Role of nitric oxide in inflammation-induced suppression of secretion in a mouse model of acute colitis

WALLACE K. MACNAUGHTON, SONYA S. LOWE, AND KELLY CUSHING
Gastrointestinal Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

MacNaughton, Wallace K., Sonya S. Lowe, and Kelly Cushing. Role of nitric oxide in inflammation-induced suppression of secretion in a mouse model of acute colitis. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1353–G1360, 1998.—The role of nitric oxide (NO) derived from the inducible isoform of NO synthase (iNOS) in epithelial transport dysfunction was studied in a model of colitis induced in mice by intrarectal 2,4,6-trinitrobenzenesulfonic acid in 30% ethanol. Expression of iNOS mRNA was determined by RT-PCR. Electrolyte transport studies were conducted in Ussing chambers in which segments of inflamed colon were incubated with or without the selective iNOS inhibitor L-NÎA-(1-iminoethyl)lysine (L-NIL). Seven days after the induction of colitis, colonic tissue exhibited increased myeloperoxidase activity compared with saline controls. There was a detectable basal expression of iNOS mRNA, but expression was increased 3.7-fold in inflamed colons. Inflammation also caused an increase in iNOS activity and a concomitant decrease in constitutive NOS activity. In Ussing chamber experiments, there was a decreased response to electrical field stimulation in inflamed tissue, which was partially reversed by pretreatment of the tissue with L-NIL. The short-circuit current response to the muscarinic agonist carbachol was also reduced in inflammation, but this was not reversed by L-NIL. In summary, NO derived from iNOS mediates, in part, inflammation-induced suppression of neurally evoked electrolyte transport.

Electrolyte transport; enteric nervous system; inducible nitric oxide synthase; mice

NITRIC OXIDE (NO) is a ubiquitous mediator of numerous physiological processes in the gastrointestinal tract, including gastric mucosal protection (20, 22, 26), regulation of blood flow (32, 33, 48), stimulation of mucus secretion (6), and regulation of motility (14, 39). The physiological functions of NO are generally ascribed to NO derived from the constitutive isoforms of NO synthase (cNOS). Neuronal NOS (nNOS) and endothelial NOS (eNOS) are both constitutively expressed enzymes regulated by the availability of intracellular Ca2+. NO has a significant pathophysiological role as well, being involved in the development of and/or exacerbation of inflammation (see Ref. 24 for review). This action of NO occurs through activity of the inducible isoform of NOS (iNOS). Expression of iNOS is triggered by inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α. The relatively large concentrations of NO produced by iNOS (compared with eNOS or nNOS) are potentially damaging to tissue (24). Recent reports have suggested that iNOS activity is increased in animal models of intestinal inflammation (30, 34, 37) and in patients with Crohn’s disease or ulcerative colitis (19, 23, 35, 43).

NO derived from iNOS is also likely to affect epithelial function. There is a growing body of evidence that implicates NO as a modulator of the transport of electrolytes and water by the intestinal epithelium. However, the effects of NO on epithelial function are conflicting, with some studies showing that NO stimulates Cl– secretion (25, 44, 45, 49), while others suggest that it is absorptive (2, 27, 28, 41). We (25) and others (44, 45, 49) have shown that segments of intestine exposed in vitro to an NO donor exhibited higher net Cl– secretion compared with controls not exposed to the NO donor. We showed in guinea pig ileum that this effect was not mediated by enteric nerves (25). Tamai and Gaginella (45) reported a Cl– secretory response in rat colon but implicated enteric nerves in the response, because the short-circuit current (Isc) change elicited by sodium nitroprusside was blocked by pretreatment of the tissue with the neurotoxin TTX. Thus the reported secretory effects of NO appear to differ mechanistically among species.

Contrary to these reports of NO-stimulated secretion, several studies have suggested that endogenous NO is proabsorptive (2, 27, 28, 41). It has been suggested that the proabsorptive capacity of endogenous NO may be mediated in part through its action on enteric nerves (41). The disparate findings of NO-mediated secretion vs. NO-mediated absorption have not been adequately explained. The difference may relate to the different methods used to measure fluid flux. Most (but not all) reports of NO-stimulated secretion involve measurement of electrolyte transport in vitro preparations exposed to relatively high concentrations of NO-donating compounds. In contrast, most reports of NO-mediated absorption involve in vivo studies of fluid flux in ligated segments of intact intestine in animals treated with a NOS inhibitor. Thus the site and mode of NO delivery, concentration, and tissue preparation are all variables that have resulted in substantial uncertainty as to the physiological and pathophysiologica roles of NO in intestinal epithelial function.

