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Involvement of K^+ channel modulation in the proabsorptive effect of nitric oxide in the rat jejunum in vivo

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Abstract

The role of K^+ channels in the mediation of the nitric oxide(NO)-induced proabsorptive effect in intestinal fluid transport was investigated in a functional study, using a model of ligated jejunal loops of anaesthetized rats in vivo. The K^+ channel opener cromakalim and the K^+ channel blocker glibenclamide were administered under basal conditions as well as under conditions, when fluid secretion was influenced by N^{ω}-nitro-L-arginine methyl ester (L-NAME), prostaglandin E_2, *Escherichia coli* heat stable enterotoxin a (*E. coli* STa) or L-arginine. Intravenous infusion of cromakalim (63.5 μg/kg per min) significantly enhanced net fluid absorption compared to controls, totally abolished net fluid secretion induced by L-NAME (0.55 mg/kg per min), reversed net fluid secretion induced by intraluminal instillation of *E. coli* STa (10 units/ml) to absorption, but did not influence fluid secretion elicited by close i.a. infusion of prostaglandin E_2 (79 ng/min). Close i.a. infusion of glibenclamide (0.16 mg/kg per min) reversed net fluid absorption to net secretion, blocked the absorptive effect of L-arginine (8.88 mg/kg per min) and reduced the proabsorptive effect of cromakalim. The secretory effect of L-NAME was not further enhanced by glibenclamide. These results suggest that modulation of basolateral K^+ channels by NO is involved in the mediation of its proabsorptive effect, since opening and closure of K^+ channels mimicked, respectively counteracted, the action of NO-donors and inhibitors of NO-synthesis on intestinal fluid transport. The role of prostaglandins in the proabsorptive effect of NO remains to be elucidated. These results furthermore support the role of K^+ channel openers as potential new antidiarrheal drugs.

Keywords: Intestinal fluid transport; Nitric oxide (NO); N^{ω}-nitro-L-arginine methyl ester (L-NAME); K^+ channels; Prostaglandin E_2; *Escherichia coli* enterotoxin-induced secretion

1. Introduction

Nitric oxide (NO) has been established as a potent messenger in the gastrointestinal system, where it regulates smooth muscle tone, mucosal blood flow, secretion of acid and mucus and mucosal protection (Whittle, 1994). A further domain of intestinal action of NO is the modulation of fluid and electrolyte transport. Although a growing body of evidence indicates that NO maintains a proabsorptive tone in the intestine (Rao et al., 1994; Barry et al., 1994; Mailman, 1994; Schirgi-Degen and Beubler, 1995; Maher et al., 1995; Hällgren et al., 1995), other studies demonstrated the opposite, that is, a prosecretory effect of NO (McNaughton, 1993; Wilson et al., 1993; Gaginella et al., 1994). Though the isoforms of NO synthase were not determined in these studies, this dilemma probably is based on the dual nature of NO, reflecting the involvement of different forms of NO synthase (constitutive and inducible) in different conditions (normal and pathological) of intestine. For example, in experimental ileitis (Miller et al., 1993) or colitis (Neilly et al., 1995) administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) reduced inflammation, but when administered to intact animals, L-NAME increased myeloperoxidase activity and caused a fluid secretory response (Miller et al., 1993). Furthermore, endotoxin-induced intestinal damage (Hutcheson et al., 1990) and basal epithelial permeability (Kubes, 1992) were enhanced after inhibition of NO synthase, again emphasizing the multifaceted role of NO.

Other possible explanations for the controversial findings concerning intestinal electrolyte and fluid transport could be the use of stripped (McNaughton, 1993; Wilson et al., 1993) versus unstripped (Rao et al., 1994) intestinal tissue in in vitro experiments or different routes of administration of drugs in in vivo experiments.

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In our investigations on fluid transport in the rat jejunum in vivo, i.v.-infusion of L-NAME elicited a dose-dependent secretory response, whereas D-NAME was without effect. Application of L-arginine, the substrate of NO synthase, or sodium nitroprusside, a NO-releasing compound, counteracted fluid secretion elicited by L-NAME, prostaglandin E_2 or *Escherichia coli* heat stable enterotoxin a (*E. coli* STa) (Schirgi-Degen and Beubler, 1995). The mechanism of action of this NO-mediated proabsorptive effect is unknown. Activation of guanylate cyclase, which accounts for e.g. the smooth muscle relaxing effect of NO, does not seem to be involved in the mediation of its proabsorptive action, since NO only activates the soluble form of this enzyme, whereas in intestinal epithelium predominantly the particulate form of guanylate cyclase is present (DeJonge, 1975).

