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Nitric oxide counteracts 5-hydroxytryptamine- and cholera toxin-induced fluid secretion and enhances the effect of oral rehydration solution

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Abstract

The effects of pharmacological modulation of the nitric oxide (NO) pathway on intestinal fluid transport were studied in a model of ligated jejunal loops of anaesthetized rats in vivo. Close intraarterial infusion of 5-hydroxytryptamine (5-HT) (0.16 μg/min) induced net fluid secretion. Intravenous infusion of the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) (0.55 mg/kg per min) reversed net fluid absorption in controls to net secretion and significantly enhanced 5-HT-induced fluid secretion. 5-HT-induced net fluid secretion was inhibited by intravenous infusion of L-arginine (8.88 mg/kg per min), sodium nitroprusside (22.2 μg/kg per min), or 3-morpholino sydnonimine (SIN-1) (22.2 μg/kg per min). Intraluminal instillation of cholera toxin (0.5 μg/ml) induced net secretion, which was significantly enhanced by L-NAME and reduced by L-arginine. Another series of experiments was performed using a model of luminally perfused jejunal loops. Cholera toxin (10 μg/ml) induced profuse net fluid secretion also in this model. L-Arginine and sodium nitroprusside significantly enhanced net fluid absorption compared to controls and abolished the secretory effect of cholera toxin. Luminal perfusion with oral rehydration solution enhanced net absorption of fluid in controls and reversed cholera toxin-induced secretion to absorption. Intravenous infusion, but not intraluminal administration, of L-arginine significantly enhanced the antisecretory effect of oral rehydration solution. These results give further support to the existence of an intestinal NO-mediated proabsorptive tone, which also downregulates fluid secretion elicited by different enterotoxins or mediators of secretion. Intravenous administration of exogenous sources of NO counteracts intestinal fluid accumulation and augments the antisecretory effect of oral rehydration solution, findings which may lead to therapeutic consequences.

Keywords: Intestinal fluid transport; Nitric oxide (NO); 5-HT (5-hydroxytryptamine, serotonin); Cholera toxin; Oral rehydration solution

1. Introduction

Nitric oxide (NO), which mediates various physiological and pathophysiological functions in the gastrointestinal tract (Whittle, 1994), also has been demonstrated to modulate the intestinal transport of fluid and electrolytes. The role of NO in intestinal transport, however, appears to be controversial considering the investigations performed in this relation: a proabsorptive effect of NO was revealed (1) in Ussing chamber experiments using full thickness preparations of intestine (Rao et al., 1994), (2) in the isolated, vascularly perfused rabbit ileum (Barry et al., 1994), (3) in the rat ileum (Mailman, 1994), (4) in the rat jejunum (Schirgi-Degen and Beubler, 1995, 1996), (5) in dogs with Thiry-Vella fistulas (Maher et al., 1995), (6) in the rat duodenum and jejunum (Schleiffer et al., 1995) and (7) in the rat duodenum (Hällgren et al., 1995).

On the other hand, in Ussing chamber experiments using stripped preparations (Wilson et al., 1993; MacNaughton, 1993; Tamai and Gaginella, 1993; Rolfe and Levin, 1994), and in one case also unstripped tissue (Rolfe and Levin, 1994), NO-donating compounds or L-arginine increased short circuit current, thus indicating a prosecretory role of NO. Corresponding with the latter results, castor oil- or bile salt-induced diarrhoea was prevented by intraperitoneal administration of NO synthase inhibitors (Mascolo et al., 1993, 1994).

The reasons for these controversial findings may be based on methodical differences (e.g., route of administration or stripped resp. unstripped tissue) or may reflect the involvement of different forms of NO synthase in normal or pathological conditions of intestine.

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In previous experiments with the rat ligated jejunal loop model in vivo, NO has been demonstrated to mediate a proabsorptive tone in the small intestine, even under conditions when the gut actively secretes fluid after challenge with prostaglandin E_2 or *Escherichia coli* heat-stable enterotoxin A (Schirgi-Degen and Beubler, 1995).

The aim of the present study was to determine whether a NO-mediated proabsorptive tone also counteracts the fluid secretion induced by cholera toxin and by 5-hydroxytryptamine (5-HT), which is an important mediator of cholera toxin-induced secretion (Beubler et al., 1989, 1993; Beubler and Horina, 1990).