Just as the effects of NO on epithelial function are not entirely clear, the mechanisms underlying inflammation-induced epithelial dysfunction have not been well described. Clearly, there are changes in permeability that may be involved in the initiation or exacerbation of the inflammatory response (15, 29). However, the effects of inflammation on epithelial transport of electrolytes and water are not well understood. In animal models of inflammatory bowel disease (3, 10), the
epithelium is hyporesponsive to stimulation by a variety of secretagogues. Given the evidence cited above, namely the activity of iNOS in inflamed intestine, the recent evidence of NO-induced suppression of secretion, and the previously described hyporesponsiveness of the inflamed intestine to secretagogues, we hypothesized that iNOS-derived NO plays a role in the decreased responsiveness to stimulation observed in inflamed intestine. Our objectives were several. First, we characterized the acute inflammatory response in the mouse colon 7 days after intrarectal 2,4,6-trinitrobenzenesulfonic acid (TNBS). Second, we determined the effects of inflammation on iNOS mRNA expression and enzyme activity. Finally, we used a selective iNOS inhibitor to determine the role of NO in inflammation-induced electrolyte transport dysfunction in vitro.

**METHODS**

**Animals.** Male mice of the C57BL strain were obtained from Charles River (Montreal, QC, Canada). They were group-housed under controlled temperature (22°C) and photoperiod (12:12-h light-dark cycle). The mice were allowed unrestricted access to standard mouse chow and tap water. They were allowed to acclimatize to these conditions for at least 5 days before inclusion in an experiment. Protocols were approved by the University of Calgary Animal Care Committee, and all procedures were conducted in accordance with the regulations of the Canadian Council on Animal Care.

TNBS-induced colitis. Colitis was induced by intrarectal administration of TNBS (60 mg/ml in 30% ethanol). For the purposes of this study, TNBS refers to the coadministration of TNBS and 30% ethanol. The total volume of injection was 0.1 ml. Controls received 0.1 ml saline. This dose of TNBS has been used previously in mice (7). Briefly, mice were anesthetized with halothane and O2, and TNBS or saline was administered via a cannula inserted 3.5 cm into the distal colon. Mice were allowed to recover in cages placed on a heating pad for 24–48 h. They were then returned to their regular housing conditions. Mice were studied 7 days after TNBS or saline administration unless otherwise stated.

Expression of colonic NOS was determined using the primer-dropping method of RT-PCR, as previously described (50). Mice were euthanized by cervical dislocation. Colon was removed and cleaned of adherent feces using cold (4°C) Krebs buffer. A segment (50–100 mg) was immediately homogenized in 500 µl homogenization buffer composed of 10 mM HEPES, 320 mM sucrose, 1 mM EDTA, 0.1 mM EGTA, 6 mM L-valine, 0.2 mM L-arginine, and 0.95 µM L-[14C]arginine (sp act, 331.2 mCi/mmol). To determine the proportion of total NOS activity that was attributable to iNOS, aliquots of supernatant were added to 50 µl of control assay buffer composed of 40 mM KH2PO4, 0.2 mM CaCl2, 1.0 mM MgCl2, 0.1 mM NADPH, 6 mM L-valine, 0.2 mM L-arginine, and 0.95 µM L-[14C]arginine. NOS activity was determined by measuring the conversion of L-[14C]arginine to L-[14C]citrulline, using a modification of the method of Salter et al. (38). Seven days after treatment with TNBS or saline, mice were killed by cervical dislocation and the colon was removed and cleaned of feces with cold (4°C) Krebs buffer. Segments of colon weighing 50–100 mg were immediately homogenized in 500 µl homogenization buffer composed of 10 mM HEPES, 320 mM sucrose, 1 mM dithiorthiolate, 0.1 mM EDTA, 0.01 mg/ml soybean trypsin inhibitor, 0.01 mg/ml leupeptin, and 0.002 mg/ml aprotinin. The samples were then centrifuged at 13,000 g in a microcentrifuge at 4°C. Aliquots (20 µl) of supernatant were added to 50 µl of control assay buffer composed of 40 mM KH2PO4, 0.2 mM CaCl2, 1.0 mM MgCl2, 0.1 mM NADPH, 6 mM L-valine, 0.2 mM L-arginine, and 0.95 µM L-[14C]arginine remains bound to the resin, while the L-[14C]citrulline formed during the production of NO is eluted. A 1-ml aliquot of the eluent was mixed with 14 ml of scintillation cocktail (Scintiverse II, Fisher Scientific, Fair Lawn, NJ) and counted in a Wallac 1214 Rackbeta liquid scintillation counter. The amount of L-[14C]citrulline was calculated after subtracting the background signal of the control sample.

