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M J Bern, … , J T Wachsman, D W Powell

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Research Article

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Immune System Control of Rat and Rabbit Colonic Electrolyte Transport

Role of Prostaglandins and Enteric Nervous System

M. Jonathan Bern, Christopher W. Sturbaum, S. Selim Karayalcin, Helen M. Berschneider, Joseph T. Wachsman, and Don W. Powell

Department of Medicine and Core Center in Diarrheal Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Abstract

The role of the immune system in controlling intestinal electrolyte transport was studied in rat and rabbit colon in Ussing chambers. A phagocyte stimulus, the chemotactic peptide FMLP, and a mast cell stimulus, sheep anti-rat IgE, caused a brief (< 10 min) increase in short-circuit current (*Isc*). Products of immune system activation, platelet-activating factor (PAF) and reactive oxygen species (ROS), caused a sustained, biphasic increase in the *Isc*. Ion replacement and flux studies indicated that these agonists stimulated electrogenic Cl secretion and inhibited neutral NaCl absorption; responses that were variably inhibited by the cyclooxygenase blockers indomethacin and piroxicam. Lesser degrees of inhibition by nordihydroguaiaretic acid could be accounted for by decreased prostaglandin synthesis rather than by lipoxygenase blockade. Tetrodotoxin, hexamethonium, and atropine also inhibited immune agonist-stimulated *Isc*, but had no effect on immune agonist-stimulated production of PGE_2 or PGI_2. These results indicate that immune system agonists alter intestinal epithelial electrolyte transport through release of cyclooxygenase products from cells in the lamina propria with at least 50% of the response being due to cyclooxygenase product activation of the enteric nervous system. The immune system, like the enteric nervous system and the endocrine system, may be a major regulating system for intestinal water and electrolyte transport in health and disease.

Introduction

AA metabolites of both the cyclooxygenase and lipoxygenase pathway are thought to play a role in the diarrheas of intestinal inflammation (1, 2) and intestinal anaphylaxis (3). Elevated levels of AA metabolites have been demonstrated in both natural diseases and experimental disease models and these products can be shown to stimulate intestinal Cl and water secretion and inhibit intestinal NaCl and water absorption (4–6). Recently, our laboratory (7) and others (8, 9) have shown fairly conclusively that the major source of eicosanoids in the intestine, particularly prostaglandins, is the subepithelial region of the gut. An important component of the lamina propria and submucosa are immune cells, including lymphocytes, phagocytes (macrophages, neutrophils, and eosinophils) and mast cells. Phagocytes and mast cells, in particular, are known to be avid producers of eicosanoids (10, 11). Therefore, stimulants of the immune system as well as products of immune cell stimulation might play important roles in intestinal secretion of inflammation and anaphylaxis through the release of eicosanoids in the lamina propria with subsequent stimulation of the enterocyte. We undertook studies of both immune cell stimulants such as the chemotactic peptide FMLP, which is thought to be specific for phagocytic cells, and anti-rat IgE immunoglobulin, which is a potent, but not entirely specific stimulus of mast cells. Similarly, we studied the transport effects of immune cell products such as reactive oxygen species (ROS),[1] which are released by phagocytes during the respiratory burst, and platelet-activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a lipid mediator released by both phagocytes and mast cells. The results reported here implicate the immune system as a regulator of intestinal electrolyte transport through a complex interaction with the enteric nervous system and the epithelium.

Methods

Transport studies. Male Sprague-Dawley rats weighing 375–500 g were killed by cervical dislocation. Male New Zealand rabbits weighing 3–4 kg were killed with intravenous pentobarbital sodium (60 mg/kg). The colon of each animal was removed, opened longitudinally, and washed of contents with oxygenated Ringer's solution. The entire rat colon or a 5-cm segment of distal rabbit colon was stripped of its outer muscle layers with a combination of blunt and sharp dissection as previously described (12–14), taking care that the submucosal plexus of the enteric nervous system remained intact as confirmed by histology.

Segments of colon were mounted in lucite half chambers with 0.5-cm^2 apertures, each side containing 5–10 ml Ringer's solution. The Ringer's solution was maintained at 37°C and pH 7.4 and was gassed with 95% O_2/5% CO_2. It contained (in millimolar): Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO_3, 25; H_2PO_4, 0.4; HPO_4, 2.4; and glucose, 10. In some studies, Cl-free and Cl-HCO_3-free solutions were made by replacing Cl and HCO_3 with isethionate. When HCO_3 was omitted, the solutions were oxygenated with 100% O_2. The bathing solutions were connected via agar bridges to calomel electrodes in

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Address reprint requests to Dr. D. W. Powell, CB #7080, 326 Burnett-Womack Bldg., University of North Carolina, Chapel Hill, NC 27599-7080.

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1. *Abbreviations used in this paper:* ACh, acetylcholine; anti-IgE, sheep anti-rat IgE; ATR, atropine; DPH, diphenhydramine; G, conductance; HEX, hexamethonium; *Isc*, short-circuit current; INDO, indomethacin; *J*, flux; J_{net}^{R}, $Isc - (J_{net}^{Na} - J_{net}^{Cl})$; MLP, methionyl-leucyl-phenylalanine; ms, mucosal-to-serosal; NDGA, nordihydroguaiaretic acid; PAF, platelet-activating factor; PXM, piroxicam; PD, potential difference; ROS, reactive oxygen species; sm, serosal-to-mucosal; TTX, tetrodotoxin; VIP, vasoactive intestinal polypeptide; X, xanthine; XO, xanthine oxidase.

order to measure the electrical potential difference (PD) across the epithelial-submucosal preparation. The tissues were short-circuited to zero PD by an automatic voltage clamp (World Precision Instruments, Inc., New Haven, CT) using Ag-AgCl electrodes connected to the bathing solution via agar bridges. Tissues were continuously short-circuited except for 5-s intervals every 15–30 min when the open-circuit PD was read. Conductances (G) were calculated from the open-circuit PD and the short circuit current (*Isc*) using Ohm's law. Unidirectional Na and Cl fluxes were measured in paired tissues which had electrical conductances differing by no more than 25%. Flux measurements were accomplished by the addition of ^{22}Na and ^{36}Cl to opposite sides of paired tissues (15). All fluxes were measured in the steady state.

Agonists were added to the serosal bathing solution after stabilization of the tissues either in Ringer's solution (control) or after the serosal addition of various AA metabolism or neural antagonists/inhibitors. At least 15 min of preincubation with inhibitors was allowed before adding the various agonists. Drug concentration-response curves were obtained by adding the various agonists to the serosal solution of four to eight tissues mounted from a single animal. Two methods were used to measure the response to agonists: (*a*) an integrated response (area under the response curve) and (*b*) the maximal change in *Isc* above baseline (ΔIsc_{MAX}) at 3–6 min after agonist (peak 1) and, if the response was biphasic, at the peak of the second response (15 min for PAF and 30 min for ROS). Because both methods gave essentially the same results, the ΔIsc_{MAX} was used. In experiments involving inhibitors, tissues pretreated with inhibitor were compared with simultaneously studied control tissues from the same animal. At varying intervals after the addition of inhibitors, antagonists, or agonists, 1-ml samples were removed from the serosal bathing solutions, placed in plastic vials, gassed with argon and stored at −20°C to await RIA of various eicosanoids. Tissues were removed from the Ussing chambers, fixed with buffered 4% formaldehyde-10% gluteraldehyde and stained with hematoxylin and eosin or with diamine silver for histological assessment of epithelial and neural integrity, respectively.

