Prolonged colonic epithelial hyporesponsiveness after colitis: role of inducible nitric oxide synthase

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Asfaha, Samuel, Cameron J. Bell, John L. Wallace, and Wallace K. MacNaughton. Prolonged colonic epithelial hyporesponsiveness after colitis: role of inducible nitric oxide synthase. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G703–G710, 1999.—Colonic epithelial secretion is an important host defense mechanism. We examined whether a bout of colitis would produce long-lasting changes in epithelial function that persisted after resolution of mucosal inflammation. Colitis was induced in rats with intracolonic trinitrobenzenesulfonic acid. Six weeks later, colonic damage and inducible nitric oxide synthase (iNOS) mRNA expression and activity were measured. Segments of distal colon were mounted in Ussing chambers for measurement of permeability and responsiveness to secretory stimuli. Basal electrolyte transport parameters and permeability were not different from untreated controls. Despite normal macroscopic and histological appearance, secretory responses to electrical field stimulation (EFS), isobutylmethylxanthine (IBMX), and carbachol were significantly depressed (by 60–70%) relative to controls. INOS mRNA expression and enzyme activity were significantly elevated. Dexamethasone reversed epithelial hyporesponsiveness and significantly reduced iNOS mRNA expression. A selective iNOS inhibitor normalized the secretory responses to EFS and IBMX but not to carbachol. These data suggest that ongoing synthesis of nitric oxide by iNOS contributes to chronic suppression of epithelial secretory function after episodes of colitis.

inflammation; secretion; rat

THE COLONIC EPITHELIUM is continually exposed to bacteria, bacterial products, and dietary antigens that are capable of inducing or exacerbating mucosal inflammation. The epithelium performs an important barrier function, preventing the entry of these factors into the lamina propria. The secretion of water by the colonic epithelium is a further defensive mechanism aimed at diminishing the ability of pathogens to adhere to the epithelium, as well as diluting any noxious luminal agents (30). Water secretion by intestinal epithelial cells occurs passively, driven by an osmotic force generated through the active transport of chloride ions. The motor activity of the intestine also contributes to this defensive response by assisting in the physical expulsion of the luminal contents. Although normally a defensive response, excessive secretion of water or impaired absorption of water (and electrolytes) can lead to diarrhea, as can impaired motor function. On the other hand, impaired electrolyte (and therefore water) secretion could predispose the colon to infection and inflammation.

There is a great deal of evidence that during an acute bout of colitis, epithelial secretory function is profoundly altered (1, 12, 15, 25). A number of inflammatory mediators, such as prostaglandins and leukotrienes, are capable of stimulating chloride secretion by colonocytes (7, 24). Despite this, however, large changes in chloride secretion have not been noted in studies of colonic secretion in inflammatory bowel disease or in animal models of colitis (9, 10, 21). It is possible that a bout of colitis may produce long-lasting changes in function (i.e., downregulation of secretory responses to inflammatory mediators) that persist after resolution of the mucosal inflammation. If such changes occurred in epithelial permeability and/or secretory function, they may render the colon more susceptible to subsequent inflammatory events.

In the present study, we have investigated the possibility that inflammation of the colon results in significant alterations in epithelial permeability and secretory function. Given recent evidence that nitric oxide (NO) can modulate intestinal permeability and secretion (2, 13, 15) and that NO derived from the inducible isofrom of NO synthase (iNOS) may contribute to altered secretory function in acute colitis (16), we have also examined the role of iNOS-derived NO in mediating prolonged epithelial dysfunction after colitis.