**Table 1. Sequences of primers used for PCR**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>iNOS</td>
<td>5′-AACAAGGAAACTACCGACCTCA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-CGAGTCTACACGAGTTGTCGAT</td>
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1. iNOS, inducible nitric oxide synthase. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Myeloperoxidase (MPO) activity was measured as an indicator of granulocyte (primarily neutrophil) infiltration into the tissue using a modification of the method of Krawisz et al. (21). Briefly, segments of colon weighing 50–150 mg were removed from mice 7 days after TNBS or saline. They were immediately homogenized (50 mg/ml) in 50 mM potassium phosphate buffer containing 5 g/l hexadecyltrimethylammonium bromide. Samples were centrifuged at 13,000 g in a microcentrifuge for 10 min. Aliquots (7 μl) of each sample were added to a 96-well microtiter plate. A 200-μl aliquot of o-dianisidine reagent was added to each well. This reagent was prepared by adding 16.7 mg o-dianisidine to 90 ml distilled water, 10 ml potassium phosphate buffer, and 50 μl 1% hydrogen peroxide. The plate was immediately read at 450 nm using a Molecular Devices UV Max kinetic plate reader. Three readings were taken 30 s apart, and activity rate was calculated using SoftMax software.

Electrolyte transport studies. Mice were euthanized by cervical dislocation. The colon was removed, opened along the mesenteric border, and rinsed clean with cold (4°C) Krebs buffer. Unstripped segments of colon were mounted in Ussing-type diffusion chambers (Navicyte, Sparks, NV), which provided an exposed surface area of 0.4 cm². In inflamed colon, the segment chosen included the area of most macroscopically severe damage. Segments for saline controls were taken a similar distance from the anus and included middle to distal colon. Once in the chambers, tissues were bathed on both the mucosal and serosal sides with Krebs buffer (pH 7.4) containing 10 mM glucose. The buffers were aerated and circulated at 5% CO₂-95% O₂. The chambers were kept at a constant temperature (37–38°C) with a heating block connected to a circulating water bath. The potential difference across the tissues was measured and clamped to zero volts with a circulating water bath. The potential difference across the temperature (37–38°C) with a heating block connected to a

RESULTS

Induction of colitis. The distal colons of mice killed 7 days after TNBS administration appeared hyperemic and thickened. There were adhesions of the colon to other segments of intestine. The colons from mice given TNBS, while obviously inflamed, did not exhibit regions of overt ulceration and necrosis as has been reported in the rat model of TNBS colitis (31). Inflammation was also assessed by measurement of MPO activity. There was a significant (P < 0.01) increase in MPO activity in TNBS-treated colons (4.27 ± 0.79 U/mg wet tissue wt; n = 5) compared with saline-treated colons (0.46 ± 0.17 U/mg wet tissue wt; n = 5).

Representative micrographs of colonic sections taken from mice 7 days after administration of TNBS or saline are shown in Fig. 1. Segments from saline-treated mice had crypts of normal, nonbranched morphology with columnar epithelial cells and no evidence of inflammatory cell influx. In contrast, segments from TNBS-treated mice had a large inflammatory cell influx into the mucosa. In these regions of cellular infiltration, crypts did not exhibit normal morphology. However, the mucosal surface was covered with a layer of squamous cells typical of newly restituted epithelium (Fig. 1). While there were some epithelial breaks, these were infrequent and minor.

Expression and activity of iNOS. There was a basal level of iNOS mRNA expression in colons from naive and saline-treated mice, as shown by RT-PCR (Fig. 2). However, this level of expression was substantially lower than that observed in colons taken from mice 1, 3, or 7 days post-TNBS. Expression of iNOS mRNA was elevated 3.6-fold in inflamed (n = 5) vs. normal (n = 5) colons taken at day 7 post-treatment (Fig. 2).

