Inhibitory effect of experimental colitis on fluid absorption in rat jejunum: role of the enteric nervous system, VIP, and nitric oxide

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Inhibitory effect of experimental colitis on fluid absorption in rat jejunum: role of the enteric nervous system, VIP, and nitric oxide. Ablation of capsaicin-sensitive (SD 14) and BAC reversed this decrease in fluid absorption: 54 (SD 13) and 44 (SD 19) were investigated. A significant decrease in jejunal net fluid absorption was evaluated by the in vivo single-pass perfusion technique. The effects of l tetradoxin (TTX), 2 benzylalkonium chloride (BAC), 3 capsaiacin, 4 vasoactive intestinal polypeptide (VIP) antagonism, 5 nitric oxide (NO) synthase (NOS) inhibition, and 6 5-hydroxytryptamine type 3 and 4 (5-HT3 and 5-HT4) receptor antagonism on the changes in fluid movement were investigated. A significant decrease in jejunal net fluid absorption was found 2 and 4 days after colitis induction: 26 (SD 14) and 28 (SD 19). No histological changes were evident in jejunal sections. TTX and BAC reversed this decrease in fluid absorption: 54 (SD 13) and 44 (SD 19) µl·min⁻¹·g dry intestinal wt⁻¹ (P = 0.0005 and P = 0.019, respectively, compared with colitis). Ablation of capsaiacin-sensitive primary afferent fibers had a partial effect: 45 (SD 5) µl·min⁻¹·g dry intestinal wt⁻¹ (P = 0.001 and P = 0.003 compared with colitis and sham, respectively). Constitutive and neuronal NOS inhibition and VIP antagonism returned jejunal net fluid absorption to normal values: 66 (SD 19), 61 (SD 5), and 56 (SD 14) µl·min⁻¹·g dry intestinal wt⁻¹, respectively. 5-HT3 and 5-HT4 receptor antagonism had no effect. Chemical colitis is associated with a significant decrease in jejunal net fluid absorption. This decrease is neurally mediated and involves VIP- and NO-related mechanisms.

experimental colitis; neural control of jejunal fluid secretion

ULCERATIVE COLITIS (UC) is a well-known chronic relapsing inflammatory bowel disease (IBD); however, its etiology has not been totally elucidated. The pathogenesis presents a wide spectrum of interactions between genetic predisposition, exogenous and endogenous triggers, and modifying factors. These interactions lead to spontaneously relapsing and remitting inflammatory processes, resulting in tissue injury mediated by the immune system (51). Accumulation of different types of immune cells (e.g., neutrophils and macrophages), in addition to increased levels of interleukins 1, 2, 6, and 8 and their mRNA, correlates with an increase in TNF-α and arachidonic acid metabolite levels in the colon (2, 51, 53). In addition, the hypothesis of neural involvement in IBD is illustrated by colonic neuronal damage and loss of mucosal neuropetopic innervation (10, 26).

Although UC is confined to the colon, many studies have shown some functional abnormalities in the small intestine of patients with UC. These abnormalities are manifested by a decrease in intestinal water, d-xylene, amino acid, and fat absorption (1, 4, 8, 11, 49). The pathophysiology of this decrease in water and nutrient absorption has not been studied. Although some investigators have found some pathological changes in the jejunum of patients with UC (11, 49), these findings have not been universally reproducible (47, 52), and the contribution of these changes to small intestinal dysfunction is not clear. In addition, clinical observations show that IBD patients have a low body weight, which could be attributed to one or a combination of the following factors: decrease in food intake, decrease in intestinal absorption of nutrients, and/or highly extensive catabolic state secondary to the chronicity of the inflammatory process.

In an animal model of iodoacetamide-induced colitis, we previously showed a significant reduction in alanine absorption from the jejunum (4). The effect of the inflammation in the colon on jejunal function is not fully understood. This discrete cross talk could be the result of a neuronal reflex or release of some neurohumoral factors. The aim of the present experiments is to study the effect of colitis on jejunal fluid transport and to investigate the role of the enteric nervous system (ENS), nitric oxide (NO), vasoactive intestinal polypeptide (VIP), and 5-hydroxytryptamine (5-HT) in this process.

MATERIALS AND METHODS

All animal experiments were approved by the Institutional Review Board Animal Care Committee and the University Research Board of the American University of Beirut.

