Significance of nitric oxide in the stimulation of intestinal fluid absorption in the rat jejunum *in vivo*

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1 The effects of inhibiting nitric oxide (NO)-synthase on fluid transport, mucosal cyclic GMP and cyclic AMP levels and intraluminal prostaglandin E₂ (PGE₂)-release were studied in a model of ligated jejunal loops of anaesthetized rats *in vivo*. Experiments were performed under basal conditions as well as under conditions, when net fluid secretion was induced by *Escherichia coli* heat stable enterotoxin a (*E. coli* STa) or PGE₂.

2 Intravenous infusion of the NO-synthase inhibitor N^ω-nitro-L-arginine methyl ester (L-NAME, 0.25–50 mg kg⁻¹, 45 min) dose-dependently reversed net fluid absorption to net secretion, whereas infusion of D-NAME, the inactive enantiomer of L-NAME, in corresponding doses did not influence net fluid transport. N^ω-nitro-L-arginine (L-NOARG, 25 mg kg⁻¹), another NO-synthase inhibitor, also elicited net secretion of fluid.

3 L-NAME (25 mg kg⁻¹)-induced net fluid secretion was reversed to net absorption by infusion of L-arginine (400 mg kg⁻¹) or sodium nitroprusside (1 mg kg⁻¹) and s.c. administration of indomethacin (10 mg kg⁻¹). Hexamethonium (1 mg kg⁻¹, s.c.), a ganglionic blocker and granisetron (100 µg kg⁻¹, s.c.), a 5-HT₃-receptor antagonist, did not influence L-NAME-induced net secretion.

4 Net fluid secretion induced by intraluminal instillation of *E. coli* STa (10 units ml⁻¹) was enhanced by infusion of L-NAME (25 mg kg⁻¹) and was inhibited by infusion of L-arginine (400 mg kg⁻¹) and sodium nitroprusside (1 mg kg⁻¹). D-Arginine (400 mg kg⁻¹) did not influence *E. coli* STa-induced fluid secretion. Likewise, net fluid secretion induced by i.a. infusion of PGE₂ (79 ng ml⁻¹, 30 min) was enhanced by infusion of L-NAME and was inhibited by L-arginine and sodium nitroprusside. D-Arginine (400 mg kg⁻¹) did not influence PGE₂-induced fluid secretion.

5 PGE₂ levels in intraluminal fluid were not elevated after infusion of L-NAME (25 mg kg⁻¹) compared to controls.

6 Mucosal cyclic GMP and cyclic AMP levels after L-NAME-treatment were not different from control values.

7 These results indicate that nitric oxide plays an important role in the regulation of intestinal fluid transport. The data suggest a nitric oxide-dependent proabsorptive tone in the intestine, which possibly involves the enteric nervous system and suppression of prostaglandin formation. This proabsorptive tone also may downregulate fluid secretion induced by *E. coli* STa or PGE₂.

Keywords: Intestinal fluid transport; nitric oxide; *Escherichia coli* enterotoxin-induced secretion; prostaglandin E₂; cyclic GMP; N^ω-nitro-L-arginine methyl ester (L-NAME); L-arginine; sodium nitroprusside; indomethacin

Introduction

Nitric oxide (NO) recently has been established as an important mediator of various physiological and pathophysiological processes in the gastrointestinal tract (Stark & Szurszewski, 1992). Endogenous NO derives from enzymatic conversion of L-arginine through NO-synthase, a family of isoenzymes, the NO-generating activity of which can be inhibited stereospecifically by L- but not by D-arginine analogues (Ignarro, 1991). In the gut, NO-synthase activity has been found throughout the intestinal wall in enteric neurones (Aimi *et al.*, 1993), enteric blood vessels (Nichols *et al.*, 1993) and enterocytes (M'Rabet-Touil *et al.*, 1993). Intestinal NO can furthermore be produced by neutrophils (McCall *et al.*, 1989), macrophages (Hibbs *et al.*, 1988) and mast cells (Salvemini *et al.*, 1990). This extensive localization of potentially NO-releasing sites supports the view that NO plays a multifaceted role in intestinal physiology and disease.