NOS activity was assessed by measuring the rate of conversion of L-[14C]arginine to L-[14C]citrulline in tissues taken from colitic and normal mice 7 days after treatment. There was no significant difference in total NOS activity between inflamed and normal colons (Fig. 3). However, in inflamed colons (n = 7), Ca²⁺-dependent NOS (cNOS) activity was reduced by 68% (P < 0.001) compared with normal colons (n = 7). Furthermore, Ca²⁺-independent NOS (iNOS) activity was increased ~15-fold in inflamed vs. normal colons (P < 0.001; Fig. 3).

Electrolyte transport studies. Inflamed and normal colonic segments mounted in Ussing-type diffusion
chambers did not exhibit any significant differences in basal \( I_{sc} \) or conductance (data not shown). EFS (10 Hz, 50 V) caused a change in \( I_{sc} \) of 53 ± 14 µA/cm² in segments of colon from control mice (n = 6; Fig. 4). In contrast, EFS evoked a change in \( I_{sc} \) of 10 ± 2 µA/cm² in inflamed colon (n = 6). Because iNOS expression and activity were increased 7 days after TNBS treatment, experiments were conducted in which tissues mounted in Ussing chambers were pretreated with the selective iNOS inhibitor L-NIL (3 and 100 µM). L-NIL (100 µM) did not affect the response to EFS in control colons (n = 6; Fig. 4). In segments of inflamed colon, pretreatment with L-NIL (3 or 100 µM; n = 9 and 7, respectively) partially, but significantly, reversed the inflammation-induced suppression of EFS-evoked transport (Fig. 4).

To determine if the effect of L-NIL pretreatment was specific to neurally evoked transport, segments of inflamed gut were pretreated with 100 µM L-NIL before exposure to the muscarinic agonist carbachol (1 µM). The response to carbachol was reduced by 97% in inflamed colon (n = 21) (Fig. 5). This suppression of responsiveness to muscarinic stimulation was not affected by pretreatment of the tissue with 3 (n = 5) or 100 µM L-NIL (n = 4) (Fig. 5).

To assess the specificity and efficacy of L-NIL administration, we conducted studies in which segments of normal and inflamed colon were assayed for NOS activity as described above. Tissue homogenates were incubated with L-NIL (3 and 100 µM) or vehicle, and NOS activities were determined as described above. L-NIL at 3 and 100 µM significantly reduced iNOS activity in colonic tissue from TNBS-treated mice (Fig. 6). L-NIL at 100 µM also reduced cNOS activity in tissue from both saline and TNBS-treated mice, suggesting that at this concentration L-NIL acts as a nonselective NOS inhibitor.

**DISCUSSION**

The mechanisms underlying the effects of inflammation on the intestinal epithelial transport of electrolytes and water remain unclear. Although it has been suggested that inflammation-induced diarrhea is due to the high mucosal concentration of inflammatory mediators that act as secretagogues stimulating the serosal-to-mucosal flux of Cl⁻ (8), direct experimental evidence fails to corroborate this contention. Earlier studies (1, 12) in human tissue suggested that inflammation-induced diarrhea was due to inhibition of Na⁺ absorption in inflamed tissue, possibly due to decreased basolateral Na⁺-K⁺-ATPase activity of enterocytes in inflamed tissue (40). Other studies have shown that while basal electrogenic ion transport is unchanged 1 wk after induction of colitis in the rat TNBS model, the in vitro response to the phosphodiesterase inhibitor IBMX was significantly lower (3). Similarly, in human tissue, the responsiveness to EFS and theophylline were significantly reduced in resected segments of inflamed jejunum compared with controls (18). While reports such as these have given us important insight into the ionic basis of inflammation-induced epithelial hyporesponsiveness, the question of underlying mecha-

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**Fig. 1.** Representative micrographs of hematoxylin-and eosin-stained cross sections of colon taken from mice 7 days after intrarectal administration of saline (A, B) or 2,4,6-trinitrobenzenesulfonic acid (TNBS) (C, D). Saline-treated colons exhibited normal crypt structure and columnar epithelium. TNBS-treated colons displayed abnormal crypt structure and large mucosal inflammatory cell influx. Surface epithelial cells in TNBS-treated colons were flattened and spread out across mucosal surface (arrowheads) compared with controls. Occasional epithelial breaks were observed (*). Scale bars, 50 µm.
nism in terms of a causative inflammatory mediator has remained largely unanswered. In the present study, we have demonstrated that colonic hyporesponsiveness to EFS-evoked secretion in the TNBS-treated mouse may be due to NO derived from iNOS.

