Dextran sodium sulfate-induced colitis reveals nicotinic modulation of ion transport via iNOS-derived NO

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Submitted 15 February 2004; accepted in final form 8 April 2004

Green, Christina L., Winnie Ho, Keith A. Sharkey, and Derek M. McKay. Dextran sodium sulfate-induced colitis reveals nicotinic modulation of ion transport via iNOS-derived NO. Am J Physiol Gastrointest Liver Physiol 287: G706–G714, 2004. First published April 15, 2004; 10.1152/ajpgi.00076.2004.—In normal colon, ACh elicits a luminally directed Cl− efflux from enterocytes via activation of muscarinic receptors. In contrast, in the murine model of dextran sodium sulfate (DSS)-induced colitis, an inhibitory cholinergic ion transport event due to nicotinic receptor activation has been identified. The absence of nicotinic receptors on enteric epithelia and the ability of nitric oxide (NO) to modulate ion transport led us to hypothesize that NO-mediated the cholinergic nicotinic receptor-induced changes in ion transport. Midportions of colon from control and DSS-treated mice were examined for inducible NO synthase (iNOS) expression by RT-PCR and immunofluorescence or mounted in Ussing chambers for assessment of cholinergic-evoked changes in ion transport (i.e., short-circuit current) with or without pretreatment with pharmacological inhibitors of NO production. iNOS mRNA and protein levels were increased throughout the tissue from DSS-treated mice and, notably, in the myenteric plexus, where the majority of iNOS immunoreactivity colocalized with the enteric glial cell marker glial fibrillary acidic protein. The drop in short-circuit current evoked by the cholinomimetic carbachol in tissue from DSS-treated mice was prevented by selective inhibitors of iNOS activity [Nω-(1-iminoethyl)-L-lysine HCl and N-[3-(aminomethyl)benzyl]acetamide] or an NO scavenger [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide] or by removal of the myenteric plexus. Thus, in this model of colitis, a “switch” occurs from muscarinic to nicotinic receptor-dominated control of cholinergic ion transport. The data indicate a novel pathway involving activation of nicotinic receptors on myenteric neurons, resulting in release of NO from neurons or enteric glia and, ultimately, a dampening of stimulated epithelial Cl− secretion that would reduce secretory diarrhea.

MATERIALS AND METHODS

Induction of colitis. Male Balb/c mice [8–12 wk old; Harlan Animal Suppliers (Indianapolis, IN) or Charles River Laboratories] were given a 4% (wt/vol) DSS (40 kDa; ICN Biomedicals, Aurora, OH) drinking water solution for 5 days followed by 3 days of normal water. 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.
drinking water. On day 3 after DSS removal (i.e., 8 days after the start of DSS treatment), mice were killed and segments of colon were excised. Colitis was assessed by three parameters: clinical score (weight loss, 0–2; colon length, 0–2; diarrhea, 0–2; and evidence of bleeding, 0 or 1: maximum score = 7), a histological damage score based on hematoxylin-and-eosin-stained sections of midcolic (loss of tissue architecture, 0–3; cellular infiltrate, 0–3; goblet cell depletion, 0–1; presence of ulcers, 0–1; edema, 0–1; muscle thickening, 0–2; crypt abscess, 0–1; maximum score = 12), and myeloperoxidase activity in the terminal 30% of the colon (7). All procedures were approved by the McMaster University and University of Calgary Animal Care Committees and were performed according to the guidelines of the Canadian Council of Animal Care.

Assessment of ion transport. Two nonmacroscopically ulcerated whole-thickness segments of middistal colon per mouse were opened along the mesenteric border and mounted in Ussing chambers (0.6-cm² opening) (24). [It is likely that tissue from DSS-treated mice would show microscopic evidence of inflammation (image in Fig. 5, bottom left, reveals a small ulcer.) Briefly, tissues were bathed in oxygenated Krebs buffer containing 10 mM glucose (serosal side) or 10 mM mannitol (luminal side) at 37°C, and net active ion transport across the epithelium was measured via an Iₑₑ (expressed in μA) injected through the tissue under voltage-clamp conditions. After a 15-min period, baseline differences in potential in (mV) were recorded and ion conductance was calculated (in mS/cm²).