Gastrointestinal functions which have been shown to be regulated and influenced by NO include gut motility (Calignano *et al.*, 1992), where NO is suggested to mediate smooth muscle relaxation as the inhibitory non-adrenergic non-cholinergic enteric neurotransmitter (Bult *et al.*, 1990), gastric

mucosal blood flow (Pique *et al.*, 1989), protection of gastric (MacNaughton *et al.*, 1989) and intestinal (Hutcheson *et al.*, 1990) mucosa, gastric mucus secretion (Brown *et al.*, 1993) and intestinal electrolyte and fluid 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, like sodium nitroprusside, to stripped intestinal tissue increases short circuit current, thus indicating a prosecretory and/or antiabsorptive role of NO (Wilson *et al.*, 1993; MacNaughton, 1993; Tamai & Gaginella, 1993). Corresponding with these results, castor-oil-induced diarrhoea was prevented by administration of NO-synthase-inhibitors in an *in vivo* study (Mascolo *et al.*, 1993). Conversely, inhibition of NO-synthase *in vivo* resulted in gastric and duodenal HCO₃⁻ (Takeuchi *et al.*, 1993) and intestinal fluid secretion (Mourad *et al.*, 1993; Miller *et al.*, 1993a,b) and in an enhancement of intestinal epithelial permeability (Kubes, 1992; Miller *et al.*, 1993c), thus pointing towards an antisecretory and/or proabsorptive role of NO in the intestine.

The mechanisms of action underlying any effect of NO on intestinal fluid and electrolyte transport remain unclear.

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Activation of guanylate cyclase and formation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) is a commonly accepted transduction mechanism of NO (Ignarro, 1991), and some authors suggest that this mechanism is responsible also for the observed intestinal effects (Wilson *et al.*, 1993; MacNaughton, 1993; Tamai & Gaginella, 1993). Moreover, the participation of enteric nerves and arachidonic acid metabolites in the mediation of NO effects is proposed (Wilson *et al.*, 1993; Tamai & Gaginella, 1993; Takeuchi *et al.*, 1993). Similar mechanisms of signal transduction have been shown to be involved in the mediation of fluid secretion induced by *Escherichia coli* heat stable enterotoxin a (*E. coli* STA): the enterotoxin elevates intestinal cyclic GMP levels (Hughes *et al.*, 1978) and involves prostaglandins (Thomas & Knoop, 1982) and the enteric nervous system (Eklund *et al.*, 1985) in its mechanism of action. In addition, 5-hydroxytryptamine (5-HT) has also been demonstrated to participate in the mediation of *E. coli* STA-induced fluid secretion (Beubler *et al.*, 1992).

The aim of the present study was to elucidate the significance of NO in the regulation of intestinal fluid transport *in vivo* under basal as well as under secretory conditions induced by the secretagogues *E. coli* STA and prostaglandin E₂ (PGE₂).

Methods

Preparation of animals and experimental design

Female Sprague-Dawley rats (180 ± 20 g body wt.) were used in this study. They were maintained on a standard laboratory diet and were deprived of food for 18 h before the experiments but had free access to water. The rats were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.), the abdomen was opened via a midline incision and a polyethylene catheter (PE60) was placed in the jejunum ~5 cm distal to the flexura duodenojejunalis and fixed by ligation. The second ligation was made ~20 cm distal to the first ligation. The loop was carefully rinsed with 20 ml of body-warm saline and the distal ligation was tied off. The jejunal loop was then returned to the abdominal cavity and the whole preparation was allowed to rest for 1 h under a heating lamp to preserve body temperature. Anaesthesia was maintained by s.c. injection of sodium pentobarbitone (20 mg kg⁻¹).

The experiments were started by i.v. infusion of saline or the following substances into the jugular vein, using a perfusion pump (Braun-Diessel, Melsungen, Germany) at a flow rate of 0.949 ml h⁻¹: N^ω-nitro-L-arginine methyl ester (L-NAME), N^ω-nitro-L-arginine (L-NOARG), inhibitors of NO-synthase; N^ω-nitro-D-arginine methyl ester (D-NAME), the inactive enantiomer of L-NAME, L-arginine, the substrate of NO synthase, that can overcome the inhibitory actions of L-arginine analogues, D-arginine, the inactive enantiomer of L-arginine, and sodium nitroprusside, a spontaneously NO-releasing compound. Infusion was maintained for 45 min; 15 min after the start of i.v. infusion, the jejunal loop was slowly filled with 2.0 ml of body warm Tyrode solution via the catheter, which was subsequently closed with a stopper. In experiments with *E. coli* STA, the same procedure was carried out except that the enterotoxin was added to the Tyrode solution to give a final concentration of 10 units ml⁻¹.