METHODS AND MATERIALS

Induction of colitis. Male Wistar rats (175–200 g) were fed standard chow and water ad libitum. Colitis was induced as previously described (20). Briefly, the rats were anesthetized with halothane, and a plastic catheter inserted rectally into the colon ~8 cm proximal to the anus was used to instill 60 mg of 2,4,6-trinitrobenzenesulfonic acid (TNBS) dissolved in 0.5 ml of 50% ethanol (vol/vol). Another group of rats was treated with intracolonic 50% ethanol (the vehicle for TNBS). A final group of rats consisted of naive controls that were left untreated during the course of the study. All procedures were approved by the Animal Care Committee of the University of Calgary and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Rats were anesthetized with halothane, then killed by cervical dislocation 6, 9, or 12 wk after intracolonic TNBS administration. Naive controls (age matched to the other rats) were killed at the same times. Rats treated with 50% ethanol were killed at the 6 wk time point. Within each group, some rats were used for assessment of colonic injury and inflammation, whereas others were used for electrolyte trans-
port studies. The minimum sample size per group in all experiments was five.

Assessment of colonic injury and inflammation. The distal colon was removed, opened by longitudinal incision, and pinned flat on a wax platform. The severity of macroscopic damage was then scored using the criteria previously described (27) by an observer unaware of the treatments the rats had received. The presence or absence of diarrhea (loose, watery stool) and the presence or absence of adhesions between the colon and other organs were noted. Samples of macroscopically inflamed tissue, or from the same sites in colons devoid of macroscopically visible inflammation, were taken for measurement of myeloperoxidase (MPO) activity and for histological assessment. MPO is an enzyme found in the azurophilic granules of neutrophils and other cells of myeloid origin. Tissue MPO activity was therefore used as an index of granulocyte infiltration. The tissue samples were weighed, frozen on dry ice, and stored at −20°C until assayed for MPO activity according to the technique described by Bradley et al. (3).

For histology, tissues were fixed in 10% buffered formalin and embedded in paraffin. Coded sections were then stained with hematoxylin and eosin and examined by light microscopy. The distal colon was excised and gently washed of fecal contents. The colon was then stripped of its external muscle by blunt dissection. A segment of mucosa from each rat, taken from macroscopically inflamed tissue or from the same site in colons devoid of macroscopic inflammation, was mounted in Ussing-type diffusion chambers (Navicyte, Sparks, NV). The tissues were bathed in a modified Krebs buffer containing (in mM) 115.0 NaCl, 2.0 KH₂PO₄, 2.4 MgCl₂, 1.3 CaCl₂, 8.0 KCl, and 25.0 NaHCO₃. The tissues were then incubated at 37°C for 10 min in 50 µl of NOS assay buffer [containing (in mmol) 50 KH₂PO₄, 1 MgCl₂, 0.2 CaCl₂, 50 valine, and 0.3 NADPH and 3.15 mg/ml l-arginine and 157 pmol l-[14C]arginine]. Addition of EGTA (1 mmol) was used to determine the activity of Ca²⁺-independent iNOS. The reaction was stopped by addition of 0.5 ml of Dowex (50W-X8 100–200) cation exchange resin, and the mixture was then loaded onto columns containing 1 ml of Dowex resin. Eluent was collected, and a 1-ml aliquot was measured for radiolabeled products using a Wallac scintillation counter (Turku, Finland).

NOS activity was defined as the rate of L-[14C]citrulline formation. NOS activity that was abolished by EGTA was taken as Ca²⁺-dependent constitutive NOS (cNOS), whereas that not inhibited by EGTA incubation was taken as Ca²⁺-independent iNOS.

Detection of iNOS mRNA expression. Colonic iNOS mRNA expression was examined using RT-PCR. Whole thickness colonic tissue (~150–200 mg) was placed in 2 ml of TRIzol reagent (GIBCO BRL, Gaithersburg, MD), and RNA was isolated according to previously described methods. The method used for RT-PCR was modified slightly from that described previously (8). The housekeeping gene for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Briefly, 1 µg of RNA from each sample was reverse transcribed at 42°C using Superscript RNAse H reverse transcriptase (GIBCO BRL) and appropriate reaction mixture (containing 2 µl of 10X PCR buffer, 2 µl of 10 mmol/l dNTP stock, and 2 µl of N⁶ random hexamer stock). The enzyme was then deactivated by heating the sample to 95°C for 10 min. After the reaction, 2 µl of cDNA was mixed with 2 µl of 2 mmol/l dNTP stock and 5 µl of 10X PCR buffer. The iNOS upstream primer (2 µl; ~20 pmol) and iNOS downstream primer (2 µl; ~20 pmol) were then added to each tube.

