Alterations in capsaicin-evoked electrolyte transport during the evolution of guinea pig TNBS ileitis

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Miceli, Paula, Gerald P. Morris, Wallace K. MacNaughton, and Stephen Vanner. Alterations in capsaicin-evoked electrolyte transport during the evolution of guinea pig TNBS ileitis. Am J Physiol Gastrointest Liver Physiol 282: G972–G980, 2002. First published December 19, 2001; 10.1152/ajpgi.00037.2001.—The efferent secretomotor activity of capsaicin-sensitive nerves was monitored during the evolution of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced ileitis in the guinea pig by recording changes in short-circuit current (ΔIsc) in response to capsaicin, substance P (SP), and carbachol. Submucosal-mucosal preparations mounted in standard Ussing chambers were studied at time 0, at 8 h, and 1, 3, 5, 7, 14, and 30 days following the intraluminal instillation of TNBS or saline. Maximal ΔIsc responses to capsaicin were dramatically attenuated (54%) by 24 h. By day 7, SP- and TTX-insensitive carbachol-stimulated ΔIsc were also significantly reduced. Similar attenuation in capsaicin and carbachol responses was observed in jejunal tissue 20 cm proximal to the inflamed site at day 7. These studies demonstrate that efferent secretomotor function of capsaicin-sensitive nerves is maintained early in TNBS ileitis but significantly reduced by 24 h. By day 7, defects in enterocyte secretory function at inflamed and non-inflamed sites also occurred, an effect that may be mediated by circulating cytokines.

capsaicin-sensitive nerves; submucosal neurons; substance P

The release of neuropeptides from capsaicin-sensitive nerve terminals within the intestine activates a number of effector systems, including mucosal electrolyte transport and fluid secretion (12, 35), motility (33), mucus secretion (28), and mucosal blood flow (1, 11, 34, 36). Two lines of evidence suggest that these capsaicin-sensitive nerves play a protective role in the intestine. First, studies have shown that stimulation of the capsaicin-sensitive nerves with capsaicin protects the integrity of the mucosal surface against the topical application of noxious agents. For example, mucosal injury induced by alcohol (12) and aspirin (11) is markedly reduced when capsaicin is acutely applied. Alternatively, when capsaicin-sensitive nerves are first selectively ablated, the magnitude of damage caused by injurious agents is markedly increased (5, 6, 23). Together, these two types of studies provide strong evidence that capsaicin-sensitive nerves protect the intestine against damage, but the mechanisms by which this occurs are unclear.

There is little known about the activity of capsaicin-sensitive nerves during the initiation and evolution of an inflammatory response. Increased blood flow to the mucosa (12, 19) and/or enhanced motility (33) and secretion (35), which may decrease contact time of noxious agents, may be important defensive mechanisms. However, the time course of these actions during inflammation is unclear. A number of studies employing a variety of inflammatory models (4, 22, 32) suggests that the synthesis and release of neurotransmitters from capsaicin-sensitive nerves is upregulated during inflammation and that this results in enhanced effector responses and faster resolution of the inflammatory response. Other studies, however, have demonstrated that substance P (SP) immunoreactivity in capsaicin-sensitive neurons falls as inflammation progresses (27), implying that the activity of capsaicin-sensitive nerves may decrease over time. Together, these data suggest that the efferent function of capsaicin-sensitive nerves may vary during the evolution of the inflammatory response.

The aim of this study was to examine the actions of capsaicin-sensitive nerves in the intestine during the establishment and resolution of intestinal inflammation. We studied capsaicin-evoked secretion in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced ileitis in the guinea pig; a model of chronic inflammation (26, 27, 29). Our previous studies (35) had established the neural secretomotor pathway activated by capsaicin-sensitive nerves in this tissue. They demonstrated that capsaicin selectively stimulates capsaicin-sensitive nerves in the submucosa to release neurotransmitter that activates cholinergic and noncholinergic secretomotor neurons. These neurons, in turn, innervate mucosal enterocytes evoking chloride secretion. This in-
formation enabled the responses to capsaicin to be followed during inflammation and identification of the specific site involved (i.e., neural or enterocyte) when changes occurred. Unlike other studies (2), inflamed tissue was taken from nonulcerated segments of intestine to ensure that results of transport studies were not altered by ulcerated epithelium.