Intestinal Perfusion

Adult Sprague-Dawley rats (180–220 g body wt, n = 5–8 in each group) were fasted for 18 h, and anesthesia was induced with an injection of pentobarbitone sodium (45 mg/kg ip) and maintained throughout the experiments by intermittent injections (15–30 mg/kg ip) as necessary. The abdomen was opened through a midline incision, and cannulas were inserted into the jejunum proximally (5 cm distal to the duodenojejunal junction) and 25 cm distally and fixed by ligation as described previously (46). The intestine was returned to the abdominal cavity, and the abdomen was closed. The femoral vein was cannulated for intravenous administration of saline or drugs. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
isolated intestinal segment was perfused in situ at 0.5 ml/min with a plasma electrolyte solution (PES; in meq/l: 140 Na\(^+\), 4 K\(^+\), 104 Cl\(^-\), and 40 CO\(_3\)\(^-\)) containing 4 μCi/l \(^{14}\)C labeled polyethylene glycol (PEG) as a nonabsorbable volume marker. After 30 min to ensure establishment of a steady state, consecutive 10-min collections of the effluent were obtained from the distal cannula for 1 h. At the end of the experiments, the animals were killed by an overdose of pentobarbitone, and the perfused intestinal segment was removed, rinsed, blotted, and desiccated in an oven at 100°C to obtain the dry weight. The samples of effluent were analyzed immediately or kept frozen at −20°C until analysis within 2 wk. Short segments of the perfused intestine were stored in 10% formalin for further histological examinations.

**Colitis Induction**

Colitis was induced by instillation of 100 μl of 6% iodoacetamide dissolved in 1% methylcellulose into the descending colon 7 cm proximal to the anal verge via a Nelaton catheter as previously described (50). Sham rats were treated with vehicle. Severity of colonic inflammation was assessed by an independent observer under a dissecting binocular microscope (×10) on a scale of 0–3, where 0 was normal, 1 was mucosal erosion, 2 was a moderate lesion, and 3 was a deep lesion (50). Intestinal perfusion was performed 2, 4, and 8 days after iodoacetamide instillation. Control experiments were performed on sham-treated rats with vehicle alone.

**Neuronal Blockade by TTX and Ablation by Benzylalkonium Chloride and Capsaicin**

To determine whether the effects of colitis on jejunal fluid movement were neurally mediated, different protocols of neuronal blockade or ablation were used.

**Neuronal blockade by TTX.** TTX (0.2 μmol/l) was added to the intestinal perfusate of PES (58).

**Ablation of the myenteric plexus with benzylalkonium chloride.** Rats were anesthetized with pentobarbitone sodium (45 mg/kg body wt), and the abdominal cavity was opened by a midline incision. A 25-cm segment of the proximal jejunum, 5 cm distal to the ligament of Treitz, was exteriorized and soaked for 30 min in a sterile petri dish containing benzylalkonium chloride (BAC) solution (3 mmol/l). The segment was thoroughly washed with saline and returned to the abdominal cavity. The abdominal wall was closed, and the animal was left to recover for 2–3 wk. This procedure results in ablation of the myenteric plexus of the treated segment (48). Sham rats underwent the same surgical procedure, and the jejunal segment was soaked with saline for 30 min. Animals had access only to water for the first 24 h after treatment. After this initial period, food and water were provided. The antibiotic penicillin G (250,000 U/kg im) was administered daily for 2 days after the surgery. The effect of chemical colitis (2 days after instillation of iodoacetamide) on jejunal fluid transport was then examined as described above.

**Chemical ablation of capsaicin-sensitive primary afferents.** Under ether anesthesia, rats were injected at time 0 with capsaicin at 25 mg/kg sc (in 10% Tween 80, 10% olive oil, and 80% distilled water) and again 8 and 32 h later (under ether anesthesia) with capsaicin at 50 mg/kg (30). After 15 days, the eye-wipe test was used to check the rats for successful ablation, and the animals were entered into the experimental protocol.

**NO Synthase Inhibition, VIP Antagonism, and 5-HT\(_3\) and 5-HT\(_4\) Receptor Antagonism**

All the following experiments were performed 2 days after iodoacetamide or vehicle treatment. The NO synthase (NOS) inhibitor nitro-L-arginine methyl ester (L-NAME, 0.1–1 mmol/l) was added to PES, and the intestine was perfused as described above. In another set of experiments, L-arginine was administered (500 mg/kg sc) before the L-NAME perfusion to determine whether the effect of L-NAME on the colitis-induced decrease in jejunal fluid absorption was specifically NO mediated.