DNA amplification was conducted under the following conditions: denaturation at 94°C for 1 min, annealing at 55°C for 30 s, and extension at 72°C for 1 min. To ensure complete denaturation of the DNA with no background polymerase activity, Taq DNA polymerase was added to the PCR mixture during the hot start of cycle 1. Optimal amplification of each primer with GAPDH was determined during preliminary trials (data not shown). The iNOS/GAPDH genes were coamplified for 26/22 cycles. Hence, the GAPDH upstream and downstream primers were added to the iNOS PCR mixture during the hot start of cycle 5.

After separation of the PCR products on a 2% agarose gel containing 10 µg of ethidium bromide, a Polaroid (Cambridge, MA) picture of the gel was taken under ultraviolet light. With the use of a densitometer and National Institutes of Health software, quantities of each product were normalized to
control levels of GAPDH and expressed as densitometry units. The iNOS and GAPDH products were made using primers based on published sequences (GenBank accession numbers: GAPDH, J02642; iNOS, D14051).

Drug treatments. Dexamethasone was tested for its ability to reverse the hyperresponsiveness of the epithelium after colitis. Five weeks after induction of colitis, dexamethasone (1 mg/kg ip) was administered once daily for 4 days. Age-matched naive rats were also administered dexamethasone or vehicle (saline) in the same manner. Rats were killed 48 h after the final dose of dexamethasone, and the colon was removed for electrolyte transport studies or for RT-PCR and MPO analyses.

Experiments examining the role of NO derived from the iNOS enzyme were performed in vitro using the Ussing chamber technique. After a 20-min equilibration period, the selective iNOS inhibitor, l-N^6-(1-iminoethyl)lysine (l-NIL; 3 µM) was added serosally. I_sc responses to EFS, IBMX, and carbachol were determined after a 10-min incubation period. The concentration of l-NIL selected was that which was found to significantly decrease iNOS activity without affecting cNOS activity (19).

Materials. TNBS was obtained from Fluka Chemika (Buchs, Switzerland). PCR buffer, dNTPs, N_t random hexamers, and RNasin were all purchased from Pharmacia Biotech (Baie d'Urfe, QC, Canada). Superscript and TRizol were from Gibco BRL (Gaithersburg, MD, USA); 50W-X8 Dowex resin was from BioRad (Hercules, CA); dexamethasone, IBMX, carbachol, EGTA, l-valine, tetrodotoxin, and L-arginine were from Sigma (St. Louis, MO). iNOS and GAPDH primers were synthesized by University Core DNA Services (Calgary, AB, Canada). 36Cl^– and 51Cr-EDTA were purchased from DuPont NEN Research Products (Mississauga, ON, Canada); 50W-X8 Dowex resin was from BioRad (Hercules, CA); dexamethasone, IBMX, carbachol, EGTA, l-valine, tetrodotoxin, and L-arginine were from Sigma (St. Louis, MO). iNOS and GAPDH primers were synthesized by University Core DNA Services (Calgary, AB, Canada). 36Cl^– and 51Cr-EDTA were purchased from DuPont NEN. 22NaCl was purchased from Amersham Pharmacia Biotech (Oakville, ON, Canada).

Statistical analysis. All data are expressed as means ± SE. Comparisons among groups of data were made using a one-way ANOVA followed by a Student-Newman-Keuls test, except for the iNOS activity data, which were compared by Student’s t-test for unpaired data. With all analyses, an associated probability (P value) < 0.05 was considered significant.