Recently a further mechanism of action of NO has been published: the activation of Ca^{2+}- and of ATP-dependent K^+ channels. NO opens ATP-sensitive K^+ channels in pancreatic β-cells (Tsuura et al., 1994) and activates Ca^{2+}-dependent K^+ channels in vascular smooth muscle (Khan et al., 1993; Bolotina et al., 1994), the latter definitely without requiring cGMP.

On the other hand, K^+ channel activators were found to decrease short circuit current in stripped rabbit distal ileal mucosa, indicating a regulatory role of K^+ channels in ileal electrolyte absorption (Homaidan and Broutman, 1994). Accordingly, K^+ channel openers have been demonstrated recently to exert antidiarrheal activity (Poggioli et al., 1995).

The present experiments were designed to test the hypothesis whether activation of K^+ channels is involved in the mediation of the NO-induced proabsorptive effect in the intestine. The K^+ channel activator cromakalim (Hamilton et al., 1986) and the K^+ channel blocker glibenclamide (Buckingham et al., 1989; Quast and Cook, 1989) were used for this purpose. The effects of these modulators of K^+ channels were tested on net fluid transport changes induced by L-arginine, which tends to enhance net fluid absorption, by the NO synthase inhibitor L-NAME, by *E. coli* STa and by prostaglandin E_2, which cause net fluid secretion, as shown before (Schirgi-Degen and Beubler, 1995).

2. Materials and methods

2.1. Preparation of animals

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, i.p.), the abdomen was opened via a midline incision and a polyethylene catheter (PE60) was placed in the jejunum about 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).

At the end of the experiments the rats were killed by a lethal dose of sodium pentobarbitone.

2.2. Experimental design

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), or cromakalim (10 μmol/kg = 2.86 mg/kg = 63.5 μg/kg per min). As shown previously, this dose of L-NAME is submaximal (Schirgi-Degen and Beubler, 1995). The dose of cromakalim was chosen according to the dose-response curve performed in vivo by Poggioli et al. (1995) as submaximal, effective dose. 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 intraluminally administered Tyrode solution to give a final concentration of 10 units/ml.

Prostaglandin E_2 (79 ng/min) and glibenclamide (10 μmol/kg = 4.93 mg/kg = 0.16 mg/kg per min) were 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 or cromakalim. In vitro, cromakalim and glibenclamide are used in a similar concentration range (Gelband and McCullough, 1993). Accordingly, the dose of glibenclamide applied in vivo was chosen as a similar dose to cromakalim. Tyrode solution was instilled intraluminally at the same time as infusion of prostaglandin E_2 or glibenclamide was started. Close i.a. administration was chosen for these drugs since it provides a possibility to applicate a substance near its location of effect and degradation in the periphery can be prevented.

2.3. Determination of net fluid transport

Net fluid transfer rates were determined gravimetrically 30 min after instillation of Tyrode solution. The catheter was removed, the proximal ligation tied off and the jejunal loop quickly withdrawn and weighed. Net fluid transport is expressed as ml/g wet wt of jejunum. Net absorption is

indicated by a negative value and net secretion by positive value.

2.4. Chemicals

N^{ω}-nitro-L-arginine methyl ester, L-arginine, prostaglandin E_2, cromakalim, glibenclamide, *Escherichia coli* heat stable enterotoxin a (Sigma, Munich), sodium pentobarbitone (Sanofi, Libourne). All other chemicals were of analytic grade (Merck, Darmstadt).

2.5. Statistics

Results are given as means ± S.E.M. and the data were analyzed by analysis of variance (ANOVA) and the Student–Newman–Keuls test. Probability values < 0.05 were considered significant.