Prostaglandin assays. PGE_2 and 6 keto $PGF_{1\alpha}$ content of incubation solutions was determined by direct immunoassay on 100–300-μl samples according to the method of Granstrom and Kindahl (16). The PG antisera had minimal cross-reactivity (< 1% for most other prostaglandins) except for 6% cross-reactivity of the E_2 antisera for PGA_2 and 2–8% cross-reactivity of the 6 keto $PGF_{1\alpha}$ antisera for $PGF_{1\alpha}$ and $PGF_{2\alpha}$. The accuracy and precision of the assays was determined under each experimental condition. Standard curves were assayed in the presence of the various inhibitors and agonists that were used in the study. It was found that indomethacin (INDO) and piroxicam (PXM) reproducibly shifted the standard curve for PGE_2, whereas tetrodotoxin (TTX) and hexamethonium (HEX) had a similar effect on the assay for 6 keto $PGF_{1\alpha}$. Therefore, in studies where these inhibitors were in the experimental samples, standards were used that contained the proper inhibitor. Among the agonists, PAF (10^{-5} M) was found to interfere with the prostaglandin assays. However, when a known amount of prostaglandin standard was added to solutions in which PAF had been previously incubated with intestinal tissue, no interference could be detected. It is likely that PAF, which is avidly bound by protein (17), became bound to tissue or albumin in the Ussing chamber and, thus, was not available for interference with the immunoassay. All assays were further checked by monitoring parallelism (linearity with sample size). As previously described, such techniques give valid assays for PGE_2 and 6 keto $PGF_{1\alpha}$ as checked by HPLC (7).

Prostaglandin production was calculated from the concentrations measured at successive 15-min periods. Reported values represent the average production in the first two 15-min periods except in the rabbit, where prostaglandin production by FMLP was only stimulated for the first 15 min. All values were expressed as ng/30 min per cm^2.

Materials. All chemicals and whole sheep sera were obtained from Sigma Chemical Co. (St. Louis, MO.) ^{22}Na and ^{36}Cl were obtained from Amersham Corp. (Arlington Heights, IL) and ICN Radiochemicals (Irving, CA), respectively. Sheep anti-rat IgE was purchased from ICN Immunobiologicals (Lisle, IL). RIA reagents for PGE_2 and for 6-Keto $PGF_{1\alpha}$ were obtained from Advanced Magnetics, Inc., (Cambridge, MA).

Methionyl-leucyl-phenylalanine (MLP) and FMLP were dissolved in DMSO, divided into aliquots, stored at −20°C and then thawed just before addition to the Ussing chambers. Lyophilized globulin or sera were reconstituted with sterile water, divided into aliquots and stored at 4°C. L-α-Lysophatidylcholine, γ-*o*-hexadecyl (lyso PAF) and L-α-phosphatidylcholine, β-acetyl-γ-*o*-hexadecyl (PAF) were obtained as dry powder, dissolved in 2.5% BSA in water and were stored in aliquots at −20°C for single time use. ROS were created in the Ussing chamber by adding varying concentrations of xanthine oxidase (XO) to 0.7 mM xanthine (X) in the serosal solution. INDO, PXM, and nordihydroguaiaretic acid (NDGA) were dissolved as stock solutions in DMSO. All reagents dissolved in DMSO were prepared so that no more than 10 μl was added to 10 ml of bathing solution in the Ussing chambers. DMSO at 0.1% concentrations does not have any effect on electrolyte transport by intestine. TTX, HEX, and atropine (ATR) were dissolved in Ringer's solution.

Statistical evaluation. If more than one study was done on the same intestine, the responses were averaged to give a single value for that animal. Hence, in the data presented here, *n* equals the number of animals. The studies were always carried out in paired fashion so that an agonist or antagonist/inhibitor could be compared with simultaneous control tissue from the same animal. To determine statistical significance, we used either the paired *t* test or, if multiple comparisons were undertaken, a parametric or nonparametric analysis of variance was used.

Results

Colonic transport response to immune system agonists. Figs. 1–5 depict the electrical response (ΔIsc) of rat and rabbit colon to the immune system stimuli and products. The concentration responses to these agonists are also shown on the appropriate graph. Two patterns of response were observed: a monophasic, transient response to immune cell stimuli and a biphasic, sustained response to immune cell products. Anti-rat IgE (Fig. 1) and FMLP (Fig. 2 and 3) caused a brief, but significant increase in *Isc* that peaked at 4–6 min and was essentially

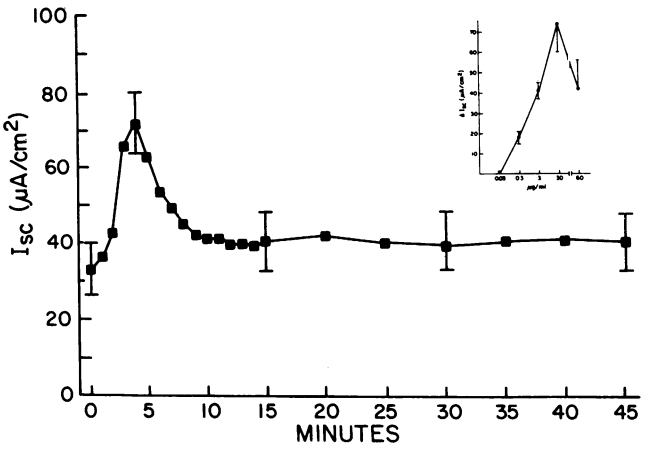

Figure 1. Short-circuit current response to sheep anti-rat IgE (3 μg/ml added to the serosal bathing solution of the Ussing chambered-rat colon (n = 6). (*Inset*) The concentration-response curve. The ED_{50} to anti-IgE was 2.7 μg/ml and the maximal change in *Isc* with 30 μg/ml was 74 μA/cm^2. The apparent elevation above baseline of the *Isc* at 10–45 min was not confirmed at other concentrations or in subsequent studies at 3 μg/ml. There was no short-circuit current response to whole sheep serum, which was used as a control (not shown).