Similar experiments were done after administration of the neuronal NOS (nNOS) inhibitor 7-nitroindazole (7-NI, 50 mg/kg in 10 mg/kg peanut oil ip) (36), instead of L-NAME.

In another group of rats, the VIP antagonist [4Cl-D-Phe\(^{6}\),Leu\(^{7}\)]VIP (2 μg·kg\(^{-1}\)·min\(^{-1}\) iv) was infused during perfusion of the intestine with PES. We previously demonstrated the specificity of this VIP antagonist and the ability of this dose to completely antagonize the secretory effect of intravenous VIP (38).

In another set of experiments, the 5-HT\(_{3}\) receptor antagonist granisetron and the 5-HT\(_{3}\) and 5-HT\(_{4}\) receptor antagonist tropisetron (75 μg/kg sc) were administered 30 min before the jejunal perfusion. The dose of these antagonists is capable of inhibiting cholera toxin-induced secretion (7, 38, 39).

**Analytic Methods and Data Analysis**

\(^{14}\)C]PEG concentrations were measured in duplicate in the effluent and perfusate by liquid scintillation spectroscopy, and net fluid movement was calculated according to the equation where \(\text{PEG}_{\text{p}}\) and \(\text{PEG}_{\text{e}}\) are the measured counts per minute of the radioactive carbon in the perfusate and effluent samples, respectively. Net fluid movement is expressed as microliters per minute per gram dry intestinal weight. Positive values denote net absorption, and negative values denote secretion. Steady-state conditions were confirmed by <5% variation in fluid movement between consecutive 10-min collections. Values were accepted only if \(^{14}\)C]PEG recovery was 95–105% (46).

Values are means (SD) and 95% confidence intervals (CI) in each group of animals. Comparisons between groups were made using unpaired \(t\)-test. ANOVA was used for multiple comparisons, with Bonferroni’s post hoc test as appropriate. GraphPad Prism 3 and Instat 3 software were used for statistics and graphics (GraphPad Software, San Diego, CA).

**Histology**

Fixed jejunal tissues were serially cut into 5- to 7-μm-thick sections and stained with hematoxylin and eosin, periodic acid-Schiff, or toluidine blue. The sections was examined using light microscopy by a pathologist unaware of the experimental protocol. The following criteria were considered for the assessment of inflammation: status of the intestinal lining, shape and alignment of enterocytes, size of villi, appearance and distribution of mast cells, changes affecting the basement membrane, inflammatory cell infiltration, and any changes in structure and shape of the intestinal layers.

**Materials**

VIP antagonist, L-arginine, L-NAME, BAC, TTX, and capsaicin were obtained from Sigma Chemical (St. Louis, MO); granisetron and tropisetron from SmithKline Beecham and Novartis Pharma, respectively; and \(^{14}\)C]PEG from Amersham International (Buckinghamshire, UK). All other reagents were supplied by British Drug House (Poole, UK).

**RESULTS**

**Effect of Iodoacetamide on Colonic Ulcer Score and Jejunal Histology**

Significant mucosal colonic damage was recorded at different intervals after iodoacetamide treatment. The mean (SD) scores were 2.8 (0.33) \((n = 78)\) and 2.6 (0.18) \((n = 8)\) at 2 and 4 days, respectively, after iodoacetamide treatment. There was
a complete recovery of the ulcerations, with the formation of a scar, at 8 days (Fig. 1).

Microscopic examination of hematoxylin-and-eosin-stained sections of colon revealed ulceration of the epithelium, with complete loss of the crypts, heavy infiltration by inflammatory cells, and tissue edema. These changes peaked at 2–3 days and resolved by 8 days after the induction of colitis. Examination of sections from sham rats and rats subjected to colitis.

Effect of Iodoacetamide on Jejunal Fluid Absorption

Basal net fluid absorption in sham rats was 61 (SD 6.5) μl·min⁻¹·g⁻¹. The iodoacetamide-induced decrease in jejunal net fluid absorption was 26 (SD 14) μl·min⁻¹·g⁻¹ (CI 5–37, n = 8, P < 0.0002; Fig. 2). This decrease was still observed 4 days after iodoacetamide treatment [28 (SD 19) μl·min⁻¹·g⁻¹, CI 13–43, n = 8, P = 0.0002], with a rebound increase 8 days after treatment [77 (SD 16) μl·min⁻¹·g⁻¹, CI 43–67, n = 7, P = 0.009 compared with sham; Fig. 2].