Another series of experiments was performed to investigate whether L-arginine, the precursor of endogenously formed NO, improves the proabsorptive effect of an oral rehydration solution in the treatment of cholera.

2. Materials and methods

2.1. Preparation of animals

Female Sprague-Dawley rats (180 ± 20 g body weight) 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, 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 either tied off ('tied-off loop') or another polyethylene catheter with a lumenal opening of 5 mm was inserted ('perfused loop'). Intact blood flow to the jejunum is maintained by this preparation. 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).

2.2. Experimental design: tied-off loop

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: N^{ω}-nitro-L-arginine methyl ester (L-NAME) (25 mg/kg = 0.55 mg/kg per min); L-arginine (400 mg/kg = 8.88 mg/kg per min); sodium nitroprusside (1 mg/kg = 22.2 μg/kg per min) and 3-morpholino sydnonimine (SIN-1) (1 mg/kg = 22.2 μg/kg per min). As shown previously, this dose of L-NAME is submaximal (Schirgi-Degen and Beubler, 1995). Infusion was maintained for 45 min in 5-HT experiments and for 4 h 15 min in cholera toxin experiments. 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 cholera toxin, the same procedure was carried out except that the enterotoxin was added to the Tyrode solution to give a final concentration of 0.5 μg/ml.

5-HT creatinine sulfate (0.16 μg 5-HT/min) 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, sodium nitroprusside or SIN-1; Tyrode solution was instilled intraluminally at the same time as the 5-HT infusion was started.

2.3. Determination of net fluid transport

Net fluid transfer rates were determined gravimetrically 30 min after instillation of Tyrode solution in 5-HT experiments or 4 h after instillation of cholera toxin. 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 (ml/g) wet weight of jejunum. Net absorption is indicated by a negative value and net secretion by a positive value.

2.4. Experimental design: perfused loop

Perfusion studies were performed according to Rolston et al. (1987) with modifications according to Pillai et al. (1994). Cholera toxin (10 μg/ml) was instilled intraluminally into the ligated jejunal loops. After 2 h the ligature was opened and luminal perfusion was started (0.5 ml/min). After an equilibration period of 40 min, four 10-min fractions of perfused fluid were collected.

The perfusion fluid was composed as 'oral rehydration solution' according to the WHO formula with reduced sodium content (Na^+ 60 mM, K^+ 20 mM, HCO_3^- 30 mM, Cl^- 50 mM, glucose 110 mM) or oral rehydration solution + L-arginine (glucose 60 mM, L-arginine 50 mM) or saline and contained [^{14}C]polyethylene glycol 4000 (10 nCi/ml). L-NAME (0.55 mg/kg per min), L-arginine (8.88 mg/kg per min), sodium nitroprusside (22.2 μg/kg per min) or saline were infused intravenously for 2 h 35 min, the infusion starting 45 min after the instillation of cholera toxin. Net fluid transport was calculated according to the following formula:

$$\mu\text{l/min per g wet weight} = \frac{\left\{\left(1 - \frac{\text{cpm}_{\text{perf}}}{\text{cpm}_{\text{effl}}}\right) \times \left(\frac{0.5\ \text{ml}}{\text{min}}\right) \times 1000\right\}}{\text{g wet weight of jejunum}}$$

where 'cpm' is the counts per minute, 'perf' is the perfusate, 'effl' is the effluent.

2.5. Chemicals

The following chemicals and reagents were used: N^{ω}-nitro-L-arginine methyl ester, L-arginine, sodium nitroprusside, cholera toxin, 3-morpholino sydnonimine, serotonin creatinine sulfate (Sigma Chemical Company, Munich, Germany), sodium pentobarbitone (Sanofi, Libourne, France). All other chemicals were of analytic grade (E. Merck, Darmstadt, Germany).

2.6. Statistics

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

3. Results

3.1. Tied-off loop

3.1.1. 5-HT experiments

Fluid was absorbed in all control rats. Close intraarterial infusion of 5-HT creatinine sulfate (0.16 μg 5-HT/min, 30 min) reversed net fluid absorption to net fluid secretion (Fig. 1). Intravenous infusion of L-NAME (25 mg/kg = 0.55 mg/kg per min, 45 min) also reversed net absorption to net secretion and significantly enhanced 5-HT-induced net fluid secretion. Infusion of L-arginine (8.88 mg/kg per min = 400 mg/kg), sodium nitroprusside (22.2 μg/kg per min = 1 mg/kg) and 3-morpholino sydnonimine (SIN-1) (22.2 μg/kg/ min = 1 mg/kg) did not significantly influence net fluid absorption compared to controls. All three NO donators, however, totally blocked 5-HT-induced fluid secretion (Fig. 1).