The data we have presented here confirm the previous reports of inflammation-induced hyporesponsiveness to neurally evoked secretion. Induction of inflammation by TNBS was confirmed by measurement of MPO activity. The increase in MPO activity measured 7 days after induction of colitis in the mouse is comparable to what has been reported in studies of the rat and even greater than that observed previously 7 days after the same dose of TNBS in the mouse (7).

Macroskopically, some differences were noted between the TNBS-induced damage that we have observed in the mouse and that which has been observed in the more commonly used rat model. In particular, the colitic mouse tissue appeared hyperemic and thickened but did not exhibit the overt ulceration and necrosis observed in the rat model of TNBS colitis (31). Adhesions of the colon to surrounding structures were observed in all TNBS-treated mice. Histologically, there was evidence of significant inflammation, with a pronounced inflammatory cell infiltrate in the mucosa of most sections studied. Interestingly, however, these inflamed regions exhibited a primarily intact epithelium. This epithelium was flatter and of a more squamous morphology than the typically columnar epithelium observed in the control sections. While not quantified in the current study, crypts appeared less numerous in inflamed colons. Thus part of the hyporesponsiveness observed in TNBS-treated tissue was likely due to inflammation-induced structural alterations in the epithelium.

Not surprisingly, iNOS mRNA expression was substantially upregulated in colitic tissues. This was observed in tissues taken from mice 1, 3, and 7 days after induction of colitis in the mouse (46) and even greater than that observed previously 7 days after the same dose of TNBS in the mouse (7). Macroscopically, some differences were noted between the TNBS-induced damage that we have observed in the mouse and that which has been observed in the more commonly used rat model. In particular, the colitic mouse tissue appeared hyperemic and thickened but did not exhibit the overt ulceration and necrosis observed in the rat model of TNBS colitis (31). Adhesions of the colon to surrounding structures were observed in all TNBS-treated mice. Histologically, there was evidence of significant inflammation, with a pronounced inflammatory cell infiltrate in the mucosa of most sections studied. Interestingly, however, these inflamed regions exhibited a primarily intact epithelium. This epithelium was flatter and of a more squamous morphology than the typically columnar epithelium observed in the control sections. While not quantified in the current study, crypts appeared less numerous in inflamed colons. Thus part of the hyporesponsiveness observed in TNBS-treated tissue was likely due to inflammation-induced structural alterations in the epithelium.

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proportions of Ca\(^{2+}\) between saline- and TNBS-treated mice, the relative expression as determined radioenzymatically, did not differ between saline- and TNBS-treated mice. Constitutive expression of iNOS mRNA has also been observed in inflamed colon and colonic tissue from humans (43) and monkeys (36). Interestingly, we observed a basal level of iNOS mRNA expression in tissue from naive and saline-treated mice. Constitutive expression of iNOS mRNA has previously been observed in mouse ileum but not in jejunum or colon (13). The difference in the regionalization of expression may be due the fact that the previous study (13) determined expression using Northern blot analysis, whereas our study used RT-PCR. Constitutive expression of iNOS has also been observed in the airway epithelium (11), suggesting that this may be a common feature of all mucosa-associated lymphoid tissues. Increased expression of iNOS mRNA coincided with increased iNOS activity. While total NOS activity, as determined radioenzymatically, did not differ between saline- and TNBS-treated mice, the relative proportions of Ca\(^{2+}\)-dependent and -independent isoforms were dramatically different between these two groups. Others have used the Ca\(^{2+}\)-dependent or -independent activities in this assay to distinguish between iNOS and cNOS (4, 38). Enhanced iNOS activity in colitic tissue occurred concomitantly with a near abolition of cNOS activity. A compensatory downregulation of cNOS activity in the face of iNOS induction has also been observed in the rat kidney (42) and has been associated with renal dysfunction in that model.