Fig. 1. Ulcer index score of rat colon 2, 4, and 8 days after instillation of iodoacetamide or vehicle. Values are means (SD). *P < 0.0001 compared with sham.

Fig. 2. Jejunal net fluid absorption 2, 4, and 8 days after treatment with iodoacetamide (colitis) or vehicle (sham). Values are means (SD) of 7–8 rats in each group. *P < 0.0002; **P = 0.009 compared with sham at the same time.

Effect of TTX, BAC, and Capsaicin on Jejunal Fluid Movement

Intraluminal perfusion of TTX (0.2 μmol/l) did not affect basal fluid absorption [60 (SD 13) μl·min⁻¹·g⁻¹, CI 44–76, n = 5, P = 0.45] but inhibited the decrease in net fluid absorption in colitic rats [54 (SD 10) μl·min⁻¹·g⁻¹, CI 45–64, n = 6, P = 0.0005; Fig. 3]. BAC treatment did not induce a significant alteration in the basal fluid transport [51 (SD 17) μl·min⁻¹·g⁻¹, CI 35–68, n = 7, P = 0.16 compared with sham BAC] but reversed the iodoacetamide-induced decrease in jejunal net fluid absorption [44 (SD 14) μl·min⁻¹·g⁻¹, CI 32–57, n = 7, P = 0.019; Fig. 4]. Capsaicin treatment in adults had no effect on basal fluid absorption [68 (SD 5) μl·min⁻¹·g⁻¹, CI 62–75, n = 5, P = 0.09]; however, it partially reversed the effect of colitis [45 (SD 5) μl·min⁻¹·g⁻¹, CI 38–51, n = 5, P = 0.001 compared with colitis and P = 0.003 compared with control capsicain; Fig. 5].

Effect of NOS Inhibition, VIP Antagonism, and 5-HT₄ Receptor Antagonism on Jejunal Fluid Movement

L-NAME dose dependently affected jejunal net fluid absorption in iodoacetamide-treated rats (P < 0.0001 by ANOVA). L-NAME at 0.5 mmol/l partially reversed the iodoacetamide-
induced decrease in jejunal net fluid absorption [49 (SD 5) μl·min⁻¹·g⁻¹, CI 40–57, n = 5, P < 0.05 compared with colitis and P < 0.05 compared with sham], and L-NAME at 1 mmol/l returned jejunal net fluid absorption to normal values [66 (SD 19) μl·min⁻¹·g⁻¹, CI 43–88, n = 5, P > 0.05]. Preadministration of L-arginine (500 mg/kg sc) completely reversed the effect of 0.5 and 1 mmol/l L-NAME [18 (SD 18) μl·min⁻¹·g⁻¹ (CI −10 to 45, n = 5) and 29 (SD 17) μl·min⁻¹·g⁻¹ (CI 7–51, n = 5, P = 0.02 and P = 0.008, respectively); Table 1]. Furthermore, L-NAME perfused in the intestine in sham rats induced a significant decrease in net fluid absorption (Table 1).

7-NI administration completely inhibited the iodoacetamide-induced decrease in jejunal net fluid absorption [61 (SD 5) μl·min⁻¹·g⁻¹, CI 53–69, n = 5, P < 0.0001]; however, it had no effect on fluid absorption in sham rats (Fig. 6).

The VIP antagonist [4Cl-d-Phe⁶,Leu¹⁷]VIP completely abolished the effect of colitis on jejunal net fluid absorption [56 (SD 14) μl·min⁻¹·g⁻¹, CI 45–67, n = 8, P = 0.0002 compared with colitis and P = 0.16 compared with sham; Fig. 7]. VIP antagonism had no effect on basal fluid transport.

The 5-HT₃ receptor antagonist granisetron and the 5-HT₄ receptor antagonist tropisetron had no effect on jejunal fluid transport in sham rats [62 (SD 6) μl·min⁻¹·g⁻¹ (CI 44–70, n = 5) and 59 (SD 7) μl·min⁻¹·g⁻¹ (CI 44–67, n = 5)] or in rats with colitis [18 (SD 10) μl·min⁻¹·g⁻¹ (CI 8–29, n = 6) and 21 (SD 6) μl·min⁻¹·g⁻¹ (CI 12–30, n = 5)].