**MATERIALS AND METHODS**

Guinea pigs (Hartley; 180–200 g) were obtained from Charles River Laboratories (Trois Rivieres, Quebec, Canada) and were maintained on standard laboratory chow and tap water ad libitum. All protocols were approved by the Queen’s University Animal Ethics Committee, and procedures involving animals followed the guidelines of the Canadian Council on Animal Care.

**Induction of ileitis.** Guinea pigs were given a preanesthetic tranquilizer (Innovar-vet: 0.5 ml/kg im) and anesthetized with pentobarbital sodium (Somnotol: 15 mg/kg sc). After a midline laparotomy, a distal section of ileum was exposed and 0.5 ml saline (sham surgical controls) or 0.5 ml TNBS (30 mg/ml in 50% EtOH) were injected intraluminally from the ileocecal junction, as previously described (26, 27). The ileum was replaced into the abdominal cavity, and the incision was sutured. Animals were killed immediately after surgery, at 8 h, or 1, 3, 5, 7, 14, or 30 days following surgery, and tissue was obtained for Ussing-chamber studies and histological evaluation.

**Ussing-chamber studies.** Animals were rendered immediately unconscious with a single blow to the head and killed by carotid and cervical transection. An 8-cm ileal segment, originating from a site 4 cm proximal to the ileocecal junction, was removed and placed in a cold, oxygenated (5% CO2-95% O2) modified Krebs buffer containing NaCl, 2.0 KH2PO4, 2.4 MgCl2, 1.3 CaCl2, 8.0 KCl, and 25.0 NaHCO3. Segments were scored along the mesenteric border, and the muscularis externa was removed. Submucosal-mucosal preparations were mounted in standard Ussing chambers (World Precision Instruments, Sarasota, FL) with 0.6 cm2 exposed surface area. Sites with grossly visible damage were excluded from these studies. The serosal side of the preparation was bathed by modified Krebs buffer containing 10 mM glucose, and the mucosal buffer contained 10 mM mannitol. The chambers were maintained at 37°C and oxygenated (5% CO2-95% O2). After a 10-min equilibration period, potential difference (PD) was recorded in the open-circuit condition and then clamped to 0 mV by applying a short-circuit current (Isc) using a voltage-clamp apparatus (EVC-4000, World Precision Instruments). The Isc was amplified via a 5110 oscilloscope and converted into a digital signal (TL-1 DMA interface, Axon Instruments, Foster City, CA) that was stored as a continuous measurement on a computerized data-acquisition system (Axotape, Axon Instruments). The Isc was monitored as an indicator of net active electrolyte transport across the tissue (20). Our previous studies (35) demonstrated that the increase in Isc reflects an increase in net secretion of predominantly chloride ions. Basal PD and Isc were recorded following equilibration, and resistance was calculated using Ohm’s law. All drugs were added to the serosal side of the chamber, and 10 min elapsed between drug applications, during which time a stable baseline was reestablished. Standard electrical field stimulation (EFS; 100 V, pulse duration 0.5 ms, 10 Hz, 5s) was delivered with a Grass stimulator via two stainless steel posts mounted at the back of both chambers. Isc responses were expressed as the maximum change in Isc from baseline (μAVCm2).

Characterization of the capsaicin-sensitive neural circuit mediating electrolyte transport (35) in this model demonstrated the specific sites of action of capsaicin, SP, and carbachol (see Fig. 6A). On the basis of these findings, capsaicin was employed to selectively stimulate neuropeptide release from capsaicin-sensitive nerves, SP was used to stimulate submucosal secretomotor nerves, and carbachol, combined with TTX, was used to directly stimulate chloride secretion by the enterocyte.