The maximum change in Iₑₑ within 10 min of the addition of various pharmacological agents to the serosal buffer was also recorded. Each tissue was challenged with 100 μM CCh (Sigma, St. Louis, MO) with or without a 10- to 15-min pretreatment with the inhibitors of inducible nitric oxide (NO) synthase (iNOS), N°-(1-iminoethyl)-lysine HCl (L-NIL, 3 μM; Sigma) or N°-3-(aminomethyl)benzylacetamide (1400w, 5 μM; AG Scientific, San Diego, CA), the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, 100 μM; Calbiochem, San Diego, CA), or the muscarinic antagonist atropine (ATR, 1 μM; Sigma). At the end of each experiment, all tissues were challenged with the cAMP-dependent secretagogue forskolin (10 μM; Sigma) to test for viability and to ensure that the tissue had been mounted in the correct orientation in the Ussing chamber.

In some experiments, tissues (colon or cecum) were challenged with the selective muscarinic agonist bethanechol (BCh, 100 μM; Sigma) with or without ATR. For analysis of putative involvement of the myenteric plexus in mediating the Iₑₑ events, the outer muscle layers, including the myenteric plexus of one colonic segment from each animal, were removed by blunt dissection. The remainder of the muscle layers was confirmed by histological assessment. These studies were performed on tissue from DSS-treated animals only. [Trials with control tissue were unsuccessful, such that attempts to remove the muscle resulted in significant tissue damage and conductance values consistently >100 mS/cm², and this may also indicate the presence of edema in the more readily stripped tissue from DSS-treated mice.]

RT-PCR for iNOS. RNA was extracted from colonic tissue using the TRIzol extraction method (Invitrogen, Burlington, ON, Canada), and cDNA was reverse transcribed from 2 μg of total RNA. cDNA was incubated in a reaction mixture that included platinum Taq polymerase (Invitrogen), 2 mM MgCl₂, and nucleotide primers as follows: 5'-AGA CCT CAA CAG AGC CCT CA-3' (forward primer) and 5'-GCA GCC TCT TGT CTT TGA CC-3' (reverse primer) for iNOS gene product (305 bp, 0.3 μM final concentration) and 5'-CCA GAG CAA GAG AGG TAT CC-3' (forward primer) and 5'-CTG TGG TGT TGA AGC AG-3' (reverse primer) for β-actin gene product (436 bp, 0.06 μM final concentration), cDNA was amplified for 35 cycles on a Techne PHC-3 thermal cycler (Mandel Scientific, Guelph, ON, Canada), with denaturing, annealing, and extending temperatures of 94°C, 57°C, and 72°C, respectively. The final PCR product was run on a 2% agarose gel, and the amplified bands were viewed via DNA binding to ethidium bromide under ultraviolet light. In addition to colonic iNOS mRNA in control and DSS-treated mice (i.e., 5 days of DSS + 3 days of water), tissue was excised and processed from animals exposed to 4% DSS for 3 days, without any switch to normal water (n = 3–4). Separate RT-PCR experiments were performed using 1 μg of RNA isolated from muscle-myenteric plexus preparations that had been stripped via blunt dissection from the mucosa/submucosa of colonic segments immediately after death (n = 3).

