Gastroenterology 2015;149:445–455

Inhibiting Inducible Nitric Oxide Synthase in Enteric Glia Restores Electrogenic Ion Transport in Mice With Colitis

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BACKGROUND & AIMS: Disturbances in the control of ion transport lead to epithelial barrier dysfunction in patients with colitis. Enteric glia regulate intestinal barrier function and colonic ion transport. However, it is not clear whether enteric glia are involved in epithelial hyporesponsiveness. We investigated enteric glial regulation of ion transport in mice with trinitrobenzene sulfonic acid– or dextran sodium sulfate–induced colitis and in *Il10*$^{-/-}$ mice. **METHODS:** Electrically evoked ion transport was measured in full-thickness segments of colon from CD1 and *Il10*$^{-/-}$ mice with or without colitis in Ussing chambers. Nitric oxide (NO) production was assessed using amperometry. Bacterial translocation was investigated in the liver, spleen, and blood of mice. **RESULTS:** Electrical stimulation of the colon evoked a tetrodotoxin-sensitive chloride secretion. In mice with colitis, ion transport almost completely disappeared. Inhibiting inducible NO synthase (NOS2), but not neuronal NOS (NOS1), partially restored the evoked secretory response. Blocking glial function with fluoroacetate, which is not a NOS2 inhibitor, also partially restored ion transport. Combined NOS2 inhibition and fluoroacetate administration fully restored secretion. Epithelial responsiveness to vasoactive intestinal peptide was increased after enteric glial function was blocked in mice with colitis. In colons of mice without colitis, NO was produced in the myenteric plexus almost completely via NOS1. NO production was increased in mice with colitis, compared with mice without colitis; a substantial proportion of NOS2 was blocked by fluoroacetate administration. Inhibition of enteric glial function in vivo reduced the severity of trinitrobenzene sulfonic acid–induced colitis and associated bacterial translocation. **CONCLUSIONS:** Increased production of NOS2 in enteric glia contributes to the dysregulation of intestinal ion transport in mice with colitis. Blocking enteric glial function in these mice restores epithelial barrier function and reduces bacterial translocation.

Keywords: IBD; Inflammation; Myenteric Plexus; Enteric Glial Cells.

Inflammatory bowel diseases (IBD), Crohn's disease, and ulcerative colitis, are characterized by diarrhea, weight loss, and malabsorption of water and nutrients, resulting in debilitating illness.[1,2] The dysregulation of water and ion transport occurs as a result of altered epithelial function and is directly involved in the symptoms of IBD. However, the mechanisms underlying this dysfunction are largely unresolved and treatment options are limited.[3]

Increased NO production has been implicated in intestinal inflammation, although the function of NO has been debated as both pro- and anti-inflammatory.[4,5] The increase in NO occurs through up-regulation of inducible NO synthase (NOS2) in epithelial and immune cells, and in neurons and enteric glial cells of the enteric nervous system (ENS).[6–11] Enteric glia, long considered to be primarily supportive cells of the ENS, are now known to be actively involved in the maintenance of epithelial barrier function.[12] NOS2-derived NO has been shown to induce epithelial hyporesponsiveness to secretagogues that normally induce apically directed chloride secretion.[13,14] We have recently described a role for enteric glia in the modulation of nicotinic cholinergic receptor–mediated ion transport.[15] However, whether enteric glia are involved in dysregulating intestinal ion transport during intestinal inflammation is not known.

During intestinal inflammation, enteric glia undergo reactive gliosis,[16,17] respond to and produce inflammatory mediators,[16,18,19] and are involved in the breakdown of epithelial barrier function.[20,21] It has been speculated that enteric glia are involved in the regulation of ion transport during experimental colitis, based on the localization and up-regulation of NOS2 in enteric glia during intestinal inflammation.[8] However, a functional role for NOS2 in enteric glia remains to be established.

We tested the hypothesis that enteric glia are involved in the dysregulation of ion transport in colitis by directly targeting them using the glial metabolic inhibitor fluoroacetate.

Abbreviations used in this paper: **1400W, N-(3-aminomethyl) benzylacetamidine; cAMP, cyclic adenosine monophosphate; DSS, dextran sodium sulfate; EFS, electrical field stimulation; ENS, enteric nervous system; IBD, inflammatory bowel disease; I_{SC}, short-circuit current; NO, nitric oxide; NOS, nitric oxide synthase; NOS1, neuronal nitric oxide synthase; NOS2, inducible nitric oxide synthase; SMTC, S-Methyl-L-thiocitrulline; TNBS, trinitrobenzene sulfonic acid; TTX, tetrodotoxin; VIP, vasoactive intestinal peptide.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2015.04.007

Fluoroacetate has been well characterized as a method of reversibly inhibiting glial metabolic function in the central nervous system, where it is preferentially taken up by astrocytes and their function is reversibly inhibited.[22–24] Enteric glia have many similarities to astrocytes,[25,26] and within the ENS, the fluoroacetate metabolite fluorocitrate has been used successfully to demonstrate their role in the regulation of motility.[27] Here we utilize fluoroacetate in conjunction with measurement of intestinal ion transport and NO release from the myenteric plexus to examine the role of enteric glia in the dysregulation of ion transport in the inflamed colon in three mouse models of colitis. We also assessed human colonic biopsies to establish whether, in the absence of the enteric plexuses, we would observe epithelial hyporesponsiveness and, if so, if that was reversed by treatment with fluoroacetate.