In a separate series of experiments, jejunal tissue was also obtained 20 cm proximal to the site of TNBS ileitis for Ussing-chamber studies. In addition, after the findings in the initial studies at days 1, 3, 5, 7, and 30, which demonstrated that agonist-evoked responses were reduced at 7 days in TNBS animals, a further set of experiments was conducted to determine whether these findings persisted at a later time point, 14 days.

**Assessment of inflammation.** In a separate series of experiments, tissue was obtained from animals receiving either saline (sham surgical controls) or TNBS, as described in **Induction of ileitis.** A 10-cm segment of ileum was removed immediately proximal to the instillation site at 4 cm from the ileocecal junction. The first and last centimeter of the segment were placed in liquid nitrogen and stored at −80°C for subsequent myeloperoxidase (MPO) assay to quantify inflammation. In the remaining 8 cm, tissue samples from sites meeting the criteria for use in electrophysiological studies were examined for epithelial integrity. Briefly, tissue was cut along the mesenteric border, pinned out mucosa-side-up, and fixed in Carnoy’s, dehydrated, and wax-infiltrated using a standard protocol. Wax blocks were sectioned at 5 μm and stained with hemotoxylin and eosin. To assess mucosal integrity, sections from sham surgical controls (saline) and TNBS-treated animals were examined by an investigator who was blinded to the treatment received.

MPO activity was measured according to the procedure of Krawisz et al. (21). Briefly, frozen intestinal segments were placed into a solution (1 ml/50 mg tissue) of hexadecltrimethylammonium bromide (0.5%, wt/vol) in potassium phosphate buffer (50 mM, pH 6.0). Samples were homogenized on ice for 30 s, centrifuged for 30 min at 10,000 rpm and 4°C, and then incubated at 60°C for 2 h. After incubation, 100 μl of supernatant were added to 2.9 ml of 0.00005% H2O2 in an o-dianisidine dihydrochloride (Sigma, St. Louis, MO) solution in a cuvette, and the absorbance was measured at 460 nm. Results were expressed as units of MPO activity per 100 milligrams of tissue (wt weight), where MPO activity is the amount of enzyme required to split 1 μmol H2O2/min at 25°C.

**Drugs.** Capsaicin (Sigma) was dissolved in Tween 80, alcohol, and saline (80:10:10); SP (Peninsula Laboratories, Belmont, CA), tetrodotoxin (Sigma), and carbachol (Sigma) were dissolved in distilled water.

**Statistics.** These data were expressed as means ± SE. For each experiment, the basal PD, Isc, resistance, and the maximal change in Isc in response to secretagogues were averaged for each treatment group. Comparisons of agonist-evoked responses in sham surgical controls (saline) and TNBS treatment groups for separate time points were analyzed using two-tailed t-tests in initial studies at days 1, 3, 5, 7, and 30 and a one-tailed t-test in follow-up studies at 14 days. The level of significance was set at P < 0.05.

**RESULTS**

**General properties.** The properties of mucosal-submucosal in vitro preparations were examined from

_AJP-Gastrointest Liver Physiol • VOL 282 • JUNE 2002 • www.ajpgi.org_
three different animal groups in this study. Control preparations (nonoperated controls) were obtained from animals that were killed without prior surgical intervention (day 0). Sham surgical controls (saline-treated preparations) and TNBS-treated preparations were obtained from killed animals that previously had undergone laparotomy at 8 h and 1–30 days before study with injection of either saline or TNBS into the terminal ileum, respectively (see MATERIALS AND METHODS).