Immunohistochemistry. Cryostat sections (10 μm) of whole-thickness tissue and whole-mount preparations were made of the longitudinally muscle-myenteric plexus and the submucosal plexus as previously described (22, 24). Tissues were fixed in Zamboni’s fixative overnight and then washed in PBS (3 times for 10 min each). Whole-mount preparations of colonic submucosal and myenteric plexus were incubated with primary antibodies for 48 h at 4°C, washed in PBS (3 times for 10 min each), and incubated with the appropriate secondary antibody at room temperature for 1 h. Primary antibodies were rabbit anti-choline acetyltransferase (ChAT, 1:500; code P3YEB, a generous gift from Dr. M. Schemann, Technical University of Munich, to K. A. Sharkey), rabbit anti-iNOS (1:500; catalog no. N-32030, Transduction Laboratories, Lexington, KY), mouse anti-neuronal NOS (bNOS, 1:500; catalog no. N-2280, Sigma), and rabbit anti-iNOS (1:500; catalog no. B7575, Biomedical Technologies, Stoughton, MA). Secondary antibodies were donkey anti-rabbit Cy3 (1:100; catalog no. 711-165-152, Biocan Scientific, Mississauga, ON, Canada) and rabbit anti-mouse FITC (1:50; catalog no. 315-095-045, Biocan Scientific). For the double-labeling studies, primary antibodies were added sequentially with the appropriate secondary antibodies. Tissues were washed in PBS (3 times for 10 min each) and mounted in bicarbonate-buffered glycerol (pH 8.6). Sections were examined using a Zeiss Axiosplan fluorescence microscope and photographed with a Sensys digital camera (Photometrics, Tucson, AZ) using V for Windows software (version 3.5, Digital Optics, Auckland, New Zealand). Montages were created in CorelDraw 11.

Data presentation and statistical analysis. Values are means ± SE, and n represents the number of mice used. Data were compared using Student’s t-test (unpaired or paired) or, for multiple group comparisons, one-way ANOVA followed by post hoc statistics with the Newman-Keuls test as appropriate. A level of statistically significant difference was accepted at P < 0.05.

RESULTS

DSS-induced colitis causes loss of muscarinic cholinergic regulation of epithelial ion transport and increased expression of ChAT. Consistent with previous reports, mice given 4% DSS-water for 5 days developed an acute colitis (7). Table 1 shows the extent of the inflammatory disease when mice were killed 3 days after DSS-water was replaced with regular water. Complementing our earlier study, Fig. 1 shows that the perturbed Iₑₑ response to CCh in tissues from mice with DSS-
Induced colitis lasts for 12 days after withdrawal of the DSS but returns to normal by 3 wk after treatment. Baseline $I_{sc}$ and conductance values were not significantly different between controls and DSS-treated mice (data not shown) (38). Moreover, tissues from DSS-treated mice were virtually unresponsive to stimulation with the selective muscarinic cholinergic agonist BCh at $10^{-5}$ M ($\Delta I_{sc} = -3.3 \pm 1.8 \mu A/cm^2$, $n = 9$), in contrast to colonic segments from control mice, which displayed a $88.4 \pm 17.8 \mu A/cm^2$ ($n = 3$) increase in $I_{sc}$ that was blocked by pretreatment with the muscarinic antagonist ATR: $\Delta I_{sc} = 5.9 \pm 5.9 \mu A/cm^2$ ($n = 3$). Moreover, although cecums of mice exposed to DSS become inflamed, as indicated by elevated myeloperoxidase activity [0.03 $\pm$ 0.02 and 0.81 $\pm$ 0.39 U/mg tissue in control and DSS mice, respectively, $n = 3$, consistent with our earlier study (38)], this tissue was still responsive to BCh: $\Delta I_{sc} = 30.1 \pm 3.9$ ($n = 3$) compared with $27.4 \pm 5.9$ ($n = 7$) $\mu A/cm^2$ in control cecum. These data, combined with those from our previous report (38), indicate a defect in muscarinic receptor-mediated control of ion transport in DSS-treated mice that is restricted to the colon and not evident in inflamed cecum.

Immunolocalization studies on cryostat and whole-mount preparations revealed abundant ChAT immunoreactivity in nerve fibers and cell bodies in colonic segments from control (36) and DSS-treated mice. However, interestingly and seemingly paradoxically, our studies indicated an increase in the intensity of ChAT expression and the number of immunoreactive cell bodies in the myenteric plexuses of colonic segments from DSS-treated mice compared with controls (Fig. 2).