PGE₂ (79 ng min⁻¹) was infused close i.a. into a branch of the superior mesenteric artery for 30 min, the infusion starting 15 min after the start of i.v. infusion of saline, L-NAME, L-arginine, D-arginine or sodium nitroprusside. Tyrode solution was instilled intraluminally at the same time as the PGE₂ infusion was started. Indomethacin (10 mg kg⁻¹), a cyclooxygenase blocker, hexamethonium (1 mg kg⁻¹), a ganglionic blocker, and granisetron (100 µg kg⁻¹), an antagonist at 5-

HT₃-receptors, were administered s.c. 10 min before i.v. infusion was started.

At the end of the experiments the animals were killed by injection of a lethal dose of the narcotic.

Determination of net fluid transport

Net fluid transfer rates were determined gravimetrically 30 min after instillation of Tyrode solution (with or without *E. coli* STA). The catheter was removed, the proximal ligation tied off and the jejunal loop quickly withdrawn and weighed. Net fluid transport is expressed as millilitres per gram wet weight (ml g⁻¹ wet wt.) of jejunum. Net absorption is indicated by a negative value and net secretion by a positive value.

Determination of cyclic nucleotides

Isobutyl methyl xanthine, a phosphodiesterase inhibitor, was added to Tyrode solution (1 mmol l⁻¹) in experiments for determination of cyclic nucleotide levels. The cyclic GMP and cyclic AMP contents were measured at the end of the experiment. The jejunal loop was removed, opened, and the mucosa scraped off and immediately frozen in liquid nitrogen. After extraction, mucosal cyclic GMP was measured with the cyclic GMP-radioimmunoassay kit (TRK 500, Amersham International, UK), cyclic AMP was measured with the cyclic AMP assay kit (TRK 432, Amersham). Cyclic nucleotide levels are expressed as pmol mg⁻¹ of protein (Protein Assay Bio-Rad Laboratories, Munich, Germany).

Determination of PGE₂

The jejunal wall was punctured with a hypodermic needle at the end of the experiment, and a small sample of fluid (~0.6 ml) was withdrawn into a polyethylene tube and immediately frozen in solid CO₂. The samples were stored at -70°C and analysed within 3 weeks.

After thawing and centrifuging the samples (800 g, 3 min), PGE₂ was determined in 400 µl aliquots of the supernatant by radioimmunoassay (Bukhave & Rask-Madsen, 1981), using rabbit anti-prostaglandin E-BSA serum (BioMakor, Rehovot, Israel) as antibody.

Chemicals

The following chemicals and reagents were used: N^ω-nitro-L-arginine methyl ester, N^ω-nitro-D-arginine methyl ester, N^ω-nitro-L-arginine, L-arginine, D-arginine, sodium nitroprusside, *Escherichia coli* heat stable enterotoxin a, isobutyl methyl xanthine, hexamethonium, prostaglandin E₂ (Sigma Chemical Company, Munich, Germany), indomethacin (Merck, Sharp & Dohme, Rahway, N.J., U.S.A.), granisetron (SmithKline Beecham Pharmaceuticals, Crawley, UK), sodium pentobarbitone (Sanofi, Libourne, France). All other chemicals were of analytic grade (E. Merck AG, Darmstadt, Germany).

Statistics

Results are given as mean ± s.e.mean, and the data were analysed by the two-sample Student's *t* test or by analysis of variance (ANOVA) and Dunnett's test or the Student-Newman-Keuls test. Probability values <0.05 were considered significant.