**DISCUSSION**

Our aim was to study the possibility of changes in jejunal fluid absorption in view of the available data on intestinal absorptive dysfunction associated with IBD. The causes and the underlying mechanisms that could be involved in these changes were also investigated. The present experiments demonstrate that experimental colitis in rats results in a significant decrease in jejunal net fluid absorption. This decrease seems to be neurally dependent and mediated by VIP and NO as neurotransmitters.

Many functional and structural abnormalities have been described in the small intestine of some patients with UC. A decrease in the absorption of fluid and electrolytes (8, 45), amino acids (57), and fat, folic acid, and D-xylose (1, 11, 52) has been documented in humans. The effect of these changes on the clinical status of dehydration and malnutrition in patients with IBD is not known. In addition, the pathophysiology of jejunal absorption of fluid and electrolytes in experimental colitis deserves further investigation.
and the role of the colonic inflammation and inflammatory mediators on these changes are far from understood.

Animal models of chemical colitis have been widely used to study the pathogenesis of IBD and the effect of some novel therapies on colonic inflammation. Very few studies have examined small intestinal disturbances in these models. A decrease in ileal fluid and electrolyte absorption has recently been described in a rat model of acetic acid-induced colitis, although the small intestinal histology remained normal, with no evidence of any inflammatory reaction (14). Other investigators have described increased permeability and myoelectric activity of the ileum in trinitrobenzene sulfonic acid (TNBS)-and ethanol-induced colitis (3, 14, 44). In addition, we recently found a decrease in jejunal amino acid absorption in iodoacetamide-induced colitis in rats (4). In the present model of colitis, ulceration in the colon was associated with a significant decrease (50%) of net fluid absorption in rat jejunum in the presence of a normal small intestinal morphology as revealed by the various staining procedures. Thus it appears that colonic inflammation has a significant effect on small intestinal function, irrespective of morphological changes. Furthermore, recent data from our group have shown detectable levels of proinflammatory cytokines in the serum and the small intestines of naive rats that were significantly increased in rats with iodoacetamide-induced colitis (6). Therefore, one may assume that the discrete presence of proinflammatory mediators and cytokines may alter the function of the gastrointestinal tract through their well-documented effects on the ENS (24) and on intestinal nutrient transport (20).

Inasmuch as fluid absorption in the small intestine is strongly influenced by the ENS and many known neurotransmitters (19), we investigated whether they play a role in this phenomenon. The ENS, one of the major parts of the autonomic nervous system, is involved in the regulation of gastrointestinal transport of fluid (19, 29) and amino acids (5, 41). Changes in the ENS and extrinsic innervation in colitis have been described, as evidenced by an increase in c-Fos in myenteric neurons, enteric glia, brain stem, and spinal cord in formalin-induced colitis in rats (31, 33) and by an increase in ileal myoelectric activity in TNBS-treated rats (3). Recently, Blandizzi et al. (9) presented convincing evidence that experimental colitis in rats leads to distinct changes in the neural circuitry of noninflamed regions of the bowel and suggested that this may underlie the changes in small bowel motility that accompany colitis. In an in vitro study on enterocyte secretory function, Miceli et al. (34) showed a defect in the noninflamed jejunum 7 days after TNBS-induced ileitis and proposed that this may be mediated by circulating cytokines. Whether the ENS plays a role in the small intestinal fluid absorptive dysfunction induced by acute colitis has not been studied in vivo. Our experiments show that the ENS plays a pivotal role in changes of fluid absorption. TTX and BAC treatment resulted in a complete reversal of the effect of colitis on jejunal net fluid absorption (Figs. 3 and 4). In general, BAC-treated rats, in which the myenteric plexus has been destroyed, have altered mucosal area due to hypertrophy of villi and crypts (18), but this did not result in a significant decrease in basal fluid absorption. However, an intact myenteric plexus is needed for the small intestinal changes observed with iodoacetamide-induced colitis. A decrease in norepinephrine release from the myenteric plexus has previously been shown in inflamed and noninflamed colonic areas in TNBS-induced colitis, a phenomenon that can be attenuated by budesonide and interleukin 1 receptor antagonist (22). Although the myenteric plexus controls intestinal motility, neural projections to the submucosal plexus may alter its absorptive and secretory functions. Inasmuch as BAC treatment does not ablate the submucosal plexus, it seems that a relay message through the myenteric plexus is important for the inhibitory effect of colitis on small intestinal net fluid absorption.