**DSS colitis is accompanied by an increase in expression of iNOS in enteric glia.** RT-PCR of whole tissue extracts revealed a time-dependent increase in iNOS mRNA. Tissues obtained from mice after 5 days of exposure to DSS and those from mice 3 days after withdrawal of DSS-water showed a significant increase in iNOS mRNA expression compared with control mice and mice that received DSS for 3 days (Fig. 3A). Increased iNOS mRNA expression was also observed in muscle layer-myenteric plexus preparations from mice exposed to DSS-water compared with those from control mice (Fig. 3C).

We used immunohistochemistry to assess the localization of iNOS protein in the wall of the colon in animals with DSS-induced colitis. Immunohistochemical studies revealed, as expected and as reported in other colitis models (17, 49), increased iNOS protein expression in patches of epithelium and submucosal/mucosal cells (presumably resident and infiltrated immune cells) in tissue from the DSS-treated mice (data not shown). Faint iNOS immunoreactivity was also observed in the myenteric plexus and scattered submucosal nerve fibers in control tissue (46) (data not shown), consistent with the expression of the PCR product (Fig. 3). iNOS positivity in submucosal plexuses in colon from DSS-treated mice (Fig. 4) was similar to that in control tissue; however, there was a striking and substantial increase in iNOS expression in the myenteric plexus in animals treated with DSS (Fig. 4). Subsequent colocalization studies confirmed that the increased iNOS expression observed in the myenteric plexus was not found in neurons expressing constitutive bNOS, the expression of which was not altered in colitis, nor did it obviously overlap with expression of the generalized neuronal marker PGP 9.5 (data not shown). Use of the specific glial cell marker GFAP revealed that the increased iNOS expression in the myenteric plexus of colons from DSS-treated mice was predominantly in the enteric glial cells (Fig. 4). Levels of iNOS expression in control tissue, although detectable, were too low for unequivocal characterization of neuronal or glial colocalization.
**CCh modulation of ion transport via nicotinic receptors in DSS tissue requires the myenteric plexus and involves NO.** Earlier studies showed that the CCh-induced reduction in \( I_{sc} \) in tissues from mice with DSS-induced colitis was tetrodotoxin and hexamethonium sensitive, indicating mediation via neuronal nicotinic receptors (38). CCh applied to the serosal side of tissue from mice with DSS-induced colitis in which the external muscle layers containing the myenteric plexus had been stripped away by blunt dissection did not elicit this characteristic drop in \( I_{sc} \) (Fig. 5).

Pharmacological studies with whole-thickness colonic segments (i.e., with an intact myenteric plexus) showed that the CCh-induced drop in \( I_{sc} \) evoked via nicotinic cholinergic receptors in the myenteric plexus was also dependent on iNOS-derived NO, because pretreatment with either inhibitor of iNOS activity, L-NIL or 1400w, blocked this ion transport event (Fig. 6A). Previously, we showed that the \( I_{sc} \) response to CCh in tissue from mice with DSS-induced colitis could be reproduced in normal tissue from control mice by blocking muscarinic receptors with ATR before CCh challenge (38). Here we show that the CCh-induced drop in colonic \( I_{sc} \) from control mice where muscarinic receptors had first been blocked by the addition of ATR (i.e., ATR + CCh) was inhibited by pretreatment with L-NIL or 1400w (Fig. 6B). Finally, serosal addition of the NO scavenger cPTIO to Ussing chamber-mounted colonic tissue from mice with DSS-induced colitis 10 min before challenge with CCh significantly inhibited the \( I_{sc} \) response (Fig. 6C).