3. Results

3.1. Effects of cromakalim

Intravenous infusion of saline resulted in net absorption of luminal fluid in all control rats (Fig. 1). Intravenous infusion of L-NAME (25 mg/kg = 0.55 mg/kg per min) for 45 min reversed net fluid absorption to net fluid secretion ($P < 0.01$) (Fig. 1). Intravenous infusion of cromakalim (2.86 mg/kg = 63.5 μg/kg per min) for 45 min significantly enhanced net fluid absorption compared to

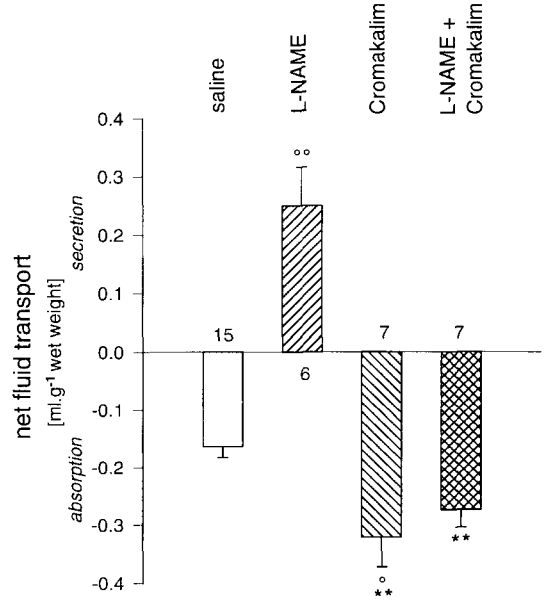

Fig. 1. Effects of saline, L-NAME (0.55 mg/kg per min, i.v.), cromakalim (63.5 μg/kg per min, i.v.) and L-NAME in the presence of cromakalim on net fluid transport in the rat jejunum in vivo. Each column represents the mean ± S.E.M. The number of experiments is given at the base of each column; $^{\circ} P < 0.05$ and $^{\circ\circ} P < 0.01$ compared to saline, $^{**} P < 0.01$ compared to L- NAME (Student–Newman–Keuls test).

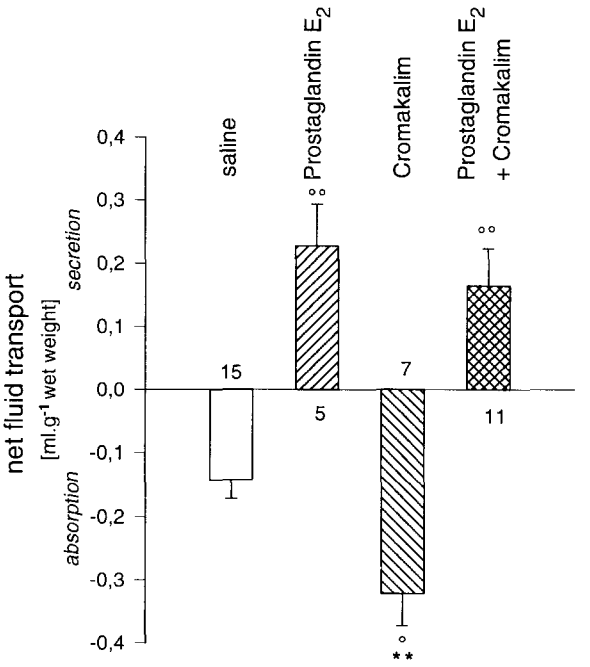

Fig. 2. Effects of saline, prostaglandin E_2 (79 ng/min, i.a.), cromakalim (63.5 μg/kg per min, i.v.) and prostaglandin E_2 in the presence of cromakalim on net fluid transport in the rat jejunum in vivo. Each column represents the mean ± S.E.M. The number of experiments is given at the base of each column; $^{\circ} P < 0.05$ and $^{\circ\circ} P < 0.01$ compared to saline, $^{**} P < 0.01$ compared to prostaglandin E_2 (Student–Newman–Keuls test).

controls ($P < 0.05$) and reversed L-NAME-induced secretion to net absorption of fluid ($P < 0.01$) (Fig. 1).

