Gut

Leading article

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.[1–4] 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.[5] Data produced by different groups over the past few years have been contradictory, some showing NO as an absorbagogue 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.[1] Being soluble in both water and lipid, it freely traverses cell membranes and passes into adjacent target cells.[6] 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.[5] Nitric oxide is formed from L-arginine by the action of a stereospecific group of enzymes called nitric oxide synthases (NOS).[7] In the gut, NOS have been localised in the myenteric and submucosal neurones in the subepithelial compartment and lamina propria including submucosal arterioles and venules, and in the apical epithelial cells.[8–12] Nitric oxide can be produced by enterocytes through both the constitutive and the inducible NOS.[13 14]

Nitric oxide has been considered as a regulator of basal intestinal water transport, as a mediator of pathological conditions where disturbance in water transport plays a role, and as an effector substance in both laxatives and antidiarrhoeal 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^G-methyl-L-arginine (L-NMA) or N^G-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 (*I*sc) which was reversed by L-arginine (0.1–10 mM) implying a net proabsorptive effect for NO on ion transport.[15] 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.[16 17] Others found that NOS inhibition decreased intestinal fluid absorption in canine Thiry-Vella fistula and rat ileum.[18 19] Furthermore, N^G-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 ($0.02–4 \times 10^{-3}$ mmol/kg/min), and in guinea pig ileum when given orally for seven days.[20–23] 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.[24] 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 *I*sc, implying that NO has a proabsorptive effect.[15] Li *et al* found that a saturated NO solution had no effect on *I*sc in rat ileum whereas other investigators demonstrated an increase in *I*sc 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).[16 25–29]

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.[30 31] In addition, Thomas *et al* showed, 15 years ago, that SNP given subcutaneously induced fluid accumulation in mouse intestine.[32] Similarly, we found that intraluminal infusion of L-arginine

Abbreviations used in this paper: NO, nitric oxide; NOS, nitric oxide synthase; L-NMA, N^G-methyl-L-arginine; L-NNA, N^G-nitro-L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; SNP, sodium nitroprusside; ISDN, isosorbide dinitrate; SNAP, S-nitroso acetyl penicillamine; STa, *Escherichia coli* heat stable toxin; CT, cholera toxin; 5-HT, 5-hydroxytryptamine; IBD, inflammatory bowel disease; IL, interleukin; ISMN, isosorbide-5-mononitrate; PG, prostaglandin; VIP, vasoactive intestinal polypeptide; SIN, 3-(morpholino) sydnonimine; GC, guanylate cyclase.

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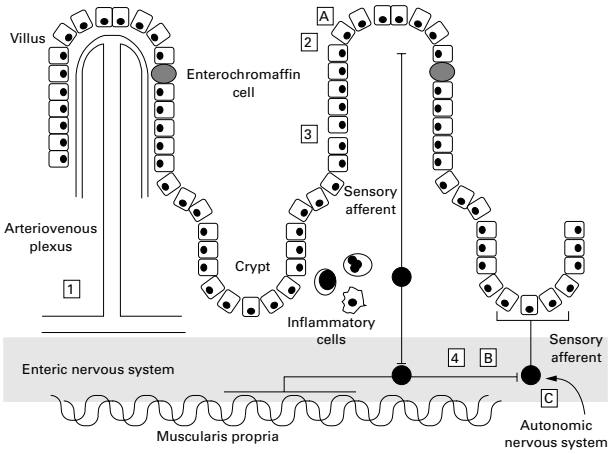

Figure 1 Putative mechanisms of action of nitric oxide (NO) as a mediator of intestinal water and electrolyte transport under resting conditions. The release of background levels of NO modulates absorption by maintaining microvascular villus perfusion (1); by a direct action on epithelial cell function (2) and tight junction integrity (3); and by the activation of neurotransmission within the enteric nervous system (4). Raised NO concentrations mediate secretion by a direct action on epithelial cell function (or by cyclooygenase- and 5-hydroxytryptamine dependent pathways) (A); and by neurotransmission within the enteric nervous system (B) and the autonomic nervous system (C).