Colonic segments were mounted in Ussing-type diffusion chambers to determine the electrolyte transport properties of the tissue. External muscle layers were not stripped due to the fragility of the tissue. We did not observe muscle thickening in histological samples taken from inflamed colons; indeed, bowel wall thickening was due almost exclusively to the inflammatory cell influx into the mucosa (data not shown). We observed a significant reduction in the responsiveness to EFS and carbachol in tissue taken from mice killed 7 days after induction of colitis. The most important finding in this study was the observation that L-NIL, a selective iNOS inhibitor (9), significantly reversed the inflammation-induced suppression of the secretory response to EFS but not that to carbachol. Using the radioenzymatic assay, we determined that the concentrations of L-NIL that restored neurally evoked secretory function also reduced iNOS activity. It should be noted that 100 µM L-NIL also significantly reduced cNOS activity, perhaps suggesting that cNOS-derived NO is also involved in the suppression of EFS-evoked secretion. This is unlikely to be the case, however, given the reduction in cNOS activity in inflamed tissue. The effect of L-NIL is not due to amelioration of colitis in these studies, since it was added to the bathing solution 10 min before stimulation of the tissues in the Ussing chambers. L-NIL has been shown to resolve spontaneous colitis in monkeys (36) but this involved a twice-a-day administration of L-NIL for 10 days and represents an anti-inflammatory effect of the chronic inhibition of iNOS activity. In our studies, the reversal of the inflammation-evoked EFS response by L-NIL represented the inhibition of an ongoing NO release that appeared to exert a tonic inhibitory effect on transport. Finally, because acute iNOS inhibition partially restored secretory responses to EFS, inflammation-induced hyporesponsiveness cannot be due solely to structural changes in the mucosa.

Although inhibition of iNOS restored, at least partially, the secretory response to EFS, it had no effect on inflammation-induced suppression of the response to carbachol. Superficially, one could surmise from this that iNOS-derived NO is acting directly on enteric neurons to suppress neurotransmitter release. Indeed, this has been suggested in the case of NO-mediated suppression of the response to bacterial toxins (41). However, it may be that the effect is at the level of the epithelium and that the observed effects of NO are on specific second messenger systems. It is interesting to note that the response to EFS is not suppressed in inflamed colon from TNBS-treated rabbits (10). It is tempting to speculate that this may due to the different neurotransmitters responsible for EFS-evoked transport in the rabbit vs. the commonly used rodent species.
such as the rat, mouse, or guinea pig. It has been well described that submucosal secretomotor neurons in rats, guinea pigs, and mice release ACh and vasoactive intestinal polypeptide (VIP), both of which are secretory (see Ref. 5 for review). In contrast, the response to EFS in rabbit intestine is not blocked by atropine (16) nor does the rabbit intestine respond with a secretory response to exogenous application of substances (i.e., 5-hydroxytryptamine, histamine, PGs, and substance P) that are potent secretagogues in other species (17).

It may be that the difference in the effects of L-NIL on the responses to EFS and carbachol represent the second messenger systems triggered by different secretagogues. For example, NO may inhibit cAMP-mediated secretion (i.e., stimulated by VIP) but not Ca2+-mediated secretion (i.e., stimulated by ACh). The response to the phosphodiesterase inhibitor IBMX is suppressed in acute (3) and chronic (unpublished results) inflammation. We have preliminary evidence to suggest that iNOS-derived NO reverses the suppression of the response to IBMX in chronic inflammation in the rat (unpublished results). Furthermore, there are reports (47) of NO-mediated suppression of adenylyl cyclase activity, which further supports this hypothesis. Obviously, more studies will have to be done to determine the mechanism underlying the effect of inhibition of iNOS activity on inflammation-induced secretory dysfunction in acute colitis.

In conclusion, we have shown that acute inhibition of iNOS activity reverses, in part, the inflammation-induced suppression of neurally evoked ion transport in the TNBS-treated mouse colon. The L-NIL insensitive component of the hyporesponsiveness is likely due to inflammation-induced structural alterations of the epithelium. The suppression of cholinerigically evoked secretion in inflamed colon was unaffected. The importance of maintaining a functional epithelium that can respond to endogenous secretagogues is important when one considers the host defense function of Cl− and water secretion, which operates in concert with stimulated motility to rid the bowel of infection (51). Future studies are required to determine the mechanism underlying the apparently different effects of iNOS-derived NO on different secretory mechanisms. These studies also provide the impetus for further investigation of the utility of selective iNOS inhibitors as therapeutic agents in inflammatory bowel disease.

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Address for reprint requests: W. K. MacNaughton, Dept. of Physiology and Biophysics, Univ. of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada, T2N 4N1.

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