminal infusion of l-arginine...
Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) from L-arginine to NO and citrulline. The release of NO is known to modulate several physiological processes through its actions on the enteric nervous system, smooth muscle, and inflammatory cells. NO can either stimulate or inhibit various cell functions.

**Role of nitric oxide in hypersecretory states**

The role of NO in intestinal secretory states has been studied in relation to enterotoxins, serotonin, some inflammatory mediators, bile acids, and laxatives. Rolfe et al. and Hayden et al. found that NOS inhibition had no effect on changes in Isc induced by Escherichia coli heat stable toxin (STa) in stripped rat ileum and pig jejunum and colon. However, 1-NAME notably decreased Isc in unstripped ileum and decreased fluid secretion caused by STa in vivo implying a significant role for NO in STa induced secretion. By contrast, Shirgi-Degen et al. found that STa induced secretion in ligated rat jejunal loops was increased by intravenous L-NAME and inhibited by intravenous L-arginine and SNP and therefore concluded that NO has a pro-absorptive tone in the intestine. Similar controversy is observed with cholera toxin (CT). Beubler et al. recently found that intravenous L-NAME enhanced CT induced fluid secretion in ligated rat jejunal loops whereas intravenous L-arginine inhibited it. Qiu et al. used a similar model of ligated rat jejunal loops but found no change in CT induced fluid secretion after administration of NOS inhibitors or NO donors. Using rat jejunum in situ, we found that the NOS inhibitors l-NAME and l-NMA, and the NO precursor l-arginine caused a reduction in CT induced secretion, implying a dual role for NO as a secretagogue and absorbagogue. Finally, Reddi et al. demonstrated that CT enhanced basal nitrite level in guinea pig ileum mounted in Ussing chambers and that l-NAME inhibited CT induced secretion during the first 30 minutes of exposure (fig 2).

5-hydroxytryptamine (5-HT) is a neurotransmitter and a potent intestinal secretagogue released from enterochromaffin cells by CT and plays an important role in the pathogenesis of CT induced secretion. Kadokawa et al. demonstrated that inhibition of NOS ameliorated 5-HT induced chloride secretion in guinea pig distal colon in vitro and noticeably decreased 5-HT induced diarrhoea in mice; both effects were reversed by the NO precursor l-arginine. These investigators and others concluded that NO may play an important role in the secretory response to 5-HT which could be partly due to the activation of neurones that generate NO. By contrast, Beubler et al. found that intraarterial 5-HT induced secretion was inhibited by l-arginine.

Nitric oxide production is increased in inflammatory bowel disease (IBD) but whether this plays a role in the pathogenesis of IBD or in the associated diarrhoea has not been elucidated. Recently, an increase in the production of interleukin-1 (IL-1) in the mucosa of patients with IBD has been demonstrated. IL-1 causes colonic water secretion in rats, and its effects on endothelial cells are mediated by local synthesis and release of NO. In this context, Bytovene et al. showed that IL-1β induced fluid secretion in rat colon could be inhibited by intraperitoneal administration of l-NMA, an effect reversed by l-arginine. These results imply that increased IL-1 production in IBD could cause colonic secretion by activating NO and releasing NO, playing a final role in IL-1 induced hypersecretion.

Bile acids stimulate fluid and electrolyte secretion in jejunum, ileum and colon in both animals and humans. Mascolo et al. showed in a rat model that bile salt induced diarrhoea and intestinal fluid secretion could be inhibited by intraperitoneal l-NAME (9–90 × 10⁻⁵ mmol/kg) and dexamethasone. This inhibition was reversed by l-arginine and isosorbide-5-mononitrate (ISMN). Induction of NO formation by bile salts has also been observed in human colon.
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The exact mechanism of intestinal secretion of many laxatives is not understood. Mascolo et al and Capasso et al demonstrated that castor oil induced diarrhoea and intestinal secretion could be notably decreased by NOS inhibition in rats, an effect that could be reversed by L-arginine, implying that NO is involved in the laxative action of castor oil. Similar results were obtained with other laxatives including magnesium sulphate, bisacodyl, phenolphthalein, senna, and cascara. By contrast, Beubler et al came to the conclusion that NO is important in the antidiarhoeic effect of loperamide and showed that loperamide could reverse the secretory effect of L-NAME. They also showed that loperamide induced NO formation in freshly prepared jejunal epithelial cells and therefore could exert its proabsorptive effect via this route.