(20 mM) into rat jejunum induced water and electrolyte secretion which could be inhibited by a low concentration of L-NAME (0.1 mM); however, subcutaneous administration of L-arginine (0.47 and 2.37 mmol/kg) had no effect.[26] Furthermore, Shirgi-Degen *et al* found that intravenous L-arginine (0.04 mmol/kg/min) or SNP (0.07×10^{-3} mmol/kg/min) had no effect on basal water transport in rat jejunum, whereas other investigators showed that L-arginine and other NO donors promoted water absorption in rabbit ileum and canine jejunum.[20 21 24] In addition, Wapnir *et al* have shown recently that L-arginine added to oral rehydration solutions could improve water absorption when perfused in rat jejunum.[33] These studies suggest that the predominant role for NO is as a secretagogue in both the small intestine and colon, an effect clearly demonstrated in vitro but with contradictory results in vivo.

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 *I*sc induced by *Escherichia coli* heat stable toxin (STa) in stripped rat ileum and pig jejunum and colon.[16 34] However, L-NAME notably decreased *I*sc in unstripped ileum and decreased fluid secretion caused by STa in vivo implying a significant role for NO in STa induced secretion.[16] 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.[21] 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.[35] 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.[36] 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.[37] 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).[38]

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.[39–41] Kadowaki *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.[42] 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.[43] By contrast, Beubler *et al* found that intra-arterial 5-HT induced secretion was inhibited by L-arginine.[35]

Nitric oxide production is increased in inflammatory bowel disease (IBD)[43 45] 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.[46] IL-1 causes colonic water secretion in rats, and its effects on endothelial cells are mediated by local synthesis and release of NO.[47 48] In this context, Eutamene *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.[17] 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.[49] Mascolo *et al* showed in a rat model that bile salt induced diarrhoea and intestinal fluid secretion could be inhibited by intraperitoneal L-NAME ($9–90 \times 10^{-3}$ mmol/kg) and dexamethasone.[49] 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.[50]

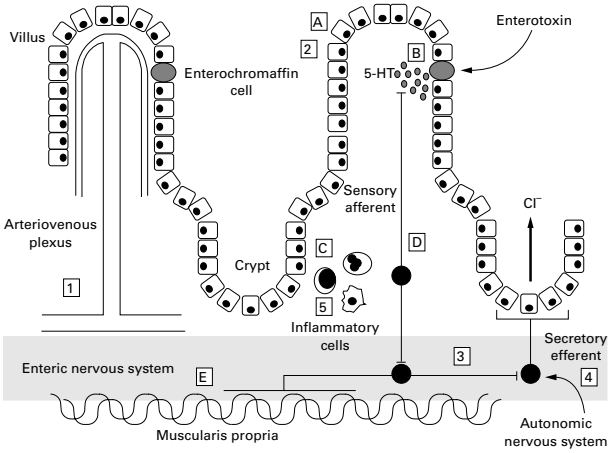

Figure 2 Putative mechanisms of action of nitric oxide (NO) as a mediator of intestinal water and electrolyte transport in hypersecretory states. The inhibition of NO release inhibits secretion by: modulating microvascular villus perfusion (1); a direct action on epithelial cell function (2); the activation of neurotransmission within the enteric nervous system (myenteric plexus) (3) and the autonomic nervous system (4); and by the inhibition of free radical formation (5). Increased levels of NO inhibit secretion by a direct action on epithelial cell function (A) by: the stabilisation of enterochromaffin cells (B) and mast cells (C); inhibiting 5-hydroytryptamine (5-HT) induced secretion (D); and by inhibiting intestinal motility (E).

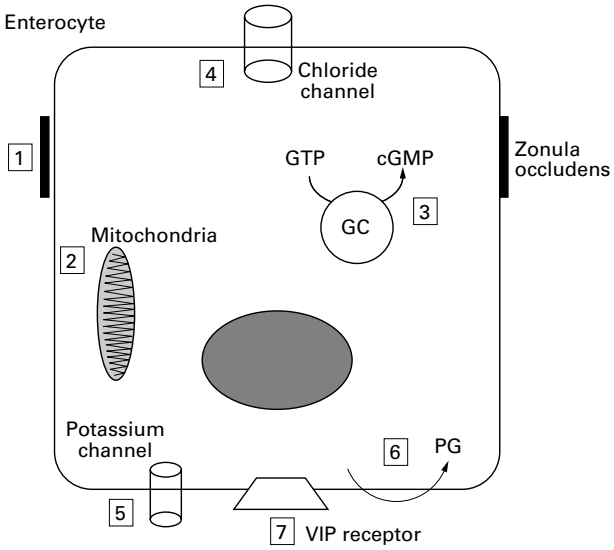

Figure 3 Putative actions of nitric oxide (NO) on the enterocyte. Actions of NO on the enterocyte include the maintenance of tight junctional integrity (1); the modulation of cell respiration (2); the activation of soluble guanylate cyclase (GC) (3); the opening of apical chloride channels (4); the activation of basolateral potassium channels (5); the stimulation of cyclooxygenase activity to generate prostaglandins (PG) (6); and the modulation of receptor activity (7).