Close i.a. infusion of prostaglandin E_2 (79 ng/min) into the superior mesenteric artery for 30 min also reversed net fluid absorption to net fluid secretion ($P < 0.01$) (Fig. 2). Simultaneous i.v. infusion of cromakalim (2.86 mg/kg = 63.5 μg/kg per min), starting 15 min prior to prostaglandin E_2 administration, was, however, without effect on prostaglandin E_2 induced fluid secretion (Fig. 2). Intraluminal instillation of *E. coli* STa (10 units/ml) for 30 min elicited net secretion of fluid ($P < 0.01$) (Fig. 3). Simultaneous intravenous infusion of cromakalim (2.86 mg/kg = 63.5 μg/kg per min), starting 15 min prior to *E. coli* STa-administration, reversed *E. coli* STa-induced net fluid secretion to net absorption ($P < 0.01$) (Fig. 3).

3.2. Effects of glibenclamide

Close i.a. infusion of glibenclamide (4.93 mg/kg = 0.16 mg/kg per min) into the superior mesenteric artery for 30 min reversed net fluid absorption to net fluid secretion ($P < 0.01$) (Fig. 4). Intravenous infusion of L-arginine (400 mg/kg = 8.88 mg/kg per min) for 45 min slightly, though not significantly, enhanced net fluid absorption compared to controls (Fig. 4). The absorptive effect of L-arginine was reversed to net fluid secretion by simultaneous administration of glibenclamide, the infusion starting

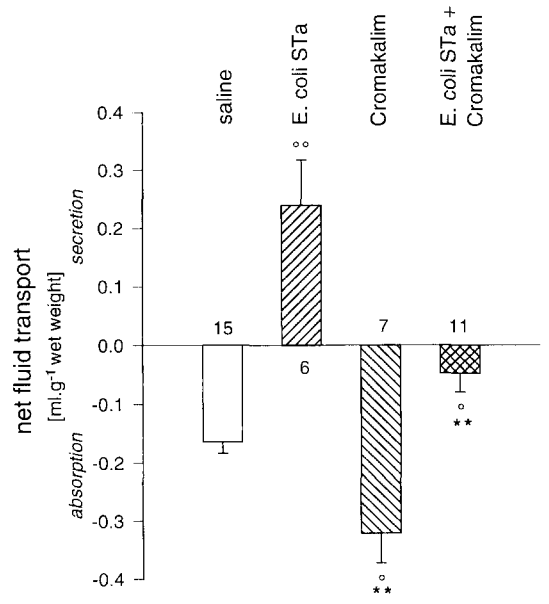

Fig. 3. Effects of saline, *E. coli* STa (10 units/ml, i.l.), cromakalim (63.5 μg/kg per min, i.v.) and *E. coli* STa in the presence of cromakalim on net fluid transport in the rat jejunum in vivo. Each column represents the mean ± S.E.M. The number of experiments is given at the base of each column; $^{\circ}P < 0.05$ and $^{\circ\circ}P < 0.01$ compared to saline, $^{**}P < 0.01$ compared to *E. coli* STa (Student–Newman–Keuls test).

15 min after L-arginine-administration ($P < 0.01$) (Fig. 4). Glibenclamide significantly ($P < 0.05$) reduced the absorptive effect of simultaneous infusion of cromakalim

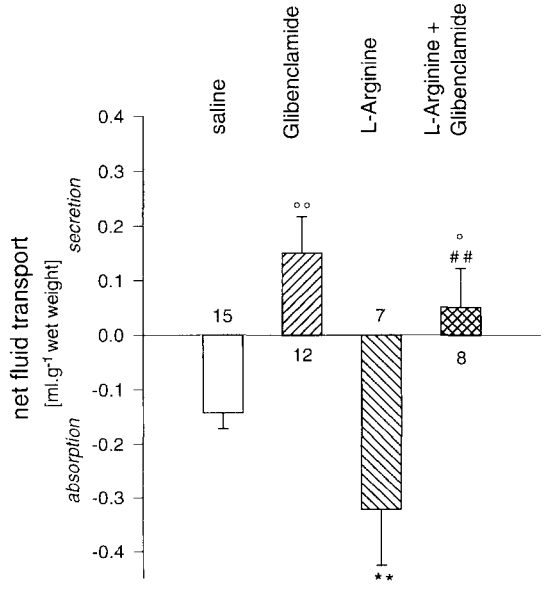

Fig. 4. Effects of saline, glibenclamide (0.16 mg/kg per min, i.a.), L-arginine (8.88 mg/kg per min, i.v.) and L-arginine in the presence of glibenclamide on net fluid transport in the rat jejunum in vivo. Each column represents the mean ± S.E.M. The number of experiments is given at the base of each column; $^{\circ}P < 0.05$ and $^{\circ\circ}P < 0.01$ compared to saline, $^{**}P < 0.01$ compared to glibenclamide, $^{\#\#}P < 0.01$ compared to L-arginine (Student–Newman–Keuls test).