Macroscopically, erythema of the mucosa and serosa was confined to the distal 8–10 cm. Mucosal ulceration was evident at and distal to the injection site in the TNBS groups on days 1, 3, 5, and 7 in most preparations. No ulceration was macroscopically evident in the proximal adjoining 10-cm segment taken for histological and electrophysiological study. Histological examination of sections of tissue confirmed the integrity of the epithelium at the selected sites (n = 4 for each group at 8 h of time; Fig. 1, days 1, 3, 5, 7, and 30). Both the sham surgical controls (saline) and TNBS had a significant mean increase in MPO activity at day 1 compared with nonoperated controls (Table 1). MPO activity in the TNBS group, however, was significantly greater than the sham surgical control group levels at days 1, 3, and 5.

Under basal resting conditions, a negative \( I_{sc} \) was maintained in all preparations, consistent with net mucosal-to-serosal anion flux, as previously reported for in vitro ileal mucosal transport (31). The basal resistance, PD, and \( I_{sc} \) of in vitro submucosal-mucosal preparations of the nonoperated controls, sham surgical controls (saline), and TNBS-treated animals are shown in Table 2. Some differences existed between groups, but these were not consistent within treatment groups. These changes likely reflect variation due to experimental technique, as described by others (30).

The addition of TTX (1 \( \mu M \)) to the serosal bath in nonoperated controls (day 0) caused a significant decrease in basal \( I_{sc} \) (mean = \(-62.3 \pm 6.7 \mu A/cm^2\)) compared with vehicle (mean = \(-0.4 \pm 0.01 \mu A/cm^2\); \( P < 0.05 \)), as previously described (13). Compared with nonoperated controls (day 0), the TTX-evoked reduction in basal \( I_{sc} \) effect in preparations from the TNBS group at day 7 was significantly less (\(-32.9 \pm 10.0 \mu A/cm^2\); \( P < 0.05 \)). The effect in the surgical control group (saline) at the same time point did not reach statistical significance (\(-37.8 \pm 9.4 \mu A/cm^2\)).

**\( I_{sc} \) response to capsaicin.** Our previous studies have demonstrated that serosal application of capsaicin evokes a dose-dependent increase in \( I_{sc} \) (35). These studies demonstrated that this increase in \( I_{sc} \) results from selective activation of capsaicin-sensitive nerves with ensuing release of neuropeptides. These neuropeptides activate submucosal secretomotor neurons, that, in turn, innervate mucosal enterocytes (see Fig. 6A), causing chloride secretion (35). The \( I_{sc} \) response, evoked by activation of this pathway, was biphasic with a brief initial phase (capsaicin \( EC_{50} = 50 \) nM) followed by a large second phase (capsaicin \( EC_{50} = 90 \) nM). In the current study, 50 nM capsaicin evoked a typical biphasic response in all nonoperated control and sham surgical control preparations (Fig. 2). Both phases of the response were unaltered by TNBS at 8 h after instillation. By day 1, however, the large second phase \( I_{sc} \) response to capsaicin in TNBS-treated preparations was almost completely abolished compared with sham surgical controls (saline). When sham surgical controls (saline) and TNBS-treated groups were compared, there was also a significant decrease in the TNBS group on days 1–7 (Fig. 2; \( P < 0.05 \)). There was also a trend toward a similar reduction at day 14, but this did not reach statistical significance. This inflammation-induced decrease in capsaicin-evoked \( I_{sc} \) was lost at day 30. The first phase of the capsaicin-evoked response was markedly reduced at day 1 in the TNBS animals compared with sham surgical controls (saline). There was also a large reduction in the magnitude of the phase 1 responses in the TNBS compared with the
sham surgical control (saline) group at days 3–7, but these changes did not reach statistical significance (data not shown).

Effect of SP, carbachol, and EFS on I_sc responses. The effects of SP and carbachol were studied to identify site(s) in the capsaicin-evoked secretomotor pathway that might account for the reduced capsaicin responses in the TNBS group. Our previous studies (35) have shown that the SP-evoked increase in I_sc is mediated by activation of the secretomotor neurons, because these responses were completely abolished by TTX. Carbachol responses, however, were largely unaffected by TTX, suggesting carbachol-evoked changes in I_sc result predominantly from direct activation of the enterocyte (see Fig. 6A).