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.[51 52] Similar results were obtained with other laxatives including magnesium sulphate, bisacodyl, phenolphthalein, senna, and cascara.[53–55] 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.[21] 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.[56–60] 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).[12 16 26–28]

Nitric oxide stimulates cyclooxygenase activity directly, independently of cGMP and Wilson *et al* reported a significant increase in cGMP and prostaglandin (PG) E_2 production in colonic mucosal strips stimulated with SNP.[12 61] Cyclooxygenase inhibitors notably attenuate changes in *I*sc induced by NO donors in animal small intestine and colon and human colon.[26–29]

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).[16 27–29 62] 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).[16]

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.[63 64] 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.[65] Similarly, in isolated perfused canine ileum, VIP output was reduced by L-NNA and increased by NO donors, so NO can exert a secretory effect by releasing VIP from nerve terminals.[66] Furthermore, VIP releases NO, establishing the scenario that these two secretagogues could act synergistically, with NO potentially amplifying VIP's biological effects.[67]

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.[68] 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 the nature of their free radical structure.[69–71] Tamai *et al* demonstrated that the chloride transport blocker, bumetanide, inhibited the NO induced increase in *I*sc in rat colon, implying that NO exerts its secretory effect by opening chloride channels (fig 3).[28]

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.[72 73] It is well known that vasoconstrictor agents, like vasopressin, cause water and electrolyte secretion in the small intestine.[74] In addition, Robinson *et al* showed that intestinal ischaemia causes net water and electrolyte 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.[75 76] This effect of NO on blood flow could play a role in modulating intestinal water transport (figs 1 and 2).[24]

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.[77] 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, Shirgi-Degen *et al* showed that NO could activate basolateral K^+ channels, an effect which may mediate its proabsorptive properties (fig 3).[78]