Methods

Animals

Wild-type male CD1 mice (6–8 weeks; Charles River, Montreal, Quebec, Canada) were used unless otherwise stated. A genetic model of colitis using *Il10*$^{-/-}$ mice generated on a 129Sv/Ev background was also used, and compared with Sv/Ev wild-type controls. *Il10*$^{-/-}$ mice develop intestinal inflammation as they age and, therefore, mice 16–20 weeks of age were used. Animal protocols were approved by the University of Calgary Animal Care Committee, and were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Animals were housed in polystyrene cages with free access to food and tap water and maintained on a 12-hour light–dark cycle in a temperature- and humidity-controlled room. All animals were killed by cervical dislocation under deep isofluorane anesthesia.

Colitis

Colitis was induced using trinitrobenzene sulfonic acid (TNBS; 0.1 mL of 30 mg/mL TNBS in 30% ethanol) administered 3 cm intrarectally in animals lightly anesthetized with isofluorane, and dextran sodium sulfate (DSS) (5% w/v) dissolved in drinking water for 5 days, followed by 2 days of normal drinking water. TNBS-treated animals were used 3 days or 21 days post treatment and DSS-treated animals were used 7 days after treatment. See Supplementary Materials for details.

Measurement of Electrogenic Ion Transport

Full-thickness distal colonic segments were mounted in Ussing chambers (0.5 cm^2 opening) and held under voltage-clamp conditions in oxygenated (95% O_2, 5% CO_2) Krebs buffer (pH 7.4) that contained the following: NaCl (117 mM), KCl (4.8 mM), $CaCl_2$ (2.5 mM), $MgCl_2$ (1.2 mM), $NaHCO_3$ (25 mM), NaH_2PO_4 (1.2 mM), and d-glucose (11 mM). Electrogenic movement of ions across the epithelium was recorded as short-circuit current (I_{SC}, $\mu A/cm^2$), where the change in I_{SC} (ΔI_{SC}) is the peak post-stimulus value subtracted from the stable pre-stimulus baseline. Drugs were added serosally unless otherwise specified and remained in the bath for the duration of the experiment. Electrical field stimulation (EFS; 50 V, 10 Hz, 3 seconds) was applied in the presence or absence of inhibitors or agonists, see Supplementary Material for details.

Electrochemical Detection of Nitric Oxide

Continuous amperometric monitoring of NO release was conducted on the mucosal surface of full-thickness colon and on myenteric ganglia in myenteric plexus-longitudinal muscle preparations, as described previously.[15,28] Briefly, a NO oxidation current was recorded using a 40-μm diameter boron-doped diamond microelectrode (described elsewhere[15,28,29]). A stainless steel wire served as the counter electrode and a "no leak" Ag|AgCl electrode (EE009; ESA Biosciences Inc., Sunnyvale, CA) was used as the reference electrode. Amperometric measurements were carried out using a BioStat multi-mode potentiostat (ESA Biosciences). See Supplementary Material for details.

For experiments investigating NO release from the mucosa, see Supplementary Material for details.

Bacterial Translocation

Examination of bacterial translocation was performed in mice with TNBS colitis or controls, with animals receiving saline or fluoroacetate injections (1 mg/kg, intraperitoneally) every 12 hours for 3 days. Mice were euthanized by cervical dislocation under deep isofluorane anesthesia and their blood, livers, and spleens were tested for translocated bacteria, as described previously.[13,30] See Supplementary Material for details.

Fluoroacetate Treatment

The glial metabolic inhibitor fluoroacetate (5 mM; Sigma, St Louis, MO) was applied for 120 min in Ussing chamber experiments before EFS or the addition of agonists and 60 minutes for dissected longitudinal muscle-myenteric plexus preparations in electrochemical detection studies.

Minimum Inhibitory Concentration Test

Bacterial growth in the presence and absence of fluoroacetate was assessed using the minimum inhibitory concentration test, as described previously.[31] See Supplementary Material for details.

Human Biopsies

Human colonic biopsy samples were acquired from the distal and rectosigmoid colon during colonoscopy or sigmoidoscopy procedures after informed consent from patients with irritable bowel syndrome (n = 2), Crohn's disease (n = 5), or ulcerative colitis (n = 4). See Supplementary Material for details.

Statistics

Data are presented as mean ± SEM and were compared using one-way analysis of variance, followed by post-hoc pairwise comparisons with Tukey's test, unless otherwise stated. $P < .05$ was accepted as a level of statistically significant difference.