In the current study, SP evoked a dose-dependent (10 nM–1 μM) increase in I_sc (EC_{50} = 90 nM). Carbachol also evoked a dose-dependent (100 pM–1 μM) increase in I_sc (EC_{50} = 800 pM; Fig. 3). The effects of 100 nM SP were studied in sham surgical control (saline) and TNBS groups. Compared with the sham surgical control group, SP-evoked responses in the TNBS group were markedly attenuated at days 7 and 14 but not at day 1, 3, or 5, as found with the capsaicin-evoked responses (see Fig. 2). When carbachol (1 μM) was applied (Fig. 3), the responses also were significantly reduced at day 7 (P < 0.05) compared with the surgical control (saline) group. These responses also displayed a marked trend toward a reduced response at 14 days and just failed to reach statistical significance (P = 0.06). In a separate series of preparations, carbachol-evoked responses on day 7 were reexamined in the presence of TTX (Fig. 4). Carbachol evoked large increases in I_sc in the sham surgical control (saline) group (mean = 70 ± 4; n = 4) but had no effect in the TNBS group (P < 0.005). Both the SP and carbachol-evoked responses returned to nonoperated control values by day 30 (Fig. 3).

Effects of TNBS inflammation on epithelial secretion at a noninflamed site. Previous studies have shown that intestinal inflammation can have significant effects on intestinal function at sites remote from the inflammation (15, 16). To examine whether this includes the capsaicin-evoked secretomotor pathway, the effects of capsaicin and carbachol were studied in parallel experiments in segments of jejunum located 20 cm proximal to the site of TNBS inflammation on day 7. Capsaicin (30 nM) evoked typical biphasic increases in I_sc at this site (n = 4). The capsaicin-evoked phase 2 response was, however, almost completely abolished in the TNBS group (Fig. 5; P = 0.028, n = 4). Macroscopically, there was no evidence of mucosal or serosal erythema. MPO activity did not differ from sham surgical control (saline) controls [mean sham surgical control (saline) = 1.15 ± 0.20 vs. TNBS = 0.17 ± 0.07 units of activity/g wet wt tissue]. The site in the capsaicin-evoked secretomotor pathway that might account for the reduced capsaicin responses in the TNBS model was examined by studying the effects of carbachol (1 μM) combined with TTX (see above and Fig. 5A). After a 3-min application of TTX (1 μM), carbachol-evoked I_sc was almost completely abolished (Fig. 5).

DISCUSSION

This study examined the secretory function of capsaicin-sensitive efferent nerves during TNBS-induced ileitis. The results suggest that inflammation dramatically attenuates capsaicin-evoked mucosal secretion within the initial 12–24 h after initiation of inflammation (see Fig. 6B). It was possible to dissect effects of

| Table 1. Changes in myeloperoxidase activity in TNBS and saline control animals |

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5 ± 1.1</td>
<td>139.3 ± 35.1</td>
<td>14.5 ± 2.5</td>
<td>15.9 ± 6.6</td>
<td>23.5 ± 7.4</td>
</tr>
<tr>
<td>Sham</td>
<td>247.2 ± 25.1*</td>
<td>29.8 ± 3.9†</td>
<td>58.6 ± 4.3‡</td>
<td>25.5 ± 10.6</td>
<td>3.5 ± 2.0</td>
</tr>
</tbody>
</table>

Values are units/100 mg tissue wt expressed as means ± SE; n = 5 for each group. *P = 0.026; †P = 0.003; ‡P = 0.006. Control, nonoperated animals; sham, operated animals (saline); TNBS, 2,4,6-trinitrobenzenesulfonic acid.