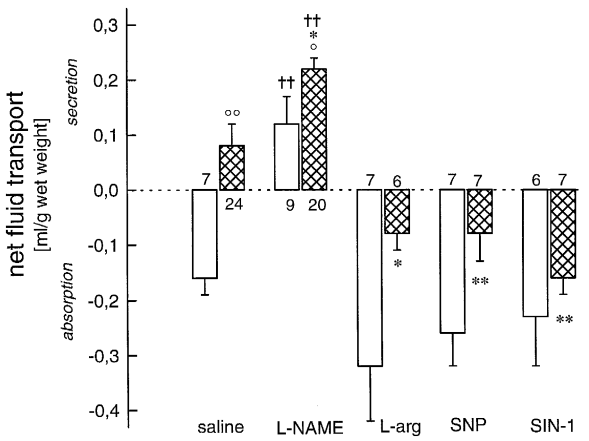

Fig. 1. Tied-off loop of the rat jejunum in vivo. Effects of i.v. infusion of saline, N^{ω}-nitro-L-arginine methyl ester (L-NAME), L-arginine (L-arg), sodium nitroprusside (SNP) and 3-morpholino sydnonimine (SIN-1) on net fluid transport in controls (open bars) and on 5-HT-induced fluid secretion (cross-hatched bars). Each column represents the mean ± S.E.M. The numerals indicate the number of experiments. $^{\dagger\dagger} P < 0.01$ compared to saline (Student's *t*-test), $^{*} P < 0.05$ and $^{**} P < 0.01$ compared to saline plus 5-HT (Dunnett *t*-test), $^{\circ} P < 0.05$ and $^{\circ\circ} P < 0.01$ compared to the respective control (Student's *t*-test).

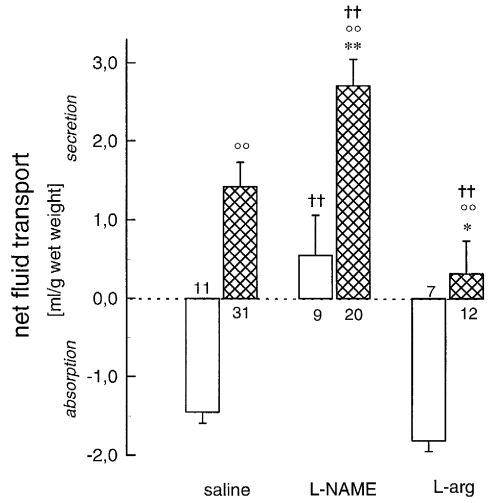

Fig. 2. Tied-off loop of the rat jejunum in vivo. Effects of i.v. infusion of saline, N^{ω}-nitro-L-arginine methyl ester (L-NAME) and L-arginine (L-arg) on net fluid transport in controls (open bars) and on cholera toxin-induced fluid secretion (cross-hatched bars). Each column represents the mean ± S.E.M. The numerals indicate the number of experiments. $^{\dagger\dagger} P < 0.01$ compared to saline (Student's *t*-test), $^{*} P < 0.05$ and $^{**} P < 0.01$ compared to saline plus cholera toxin (Dunnett *t*-test), $^{\circ\circ} P < 0.01$ compared to the respective control (Student's *t*-test).

3.1.2. Cholera toxin experiments

Fluid was absorbed in all control rats. A 4 h exposure to cholera toxin (0.5 μg/ml) caused profuse net fluid secre-