Results

Effects on basal fluid transport

Infusion of saline resulted in net absorption of luminal fluid (Figure 1). L-NAME dose-dependently (0.25–50 mg kg⁻¹; 5.55 µg kg⁻¹ min⁻¹–1.11 mg kg⁻¹ min⁻¹) reversed net absorp-

tion to net fluid secretion, whereas infusion of D-NAME in corresponding doses did not influence control net absorption significantly (Figure 1). Further experiments with L-NAME were performed at the dose of 25 mg kg^{-1} ($= 0.55 \text{ mg kg}^{-1} \text{ min}^{-1}$).

L-NOARG (25 mg kg^{-1}) also elicited net fluid secretion of $+0.14 \pm 0.04 \text{ ml g}^{-1} \text{ wet weight}$ ($n = 6$), secretion which corresponds to the value obtained with an equivalent dose of L-NAME (data not shown).

Infusion of L-arginine ($400 \text{ mg kg}^{-1} = 8.88 \text{ mg kg}^{-1} \text{ min}^{-1}$) slightly but not significantly enhanced net fluid absorption compared to controls (Figure 2). L-Arginine reversed L-NAME-induced fluid secretion to net absorption (Figure 2).

Infusion of sodium nitroprusside ($1 \text{ mg kg}^{-1} = 22.2 \mu\text{g kg}^{-1} \text{ min}^{-1}$) showed no effect on control net absorption and also inhibited the effect of L-NAME (Figure 2). Administration of indomethacin (10 mg kg^{-1} , s.c.) did not influence control net fluid absorption (Figure 2). L-NAME-induced fluid secretion was significantly reduced by indomethacin-pretreatment (Figure 2).

Neither s.c. pretreatment with hexamethonium (1 mg kg^{-1}) nor granisetron ($100 \mu\text{g kg}^{-1}$) influenced basal fluid absorption or L-NAME-induced fluid secretion (Figure 2).

Effects on *E. coli* STa-induced fluid secretion

Intraluminal instillation of *E. coli* STa (10 units ml^{-1}) reversed net fluid absorption to net secretion (Figure 3). Infusion of L-NAME resulted in a pronounced enhancement of *E. coli* STa-induced secretion (Figure 3). Infusion of L-arginine (400 mg kg^{-1}) as well as infusion of sodium nitroprusside (1 mg kg^{-1}) significantly inhibited *E. coli* STa-induced net fluid secretion, but control levels of net absorption were not reached (Figure 3). Infusion of D-arginine (400 mg kg^{-1}) did not significantly influence *E. coli* STa-induced net fluid secretion ($+0.10 \pm 0.06 \text{ ml g}^{-1} \text{ wet weight}$, $n = 10$).

Effects on PGE₂-induced fluid secretion

Close i.a. infusion of PGE₂ (79 ng ml^{-1}) reversed net fluid absorption to net secretion (Figure 4). Infusion of L-NAME markedly enhanced PGE₂-induced fluid secretion (Figure 4). Administration of L-arginine (400 mg kg^{-1} , i.v.) as well as

administration of sodium nitroprusside (1 mg kg^{-1} , i.v.) significantly reduced PGE₂-induced fluid secretion (Figure 4). Infusion of D-arginine (400 mg kg^{-1}) did not significantly influence PGE₂-induced fluid secretion ($+0.21 \pm 0.04 \text{ ml g}^{-1} \text{ wet weight}$, $n = 9$).

Effects on cyclic nucleotide-levels

L-NAME (25 mg kg^{-1} , i.v.), D-NAME and L-NAME in combination with L-arginine (400 mg kg^{-1} , i.v.) did not influence

Figure 2 Effects of L-arginine (L-Arg) (400 mg kg^{-1} , i.v.), sodium nitroprusside (SNP) (1 mg kg^{-1} , i.v.), indomethacin (Indo) (10 mg kg^{-1} , s.c.), hexamethonium (Hexa) (1 mg kg^{-1} , s.c.) and granisetron (Grani) ($100 \mu\text{g kg}^{-1}$, s.c.) on basal (open columns) and on L-NAME (25 mg kg^{-1})-influenced net fluid transport (crosshatched columns) in the rat jejunum *in vivo* in comparison with the influence of saline and L-NAME (solid columns) on basal net fluid transport. Each column represents the mean $\pm$ s.e.mean. The numerals indicate the number of experiments; $^{\circ\circ}P < 0.01$ compared to saline; $^{**}P < 0.01$ compared to the respective control; $^{\dagger\dagger}P < 0.01$ compared to L-NAME (Student's *t* test).