| Table 2. Basal R, PD, and Isc in saline and TNBS animals |

<table>
<thead>
<tr>
<th>PD, mV</th>
<th>0 h</th>
<th>8 h</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9 ± 0.3</td>
<td>1.3 ± 0.0</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>TNBS</td>
<td>2.3 ± 0.1‡</td>
<td>2.2 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>3.9 ± 0.4</td>
<td>2.0 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>Isc, μA/cm²</td>
<td>82.4 ± 7.4</td>
<td>74.4 ± 3.1</td>
<td>66.0 ± 4.2</td>
<td>76.8 ± 4.3</td>
<td>56.4 ± 4.8</td>
<td>57.3 ± 5.9</td>
<td>52.9 ± 4.9</td>
<td>92.8 ± 5.5</td>
</tr>
<tr>
<td>R, Ω/cm²</td>
<td>99.7 ± 5.0</td>
<td>62.0 ± 6.9</td>
<td>98.1 ± 11.7</td>
<td>76.8 ± 6.1</td>
<td>108.7 ± 17.6</td>
<td>50.5 ± 13.0</td>
<td>55.2 ± 4.3‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 for each group, *P < 0.03; †P < 0.05; ‡P < 0.001. R, resistance; PD, potential difference; Isc, short-circuit current. Control: 0 h, nonoperated animals; 8 h—Day 30, operated animals (saline).
altered neural function from changes occurring at the level of the enterocyte, because we had carefully defined the components of this neural pathway (see Fig. 6A) in previous studies (35) and the current study examined inflamed tissue with nonulcerated epithelium. To determine whether this effect was confined to alterations in capsaicin-sensitive nerves, we examined the effects of SP and carbachol-evoked responses (see Fig. 6A). These studies showed that the attenuated capsaicin-evoked secretion was confined to capsaicin-sensitive nerves during the first 5 days, because there was no concomitant alteration in SP- and carbachol-evoked electrolyte transport. However, by 7 days, carbachol-evoked responses in the presence of TTX were also significantly reduced, demonstrating changes at the level of the enterocyte (see Fig. 6C). These findings persisted for at least an additional 7 days. The reduction in SP responses, which occurred at these same time points, may also solely reflect changes at the level of the enterocyte but could also include alterations in submucosal secretomotor neurons. The reduction in

TTX-sensitive baseline secretion (i.e., spontaneous submucosal secretomotor-evoked secretion) seen at 7 days may reflect this effect. Together, these studies demonstrate that both neural and nonneural secretory mechanisms are affected during the evolution of the ileitis.

There are several possible mechanisms that might underlie the inflammation-induced attenuation of capsaicin-sensitive nerve function. One possibility is that local release of inflammatory cytokines, such as interleukin (IL)-1β (14, 16, 24), may inhibit release of neurotransmitter. This inhibitory effect appears to affect multiple neural pathways, including cholinergic nerves, as suggested in preliminary studies (24). This action is an unlikely explanation of the capsaicin attenuation, given that the submucosal secretomotor pathways, which include cholinergic nerves (35), were unaffected. An alternative explanation is that tachyphylaxis occurs following supramaximal stimulation from cytokines, rendering the tissue hyporesponsive to additional stimuli. This is also unlikely, given that basal secretion did not differ between sham surgical controls (saline) and TNBS-treated animals and that SP and carbachol stimulation was initially unaffected.

The most likely possibility is that inflammation results in constant stimulation of capsaicin-sensitive nerve terminals resulting in eventual depletion of neurotransmitter. This concept is directly supported by a related study that examined SP tissue levels and immunoreactivity in the same model of guinea pig TNBS ileitis and studies in the colon using an immune complex model (7, 25, 26). In the guinea pig TNBS-ileitis studies, SP immunoreactivity in the mucosa and submucosa was reduced by day 1 of inflammation and whole tissue SP content by day 3 (7, 25, 26). The reduced immunoreactivity was particularly marked in perivascular nerves, which are almost solely capsaicin-sensitive afferent nerves (8, 34). In a similar fashion, studies in the colon (7) have shown that inflammation reduces the SP content in whole thickness colonic extracts as early as 8 h. Together, these findings show that SP neurotransmitter content falls during the same time period that the capsaicin nerve-evoked responses are attenuated, strongly supporting the notion that the reduced secretory responses result from neurotransmitter depletion from the capsaicin-sensitive nerves.