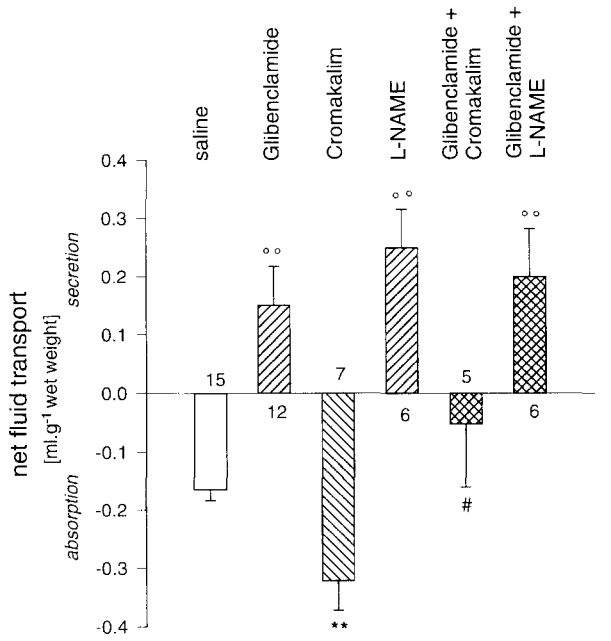

Fig. 5. Effects of saline, glibenclamide (0.16 mg/kg per min, i.a.), cromakalim (63.5 μg/kg per min, i.v.), L-NAME (0.55 mg/kg per min, i.v.) and cromakalim or L-NAME, respectively, in the presence of glibenclamide on net fluid transport in the rat jejunum in vivo. Each column represents the mean ± S.E.M. The number of experiments is given at the base of each coumn; $^{\circ\circ}P < 0.01$ compared to saline, $^{**}P < 0.01$ compared to glibenclamide, $^{\#}P < 0.05$ compared to cromakalim (Student–Newman–Keuls test).

(2.86 mg/kg = 63.5 μg/kg per min) (Fig. 5). The secretory effect of L-NAME (25 mg/kg = 0.55 mg/kg per min) was not influenced by infusion of glibenclamide (Fig. 5).

4. Discussion

The present results further support the concept of an intestinal proabsorptive effect of NO, which has been demonstrated earlier by several studies both in vitro and in vivo (for references, see Section 1). The mechanism of this proabsorptive action of NO, however, has not been elucidated yet, though the involvement of neural mechanisms has been suggested (Rao et al., 1994; Hällgren et al., 1995)

Until recently, the activation of guanylate cyclase with subsequent formation of cGMP was considered to represent the major mechanism of action of NO, except some interactions of highly concentrated NO with different proteins (Schmidt et al., 1993). Activation of guanylate cyclase also is known to be one of the second messenger systems that can influence intestinal fluid transport: *E. coli* STa belongs to a group of enterotoxins which have been shown to elicit their secretory effect by elevation of mucosal cGMP (Field et al., 1978). The enzyme that is stimulated by these enterotoxins is the particulate and not

the soluble form (Rao et al., 1980). An increase in mucosal cGMP in stripped guinea-pig ileal tissue also has been demonstrated after administration of the unspecific NO-donor sodium nitroprusside (McNaughton, 1993), but this result seems to be questionable, since it has been shown earlier 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 our own studies, soluble guanylate cyclase was not detected in a preparation of scraped jejunal mucosa and the particulate guanylate cyclase, which was well stimulated by *E. coli* STa, was not sensitive to sodium nitroprusside (Beubler et al., 1993). L-NAME, moreover, did not alter mucosal cyclic GMP levels in vivo (Schirgi-Degen and Beubler, 1995). Furthermore, elevation of intestinal cGMP rather enhances fluid and electrolyte secretion, whereas administration of NO or NO-donating compounds causes fluid absorption. Therefore, another mechanism seems to account for the proabsorptive properties of NO.