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 capable of evoking net secretion. The development of selective NOS inhibitors will assist in dissecting 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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1 Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;**327**:524–6.
2 Ignarro LJ, Buga GM, Wood KS, *et al*. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987;**84**:9265–9.
3 Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;**43**:109–42.
4 Moncada S, Higgs EA. Endogenous nitric oxide: physiology, pathology and clinical relevance. *Eur J Clin Invest* 1991;**21**:361–74.
5 Salzman AL. Nitric oxide in the gut. *New Horizons* 1995;**3**:33–45.
6 Henry Y, Lepoivre M, Drapier J, *et al*. EPR characterisation of molecular targets for NO in mammalian cells and organelles. *FASEB J* 1993;**1**:1124–34.
7 Lowenstein C, Dinerman J, Snyder S. Nitric oxide, a physiologic messenger. *Ann Intern Med* 1994;**120**:227–37.
8 Kostka P, Jang E, Watson EG, *et al*. Nitric oxide synthase in the autonomic nervous system of canine ileum. *J Pharmocol Exp Ther* 1993;**264**:234–9.
9 Llewellyn-Smith IJ, Song Z-M, Costa M, *et al*. Ultrastructural localisation of nitric oxide synthase immunoreactivity in guinea-pig enteric neurons. *Brain Res* 1992;**577**:337–42.
10 Nichols K, Staines W, Rubin S, *et al*. Distribution of nitric oxide synthase activity in arterioles and venules of rat and human intestine. *Am J Physiol* 1994;**267**:G270–5.
11 Nichols K, Staines W, Krantis A. Nitric oxide (NO) synthase distribution in the rat intestine: a histochemical analysis. *Gastroenterology* 1993;**105**:1651–61.
12 Wilson KT, Vaandrager AB, De Vente J, *et al*. Production and localisation of cGMP and PGE_2 in nitroprusside-stimulated rat colonic ion transport. *Am J Physiol* 1996;**270**:C832–40.
13 Blachier F, Darcy-Vrillon B, Sener A, *et al*. Arginine metabolism in rat enterocytes. *Biochim Biophys Acta* 1991;**1092**:304–10.
14 Tepperman BL, Brown JF, Whittle BJR. Nitric oxide synthase induction and intestinal cell viability in rats. *Am J Physiol* 1993;**265**(2 pt 1):G214–18.
15 Rao R, Riviere P, Pascaud X, *et al*. Tonic regulation of mouse ileal ion transport by nitric oxide. *J Pharmacol Exp Ther* 1994;**269**:626–31.
16 Rolfe V, Levin RJ. Enterotoxin Escherichia coli STa activates a nitric oxide dependent myenteric plexus secretory reflex in rat ileum. *J Physiol* 1994;**475**:531–7.
17 Eutamene H, Theodorou V, Fioramonti J, *et al*. Implication of NK1 and NK2 receptors in rat colonic hypersecretion induced by interleukin 1β: role of nitric oxide. *Gastroenterology* 1995;**109**:483–9.
18 Maher MM, Gontarek JD, Jimenez RE, *et al*. Endogenous nitric oxide promotes ileal absorption. *J Surg Res* 1995;**58**:687–92.
19 Mailman D. Differential effects of lumenal L-arginine and N^G-nitro-L-arginine on blood flow and water fluxes in rat ileum. *Br J Pharmacol* 1994;**112**:304–10.
20 Barry M, Aloisi J, Pickering S, *et al*. Nitric oxide modulates water and electrolyte transport in the ileum. *Ann Surg* 1994;**219**:382–8.
21 Shirgi-Degen A, Beubler E. Significance of nitric oxide in the stimulation of intestinal fluid absorption in the rat jejunum in vivo. *Br J Pharmacol* 1995;**114**:13–18.
22 Beubler E, Shirgi-Degen A. Loperamide induces intestinal absorption in the rat jejunum via nitric oxide [abstract]. *Gastroenterology* 1997;**112**:A348.
23 Miller MJS, Sadowska-Krowicka H, Chotinaruemol S, *et al*. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J Pharmacol Exp Ther* 1993;**264**:11–16.
24 Mourad FH, O'Donnell LJD, Andre EA, *et al*. L-arginine, nitric oxide, and intestinal secretion: studies in rat jejunum in vivo. *Gut* 1996;**39**:539–44.
25 Li Y, Weisbrodt N, Lodato R, *et al*. Nitric oxide is involved in muscle relaxation but not in changes in short-circuit current in rat ileum. *Am J Physiol* 1994;**266**:G554–9.
26 MacNaughton W. Nitric oxide donating compounds stimulate electrolyte transport in the guinea pig intestine in vitro. *Life Sci* 1993;**53**:585–93.
27 Wilson KT, Xie Y, Musch MW, *et al*. Sodium nitroprusside stimulates anion secretion and inhibits sodium chloride absorption in rat colon. *J Pharmacol Exp Ther* 1993;**266**:224–30.
28 Tamai H, Gaginella TS. Direct evidence for nitric oxide stimulation of electrolyte secretion in the rat colon. *Free Radic Res Commun* 1993;**19**:229–39.
29 Stack WA, Filipowicz, Hawkey CJ. Nitric oxide donating compounds stimulate human colonic ion transport in vitro. *Gut* 1996;**39**:93–9.
30 Hellier M, Thirumalai C, Holdsworth C. The effect of amino acids and dipeptides on sodium and water absorption in man. *Gut* 1973;**14**:41–5.
31 Hegarty J, Fairclough P, Clark M, *et al*. Jejunal water and electrolyte secretion by L-arginine in man. *Gut* 1981;**22**:108–13.
32 Thomas DD, Knoop FC. The effect of calcium and prostaglandin inhibitors on the intestinal fluid response to heat-stable enterotoxin of Escherichia coli. *J Infect Dis* 1982;**145**:141–6.
33 Wapnir RA, Wingertzahn MA, Teichberg S. L-arginine in low concentration improves rat intestinal water and sodium absorption from oral rehydration solutions. *Gut* 1997;**40**:602–7.
34 Hayden UL, Greenberg RN, Carey HV. Role of prostaglandins and enteric nerves in Escherichia coli heat-stable enterotoxin (STa)-induced intestinal secretion in pigs. *Am J Vet Res* 1996;**57**:211–15.
35 Beubler E, Schirgi-Degen A. Nitric oxide counteracts 5-hydroxytryptamine- and cholera toxin-induced fluid secretion and enhances the effect of oral rehydration solution. *Eur J Pharmacol* 1997;**326**:223–8.