In all experiments, tissues were challenged with forskolin, which consistently resulted in a sustained increase in \( I_{sc} \) as a result of luminally directed active Cl\(^{-}\)/H\(^+\) secretion: the magnitude of this event was significantly reduced in tissues from mice with DSS-induced colitis: \( 238 \pm 19 \mu A/cm^2 \) (\( n = 16 \)) vs. \( 26 \pm 6 \mu A/cm^2 \) (\( n = 6 \)). In agreement with results from other investigators (1), pretreatment with the iNOS inhibitor 1400w partially prevented the reduction in magnitude of the \( \Delta I_{sc} \) induced by forskolin in tissue from mice with DSS-induced colitis: \( 86 \pm 28 \mu A/cm^2 \) (\( n = 7 \), \( P < 0.05 \) compared with control and DSS tissue without 1400w).

**DISCUSSION**

Of the many factors that influence ion transport, the neurotransmitter ACh is of particular importance. In normal mammalian colon, the ion transport response to ACh or to synthetic derivatives (e.g., CCh) is dominated by activation of epithelial M\(_3\) cholinergic receptors, the ligation of which causes a Cl\(^{-}\) efflux from the cell (8, 38). We previously showed that cholinergic control of ion transport is perturbed in tissue from mice with DSS-induced colitis (38), complementing observations...
from other studies (21, 34). However, the DSS model is intriguing, because CCh produced a sustained reduction in \( I_{sc} \), rather than a diminished transient increase in \( I_{sc} \) observed in other models, such as those evoked by 2,4,6-trinitrobenzenesulfonic acid, 2,4-dinitrobenzenesulfonic acid (21, 34), or oxazolone (unpublished observation). Underlying differences in the models may contribute to this discrepancy; for example, 2,4,6-trinitrobenzenesulfonic acid and 2,4-dinitrobenzenesulfonic acid elicit a polarized Th1-type inflammatory response and oxazolone produces a Th2-type response, whereas DSS-induced colitis involves a Th1- and a Th2-type inflammatory profile (reviewed in Ref. 3). Further analyses attributed this altered \( I_{sc} \) response in colonic tissue from DSS-treated mice to the activation of neuronal nicotinic cholinergic receptors (38).

Here we extend these observations by demonstrating a role for iNOS-derived NO in the modulation of cholinergic ion transport that is unmasked during DSS-induced colitis.

There is evidence of nonneuronal expression of nicotinic receptor mRNA or protein on immune cells and airway epithelial cells (37, 48). However, the response to CCh in DSS-treated animals was tetrodotoxin and hexamethonium sensitive (38), suggesting that the cholinergic receptor involved in this response was expressed on enteric neurons. This postulate is supported by the inability of nicotine to elicit any direct change in \( I_{sc} \) in the T84 and HT-29 human colonic epithelial cells (unpublished observation). We reasoned that an intermediate
molecule was mobilized to affect the epithelium subsequent to CCh challenge; previous pharmacological studies with this model ruled out the involvement of norepinephrine and opiates (38). NO was hypothesized as the intermediate factor, because 1) it can modulate ion transport, both directly and indirectly (18, 20, 24); 2) it acts as an intermediate in other cholinergic events (16, 42); and 3) it colocalizes with ChAT in the mouse colon (36).

Initial studies revealed, as expected, increased iNOS mRNA transcripts in colonic tissues from DSS-treated mice (38), a finding supported by the immunohistochemical expression of iNOS. Consistent with analyses of inflamed human colon and other rodent models of colitis (22, 40, 49), patches of epithelium and cells in the mucosa and submucosa displayed iNOS immunoreactivity in the colon of DSS-treated mice. A similar pattern of iNOS immunoreactivity was not found in control tissue but has been observed in other studies with the DSS model of colitis (17). Moreover, there was an obvious increase in iNOS expression, both mRNA and protein, in the myenteric plexuses of colons from DSS-treated mice, again, a finding that is not unprecedented (25, 44). Colocalization studies confirmed that the immunoreactivity detected in the myenteric plexus of tissue from DSS-treated and control mice was, in fact, iNOS, which was revealed by double labeling to be distinct from constitutively expressed bNOS. iNOS expression in control tissue may at first appear unusual, but it is consistent with the status of “physiological inflammation” that exists in the colon, which is constantly exposed to an immense microbial flora (9).