Possible mechanisms of action of nitric oxide

Nitric oxide could be involved in intestinal water transport either by acting directly on the epithelium or indirectly by stimulating neuronal reflexes, or by stimulating the release of other agents from the epithelium or the enteric nervous system that can modify water transport, or by affecting mucosal blood flow.

Nitric oxide can activate soluble guanylate cyclase resulting in an increase in cGMP, a potent activator of intestinal secretion. SNP and NO gas evoke electrolyte secretion in rat small bowel and colon associated with increased cGMP levels; this phenomenon is inhibited by the guanylate cyclase inhibitor, methylene blue, implying a significant stimulation of soluble guanylate cyclase in the intestine by NO (fig 3).

Nitric oxide stimulates cyclooxygenase activity directly, independently of cGMP and Wilson et al reported a significant increase in cGMP and prostaglandin (PG) E2 production in colonic mucosal strips stimulated with SNP. Cyclooxygenase inhibitors notably attenuate changes in Isc induced by NO donors in animal small intestine and colon and human colon.

Nitric oxide could also produce effects on water transport by its action on enteric neurones. Neuronal inhibition by tetrodotoxin inhibits the secretory effect of NO donors, but whether NO has a direct stimulatory effect on the enteric nervous system or through activation of cGMP as a second neuronal messenger needs to be investigated further (fig 1B). Rolfe et al concluded that STa increases electrogenic Cl− secretion across intact rat ileum in vitro by activating a capsaicin sensitive (afferent) and a NO dependent (efferent) myenteric plexus secretory reflex, proposing NO as a neuronal secretagogue (fig 2).

Vasoactive intestinal polypeptide (VIP) is present in enteric neurones and has been proposed as a stimulatory transmitter of secretory processes in the submucous plexus and the mucosa. It has been demonstrated that VIP release from rat enteric synaptosomes can be stimulated by the NO donors SNP and 3-(morpholino) sydnonimine (SIN-1), as well as by L-arginine. Similarly, in isolated perfused canine ileum, VIP output was reduced by L-NAME and increased by NO donors, so NO can exert a secretory effect by releasing VIP from nerve terminals. Furthermore, VIP releases NO, establishing the scenario that these two secretagogues could act synergistically, with NO potentially amplifying VIP's biological effects.

Nitric oxide is also known to modulate free radical generation and is capable of combining with free radicals such as peroxide to form the highly toxic peroxynitrite which may have an effect on epithelial cell membrane lipid peroxidation. This could alter normal physiological regulation of electrolyte transport in the small intestine and colon. Oxyradicals have been shown to stimulate intestinal electrolyte transport in rabbit and rat intestine and thus NO and other nitrogen oxides could stimulate intestinal secretion by their free radical structure. NO and other nitrogen oxides could stimulate intestinal secretion secondary to a profound inhibition of water and sodium absorption by villus whereas the intact crypt cells continue to secrete sodium, chloride and water. This effect of NO on blood flow could play a role in modulating intestinal water transport (figs 1 and 2).

Nitric oxide is known to inhibit mast cell degranulation, and as mast cells and enterochromaffin cells degranulate by a similar calcium dependent mechanism, it is postulated that NO would also inhibit enterochromaffin cell degranulation. CT induced depletion of tissue 5-HT concentrations was prevented by L-arginine implying that the elevated levels of NO inhibit enterochromaffin cell degranulation and may actually, under these circumstances, result in the failure of activation of 5-HT dependent secretory pathways (fig 2B) (personal communication).

Finally, Shirigi-Degen et al showed that NO could activate basolateral K+ channels, an effect which may mediate its proabsorptive properties (fig 3).

Summary

Nitric oxide acting at many different sites in the intestine with some opposing effects could explain the controversy in the literature on the final effect of NO on water transport. It seems that NO could act as both an absorbagogue and a secretagogue depending on the circumstances.
and on the site of delivery. Studies suggest that physiologically NO promotes fluid absorption, but in pathophysiological states it may be produced in high concentrations leading to net secretion. The development of selective NO inhibitors will assist in distinguishing out these different aspects of NO function. Ultimately, the inhibition of the pathological rather than protective effects may yield therapeutic benefits. Until that time, however, it remains to be seen whether NO is a mucosal “friend” or “foe”.

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