Similarly, TTX totally reversed the effect of iodoacetamide but had no effect on basal fluid transport, suggesting that colitis effects require an intact transmission of neuronal messages. It has been previously shown that this concentration of TTX (0.2 μmol/l) does not alter the ionic movements in rat colon and small intestine (23, 58) but interferes with synaptic mechanisms activated by high doses of carbachol (58) or cholera toxin (23). In addition, ablation of capsaicin-sensitive primary afferent (CSPA) fibers partially reversed the decrease in jejunal fluid absorption in rats with colitis, providing evidence for the importance of these afferent fibers in initiating this reflex alteration of jejunal net fluid absorption. Although Miceli et al. (34) showed in vitro that TTX does not prevent the effect of carbachol on changes in short-circuit current in rats with TNBS-induced ileitis, this observation does not exclude the possible involvement of other neurotransmitters and neural mechanisms. Furthermore, the decreased effect of capsaicin on changes in short-circuit current in noninflamed jejunum are consistent with our in vivo findings showing the involvement of CSPA fibers in the decreased jejunal net fluid absorption during colitis.

To further examine the mediators involved in these changes, we studied the effect of three known intestinal secretagogues and neurotransmitters: 5-HT, VIP, and NO. 5-HT has been found to be increased in the colon of patients with chronic UC (16, 54) as well as in rats and guinea pigs with experimental colitis (28, 42). However, it seems that 5-HT does not play a role in the decrease of jejunal fluid absorption in colitic rats, inasmuch as the 5-HT3 receptor antagonist granisetron and the 5-HT1 and 5-HT2 receptor antagonist tropisetron had no effect.

VIP is a neurotransmitter found in the myenteric plexus, lamina propria, and mucosa of the small and large intestine in different animal species. It is released from the intestine in response to secretomotor reflexes, where it acts on the mucosa to activate adenylate cyclase (56). It has been found that plasma and colonic VIP levels and VIP mRNA, as well as colonic VIPergic neurons, are increased in patients with severe UC compared with controls (15, 17, 55). A positive association between colitis activity and an increase in colonic VIP nerves and VIP content in rats with chemical colitis has been also shown (25, 32). Our results demonstrate that VIP plays an important role in causing a decrease in net intestinal fluid absorption in chemical colitis, inasmuch as this decrease was completely inhibited by the VIP antagonist [4Cl-β,Leu17]VIP.

NO production has been found to be increased in IBD and experimental colitis (33, 35, 43), but its role in the pathogenesis or in the associated diarrhea has not been elucidated. The effect of NO on intestinal fluid transport has been controversial, with many studies showing a proabsorptive and/or a prosecretory effect (21, 40). Whether the release of NO in colitis would affect fluid and nutrient absorption in a distant
part of the intestine has not been investigated. We have found that \(\gamma\)-L-NAME administration resulted in a significant inhibition of the effect of colitis on small intestinal net fluid absorption, an effect that could be reversed by the NO precursor \(\gamma\)-arginine. Similar results were obtained with the nNOS inhibitor 7-NI, demonstrating that the decrease in fluid absorption was through neuronal NO. It is unlikely that NO produced in the colon affects jejunal fluid absorption through the systemic circulation, inasmuch as it has a very short half-life and is quickly inactivated by hemoglobin. Thus the effect of NO is secondary to an increase in NO-containing neurons in the jejunum or to a reflex stimulation of nitricergic neurons induced by colonic inflammation. A similar argument can be added to explain the effect of VIP, especially that VIP and NO coexist in the ENS.

It has been well established that VIP and nNOS are colocalized mainly in the myenteric neurons (13, 27), although some recent data demonstrated their coexistence in rat submucosal neurons, the axons of which could secrete VIP or NO (12). On the other hand, VIP/NOS terminals in the myenteric plexus synapse on VIP and non-VIP secretomotor neurons that do not contain NOS (13, 27). This may imply that NO produced in the myenteric plexus and, to a lesser extent, in the submucosal plexus could induce VIP release from either plexus to stimulate secretion. We previously demonstrated an interaction between VIP and NO in causing intestinal fluid secretion (37), which may explain why inhibition of each neurotransmitter resulted in a complete inhibition of the effect of colitis on jejunal fluid transport.

In conclusion, our experiments demonstrate that experimental colitis in rats induces distant alteration in the normal function of the gastrointestinal tract, as illustrated by the significant decrease in jejunal fluid absorption. This pathology may involve extrinsic and intrinsic neuronal pathways that are mediated by the activation of VIP and NO synaptic mechanisms.

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