RESULTS

Colonic injury and inflammation. As shown in Fig. 1, the severe inflammation that was apparent 3 days after intracolonic administration of TNBS-ethanol or ethanol alone had resolved within 6 wk to a point where the colonic damage score was not significantly different from that of age-matched, naive control rats. Similarly, the colons of TNBS- and ethanol-treated rats were not histologically different from naive controls (data not shown). Likewise, colonic MPO activity in rats treated with TNBS-ethanol or ethanol alone had recovered to the levels seen in naive control rats. In the rats killed 9 or 12 wk after TNBS administration, the colon was macroscopically and histologically indistinguishable from naive controls.

Epithelial permeability. Measurement of unidirectional fluxes of 51Cr-EDTA, summarized in Table 1, demonstrated that there were no significant differences in the flux of this permeability marker in either direction between the rats treated with TNBS and controls at either time point examined. These data, taken together with the lack of significant differences in G of the epithelium in these two groups (Table 1), strongly suggest that there was no difference between the groups in terms of epithelial permeability.

Electrolyte transport. Basal transport parameters (I_sc, PD, and G) of the distal colon taken from rats killed 6 wk after TNBS administration were not different from naive controls (G data shown in Table 1). Tissue from rats killed 6 wk after intracolonic administration of 50% ethanol also exhibited basal transport parameters not significantly different from those of naive controls. Furthermore, basal colonic Na^+ and Cl^- ion fluxes of rats receiving TNBS 6 wk earlier were not

Table 1. Permeability of colonic epithelium in rats 6 and 9 wk after induction of colitis with TNBS

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>TNBS</th>
</tr>
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<tbody>
<tr>
<td>6 wk Post-TNBS</td>
<td></td>
<td></td>
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<tr>
<td>Mucosal to serosal EDTA flux</td>
<td>1.69 ± 0.22</td>
<td>2.68 ± 0.51</td>
</tr>
<tr>
<td>Serosal to mucosal EDTA flux</td>
<td>2.29 ± 0.42</td>
<td>3.57 ± 1.45</td>
</tr>
<tr>
<td>Conductance</td>
<td>32 ± 4</td>
<td>33 ± 6</td>
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<tr>
<td>9 wk Post-TNBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosal to serosal EDTA flux</td>
<td>2.72 ± 0.53</td>
<td>3.12 ± 0.19</td>
</tr>
<tr>
<td>Serosal to mucosal EDTA flux</td>
<td>1.65 ± 0.15</td>
<td>2.47 ± 0.53</td>
</tr>
<tr>
<td>Conductance</td>
<td>36 ± 4</td>
<td>29 ± 4</td>
</tr>
</tbody>
</table>

Results for flux are expressed as amount of 51Cr-EDTA that moved across epithelium as a percentage (× 10^-8) of amount added to 1 side of chamber ± SE. Results for conductance are expressed as mS/cm² ± SE. Each group consisted of 5 or 6 rats. There were no significant differences among groups. Control rats were untreated but were age-matched to the 2,6,6-trinitrobenzensulfonic acid (TNBS)-treated rats.
significantly different from those of control rats (Table 2). However, the distal colon from rats killed 6 wk after TNBS administration had significantly depressed $I_{sc}$ responses to EFS, IBMX, and carbachol (Fig. 2). Moreover, at both 9 and 12 wk post-TNBS administration, the $I_{sc}$ responses to EFS and carbachol remained significantly depressed, whereas the response to IBMX had recovered to that observed in naive controls. The hyporesponsiveness observed after induction of colitis with TNBS was not a nonspecific response to tissue injury, as tissues taken from rats 6 wk after intracolonic administration of 50% ethanol, which caused extensive colonic injury (Fig. 1), responded to EFS and carbachol stimulation in a comparable manner to that observed in naive controls (e.g., response to EFS: 50% ethanol: $21.6 \pm 3.6$ vs. $22.7 \pm 6.1 \mu A/cm^2$ in controls; response to IBMX: 50% ethanol: $71.7 \pm 15.8$ vs. $68.8 \pm 10.7 \mu A/cm^2$ in controls; response to carbachol: 50% ethanol: $28.9 \pm 5.4$ vs. $31.2 \pm 7.3 \mu A/cm^2$ in controls). The hyporesponsiveness observed after induction of colitis was also not a result of neuronal changes, as tissues from both naive and TNBS-treated rats preincubated with the sodium channel blocker TTX (1 µM) did not exhibit altered $I_{sc}$ responses to IBMX and carbachol compared with tissues preincubated with vehicle (Table 3).