The role of hypersecretion of the epithelium during inflammation within the small intestine has not been fully resolved. In the colon, diarrhea induced by TNBS colitis appears to be secondary to loss of electrolyte absorption attributable to epithelial damage and not altered electrolyte secretion (2). In the current study, the effects of TNBS-ileitis were examined at sites that were inflamed but the mucosa was intact. There was some variability in basal $I_{sc}$ measurements, as shown in Table 2, but these did not display any pattern or distribution within the data that would suggest a physiological effect. Such variation is typical in Ussing chamber studies (30), and as a result, $I_{sc}$ measurements are traditionally reported as $\Delta I_{sc}$. Table 2 also

![Fig. 2. Capsaicin-evoked short-circuit current ($I_{sc}$) is markedly attenuated by 24 h of inflammation. A: a representative trace showing that 50 nM capsaicin evoked a typical biphasic increase in $I_{sc}$ in sham surgical control (saline) tissue, as previously described (34). A brief phase I response was followed by a prolonged phase II response. B: capsaicin-evoked phase II responses (open bars) were markedly attenuated by 24 h following installation of TNBS compared with sham surgical control (saline) animals (filled bars). This effect persisted until day 7 and returned to control values by day 30. Phase I responses exhibited a similar pattern (data not shown). Each point represents the mean ± SE for 9 experiments. *P < 0.05.](http://ajpgi.org/)

AJP-Gastrointest Liver Physiol • VOL. 282 • JUNE 2002 • www.ajpgi.org
shows that baseline resistance was not reduced by the inflammation. This finding is consistent with previous reports (2) in which inflammation was shown to reduce intestinal secretion while the epithelial barrier was maintained. We also found that by day 7, epithelial electrolyte transport was hyporesponsive to secretagogues (see Fig. 3). Previous studies of TNBS colitis and mitomycin-C colitis have also shown the epithelium is hyporesponsive to secretagogues (2, 17). Our studies and others (2) suggest this effect is not due to a loss of epithelial integrity but rather, as previously suggested (17), may be due to a receptor-coupling defect or desensitization of receptor-linked secretory mechanisms.

Previous studies (15, 16) have shown that the acute local inflammatory response to TNBS can alter neural function in the myenteric plexus at both inflamed and noninflamed sites in the intestine. These actions appeared to be due to the local release of cytokines, which act at the site of inflammation and at the distant noninflamed sites (15). IL-1β has been shown to be released at the site of intestinal inflammation (18), and exogenous application of IL-1β in control tissue can mimic alterations in neural function seen during the inflammatory response (14). Furthermore, the suppression in neural function observed during the inflammatory response could be attenuated by infusion of...
sham surgical controls (saline). Although these values were significantly less than the TNBS group until day 7, they were greater than nonoperated controls until between days 7 and 30. This effect in the sham surgical controls (saline) presumably resulted from handling of the ileum during surgery. When this sham effect was removed (by subtracting from the TNBS-induced rise in MPO), it was evident that TNBS-induced MPO activity had fallen almost 100% by day 7. In contrast, MPO levels continued to rise by day 7 in previous ileitis studies (26) and remained elevated at day 30. However, this study and others reporting sustained elevations in MPO activity used a similar or greater concentration of TNBS in 50% ethanol (2, 3, 26, 27) in contrast to the 30% ethanol used in the present study. Ethanol-induced perturbation of the mucosa is the first step in the induction of inflammation, and thus increased concentrations of ethanol used in other studies may facilitate increased permeation of TNBS into the mucosa and produce both acute and chronic inflammation. Moreover, the ileal segment used in Ussing chamber studies and histological analyses was up to 8 cm removed from the site of TNBS administration. This site was selected based on reports (2) that TNBS disrupts the mucosal integrity at the site of instillation. Thus