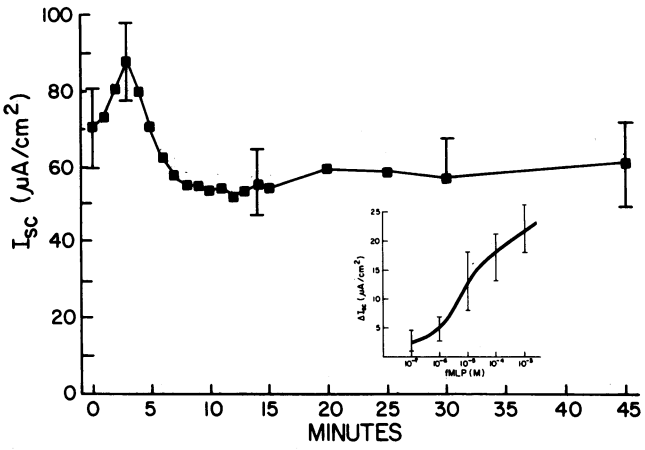

Figure 2. *Isc* response to FMLP (0.1 mM) in Ussing chambered-rat colon (n = 6). The concentration-response curve shows an ED_{50} of ~ 8×10^{-6} M and an ΔIsc_{MAX} of ~ 22±4 μA/cm². The apparent suppression of *Isc* at 10–45 min was not confirmed in subsequent studies. The nonformylated tripeptide MLP was used as a control and in concentrations up to 1 mM had no effect on the *Isc* (not shown).

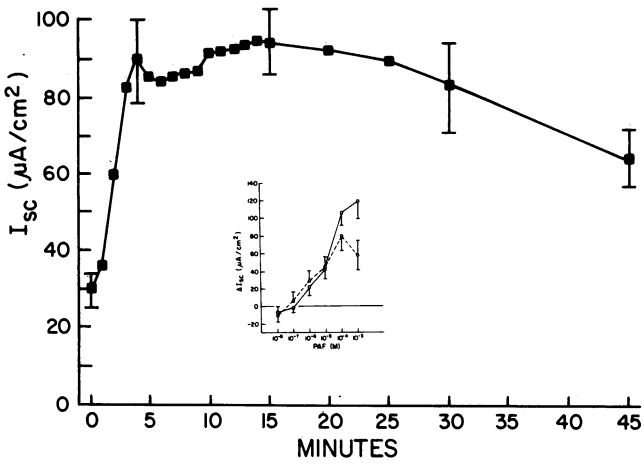

Figure 4. *Isc* response of the rat colon to PAF (10 μM, n = 6). Note the biphasic response to PAF with an initial peak at ~ 4 min and a delayed ΔIsc peak at ~ 15 min. The concentration-response curve revealed an ED_{50} of 20 μM and a ΔIsc_{MAX} of 110±25 μA/cm² for peak 1 (——) and 80±14 μA/cm² for peak 2 (-----). LysoPAF was used as a control and in concentrations up to 1 mM had no effect on *Isc* (not shown).

completed by 10 min. The concentration response to anti-IgE was similar (maximal response 3–30 μg/ml) to that reported for eicosanoid secretion by isolated human intestinal mast cells (18). However, the concentration of FMLP (> 0.1 mM) necessary for substantial stimulation of the *Isc* in the rat colon far exceeded the concentration necessary to stimulate the phagocytic cells of most other species (19). In addition, on occasion, the rat colon of an individual animal did not respond to FMLP at all. However, it is known that rat phagocytes contain a paucity of FMLP receptors. Therefore, we repeated these experiments with rabbit colon, a species with ~ 10^5 receptors/neutrophil. In rabbit colon (Fig. 3), not only was there a consistent response to much lower levels of FMLP (10 nM) but, the response was also significantly greater; ΔIsc_{MAX} of 92 μA/cm² as compared with 22 μA/cm² in the rat. Furthermore, the ED_{50} response for the rabbit was ~ 3 nM, which is similar to that reported for FMLP degranulation of rabbit neutrophils (19). Our findings with FMLP resemble those reported in preliminary studies by others (20).

In contrast to the immune system stimuli, the immune cell products PAF and ROS (created by the X/XO reaction) both demonstrated a biphasic response (Figs. 4 and 5) with an initial peak at 3–6 min followed by a second *Isc* peak at ~ 15 min for PAF and 30–40 min for X/XO. The ED_{50} for PAF stimulation of rat colon was 20 μM, similar to that obtained in rabbit colon (5 μM, n = 3, not shown). This is considerably greater than that necessary to stimulate chemotaxis and degranulation of phagocytes (0.01–100 nM) (20). A distinct circadian response was noted with PAF as has been noted by others in studies of rat small intestine (21). Animals studied in the afternoon had

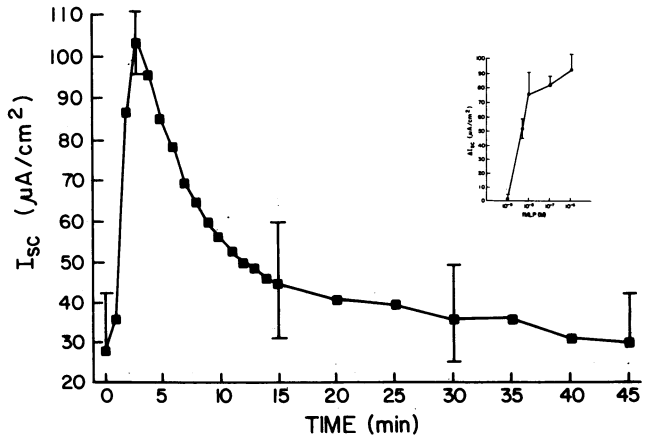

Figure 3. *Isc* response of rabbit colon to FMLP (10 nM, n = 6). The concentration response curve in the *inset* shows an ED_{50} in rabbit colon of 3 nM and a ΔIsc_{MAX} of 92 μA/cm².

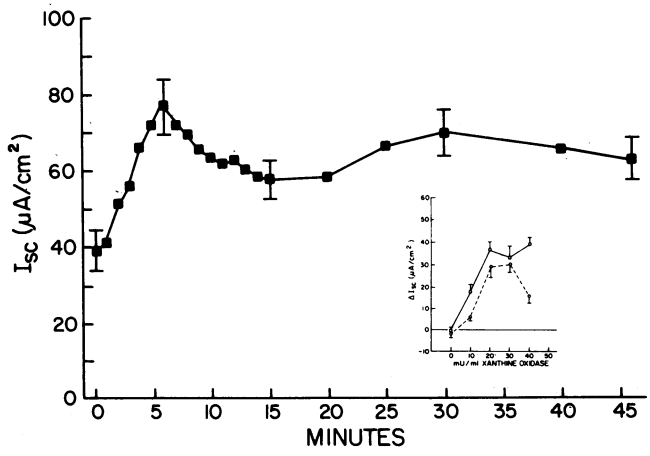

Figure 5. *Isc* response of rat colon to reactive oxygen metabolites created by the X (0.7 mM)/XO (20.8 mU/ml) reaction (n = 5). Note the biphasic response with the initial peak at ~ 6 min and a delayed peak at 30 min. The concentration response curve demonstrates an ED_{50} for XO of 10–13 mU/ml when added to 0.7 mM X. The ΔIsc_{MAX} were 38±5 and 30±6 μA/cm² for peaks 1 (——) and 2 (-----), respectively. The separate addition of X or of XO alone had no effect on *Isc* (not shown).