Figure 1 Effects of infusion of saline ($\square$) N^o-nitro-L-arginine methyl ester (L-NAME, $\bullet$) and D-NAME ($\circ$) (0.25 – 50 mg kg^{-1}) on net fluid transport in the rat jejunum *in vivo*. Each point represents the mean $\pm$ s.e.mean. The numerals indicate the number of experiments; $^{\circ\circ}P < 0.01$ compared to saline (Dunnett's test), $^{**}P < 0.01$ compared to D-NAME (Student's *t* test).

Figure 3 Effects of i.v.-infusion of saline, N^o-nitro-L-arginine methyl ester (L-NAME) (25 mg kg^{-1}), L-arginine (L-Arg), (400 mg kg^{-1}) and sodium nitroprusside (SNP) (1 mg kg^{-1}) on *E. coli* STa (10 units ml^{-1})-influenced net fluid transport in the rat jejunum *in vivo* (crosshatched columns) in comparison with the influence of saline (open column) and L-NAME (25 mg kg^{-1}) (solid column) on basal net fluid transport. Each column represents the mean $\pm$ s.e.mean. The numerals indicate the number of experiments; $^{\circ\circ}P < 0.01$ compared to saline (open column); $^{\dagger\dagger}P < 0.05$, $^{**}P < 0.01$ compared to L-NAME (solid column); $^{*}P < 0.05$ compared to *E. coli* STa (saline) (Student-Newman-Keuls' test).

cyclic GMP-levels (Table 1). L-NAME furthermore did not influence mucosal cyclic AMP levels compared to control (Table 1).

Effects on PGE₂-levels

Infusion of L-NAME (25 mg kg⁻¹), without and with indomethacin-pretreatment (10 mg kg⁻¹, s.c.), did not influence PGE₂-contents in intraluminal fluid compared to controls. The luminal PGE₂-contents were 0.12 ± 0.03 ng g⁻¹ wet weight of jejunum (saline, *n* = 8), 0.13 ± 0.05 (L-NAME, 25 mg kg⁻¹, *n* = 12), 0.03 ± 0.02 (saline + indomethacin, 10 mg kg⁻¹, *n* = 6), 0.12 ± 0.05 (L-NAME + indomethacin, *n* = 6).

Discussion

The present results provide further support for the concept that NO is an important mediator of intestinal physiological and pathophysiological functions. Infusion of L-NAME i.v. stimulated in a dose-dependent manner jejunal fluid secretion, an effect that is attributed to inhibition of NO-synthase, since it was overcome by L-arginine, the physiological precursor of NO (Palmer *et al.*, 1988), was mimicked by L-NOARG, another NO-synthase-inhibitor and did not occur when D-NAME, the inactive enantiomer of L-NAME, was infused. Corresponding to these findings, sodium nitroprus-

side, a compound that spontaneously releases NO, did not influence basal fluid transport but completely blocked L-NAME-induced fluid accumulation. These results indicate a proabsorptive role of NO in the regulation of jejunal fluid transport.

Assuming that a proabsorptive effect is synonymous with a beneficial action, the data are in accordance with several studies, which have demonstrated a beneficial role of NO in intestine: inhibition of NO-synthase *in vivo* enhanced *E. coli* lipopolysaccharide-induced jejunal damage and plasma leakage, whereas administration of a NO-generating compound attenuated these effects (Hutcheson *et al.*, 1990; Boughton-Smith *et al.*, 1990). NO has furthermore been demonstrated to have an important role in maintaining the integrity of the mucosal barrier, derived from the observation, that i.a. infusion of L-NAME resulted in an increase in epithelial permeability of the feline ileum. This effect was reversed by sodium nitroprusside and L-arginine and appeared to involve neither tissue injury nor alterations in blood flow (Kubes, 1992). In another study, oral administration of L-NAME to naive guinea-pigs resulted in ileal fluid secretion, whereas the same treatment ameliorated chronic ileitis (Miller *et al.*, 1993a,b). The authors proposed that NO may be protective under basal and acute pathological conditions, but deleterious under chronic pathological conditions and suggested this phenomenon might be due solely to the presence of the constitutive form of NO-synthase under basal and acute conditions and the expression of the inducible form under chronic conditions. It cannot be ruled out, that the ability of NO to participate in inflammatory processes could play a role in the observed secretory response to NO-synthase-inhibition. NO has been demonstrated to mediate inflammation (Ialenti *et al.*, 1992; Middleton *et al.*, 1993) by its cytostatic and cytotoxic properties, but its role in inflammation seems to be a dual one (Moncada & Higgs, 1993; Miller *et al.*, 1993a,b,c). We did not measure parameters of inflammation but macroscopically we did not observe reddening of the jejunum or intraluminal haemorrhage after L-NAME-treatment as an indicator of inflammation. Accordingly, Hutcheson and coworkers (1990) also did not observe jejunal damage after i.v. administration of an NO-synthase inhibitor.