IL-1-receptor antagonist, a selective antagonist of IL-1β (16). In the present study, TNBS-evoked ileitis also attenuated secretory responses at noninflamed sites in the proximal jejunum. These actions, however, were not confined to neural pathways, because when carbachol in the presence of TTX was employed to directly stimulate the enterocyte (see Fig. 5), these responses were dramatically attenuated. Together, these data suggest that cytokines can act at noninflamed sites to alter intestinal secretion through neural and nonneural mechanisms. Further studies are needed to determine whether this action is predominately mediated by IL-1β or whether additional or alternative cytokines are involved.

In the present study, the time course and severity of the inflammatory response produced by TNBS differ from the characteristics of TNBS-induced ileitis reported previously (26). The determination of the duration of the TNBS-evoked rise in MPO in the current study was confounded by a parallel rise in MPO in the

Fig. 5. Secretory responses are attenuated at intestinal sites proximal to the inflammation. A: the effects of capsaicin (30 nM) were examined on day 7 in the terminal ileum, as described in Fig. 2, and in the jejunum 20 cm proximal to the site of TNBS instillation. Myeloperoxidase (MPO) levels were normal in the jejunum in both sham surgical controls (saline) and TNBS-treated animals (see text). Capsaicin-evoked I\textsubscript{s}CCH responses (filled bars) were significantly attenuated in TNBS-treated animals at both the site of inflammation in the ileum and at the noninflamed site in the jejunum compared with sham surgical controls (saline; open bars). Each bar represents the means ± SE (n = 4; P < 0.01). B: carbachol (1 μM) was also examined in combination with TTX (1 μM), as described in Fig. 2. Carbachol-evoked responses were also almost completely attenuated in the jejunum. Each bar represents the mean ± SE (n = 4; P < 0.01).

Fig. 6. Schematic drawing of capsaicin-evoked secretory pathway and the sites of disruption during evolution of TNBS ileitis. A: schematic drawing of the secretory pathway, based on our previous studies with this model (35), showing that capsaicin-sensitive nerves innervate submucosal secretomotor neurons. These neurons, in turn, innervate the enterocyte and evoke chloride and water secretion. The pathway can be activated at multiple sites: capsaicin (Cap) selectively activates capsaicin-sensitive nerves, SP activates the cell bodies of submucosal secretomotor neurons, and carbachol (CCh) predominantly stimulates the enterocyte directly but also has some effect on the submucosal neuron. In the presence of TTX, which blocks action potential conduction in submucosal neurons, CCh acts selectively at the level of the enterocyte. B: After 24 h of TNBS-induced ileitis, the actions of Cap-sensitive nerves are markedly attenuated, as depicted by X. C: at day 7 of TNBS ileitis, there is a marked attenuation in the secretory pathway due to changes at the level of the enterocyte (see X). There are likely also changes in the secretory pathway at neural site(s) proximal to this (see DISCUSSION).
the ileal segment used in this study might exhibit less inflammatory change compared with previous studies (27) in which the site of TNBS-induced damage was directly examined.

In summary, the findings of the current study demonstrate that the efferent actions of capsaicin-sensitive nerves remain functional in the very early stages of inflammation (i.e., 8 h) but not during more established inflammation. Consequently, in models in which the mucosa is acutely challenged by a noxious agent, release of neurotransmitter, which appears to protect against mucosal damage (12), is limited to the time frame of the initial challenge. The loss of function during established inflammation may suggest that this defense mechanism can be functionally ablated by continued stimulation. This relationship may, however, not apply to all inflammatory settings, because SP expression in capsaicin-sensitive nerves remains functional in the very early stages of inflammation (10, 30), suggesting different mechanisms may be involved.

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