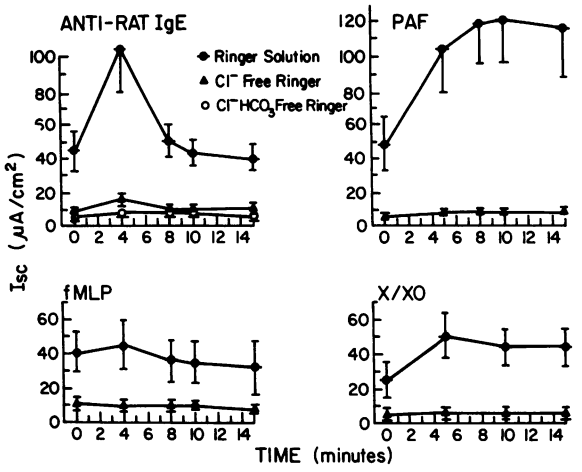

Figure 6. The *Isc* response to various immune cell stimulants (anti-rat IgE and FMLP) and immune cell products (PAF and ROS created by the X/XO reaction) in the rat colon ($n = 6$) bathed in either Ringer's solution or Cl-free and Cl-HCO_3-free Ringer's solutions. The *Isc* response to FMLP, PAF, and X/XO were abolished in Cl-free solutions, whereas it requires removal of both Cl and HCO_3 to abolish the *Isc* response to anti-rat IgE. Note also the dependence of basal *Isc* in the rat colon on the presence of Cl.

blunted or absent *Isc* responses and were not included in the data reported here. There is no clear way to compare the concentration response to X/XO. However, as we have shown in preliminary studies, the maximum response to X/XO is equivalent to that produced by 1 mM H_2O_2 (22).

Fig. 6 demonstrates that the ΔIsc's to these various agonists were essentially abolished when the studies were repeated in Cl-free Ringer's solution. The small *Isc* response to anti-rat IgE in Cl-free Ringer's was completely abolished when experiments were repeated in solutions free of HCO_3 as well as Cl. Fig. 6 also demonstrates that the basal *Isc* of rat colon is Cl dependent, probably representing some level of basal electrogenic Cl secretion.

The effect of anti-IgE on Na and Cl fluxes are shown in Table I (section *A*). Because the response to anti-IgE was short lived, steady-state fluxes could only be determined after the *Isc* had returned to baseline levels. Nonetheless, when fluxes were measured even after the anti-IgE-induced *Isc* response (electrogenic Cl secretion) was dissipated, a significant alteration in Na and Cl transport could be demonstrated, (decreased J_{ms}^{Na}, J_{Net}^{Na}, J_{ms}^{Cl}, and J_{Net}^{Cl}).

In contrast, the sustained *Isc* response of X/XO allowed flux measurements during the increase in *Isc*. As shown in Tables II (section A) and III (section A), there was a significant decrease in J_{ms}^{Na} and J_{ms}^{Cl}, as well as (in Table III [section A]) a significant increase in J_{sm}^{Cl} and *Isc*. The net result of these changes in unidirectional fluxes was an inhibition of Na and Cl absorption (decreased J_{Net}^{Na} and J_{Net}^{Cl}) and, depending on the magnitude of the decrease in J_{ms}^{Cl} and the increase in J_{sm}^{Cl} there was either complete inhibition of net Cl absorption (Table II [section A]) or significant net Cl secretion (Table III [section A]). Together, these three tables demonstrate that the response to immune cell stimuli and products was one of inhibition of neutral NaCl absorption and a variable degree and duration of stimulation of electrogenic Cl secretion.

Inhibition of immune system-mediated colonic electrolyte transport. Because these immune cell stimulants and products are known to release eicosanoids from white blood cells and from other mesenchymal tissues, we investigated the effect of cyclooxygenase and lipoxygenase inhibitors on electrolyte transport. Table IV compares the ΔIsc_{MAX} of these four immune system agonists in the presence of a cyclooxygenase blocker, INDO (10^{-6} M), a lipoxygenase blocker, NDGA (10^{-6} M), and to the combination of INDO and NDGA with the inhibitor responses normalized to the percent of control response. Preincubation with INDO significantly inhibited the ΔIsc_{MAX} to these four agonists by ~ 60–90%. There was a smaller and not always significant inhibition of the ΔIsc_{MAX} by NDGA (~ 50%). Except in the studies of rat colon with FMLP, the combination of INDO plus NDGA did not inhibit the ΔIsc to a greater extent than did INDO alone.

Table I. Effect of (A) Sheep Anti-Rat IgE (3 μg/ml) on Rat Colonic Electrolyte Transport and (B) Response to INDO (10^{-6} M) ($n = 8$)

	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	*Isc*	J_{net}^{R}	PD	G
	μeq/h per cm²								*mV*	*mS/cm²*
A										
(*i*) Basal	18.1±0.8	9.2±0.9	8.9±0.7	22.7±0.6	14.2±0.8	8.5±1.0	1.3±0.1	0.9±0.4	−1.9±0.3	20.3±2.5
(*ii*) Anti-Rat IgE	16.3±0.6	9.6±1.2	6.6±0.9	19.9±0.5	13.7±0.9	6.2±0.7	1.4±0.2	1.0±0.4	−2.0±0.4	23.4±3.6
(*ii* vs. *i*)	$P < 0.05$	NS	0.05	0.01	NS	0.05	NS	NS	NS	NS
B										
(*iii*) INDO	18.9±0.7	10.9±0.9	8.0±0.5	24.3±0.9	16.7±1.0	7.6±0.6	0.8±1.0	0.5±0.3	−1.0±0.1	21.2±2.1
(*iv*) INDO + anti-Rat IgE	17.1±0.7	11.2±1.2	5.9±0.9	21.7±0.8	15.3±1.1	6.4±0.7	0.8±0.1	1.3±0.5	−1.1±0.1	22.0±2.4
(*iv* vs. *iii*)	$P < 0.01$	NS	0.05	0.001	0.05	0.05	NS	NS	NS	NS
(*iii* vs. *i*)	$P <$ NS	NS	NS	0.05	0.05	NS	0.01	NS	0.01	NS
(*iv* vs. *ii*)	$P <$ NS	NS	NS	NS	NS	NS	0.01	NS	0.01	NS

Four pairs of tissue for each animal were studied simultaneously. Two pairs had INDO in the Ringer's solution. After a basal measurement (mean of two 15-min flux periods), the agonist (anti-rat IgE) was added to the serosal solution. After a 15-min equilibration, two additional flux periods were measured and meaned. (*ii* vs. *i*) = response of control colon to agonist; (*iv* vs. *iii*) = response of the inhibitor- (INDO) treated colon to the agonist; (*iii* vs. *i*) = response of the control colon to the inhibitor (INDO); (*iv* vs. *ii*) = effect of the inhibitor (INDO) on the agonist response.