### Table 2. Colonic sodium and chloride electrolyte fluxes

<table>
<thead>
<tr>
<th>Group</th>
<th>$J_{Na}$</th>
<th>$J_{Cl}$</th>
<th>Net</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m→s</td>
<td>s→m</td>
<td>Net</td>
</tr>
<tr>
<td>Control</td>
<td>5.39 ± 0.42</td>
<td>4.35 ± 0.33</td>
<td>0.72 ± 0.59</td>
</tr>
<tr>
<td>TNBS</td>
<td>4.51 ± 0.48</td>
<td>3.89 ± 0.53</td>
<td>0.61 ± 0.75</td>
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</tbody>
</table>

*All $Na^+$ and $Cl^-$ flux ($J$) values are expressed in $\mu eq \cdot cm^{-2} \cdot h^{-1}$ (means ± SE; n ≥ 5). There were no significant differences among the groups. m→s, Mucosal to serosal flux; s→m, serosal to mucosal flux. TNBS, rats 6 wk after induction of colitis with TNBS.*

NOS activity and mRNA expression. In naive rats, iNOS activity in the colonic tissue was very low or undetectable. In rats killed 6 wk after induction of colitis, iNOS activity was variable but significantly (P < 0.05) elevated above that in the controls (Fig. 3). These rats also exhibited significantly (P < 0.01) elevated iNOS mRNA expression in the distal colon compared with controls (Fig. 4). Expression of iNOS mRNA was not observed in rats killed 6 wk after intracolonic 50% ethanol.

Effects of dexamethasone. Treatment with dexamethasone 5 wk after intracolonic TNBS administration had no effect on basal $I_{sc}$. The $I_{sc}$ response to IBMX in rats treated with dexamethasone was not significantly different from naive rats and significantly elevated compared with TNBS-treated rats receiving saline. Interestingly, the $I_{sc}$ response to EFS after dexamethasone treatment was not significantly different from either naive controls or TNBS-treated animals receiving saline. In contrast, the $I_{sc}$ response to carbachol after dexamethasone treatment was not significantly different from TNBS-treated rats receiving saline and remained significantly depressed compared with naive rats (Fig. 5). Dexamethasone treatment had no effect on the basal $I_{sc}$ of TNBS-treated rats and had no effect on transport responses in tissues from naive rats (e.g.,
response to EFS: rats treated with vehicle: 25.8 ± 4.8 vs. 31.2 ± 4.9 µA/cm² in rats treated with dexamethasone; response to IBMX: rats treated with vehicle: 80.8 ± 15.8 vs. 83.1 ± 8.8 µA/cm² in rats treated with dexamethasone; response to carbachol: rats treated with vehicle: 41.0 ± 9.1 vs. 47.7 ± 7.9 µA/cm² in rats treated with dexamethasone). Treatment with dexamethasone abolished the increase in iNOS mRNA expression (Fig. 4).

**DISCUSSION**

An acute bout of colitis is associated with substantial alterations in epithelial function, including a profound hyporesponsiveness to secretagogues (1, 10, 16). Few studies have investigated intestinal function during the postinflammatory period. In the present study we demonstrated that epithelial hyporesponsiveness to a variety of secretagogues extends for up to 12 wk beyond the acute phase of the inflammatory response.

