Role of nitric oxide in intestinal water and electrolyte transport

Since Palmer et al andIgnarro 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 absorptive 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, neurons), 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 neurons in the subepithelial compartment and lamina propria including submucosal arterioles and venules, and in the apical epithelial cells. Nitric oxide can be produced by enterocytes 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 role, and as an effector substance in both lactic and anti-diarrhoeal agents.

Role of nitric oxide in basal water transport

Our understanding of the physiological action of NO comes from studies on the effects on water and electrolyte movement of substances that inhibit NOS and thus prevent NO release. Serosal addition of the NOS inhibitors N\(^{-}\)-nitro-L-arginine (L-NAME) or N\(^{-}\)-nitro-L-arginine (L-NNA) (0.01–0.3 mM) to unstripped mouse ileum mounted in Ussing chambers resulted in an increase in short circuit current (Isc) which was reversed by L-arginine (0.1–10 mM) implying a net proabsorptive effect for NO on ion transport. However, Rolfe et al and Eutamene et al found no effect of NOS inhibition on water transport in rat ileum in vitro and in rat colon in vivo, respectively. Others found that NOS inhibition decreased intestinal fluid absorption in canine Thirty-Vella fistula and rat ileum. Furthermore, N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME) induced net water secretion in rabbit ileum when given intra-arterially (0.07 × 10\(^{-3}\) mmol/kg/min), in rat jejunum when given intravenously (0.02–4 × 10\(^{-3}\) mmol/kg/min), and in guinea pig ileum when given orally for seven days. Intraperitoneal and intraluminal administration of a high dose of L-NAME (0.37 mmol/kg and 1–20 mM, respectively) resulted in net water secretion in rat jejunum which was associated with intestinal ischaemia, both effects being prevented by L-arginine. It seems from these studies that NO has a physiological proabsorptive effect in the small intestine but whether this is a direct effect on enterocytes, or indirect through the enteric nervous system or the regulation of intestinal blood flow is still not completely clear (fig 1, 1–4).

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 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 small intestine, rat ileum, rat 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

**Abbreviations used in this paper:** NO, nitric oxide; NOS, nitric oxide synthase; 1-NAME, N\(^{-}\)-nitro-L-arginine; 1-NNA, N\(^{-}\)-nitro-L-arginine methyl ester; SNP, sodium nitroprusside; ISDN, isosorbide dinitrate; SNAP, S-nitroso acetyl penicillamine; StA, Enterobacter cloacae heat stable toxin; CT, choleragenoid; 5-HT, 5-hydroxytryptamine; IBD, inflammatory bowel disease; IL, interleukin; ISM, isosorbide-5-mononitrate; PG, prostanoid; VIP, vasoactive intestinal polypeptide; SIN, 3-(morpholino) sydnonimine; GC, guanylate cyclase.
The 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, l-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 proabsorptive 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. Qi 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, Reddix et al demonstrated that CT increased 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 intra-arterial 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, Butamene 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 NOS 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 × 10⁻³ mmol/kg) and dexamethasone. This inhibition was reversed by l-arginine and isosorbide-5-mononitrile (ISMN). Induction of NO formation by bile salts has also been observed in human colon.

![Figure 1: Putative mechanisms of action of nitric oxide (NO) as a mediator of intestinal water and electrolyte transport under resting conditions.](http://gut.bmj.com/)

![Figure 2: Putative mechanisms of action of nitric oxide (NO) as a mediator of intestinal water and electrolyte transport in hypersecretory states.](http://gut.bmj.com/)
Role of nitric oxide in intestinal water and electrolyte transport

The exact mechanism of intestinal secretion of many laxatives is not understood. Mascoco 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 antidiarrhoeic 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 enterochromaffin system that can modify water transport, or by a direct effect on the epithelium. Nitric oxide is a vasodilator and its continuous effect on blood flow could play a role in modulating intestinal water transport (figs 1 and 2).

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 a free radical mechanism. Tamai et al demonstrated that the chloride transport blocker, bumetanide, inhibited the NO induced increase in Isc in rat colon, implying that NO exerts its secretory effect by opening chloride channels (fig 3).

Nitric oxide is a vasodilator and its continuous endogenous production is important in maintaining mesenteric microcirculation and mucosal integrity. NOS inhibition leads to a significant decrease in mesenteric blood flow. It is well known that vasoconstrictor agents, like angiotensin II and noradrenaline, cause decreased intestinal perfusion, and therefore could exert its proabsorptive effect via this route. 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 absorptive and a secretagogue depending on the circumstances.
and on the site of delivery. Studies suggest that physiologically 
NO promotes fluid absorption, but in pathophysi- 
ological states it may be produced in high concentrations 
leading to net secretion. The development of 
selective NOS inhibitors will assist in dissecting out the 
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”.

F H MOURAD
Department of Medicine,
American University of Beirut,
Beirut, The Lebanon

Dietary Digestes Research Centre,
St Bartholomew’s and The Royal London
School of Medicine and Dentistry,
Turner Street, London E1 2AD, UK

Correspondence to: Dr Turvill.

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