*Table II. Effect of (A) ROS Created by the X (0.7 mM)/XO (20.8 mU/ml) Reaction on Rat Colonic Electrolyte Transport and (B) Response to INDO (10^{-6} M) (n = 8)**

	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	Isc	J^{R}_{net}	PD	G
					μeq/h per cm²				*mV*	*mS/cm²*
A										
(*i*) Basal	17.8±1.3	11.3±1.0	6.5±1.3	22.4±1.5	15.9±1.1	6.4±1.4	1.1±0.1	1.1±0.5	−1.5±0.2	20.0±2.3
(*ii*) X/XO	15.1±0.8	13.1±0.8	1.9±0.8	17.6±0.7	17.6±1.0	0.0±0.8	2.2±0.2	0.3±0.5	−2.5±0.4	26.6±2.7
(*ii* vs. *i*)	$P < 0.05$	0.05	0.01	0.01	NS	0.001	0.001	0.05	0.05	0.01
B										
(*iii*) INDO	16.7±0.7	10.2±0.4	6.4±0.6	22.1±1.5	15.9±1.1	6.2±0.8	0.7±0.1	0.4±0.6	−0.9±0.2	20.4±1.5
(*iv*) INDO + X/XO	15.4±0.6	10.6±0.7	4.8±0.9	19.7±1.3	15.3±1.2	4.4±0.6	1.1±0.2	0.7±0.5	−1.5±0.3	21.3±2.5
(*iv* vs. *iii*)	$P < 0.05$	NS	0.05	0.001	NS	0.05	0.01	NS	0.01	NS
(*iii* vs. *i*)	$P <$ NS	NS	NS	NS	NS	NS	0.05	NS	0.05	NS
(*iv* vs. *ii*)	$P <$ NS	0.001	0.05	NS	NS	0.001	0.01	NS	0.05	0.05

* See footnote to Table I.

The effect of INDO on basal and experimental Na and Cl fluxes in control and agonist-treated colons are shown in Tables I (section B) and II (section B). INDO had no effect on basal net Na and Cl fluxes by the control rat colon. However, INDO significantly decreased the basal *I*sc. This suggests that INDO affected the basal rate of transport of other ions (K, HCO_3, Ca, etc.), although this was not confirmed with corresponding changes in J_{Net}^{R}. The lack of effect of INDO on anti-IgE-induced inhibition of neutral NaCl absorption is also shown in Table I (section B). The inhibition of the neutral NaCl absorption noted with ROS, however, was reduced by INDO pretreatment (Table II [section B]), as was the electrogenic secretion of Cl. The blockade of prostaglandin synthesis with INDO thus inhibited the electrogenic Cl secretion stimulated by these immune system agonists and had a variable ability to prevent the inhibition of neutral NaCl absorption.

Because degranulating mast cells also release histamine, we studied the effect of the H_1-receptor antagonist diphenhydramine (DPH) on the Δ*I*sc to anti-IgE. As shown in Fig. 7, DPH inhibited the Δ*I*sc by ~ 50% as compared with 70% inhibition with INDO. The inhibition with a combination of DPH and INDO was not statistically different from that with INDO alone. These studies suggest that both histamine and eicosanoids were being released by anti-IgE stimulation and that histamine can trigger an increased Δ*I*sc either directly or through the release of eicosanoids. Furthermore, because phagocytes also have low-affinity IgE receptors (23), but don't produce histamine, this indicates that mast cells were being degranulated in rat colon.

Eicosanoid production stimulated by immune system agonists. The lack of an additive effect of INDO and NDGA on the ΔIsc_{MAX} (Table IV) suggests that NDGA, which is not specific for the lipoxygenase pathway of AA metabolism, may have also interfered with the formation of a cyclooxygenase product. To investigate this possibility, PGE_2 production was measured in control and agonist-stimulated tissues with or without prior pretreatment with either INDO or NDGA. Prostaglandin production peaked in the first or, more commonly, in the second 15-min period after addition of the immune system agonists and then declined toward, but not to, basal

*Table III. Effect by (A) ROS Created by the X (0.7 mM)/XO (20.8 mU/ml) Reaction on Rat Colonic Electrolyte Transport and (B) Response to TTX (10^{-7} M), (n = 5)**

	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	Isc	J^{R}_{net}	PD	G
					μeq/h per cm²				*mV*	*mS/cm²*
A										
(*i*) Basal	15.0±1.4	10.1±0.9	4.8±0.8	18.8±1.9	15.1±0.8	3.7±1.2	1.5±0.3	0.3±0.3	−1.7±0.3	22.3±2.9
(*ii*) X/XO	12.0±1.4	10.9±1.2	1.1±0.8	15.1±1.3	17.3±0.7	−2.3±0.8	3.1±0.2	−0.3±0.9	−3.9±0.5	22.4±2.4
(*ii* vs. *i*)	$P < 0.01$	NS	0.01	0.01	0.001	0.01	0.001	NS	0.01	NS
B										
(*iii*) TTX	16.7±1.1	10.5±1.5	6.2±0.8	21.6±1.5	14.9±2.0	6.7±0.7	0.8±0.2	1.3±0.2	−0.9±0.2	23.4±2.9
(*iv*) TTX + X/XO	15.5±1.5	11.1±1.7	4.5±0.9	18.7±1.6	15.3±2.3	3.4±0.9	1.4±0.3	0.4±0.3	−1.7±0.3	24.5±4.1
(*iv* vs. *iii*)	$P < 0.05$	NS	0.01	0.001	NS	0.001	0.01	NS	0.05	NS
(*iv* vs. *i*)	$P <$ NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS
(*iv* vs. *ii*)	$P <$ NS	NS	0.05	NS	NS	0.01	0.01	NS	0.05	NS

* See footnote to Table I.

Table IV. Effect of Cyclooxygenase and Lipoxygenase Inhibitors on Colonic Short-Circuit Current Response (ΔIsc_{MAX}) to Immune System Agonists

Agonist	ΔIsc_{MAX}* control (n)	Percent of control response		
		INDO (10^{-6} M)	NDGA (10^{-6} M)	NDGA + INDO (10^{-6} M each)
	$\mu A/cm^2$		%	
Anti-IgE (*3 μg/ml*)	63.7±8.6 (7)	36.0±7.7‡	70.9±8.4‡	42.3±9.7‡
FMLP (10^{-4} *M*)	20.0±6.6 (5)	11.6±10.6‡	44.6±33.2	0.0±9.1‡
PAF (10^{-5} *M*)	57.0±8.7 (7)	11.1±4.1‡	38.1±9.7‡	17.7±8.8‡
X (*0.7 mM*)/XO (*20.8 mU/ml*)	59.7±5.7 (6)	27.8±10.7‡	56.7±11.6‡	23.2±15.1‡

* ΔIsc_{MAX} = Peak change in Isc at 3–6 min after addition of agonists to the serosal bathing solution. ‡ $P < 0.02$ agonist versus agonist plus inhibitors.

levels at 30–45 min after addition of the agonist. Except for FMLP, which is a poor *I*sc stimulant in rat colon, the other three agonists all significantly increased PGE_2 production two to threefold (Fig. 8). Pretreatment with INDO not only prevented this increase in PGE_2, but also reduced PGE_2 production below basal levels as well. NDGA was moderately effective as a cyclooxygenase inhibitor. It did not suppress PGE_2 production below basal levels, but NDGA did inhibit the stimulation of PGE_2 production by these agonists. This lends support to the idea that the inhibition of the *I*sc response by NDGA was the result of partial inhibition of prostaglandin synthesis by this nonspecific eicosanoid pathway inhibitor.