36 Qiu B, Pothoulakis C, Castagliuolo I, *et al*. Nitric oxide inhibits rat intestinal secretion by Clostridium difficile toxin A but not Vibrio cholerae enterotoxin. *Gastroenterology* 1996;**111**:409–18.
37 Turvill JL, Farthing MJG. Nitric oxide (NO) mediates cholera toxin (CT)-induced secretion in rat small intestine in vivo [abstract]. *Gastroenterology* 1996;**110**:A368.
38 Reddix R, Ceasar S, Niu X. Stimulation of endogenous nitric oxide by cholera toxin in guinea pig ileum [abstract]. *Gastroenterology* 1997;**112**:A396.
39 Nilsson O, Cassuto J, Larsson PA, *et al*. 5-Hydroxytryptamine and cholera secretion: a histochemical and physiologic study in cats. *Gut* 1983;**24**:542–8.
40 Beubler CP, Horina G. 5-HT_2 and 5-HT_3 receptor subtypes mediate cholera toxin-induced intestinal fluid secretion in the rat. *Gastroenterology* 1990;**99**:83–9.
41 Mourad FH, O'Donnell LJD, Dias JA, *et al*. Role of 5-hydroxytryptamine type 3 receptors in rat intestinal fluid and electrolyte secretion induced by cholera and Escherichia coli enterotoxins. *Gut* 1995;**37**:340–5.
42 Kadowaki M, Gershon MD, Kuwahara A. Is nitric oxide involved in 5-HT-induced fluid secretion in the gut? *Behav Brain Res* 1996;**73**:293–6.
43 Franks CM, Hardcastle J, Hardcastle PT. Involvement of nitric oxide in the response to 5-hydroxytryptamine in the rat in-vivo. *J Pharm Pharmacol* 1994;**46**:387–9.
44 Middleton SJ, Shorthouse M, Hunter JO. Increased nitric oxide synthesis in ulcerative colitis. *Lancet* 1993;**341**:465–6.
45 Boughton-Smith NK, Evans SM, Hawkey CJ, *et al*. Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet* 1993;**342**:338–40.
46 Stevens C, Walz G, Singaram C, *et al*. Tumor necrosis factor α, interleukin 1 and interleukin-6 expression in inflammatory bowel diseases. *Dig Dis Sci* 1992;**6**:818–26.
47 Theodorou V, Eutamene H, Floramonti J, *et al*. Interleukin-1 induces a neurally mediated colonic secretion in rats: involvement of mast cells and prostaglandins. *Gastroenterology* 1994;**106**:1494–500.
48 Beasley D, Schwartz JH, Brenner BM. Interleukin-1 induces prolonged L-arginine dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. *J Clin Invest* 1991;**87**:602–8.
49 Mascolo N, Gaginella TS, Izzo AA, *et al*. Nitric oxide involvement in sodium choleate-induced fluid secretion and diarrhoea in rats. *Eur J Pharmacol* 1994;**264**:21–6.
50 Casellas F, Mourelle M, Papo M, *et al*. Bile acid-induced colonic irritation stimulates intracolonic nitric oxide release in humans. *Gut* 1996;**38**:719–23.
51 Mascolo N, Izzo A, Barbato F, *et al*. Inhibitors of nitric oxide synthetase prevent castor oil-induced diarrhoea in the rat. *Br J Pharmacol* 1993;**108**:861–4.
52 Capasso F, Mascolo N, Izzo AA, *et al*. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of N^G-nitro-L-arginine methyl ester. *Br J Pharmacol* 1994;**113**:1127–30.
53 Izzo A, Gaginella T, Mascolo N, *et al*. Nitric oxide as a mediator of the laxative action of magnesium sulphate. *Br J Pharmacol* 1994;**113**:228–32.
54 Gaginella TS, Mascolo N, Izzo AA, *et al*. Nitric oxide as a mediator of bisacodyl and phenolphthalein laxative action: induction of nitric oxide synthase. *J Pharmacol Exp Ther* 1994;**270**:1239–45.
55 Izzo A, Gaginella T, Mascolo N, *et al*. N^G-nitro-L-arginine methyl ester reduces senna- and cascara-induced diarrhoea and fluid secretion in the rat. *Eur J Pharmacol* 1996;**301**:137–42.
56 Murad F, Mittal CK, Arnold WP, *et al*. Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. In: George WJ, Ignarro LJ, eds. *Advances in cyclic nucleotide research*. New York: Raven, 1978.
57 Arnold WP, Mittal CK, Katsuki S, *et al*. Nitric oxide activates guanylate cyclase and increases guanosine 3', 5'-cyclic monophosphate levels in various tissue preparations. *Proc Natl Acad Sci USA* 1977;**74**:3203–7.
58 Schmidt HHHW. NO, CO and OH endogenous soluble guanylate cyclase-activating factors. *FEBS* 1992;**307**:102–7.
59 Guandalini S, Migliavacca M, de Campora E, *et al*. Cyclic guanosine monophosphate effects on nutrient and electrolyte transport in rabbit ileum. *Gastroenterology* 1982;**83**:15–21.
60 Brasitus TA, Field M, Kimberg DV. Intestinal mucosal cyclic GMP: regulation and relation to ion transport. *Am J Physiol* 1976;**231**:G275–82.
61 Salvemini D, Misko TP, Masferrer JL, *et al*. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA* 1993;**90**:7240–4.
62 Waed SM, Dalziel HH, Bradley ME, *et al*. Involvement of cyclic GMP in non-adrenergic, non-cholinergic inhibitory neurotransmission in dog proximal colon. *Br J Pharmacol* 1992;**107**:1075–82.
63 Bryant MG, Polak JM, Modlin I, *et al*. Possible dual role for vasoactive intestinal polypeptide as gastrointestinal hormone and neurotransmitter substance. *Lancet* 1976;i:991–3.
64 Costa M, Furness JB, Buffa R, *et al*. Distribution of enteric nerve cell bodies and axons showing immunoreactivity for vasoactive intestinal polypeptide in the guinea pig intestine. *Neuroscience* 1980;**5**:587–96.
65 Allescher HD, Kurjak M, Huber A, *et al*. Regulation of VIP release from rat enteric nerve terminals: evidence for a stimulatory effect of NO. *Am J Physiol* 1996;**271**:G568–74.
66 Daniel EE, Haugh C, Woskowska Z, *et al*. Role of nitric oxide-related inhibition in intestinal function: relation to vasoactive intestinal polypeptide. *Am J Physiol* 1994;**266**:G31–9.
67 Murthy KS, Zhang K-M, Jin J-G, *et al*. VIP-mediated G protein-coupled Ca^{2+} influx activates a constitutive NOS in dispersed gastric muscle cells. *Am J Physiol* 1993;**265**:G660–71.