Evidence was given by Bolotina et al. (1994) that NO can use a further mechanism of action to mediate its smooth-muscle relaxing effect without requiring cGMP: the activation of Ca^{2+}-dependent K^+ channels.

On the other side, in rabbit ileal mucosa, activation of K^+ channels decreased short circuit current which was correlated with an increase in NaCl absorption (Homaidan and Broutman, 1994). The increase in basolateral K^+ permeability represents a homeostatic mechanism by which K^+ ions, that have entered via the basolateral Na^+/K^+-ATPase, leave the cell. So the maintenance of the K^+ outward transport is a prerequisite for the function of the Na^+/K^+-ATPase, which transports Na^+ at the basolateral side out of the cell, and subsequently also for apical Na^+-absorption. Accordingly, K^+ channel openers have been shown recently to delay intestinal transit and to inhibit castor–oil-induced diarrhea (Poggioli et al., 1995). However, the homeostatic mechanism of outward K^+ transport is important not only for absorptive villus cells but also for secretory crypts and the function of the basolateral $Na^+/K^+/2Cl^-$ cotransporter. The net effect of altering potassium conductance in villus versus crypt enterocytes remains to be elucidated. Basolateral K^+ channels have been identified by patch–clamp studies in enterocytes from rabbit (Loo and Kaunitz, 1989), rat (Morris et al., 1986) and guinea-pig (Walters and Sepulveda, 1991) as well as in T_{84} cells (Devor et al., 1990; Devor and Frizzell, 1993).

In the present study our intention was to test the following hypothesis: NO activates basolateral K^+ channels in enterocytes and increases K^+ permeability, resulting in enhanced NaCl and fluid absorption.

According to this hypothesis K^+ channel activators and K^+ channel blockers would be expected to alter net fluid transport in a similar way as NO-donors and inhibitors of NO synthase. Furthermore, opening of K^+ channels would inhibit the fluid secretion induced by inhibition of NO synthase and closure of K^+ channels would block the proabsorptive effect of a NO-donor.

The results obtained in the present study, representing functional changes in intestinal fluid transport after different treatments, fit well in the concept outlined above: Cromakalim, a well known opener of K^+ channels, enhanced absorption of fluid under basal conditions, thus mimicking the effect of administration of NO. It furthermore inhibited fluid secretion induced by L-NAME. So the reduced NO-formation, being the consequence of L-NAME-administration, possibly leaves K^+ channels inactivated and results in net secretion. This secretion can be counteracted by opening of K^+ channels with cromakalim.

Likewise, *E. coli* STa-induced secretion was inhibited by cromakalim, thus mimicking the effect of NO-donors in this kind of secretion (Schirgi-Degen and Beubler, 1995). This result also confirms the observations by Poggioli et al. (1995), who demonstrated an inhibition of castor–oil-induced diarrhea in mice by cromakalim and pinacidil.

Prostaglandin E_2-induced fluid secretion, however, which also had been shown to be inhibited by NO-donors previously (Schirgi-Degen and Beubler, 1995), was not influenced by activation of K^+ channels with cromakalim in the present experiments. This discrepancy has to remain unexplained at present.

Administration of glibenclamide, which is a specific inhibitor of ATP-sensitive K^+ channels, elicited net fluid secretion in the present study, thus mimicking the effect of a NO synthase inhibitor. Glibenclamide counteracted the absorptive effect of L-arginine, the substrate of NO synthase and did not enhance L-NAME-induced secretion. This result provides further evidence for an identical site of action of L-NAME and glibenclamide: If K^+ channels are already inactivated in the absence of NO, that is in L-NAME treated animals, administration of a substance that inactivates glibenclamide- (ATP-) sensitive K^+ channels would not be expected to exert a further effect on fluid transport. Glibenclamide finally reduced cromakalim-induced fluid absorption, indicating the antagonism of these substances on fluid transport.

We are well aware that the presented concept is solely based on functional changes in intestinal net fluid transport, challenged by different treatments. Taking this into account, however, the data presented fit well into the concept, that NO-mediated fluid absorption involves opening of basolateral K^+ channels. The present study furthermore supports the significance of K^+ channel openers as potential new antidiarrheal drugs.

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