Enteric nervous system–prostaglandin interactions. Because it has recently been suggested that prostaglandins, particularly PGI_2, might activate enteric nerves (24–29), we correlated the effects of enteric nervous system and cyclooxygenase inhibitors on immune system agonist-stimulated colonic ΔIsc_{MAX} and prostaglandin production. Table V compares the control *I*sc response with the various agonists with the response after preincubation with neural inhibitors such as TTX, HEX, and ATR. In the same tissues, we determined the response to the cyclooxygenase blockers INDO and PXM. The neural inhibitors and cyclooxygenase blockers reduced the ΔIsc to 60–90% of control levels. ATR at 10^{-6} M decreased the ΔIsc_{MAX} by only 10–20%; it required a large concentration of 50 μM to accomplish a significant degree of inhibition. The neural inhibitors and cyclooxygenase blockers reduced the ΔIsc_{MAX} of both peaks of the biphasic response to PAF and X/XO.

The effects of TTX on X/XO-induced alterations in colonic electrolyte transport are shown in Table III (section B). TTX had no statistically significant effect on basal Na and Cl absorption in the control tissues. TTX did effectively prevent the reduction in neutral NaCl absorption (J^{Na}_{Net} and J^{Cl}_{Net}) and prevented the stimulation of electrogenic Cl secretion (ΔIsc) brought about by X/XO. These data and those given in Table V indicate that a significant portion of the immune system agonist effect is mediated by the enteric nervous system.

It is conceivable that the enteric nervous system could be releasing prostaglandins that in turn might directly stimulate the enterocyte. Alternatively, prostaglandins released from immune and mesenchymal cells could be releasing neurotransmitters from the enteric nervous system that then stimulate the enterocyte. To determine which of these sequences was more likely, we measured prostaglandin production in the

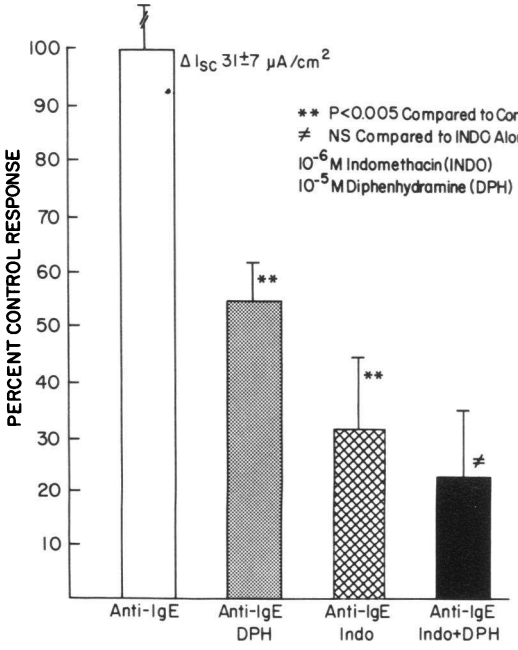

Figure 7. Isc response of rat colon to sheep anti–rat IgE (3 μg/ml, n = 6) is significantly inhibited by DPH (10^{-5} M) and by INDO (10^{-6} M). The inhibition of ΔIsc by the combination of the INDO and DPH was not statistically different from that shown with INDO alone.

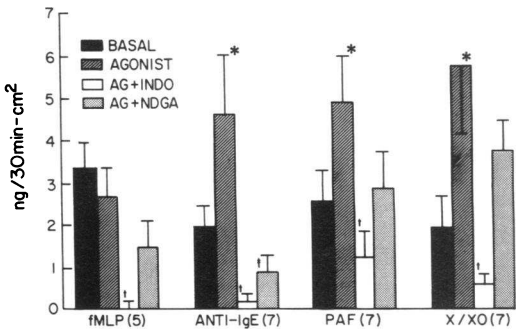

Figure 8. Effect of INDO (10^{-6} M) and NDGA (10^{-6} M) on PGE_2 secretion into the serosal bathing solution of Ussing chambered-rat colon in response to the various immune system agonists. *$P < 0.01$ comparing basal with agonist; †$P < 0.01$ comparing agonist to INDO plus agonist; ‡$P < 0.01$ comparing agonist to NDGA plus agonist.

Table V. Effect of Cyclooxygenase and Neural Inhibitors on Colonic Short-Circuit Current Response (ΔIsc_{MAX}) to Immune System Agonists

Agonist	ΔIsc_{MAX} control (n)*	Percent of control response				
		INDO (10^{-6} M)	PXM (10^{-6} M)	TTX (10^{-7} M)	HEX (10^{-5} M)	ATR (5×10^{-5} M)
	$\mu A/cm^2$			%		
Anti-rat IgE (*3 μg/ml*)	51.8±5.5 (8)	28.8±8.5‡	20.6±4.8‡	22.6±8.2‡	64.1±6.0‡	28.5±8.2‡
FMLP						
Rat (10^{-4} *M*)	28.8±3.9 (5)	4.5±2.0‡	15.4±3.2‡	33.3±10.4‡	41.3±11.5‡	24.6±10.2‡
Rabbit (10^{-8} *M*)	49.7±5.4 (6)	0.6±1.9§	—	58.3±15.9§	—	45.8±12.2§
PAF (10^{-5} *M*)						
Peak 1	65.6±7.4 (5)	10.0±3.4‡	14.0±5.4‡	26.7±12.4‡	56.5±12.8‡	34.6±10.4‡
Peak 2	65.6±10.2 (5)	19.6±10.3‡	24.1±14.4‡	9.8±3.3‡	42.5±23.1‡	19.9±13.1‡
X (*0.7 mM*)/XO (*20.8 mU/ml*)						
Peak 1	68.4±8.7 (5)	26.0±7.6‡	17.1±8.6‡	13.5±2.7‡	42.5±14.6‡	28.0±6.7‡
Peak 2	74.4±20.8 (5)	11.3±10.7‡	3.4±5.9‡	33.7±17.6‡	65.0±29.8	39.7±12.6

* ΔIsc_{MAX} = peak change in Isc from baseline at either 3–6 min after addition of agonists to the serosal bathing solution (peak 1) or at 15 min (PAF) or 30 min (X/XO) after the addition of agonist (peak 2). ‡ $P < 0.005$ agonist versus agonist plus inhibitors. § $P < 0.02$ agonist versus agonist plus inhibitors.