Exposure of the rat colonic lumen to TNBS in 50% ethanol caused an acute inflammatory response characterized by mucosal ulceration, bowel wall thickening, and adhesion formation as previously described (27). Furthermore, there was an acute granulocyte influx as shown by the significant increase in colonic MPO activity. By 6 wk after the initial administration of TNBS the colonic damage score and MPO had returned to the levels of control rats. Tissue G and the serosal-to-mucosal flux of 51Cr-EDTA were similar to those observed in naive animals, indicating that there was no persistent permeability defect after resolution of the acute phase of inflammation. Despite the fact that, on the basis of these parameters, the colon was not inflamed at 6 wk post-TNBS, segments of this tissue mounted in Ussing chambers exhibited significant hyporesponsiveness to EFS or to serosal application of IBMX and carbachol. Furthermore, compared with naive animals, iNOS expression and iNOS activity were increased 6 wk post-TNBS. The effects on responsiveness to secretagogues and on iNOS expression and activity were specific to the inflammatory response and were not simply a function of generalized tissue injury. Intracolonic administration of 50% ethanol alone caused an acute increase in colonic damage and MPO activity but did not cause epithelial hyporesponsiveness after 6 wk. Furthermore, iNOS mRNA expression was not elevated 6 wk after intracolonic 50% ethanol.

Prolonged epithelial dysfunction after acute inflammation may be due to structural changes that persist after resolution of the acute phase of the inflammatory response or to ongoing mediator release. Although we have not investigated the former possibility in the present study, the fact that L-NIL reversed the hyporesponsiveness suggests that a "structural change" did not account for the hyporesponsiveness, at least in the case of EFS- and IBMX-evoked secretion. The role of NO in epithelial function is controversial. Several studies have shown that in vitro application of NO-donating compounds to intestinal tissue in Ussing chambers stimulates chloride secretion (15, 26, 29). Furthermore, endogenous NO has been implicated in the diarrhea caused by administration of castor oil (17) or magnesium sulfate (11), although whether this was a secretory or malabsorptive diarrhea was not determined. In contrast to these studies, it has also been shown that endogenous NO may be antiselective. Inhibition of NOS activity exacerbates cholera toxin- and prostaglandin-induced secretion in rats (2, 23) perhaps through effects on enteric neurons. Our data are in keeping with

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**Table 3. Effect of TTX on colonic Isc responses**

<table>
<thead>
<tr>
<th>Group</th>
<th>EFS</th>
<th>IBMX</th>
<th>Carbachol</th>
</tr>
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<tbody>
<tr>
<td>Control-vehicle</td>
<td>100.0±10.8</td>
<td>99.9±32.5</td>
<td>100.0±26.3</td>
</tr>
<tr>
<td>Control-TTX</td>
<td>25.8±7.3‡</td>
<td>104.2±24.7</td>
<td>124.4±32.7</td>
</tr>
<tr>
<td>TNBS-vehicle</td>
<td>57.9±12.3†</td>
<td>32.7±12.8*</td>
<td>33.3±13.7*</td>
</tr>
<tr>
<td>TNBS-TTX</td>
<td>9.5±4.6‡</td>
<td>25.4±9.1*</td>
<td>32.8±9.7*</td>
</tr>
</tbody>
</table>

Results are expressed as %control-vehicle short-circuit current (Isc) response to each stimulant (means ± SE; n = 5). *P < 0.05, †P < 0.01, ‡P < 0.001 compared with control-vehicle response to each stimulant. TNBS, rats 6 weeks after induction of colitis with TNBS. EFS, electric field stimulation.