Additional immunohistochemical studies revealed that the in-

Fig. 5. Myenteric plexus is essential for nicotinic cholinergic $I_{\text{sc}}$ response elicited by carbachol (CCh). Colonic tissues from mice treated with 4% DSS for 5 days and normal drinking water for 3 days, from which external muscle layers and attendant myenteric plexus have been removed, do not display a downward deflection of $I_{\text{sc}}$ in response to 100 µM CCh. Values are means ± SE; n = 5. *P < 0.05 vs. full-thickness intact tissue. Insets: hematoxylin-and-eosin-stained sections of full-thickness (intact) and muscle-stripped segments of midcolon from DSS-treated mice.

Fig. 6. iNOS-derived NO mediates $I_{\text{sc}}$ response to CCh in DSS-treated tissue. A: pretreatment of DSS colonic tissue with 3 µM Nω-(1-iminoethyl)-lysine HCl (t-NIL) or 5 µM N-(3-(aminomethyl)benzyl)acetamidine (1400w) prevents decrease in $I_{\text{sc}}$ evoked by 100 µM CCh (n = 6). B: tissue from control mice pretreated with 1 µM atropine (ATR) to block cholinergic muscarinic receptors does not respond to CCh with a decrease in $I_{\text{sc}}$ when pretreated with t-NIL or 1400w (n = 5). Insets: sample current traces in which 1 colonic segment received 1400w and CCh, and the other segment received only CCh. C: pharmacological scavenging of NO with 100 µM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) also prevents decrease in $I_{\text{sc}}$ after CCh application to tissue from mice treated with 4% DSS for 5 days and normal drinking water for 3 days (n = 9). Values are means ± SE; *P < 0.05 vs. CCh or ATR + CCh.
increased iNOS expression in the myenteric plexus of DSS-treated mice was predominantly found in enteric glial cells, a cell type that can be an active participant in neuroimmuno-physiological events (4, 32). Enteric glia are of neural crest lineage and are similar to central nervous system (CNS) astrocytes (33). Astrocytes have been identified as a source of NO when stimulated with proinflammatory cytokines or in inflammatory pathologies of the CNS (26). The expression of iNOS in enteric glia in intestinal inflammation suggests that their response to inflammation is similar to that of astrocytes in the CNS. Astrocytes express nicotinic receptors in the CNS, and the expression profile of the subunits that compose the functional receptors differs between the astrocytes and the neurons (13). There is no evidence that enteric glia express cholinergic nicotinic receptors (50), and indeed our analyses indicated only neural expression of nicotinic receptors in the colon of control and DSS-treated mice (unpublished observation).

Use of two inhibitors of iNOS activity and a pharmacological scavenger of NO revealed that iNOS-derived NO was required for the drop in $I_{sc}$ after cholinergic stimulation of colonic tissue from DSS-treated mice. Furthermore, the drop in $I_{sc}$ evoked by CCh in control tissues after blockade of muscarinic receptors by ATR was also prevented by use of either iNOS inhibitor. However, neither iNOS nor NO inhibition restored a normal secretory response to CCh, suggesting that NO was not blocking epithelial muscarinic receptors. These data suggest that NO plays a physiological role in modulating nicotinic receptor-mediated cholinergic ion transport in the mouse colon and that this role becomes more prominent during inflammation associated with dysfunctional epithelial muscarinic cholinergic receptors. Such a possibility is supported by evidence showing that NO suppresses electrically evoked ion transport mediated by submucosal neurons in the guinea pig colon (31).

Colonic tissues from DSS-treated mice that had been stripped of the external muscle layers and attendant myenteric plexus did not display a CCh-induced drop in $I_{sc}$. This clearly indicates that the nicotinic receptors that initiate the $I_{sc}$ response reside in the myenteric plexus and suggests that iNOS-positive neurons or glial cells are the source of the NO. Should enteric glia prove to be a significant source of the iNOS-derived NO, as our immunohistochemical data suggest, then the lack of demonstrable nicotinic receptors on the enteric glia indicate neuron-to-glial communication in the modulation of colonic ion transport, an unprecedented observation.