presence of both neural inhibitors and cyclooxygenase blockers (Tables VI and VII). Basal PGE_2 and PGI_2 production (as measured by assaying for the 6 keto-$PGF_{1\alpha}$ metabolite) were variable from study to study, but PGE_2 production was significantly less than 6 keto-$PGF_{1\alpha}$ in the rat. Both prostaglandins were increased significantly by anti-IgE, PAF, and X/XO. Although no increases in PG could be demonstrated in the rat colon after exposure to FMLP, this peptide increased rabbit colonic PGE_2 production 10-fold. In the rat colon, the immune cell products PAF and ROS were better stimulants of PG production (5–17-fold) than the immune cell stimuli anti-IgE and FMLP (0–1.5-fold). PAF and ROS stimulated more 6 keto-$PGF_{1\alpha}$ production (~ 15-fold) than PGE_2 production

Table VI. PGE_2 and Prostacyclin (measured as 6 keto-$PGF_{1\alpha}$) Production by Rat and Rabbit Colon in Response to Immune Cell Stimulants and Neural or Cyclooxygenase Inhibitors

Agonist (concentration) (n) + inhibitors (concentration)	PGE_2	6 keto-$PGF_{1\alpha}$
	ng/30 min per cm²	
Anti-rat IgE (*3 μg/ml*) (n = 8)		
Control	1.4±0.2	2.4±0.4
Agonist alone	2.2±0.2	3.7±0.7
(+)TTX (10^{-7} *M*)	2.0±0.3	2.5±0.5
(+)HEX (10^{-5} *M*)	1.7±0.6	2.8±0.6
(+)ATR (5×10^{-5} *M*)	1.8±0.4	3.4±0.7
(+)INDO (10^{-6} *M*)	0.1±0.1*‡	−0.1±0.7*‡
(+)PXM (10^{-6} *M*)	0.7±0.4‡	0.8±0.04‡
FMLP		
Rat (10^{-4} *M*) (n = 3)		
Control	0.9±0.1	2.2±0.8
Agonist alone	0.4±0.4	−4.1±2.4
(+)TTX (10^{-7} *M*)	1.1±1.2	−6.1±4.7
(+)HEX (10^{-5} *M*)	1.6±0.2	−1.7±1.7
(+)ATR (5×10^{-5} *M*)	1.2±0.5	−2.8±2.9
(+)INDO (10^{-6} *M*)	0.2±0.2	−8.7±9.0
(+)PXM (10^{-6} *M*)	0.0±0.1	−3.8±2.0
Rabbit (10^{-8} *M*) (n = 6)		
Control	1.1±2.8	0.1±0.8
Agonist alone	10.6±2.6*	3.7±1.0*
(+)TTX (10^{-7} *M*)	7.2±3.4*	3.1±1.3*
(+)INDO (10^{-6} *M*)	−2.6±3.2‡	0.2±0.6‡

* $P < 0.01$ control versus agonist or agonist plus inhibitor.
‡ $P < 0.01$ agonist versus agonist plus inhibitor.

Table VII. PGE_2 and Prostacyclin (measured as 6 keto-$PGF_{1\alpha}$) Production by Rat Colon in Response to Immune Cell Products and Neural or Cyclooxygenase Inhibitors

Agonist (concentration) (n) plus inhibitors (concentration)	PGE_2	6 keto-$PGF_{1\alpha}$
	(ng/30 min per cm²)	
PAF (10^{-5} *M*) (n = 5)		
Control	0.5±0.2	1.9±0.6
Agonist alone	2.6±0.5*	26.7±6.4*
(+)TTX (10^{-7} *M*)	2.0±0.6	30.2±7.7*
(+)HEX (10^{-5} *M*)	2.7±0.6*	41.2±12.0*
(+)ATR (5×10^{-5} *M*)	3.5±1.3*	36.2±9.0*
(+)INDO (10^{-6} *M*)	−0.4±0.2‡	2.1±1.0‡
(+)PXM (10^{-6} *M*)	−0.2±0.1‡	0.4±1.0‡
X (*0.7 mM*)/XO (*20.8 mU/ml*) (n = 5)		
Control	1.3±0.5	1.5±0.2
Agonist alone	5.9±0.2*	26.6±3.6*
(+)TTX (10^{-7} *M*)	6.6±0.6*	22.0±3.6*
(+)HEX (10^{-5} *M*)	6.1±0.1*	23.8±4.8*
(+)ATR (5×10^{-5} *M*)	6.3±0.5*	19.4±4.5*
(+)INDO (10^{-6} *M*)	1.5±0.3‡	4.9±1.4‡
(+)PXM (10^{-6} *M*)	1.8±0.6‡	5.1±1.8‡

* $P < 0.005$ control versus agonist or agonist plus inhibitor.
‡ $P < 0.005$ agonist versus agonist plus inhibitor.

(increased fivefold). Of importance is the lack of inhibition of PG production by the neural inhibitors while in the same tissues significant inhibition was demonstrated with INDO and PXM. When viewed in conjunction with the simultaneously measured ΔIsc_{MAX} (Table V), these data suggest that the alteration in electrolyte transport was the result of the enteric nervous system being stimulated by cyclooxygenase products rather than prostaglandins being released in response to neural stimulation.

Discussion

Three main classes or groups of agents have been identified that alter intestinal electrolyte transport: neurotransmitters, hormones, and bacterial enterotoxins (30). The enteric nervous system stimulates intestinal secretion through neurotransmitters such as acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) and stimulates intestinal absorption with neurotransmitters such as α_2-adrenergic agents, enkephalins and somatostatin. An example of endocrine or hormonal control is aldosterone-enhancement of Na and water absorption from the colon. The third group is represented by enterocyte receptors for bacterial enterotoxins, such as those elaborated by *Escherichia coli* and *Vibrio cholerae,* which are important causes of pathologic alterations of water and electrolyte transport. We believe the data presented here are confirmatory evidence for an additional regulatory system: the immune system (31). We have shown that stimuli of immune cells, as well as products of immune cell stimulation, are capable of altering intestinal electrolyte transport. Furthermore, the evidence suggests that cyclooxygenase products of AA metabolism and the enteric nervous system play a central role in mediating this effect.

Mast cells and phagocytes are two types of immune effector cells that release soluble mediators in the lamina propria in response to stimuli such as anti-IgE and FMLP, which are reasonably specific for these two classes of effector cells. These stimuli activate immune effector cells through receptor-mediated phosphoinositol metabolism (32, 33). Mucosal mast cells, which play an important role in hypersensitivity responses such as those demonstrated after sensitization to egg or milk protein or to nematodes (34), are degranulated when receptor bound IgE molecules are cross-linked by antigen. In a laboratory setting, a convenient antigen for cross-linking IgE receptors is antibody raised in one animal species against the IgE of the experimental species. Here, we have used sheep anti–rat IgE. Since phagocytic cells have low-affinity IgE receptors, the response to anti-IgE may not be specific for the mast cell. However, our Cl-secretory response was partially inhibited by a H_1 antagonist, indicating that histamine was being released from mast cells after anti-IgE application to rat colon. Phagocytes, which include eosinophils, neutrophils, and macrophages, play important roles in infectious or idiopathic inflammation. To stimulate phagocytic cells, we have used the chemotactic peptide FMLP. This formylated tripeptide seems to be specific for phagocytes; mast cells do not respond to FMLP (18). Both anti-IgE and FMLP proved capable of altering intestinal electrolyte transport.