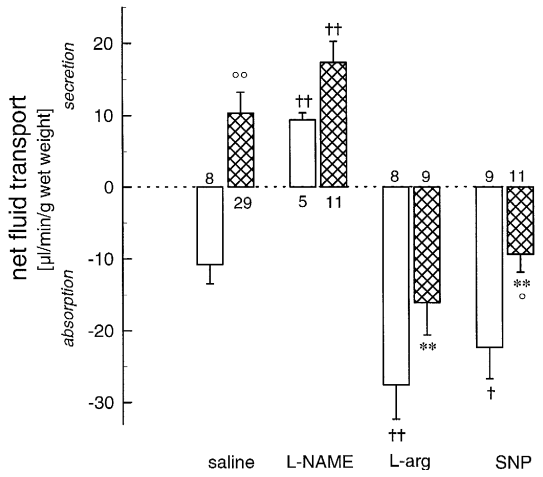

Fig. 3. Perfused loop of the rat jejunum in vivo. Effects of i.v. infusion of saline, N^{ω}-nitro-L-arginine methyl ester (L-NAME), L-arginine (L-arg) or sodium nitroprusside (SNP) on net fluid transport in controls (open bars) and on cholera toxin-induced fluid secretion (cross-hatched bars). Each column represents the mean ± S.E.M. The numerals indicate the number of experiments. $^{\dagger} P < 0.05$ and $^{\dagger\dagger} P < 0.01$ compared to saline (Student's *t*-test), $^{**} P < 0.01$ compared to saline plus cholera toxin (Dunnett *t*-test), $^{\circ} P < 0.05$ and $^{\circ\circ} P < 0.01$ compared to the respective control (Student's *t*-test).

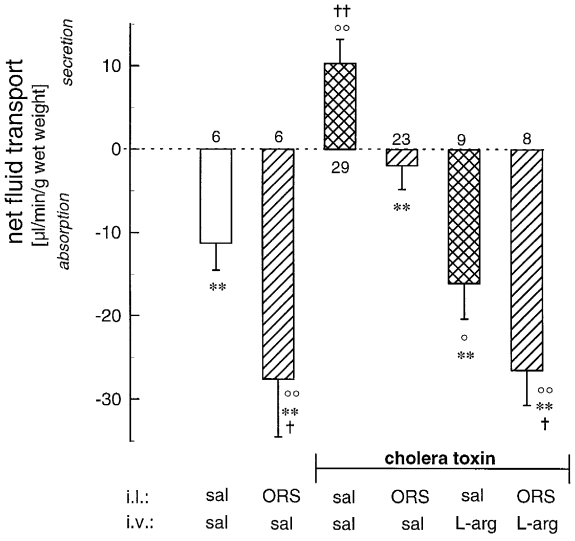

Fig. 4. Perfused loop of the rat jejunum in vivo. Effect of luminal perfusion with saline (sal) or oral rehydration solution (ORS) with or without i.v. infusion of L-arginine (L-arg) on net fluid transport in controls and on cholera toxin-induced secretion. Each column represents the mean ± S.E.M. The numerals indicate the number of experiments. † $P < 0.05$ and †† $P < 0.01$ compared to saline (Student's *t*-test), ** $P < 0.01$ compared to cholera toxin plus intraluminal (i.l.) saline plus i.v. saline, ° $P < 0.05$ and °° $P < 0.01$ compared to cholera toxin plus i.l. ORS plus i.v. saline (Student's *t*-test).

tion (Fig. 2). Intravenous infusion of L-NAME (0.55 mg/kg per min = 142 mg/kg) reversed net absorption to net secretion and significantly enhanced the secretory effect of cholera toxin. Infusion of L-arginine (8.88 mg/kg per min = 2.27 g/kg) did not influence net fluid absorption compared to controls but significantly reduced cholera toxin-induced fluid secretion (Fig. 2).

3.2. Perfused loop

Using saline as perfusion fluid, cholera toxin (10 µg/ml) caused profuse net fluid secretion. Intravenous infusion of L-NAME (0.55 mg/kg per min = 86 mg/kg) also reversed net absorption to net secretion and enhanced the secretory effect of cholera toxin; the enhancement, however, was not significant (Fig. 3). Infusion of L-arginine (8.88 mg/kg per min = 1.38 g/kg) and of sodium nitroprusside (22.2 µg/kg per min = 3.44 mg/kg) significantly enhanced net absorption compared to controls and both totally inhibited cholera toxin-induced fluid secretion (Fig. 3).

Intraluminal perfusion with oral rehydration solution significantly enhanced fluid absorption compared to perfusion with saline (Fig. 4). In cholera toxin experiments, intraluminal perfusion with oral rehydration solution reversed net fluid secretion to fluid absorption. Additional intravenous infusion of L-arginine (8.88 mg/kg per min = 1.38 g/kg) totally blocked cholera toxin-induced secretion and markedly enhanced the proabsorptive effect of oral rehydration solution during challenge with cholera toxin (Fig. 4). L-Arginine administered luminally as a component of the oral rehydration solution did not enhance the proabsorptive effect of oral rehydration solution alone (data not shown).