NO produced in the myenteric plexus is unlikely to reach and directly affect the epithelium and so must mobilize an intermediate mediator to influence epithelial ion transport. We can only speculate as to the nature of this mediator [e.g., neuropeptide Y (23), somatostatin (6), prostaglandin D$_2$ (30)], but it is apparent that cholinergic control of colonic ion transport is more complex than simple interaction of ACh with epithelial M$_3$ receptors. Perturbation of the cholinergic system is not uncommon in inflammatory conditions (12, 29, 39), and it is clear that the DSS-induced colitis has resulted in dysfunctional muscarinic receptors on the epithelium. This could be the result of reduced receptor expression, altered receptor affinity, or failure of the intracellular signaling pathway. Evidence from other model systems can be advanced in support of each scenario (10, 19, 43). Although this study has focused on the cholinergic nicotinic-NO pathway of controlling epithelial ion transport, the failure of the muscarinic system is an issue that needs to be investigated. Our preliminary data indicate no obvious changes in the density of M$_3$ receptor expression in colonic tissue from DSS-treated mice.

As noted, enteric inflammation can be associated with altered cholinergic responses, and in accordance with this study, others have reported increased ChAT levels in inflamed gut tissue (5). This presents the seemingly paradoxical situation in which tissues from mice with DSS-induced colitis have the ability to make increased amounts of ACh while simultaneously having dysfunctional muscarinic receptors. However, increased availability of ACh to bind to nicotinic receptors may represent a braking strategy to counter the prosecretory effects of the cholinergic muscarinic system that would contribute to diarrhea. Should the findings in the DSS model system extrapolate to human colonic inflammation, then nicotinic receptor-mediated NO regulation of epithelial ion transport represents another facet of the putative therapeutic action of nicotine, along with nicotine-NO-mediated muscle relaxation (14), direct immunosuppression (45), and stimulation of mucin synthesis (11).

Collectively, the data reveal in the colon, but not the cecum, a shift in the balance of cholinergic control from predominantly muscarinic to nicotinic receptor-driven ion transport events during DSS-induced colitis and likely involves enteric glia. The results fit a model where cholinergic stimulation activates nicotinic receptors expressed on myenteric plexus neurons, resulting in NO production from iNOS in enteric glia or neurons. The NO then activates or inhibits other neurons or stromal cells, which signal the epithelium to alter its ion transport properties in a manner that counters a hypersecretory state (Fig. 7). The ability to recreate the ion transport events observed in tissue from DSS-treated mice in control tissue using pharmacological agonists and antagonists suggests that this pathway is present in normal tissue but has not been detected in studies that report net changes in $I_{sc}$ induced by cholinomimetics, where M$_3$ events will dominate. Thus the unique attributes of the DSS model of colitis have revealed a novel pathway of cholinergic regulation of epithelial electrolyte transport that may help define targets for therapeutic interventions to relieve the inflammation and symptoms associated with human inflammatory bowel diseases.

Fig. 7. Model illustrating pathways and cellular mediators of responses to CCh observed in DSS-induced colitis. CCh activates nicotinic receptor (NicR) on myenteric neurons, which liberate a mediator that initiates NO release from surrounding enteroglia or neurons that express iNOS. NO can then act on stromal cells (I), directly at the enterocyte (II), or at the level of the submucosal plexus (III) to inhibit secretion. –ve, Negative signal or inhibition.
ACKNOWLEDGMENTS

Technical assistance from Jun Lu (McMaster University) is greatly appreciated.

GRANTS

This work was funded by operating grants from the Crohn’s and Colitis Foundation of Canada (to D. M. McKay) and the Canadian Institutes of Health Research (to D. M. McKay and K. A. Sharkey). C. L. Green is a recipient of a Research and Development Scholarship from the Canadian Institutes of Health Research, and K. A. Sharkey is a recipient of a Medical Scientist award from the Alberta Heritage Foundation for Medical Research.

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