In contrast to a beneficial role of NO are *in vivo* experiments, where NO-synthase inhibition prevented castor-oil-induced diarrhoea (Mascolo *et al.*, 1993). This finding seems unexplainable in the light of the present results, but it should be considered, that L-NAME was administered i.p. in the study of Mascolo *et al.* (1993) and i.v. in the present experiments. In most studies, which have demonstrated a beneficial role of NO in intestine, the route of administration of the substances was via the blood, either i.v. or i.a. (Hutcheson *et al.*, 1990; Boughton-Smith *et al.*, 1990; Kubes, 1992). When considering this fact, the question arises, whether the observed secretory effects of NO inhibitors may just reflect their influence on blood flow i.e. be the result of their vasoconstrictor effects. Conclusive evidence for a relationship between any vasoactive and secretagogue action has, however, not been demonstrated. Some secretagogues are vasodilators (Granger *et al.*, 1987) (e.g. PGE₂), whereas others are vasoconstrictors (e.g. 5-HT).

Figure 4 Effects of i.v.-infusion of saline, N^ω-nitro-L-arginine methyl ester (L-NAME) (25 mg kg⁻¹), L-arginine (L-Arg), (400 mg kg⁻¹) and sodium nitroprusside (SNP) (1 mg kg⁻¹) on prostaglandin E₂ (PGE₂)-influenced net fluid transport in the rat jejunum *in vivo* (crosshatched columns) in comparison with the influence of saline (open column) and L-NAME (25 mg kg⁻¹) (solid column) on basal net fluid transport. Each column represents the mean ± s.e.mean. The numerals indicate the number of experiments; ***P* < 0.01 compared to saline (open column); †*P* < 0.05, ††*P* < 0.01 compared to L-NAME (solid column); ***P* < 0.01 compared to PGE₂ (saline) (Student-Newman-Keuls' test).

Table 1 Influence of i.v. infusion of N^ω-nitro-L-arginine methyl ester (L-NAME, 25 mg kg⁻¹ = 0.55 mg kg⁻¹ min⁻¹), D-NAME (25 mg kg⁻¹) and L-NAME (25 mg kg⁻¹) + L-arginine (400 mg kg⁻¹ = 8.88 mg kg⁻¹ min⁻¹) on mucosal levels of cyclic GMP and cyclic AMP in the rat jejunum

	Cyclic GMP level (pmol mg ⁻¹ protein)		Cyclic AMP level (pmol mg ⁻¹ protein)	
		<i>n</i>		<i>n</i>
Saline	0.22 ± 0.03	17	6.36 ± 0.41	9
L-NAME	0.16 ± 0.01	17	5.77 ± 0.16	9
D-NAME	0.24 ± 0.04	11	Not tested	
L-NAME + L-arginine	0.26 ± 0.04	11	Not tested	

Values are mean ± s.e.mean; *n* refers to the number of animals studied.