68 Radi R, Beckman JS, Bush KM, *et al*. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991;**288**:481–7.
69 Bern MJ, Sturbaum CW, Karayalcin SS, *et al*. Immune system control of rat and rabbit colonic electrolyte transport: role of prostaglandins and enteric nervous system. *J Clin Invest* 1989;**83**:1810–20.
70 Karayalcin SS, Sturbaum CW, Wachsman JT, *et al*. Hydrogen peroxide stimulates rat colonic prostaglandin production and alters electrolyte transport. *J Clin Invest* 1990;**86**:60–8.
71 Gaginella TS, Kachur JF, Tamai H, *et al*. Reactive oxygen and nitrogen metabolites as mediators of secretory diarrhea. *Gastroenterology* 1995;**109**: 2019–28.
72 Gardiner SM, Compton AM, Bennett T, *et al*. Regional haemodynamic changes during oral ingestion of N-monomethyl-L-arginine or N-nitro-L-arginine methyl ester in conscious Brattleboro rats. *Br J Pharmacol* 1990;**101**:10–12.
73 Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990;**99**:607–11.
74 Donowitz M, Welsh MJ. Regulation of mammalian small intestine electrolyte secretion. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. New York: Raven Press, 1993:1351–88.
75 Robinson JWL, Mirkovitch V, Winistorfer B, *et al*. Response of the intestinal mucosa to ischaemia. *Gut* 1981;**22**:512–27.
76 Robinson JWL, Winistorfer B, Mirkovitch V. Source of net water and electrolyte loss following intestinal ischaemia. *Res Exp Med (Berl)* 1980;**176**: 263–75.
77 Kanwar S, Wallace J, Befus DE. Nitric oxide synthesis inhibition increases permeability via mast cells. *Am J Physiol* 1994;**266**:G222–9.
78 Shirgi-Degen A, Beubler E. Nitric oxide exerts its proabsorptive effect in the rat jejunum by opening of potassium channels [abstract]. *Gastroenterology* 1996;**110**:A357.