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**Fig. 3. Inducible nitric oxide synthase (iNOS) enzyme activity in rats 6 wk after induction of colitis with TNBS. Each bar represents mean ± SE, with 6 rats in control group (naive rats) and 8 rats in TNBS group. *P < 0.05 compared with control group.**

**Fig. 4. Effects of dexamethasone (Dex) treatment (1 mg/kg; once daily for 4 days) 5 wk after intracolonic TNBS administration on iNOS mRNA expression in distal colon. Representative gel of iNOS expression as determined by RT-PCR of tissue from naive rats, TNBS-treated rats receiving saline, and TNBS-treated rats receiving dexamethasone. MW, molecular weight markers. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.**
We have shown that elevation of iNOS mRNA expression and iNOS activity persist for at least 6 wk post-TNBS. Inhibition of iNOS mRNA expression by dexamethasone and of iNOS activity using the selective iNOS inhibitor L-NIL resulted in responses to EFS and

Fig. 5. Effects of dexamethasone treatment (1 mg/kg; once daily for 4 days) 5 wk after intracolonic TNBS administration on colonic epithelial responses to EFS (A), IBMX (B), and carbachol (C) stimulation. Each bar represents mean ± SE, with 6–10 rats per group. *P < 0.05, **P < 0.01 compared with naive group response to same secretagogue. *P < 0.05 compared with control rats that received intracolonic TNBS and 5 wk later received vehicle (saline) in place of dexamethasone.

these latter observations and suggest that chronic elevation of iNOS activity may exert a potent antisecretory tone on the colonic epithelium.

iNOS is expressed during periods of active inflammation in animal models of colitis (22) and in inflammatory bowel disease (14). The evidence for iNOS expression after resolution of acute inflammation is less clear.

Fig. 6. Effects of the selective iNOS inhibitor L-N(6)-(1-iminoethyl)lysine (L-NIL; 3 µM) on in vitro epithelial responses to EFS (A), IBMX (B), and carbachol (C) stimulation. Each bar represents mean ± SE, with 5–10 rats per group. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with naive group response to same secretagogue. *P < 0.05 compared with tissue from rats that received intracolonic TNBS 6 wk earlier and were treated with vehicle (distilled water) in place of L-NIL. Basal $I_{sc}$ values in presence and absence of L-NIL were not significantly different from each other (e.g., 70.7 ± 18.5 for control-vehicle group vs. 61.9 ± 17.1 µA/cm² for control-L-NIL and 51.7 ± 13.6 for TNBS-vehicle vs. 53.4 ± 13.3 µA/cm² for TNBS-L-NIL).
IBMX that were similar to those observed in control animals. Dexamethasone inhibits iNOS mRNA expres-
sion by increasing I-κB and decreasing nuclear fac-
tor-κB (6). L-NIL is a selective inhibitor of TNBS-
induced iNOS activity in mouse colon at the
concentration used in this study (16). Interestingly,
L-NIL inhibition of NOS expression and activity did not
reverse the hyporesponsiveness to carbachol. This sug-
gests that iNOS-derived NO selectively inhibits CAMP-
mediated secretion. NO has been shown in other sys-
tems to reduce intracellular cAMP (28). Neurally evoked
chloride secretion is a composite response to neurotrans-
tmitter release from cholinergic and vasoactive intesti-
nal peptidergic (VIPergic) submucosal secretomotor
neurons, implying that both Ca2+-dependent (acetylcho-
line) and CAMP-dependent (VIP) mechanisms are in-
volved in the response to EFS (4). Furthermore, these
data indicate that the hyporesponsiveness to carbachol
is mediated by an as yet undetermined NO-indepen-
dent mechanism.

The NO-mediated prolonged hyporesponsiveness to
secretagogues may have important implications for
host defense against invading pathogens and antigens.
Physiological secretion of chloride ions and water is
characteristic of enterocytes near the base of the crypt
region and is thought to “flush” bacteria, bacterial
products, and antigens away from the epithelium. The
intestinal neuroimmune system links this secretory
function to motility to effectively remove harmful micro-
organisms and antigens from the intestinal lumen (30).
An inability to respond to these luminal stimuli would
compromise this defense mechanism, potentially lead-
ing to translocation of bacteria, toxins, or antigens
across the epithelium, with subsequent inflammation
or endotoxemia. Our data are consistent with the
hypothesis that chronic synthesis of NO through iNOS
may also predispose the intestine to recurrent bouts of
inflammation and injury.

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