4. Discussion

Absorption of water and electrolytes in the small intestine is a very important process in the maintenance of normal digestion (Spiller, 1994). The final mediators of absorption, however, are mostly unknown. Recently it has been demonstrated both in vivo and in vitro that nitric oxide (NO) acts as transmitter of absorption, but its proabsorptive role is not yet established (see Section 1).

The present results give further support to the concept that NO is a mediator of intestinal absorption. Inhibition of NO synthase with L-NAME elicited net secretion of fluid in the tied-off loop as well as in the perfused loop model, thus suggesting that NO, produced continuously in small amounts, maintains a proabsorptive tone. L-NAME furthermore significantly enhanced the secretory effect of 5-HT and of cholera toxin in the tied-off loop. An enhancement of the effect of cholera toxin by L-NAME in the perfused loop model, however, did not reach statistical significance. 5-HT- as well as cholera toxin-induced secretion was totally blocked, respectively significantly reduced, by L-arginine or NO donors in both models used. These results are in accordance with earlier findings, where prostaglandin E_2 and *Escherichia coli* heat-stable enterotoxin A as secretagogues were used (Schirgi-Degen and Beubler, 1995), and suggest that provision of NO, either as substrate for NO synthase or as exogenous NO, can counteract fluid secretion elicited by different stimuli.

Corresponding observations were published by Qiu et al. (1996), who found that subcutaneous administration of L-NAME significantly enhanced, whereas L-arginine and the NO donor *S*-nitroso-*N*-acetyl-L-cysteine reduced *Clostridium difficile* toxin A-induced fluid secretion in the rat ileum. The authors, however, did not see these effects with cholera toxin-induced secretion and furthermore L-NAME alone elicited no effect in these experiments. Their conclusion was that NO, produced by constitutive NO synthase, inhibits intestinal mast cell release and consequently toxin A-induced effects. Kubes (1992) and Kanwar et al. (1994), on the contrary, observed an enhancement in intestinal permeability after intravenous administration of L-NAME and also suggested this effect to be elicited by activation of mucosal mast cells. The present results do not contradict a possible effect on mast cell degranulation or intestinal permeability of NO donors and NO synthase inhibitors. Nevertheless, alternative proposals for the mechanism of the intestinal transport effects of NO were published by Hällgren et al. (1995), who found an involvement of neural mechanisms, and by Schirgi-Degen and

Beubler (1996), who suggested that NO may exert its proabsorptive effect by opening of basolateral K^+ channels in enterocytes.

Substances interfering with the NO pathway also affect intestinal blood flow, but an involvement of blood flow alterations in the transport effects is unlikely, since any dependence of intestinal transport and changes in blood flow has not been proven (Granger et al., 1987). Some secretagogues are vasodilators (e.g., prostaglandin E_2), whereas others are vasoconstrictors (e.g., 5-HT).

The oral rehydration solution used in the present experiments to investigate whether addition of L-arginine improves its antisecretory efficacy was based on the standard WHO formula, modified according to Pillai et al. (1994) with 60 mM sodium content instead of 90 mM. This oral rehydration solution, intraluminally applied, caused a marked absorption of fluid in controls and significantly reduced the secretory effect of cholera toxin in the perfused jejunal loop. Addition of L-arginine to the oral rehydration solution, however, did not augment the antisecretory effect of oral rehydration solution. This is in agreement with the observations that intraluminal L-arginine even slightly caused secretion of fluid in the rat jejunum (Mourad et al., 1995) and in human perfused jejunal loops (Hinterleitner and Beubler, unpublished). Intravenous administration of L-arginine, on the other hand, significantly enhanced the proabsorptive effect of the oral rehydration solution, thus emphasizing the importance of the route of administration of pharmacological agents interfering with the NO pathway and furthermore providing another evidence for the proabsorptive efficacy of the substrate of NO synthase, L-arginine.

In conclusion, the present results support the concept that endogenous production of NO exerts a proabsorptive tone in the intestine, which also downregulates fluid secretion elicited by different secretagogues. Administration of exogenous NO by L-arginine or NO donors furthermore totally blocks fluid secretion induced by 5-HT or cholera toxin. L-Arginine, applied intravenously, finally markedly enhances the proabsorptive effect of the WHO oral rehydration solution, a finding which may lead to therapeutic consequences.

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