Sodium nitroprusside or exogenous NO, administered to stripped colonic (Wilson *et al.*, 1993; Tamai & Gaginella, 1993) or ileal (MacNaughton, 1993) tissue, induced electrolyte secretion *in vitro*, whereas application of NO-synthase inhibitors did not alter the basal response in short circuit current (Wilson *et al.*, 1993; MacNaughton, 1993). These studies also seem to be in conflict with the present results. The most probable explanation of the differences of *in vivo* and *in vitro* findings may be the preparation of the tissue, which was dissected from muscle layers and neuronal influences in the Ussing chamber studies. The observed effects on fluid transport *in vivo* may involve an influence of NO-synthase inhibition on nervous transmission, comparable to the effect of NO-synthase inhibition on gut motility. Application of NO-synthase inhibitors induces intestinal contractions and increases the basal tension due to inhibition of non-adrenergic non-cholinergic neurotransmission (Boeckxstaens *et al.*, 1990; Calignano *et al.*, 1992; Rand, 1992). It can be speculated, that a NO-dependent proabsorptive tone exists in the intestine, similar to the proposed intestinal NO-dependent dilator tone (Moncada & Higgs, 1993). Hexamethonium, however, did not influence L-NAME-induced secretion in the present experiments thus excluding the participation of nicotinic receptors in the secretory response.

5-HT₃ receptors, that are involved in the mediation of *E. coli* STa- (Beubler *et al.*, 1992) and cholera toxin-induced secretion (Beubler & Horina, 1990; Bhardan *et al.*, 1993), are obviously not involved in the secretory response to L-NAME, since granisetron, an antagonist at these receptors, did not influence L-NAME-induced secretion, indicating that the involvement of 5-HT seems unlikely.

Indomethacin significantly reduced L-NAME-induced fluid secretion, suggesting the involvement of prostaglandins in the secretory response to NO-synthase-inhibition. Comparable findings have been reported by Takeuchi and coworkers (1993), who demonstrated that indomethacin-pretreatment inhibited L-NAME-induced duodenal HCO₃-secretion. The authors proposed, that NO plays a suppressive role in the regulation of HCO₃-secretion and that the action of L-NAME involves both prostaglandin-sensitive and neuronal pathways. In the present experiments, however, enhanced levels of PGE₂ in the jejunal lumen after infusion of L-NAME could not be detected. This negative result could also be due to a transient release of small amounts of PGE₂ and/or a fast degradation, so that a spill-over of the prostaglandin into the lumen is prevented.

Infusion of L-NAME exerted an additive effect on both *E. coli* STa and PGE₂-induced secretion in the present experiments. These results again argue for a proabsorptive tone, which is present also when the intestine secretes fluid due to the action of these secretagogues, and which is prevented by NO-synthase inhibition. Interestingly, infusion of L-arginine as well as sodium nitroprusside inhibited both *E. coli* STa and PGE₂-induced secretion. Thus, reinforcement of the NO-dependent proabsorptive action by exogenous NO may reverse active secretion of fluid to absorption.

The existence of an endogenously regulated proabsorptive tone, dependent on endogenous opiates, was also demonstrated previously by Beubler & Lembeck (1980). The authors reported, that blockade of morphine receptors by naloxone enhanced PGE₁-induced intestinal fluid secretion.

The mechanism of action of NO in the intestine has not yet been elucidated. MacNaughton (1993) found a 44 fold increase in mucosal cyclic GMP levels after application of sodium nitroprusside to stripped ileal tissue. This result seems to be questionable, since it has been reported that NO activates the soluble, but not the particulate form of guanylate cyclase (DeJonge, 1984) and that in the intestine predominantly the particulate form of the enzyme is present (DeJonge, 1975). Accordingly, in a previous study, soluble guanylate cyclase could not be detected in a preparation of scraped jejunal mucosa, and the particulate guanylate cyclase, which was present, could not be activated by sodium nitroprusside (Beubler *et al.*, 1993). However, elevation of mucosal cyclic GMP levels occurred in the same preparation after challenge with *E. coli* STa (Beubler *et al.*, 1992). In the present experiments we did not find alterations of mucosal cyclic GMP levels after administration of L-NAME *in vivo*.

In conclusion we suggest that there exists an endogenous proabsorptive tone in the intestine, which is dependent on NO and possibly involves the enteric nervous system and suppression of prostaglandin formation. This proabsorptive tone also seems to be present when the gut secretes fluid into the lumen after challenge with secretagogues like *E. coli* STa or PGE₂. Provision of exogenous NO may be a possible tool to reverse net fluid secretion to net absorption.

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