Once activated, mast cells and phagocytes release many products capable of stimulating other immune or mesenchymal cells or the enterocyte. For example, phagocytes and mast cells release prostaglandins, leukotrienes, PAF, ROS, and cytokines (34, 35). Mast cell also release histamine, serotonin, and adenosine, all of which are secretogogues in the intestine (30). We have investigated two of the less well-studied mediators produced by mast cells and phagocytes; PAF and ROS. Both appear to be potent stimuli of intestinal Cl secretion. PAF is a phosphatidylcholine species with an *O*-alkyl ether residue at the *sn*-1 position and an acyl-acetyl group at the *sn*-2 position of the glycerol. It is a potent activator of phagocytes, but also is believed to stimulate other cells, including those in the gastrointestinal tract (17). We have created ROS by the X/XO reaction which initially forms superoxide anion (O_2^-), a species that is rapidly changed to hydrogen peroxide (H_2O_2) through both spontaneous and enzymatic dismutation (36). Preliminary evidence suggests that it is H_2O_2 rather than O_2^- or the hydroxyl radical that is responsible for the Cl secretion in rat colon (22).

Although it is possible that these immune cell stimulants and products might directly affect the epithelium, we present evidence here that prostaglandins play a central role in the changes in electrolyte transport brought about by these agonists. The responses to these agonists were inhibited by 50–75% by two classes of cyclooxygenase blockers, INDO and PXM. Furthermore, we were able to demonstrate significant increases in PGE_2 and 6 keto $PGF_{1\alpha}$, the latter being the major degradation product of PGI_2. The PG responses to FMLP (rabbit) and anti-IgE (rat) were not as profound as that with the immune cell products PAF and ROS. We could not demonstrate FMLP-stimulated synthesis of either in the rat. However, as noted above, the rat is a poor responder to chemotactic peptide. The interpretation of the role of the prostaglandins in the FMLP-mediated response is further complicated by the fact that both INDO and PXM may block the FMLP receptor (37–39). Therefore, the decrease in response demonstrated after preincubation with these cyclooxygenase inhibitors could come about through blockade of FMLP receptors rather than blockade of prostaglandin synthesis. However, it requires considerably higher drug concentrations to block FMLP receptors (50% blockade at 50 μM) than to block prostaglandin synthesis ($\sim$ 100% at 1 μM), so it seems likely that a considerable part of the inhibition of the ΔIsc seen with PXM and INDO was due to decreased prostaglandin synthesis.

The stimulation of PGI_2 production by anti-IgE was not as great as that seen by PAF and ROS (contrast Tables VI with VII). Mast cell degranulation might result in Cl secretion through release of some of its other mediators such as histamine, serotonin or adenosine. Indeed, H_1-receptor blockade effectively reduced the ΔIsc to anti-IgE in the rat (Fig. 7). However, the ability of INDO and PXM to significantly inhibit the ΔIsc to anti-IgE suggest that prostaglandins are more important intermediates. Furthermore, there is evidence that histamine releases prostaglandins in the rat colon (40), an event that would explain the lack of an additive effect between H_1-antagonists and cyclooxygenase blockade. PGD_2 is reported as the major prostaglandin synthesized by the mast cell (3), whereas thromboxane A_2 is the major prostaglandin produced by phagocytes (41). It is conceivable that these might also be important prostaglandins mediating the Cl secretion of anti-IgE and FMLP stimulation in the rat.

The two immune cell products PAF and ROS stimulated significant increases in both PGE_2 ($\sim$ 5-fold) and in 6 keto $PGF_{1\alpha}$ ($\sim$ 15-fold). The ability of PAF to stimulate eicosanoid

production by immune and mesenchymal cells is well known (17). However, the eicosanoid response to ROMs is less well recognized.

Our studies do not implicate any significant role for lipoxygenase products in the stimulation of Cl secretion by the immune system. There was minimal inhibition of the ΔIsc by NDGA and that response was not additive to that observed with INDO alone. Furthermore, we found that NDGA inhibited agonist-induced increases in PGE_2, in keeping with its known lack of specificity for the lipoxygenase pathway. These studies, however, do not rule out a role for lipoxygenase products in immune system–mediated alterations in electrolyte transport. Lipoxygenase products do alter intestinal electrolyte transport in the Ussing Chamber (6, 42). However, a good part of that effect is inhibited by INDO suggesting that lipoxygenase products act primarily on immune or mesenchymal cells stimulating release prostaglandins, which then cause Cl secretion. We have documented two responses to the immune system agonists: electrogenic Cl secretion (ΔIsc) and inhibition of neutral NaCl absorption. INDO did not completely abolish the inhibition of neutral NaCl absorption brought about by anti-IgE (see Table I [section B]), but it reduced the ΔIsc by > 70%. Perhaps prostaglandins are responsible for the electrogenic Cl secretion from the colonic crypts, whereas lipoxygenase products might be responsible for the inhibition of neutral NaCl absorption from the colonic surface epithelium. This has been suggested by others (42), but remains to be demonstrated by more rigorous studies. It is also equally possible that serotonin, adenosine or some other secretagogue released from mast cells might also be responsible for the inhibition of neutral NaCl absorption.

The alterations in transport reported here could come about through receptor-mediated stimulation of enterocyte adenylate cyclase (43). However, there is emerging evidence that the effects of prostaglandins on intestinal electrolyte transport are not all mediated through activation of enterocyte adenylate cyclase. Rask-Madsen and colleagues have shown that prostaglandins can stimulate intestinal secretion at concentrations far below those necessary to stimulate adenylate cyclase in the intestine (44, 45). Furthermore, this stimulatory process is sensitive to the level of ambient Ca^{2+} and is inhibited by calcium channel blockers. Several studies have demonstrated that prostaglandins will release neurotransmitters, particularly ACh, from the enteric nervous system (24–29, 44, 45). Furthermore, the ΔIsc response of rat colon to exogenous prostaglandins, particularly PGI_2, is inhibited by removal of the enteric nervous system or TTX blockade (28, 29), a finding we have confirmed (unpublished observations).

We have shown here that TTX, HEX, and ATR can inhibit the responses to immune cell stimuli and products. Furthermore, the release of prostaglandins by various immune agonists was not inhibited by neural blockade, indicating that it is the PGs which stimulate the enteric nervous system and not vice versa. The 50–90% inhibition of ΔIsc after preincubation with TTX, HEX, and ATR implies that prostaglandin-mediated release of neurotransmitters from the enteric nervous system is a major, ultimate stimulant of electrogenic Cl secretion in rat colon. The inhibition of the ΔIsc response with ATR suggests that ACh is an important neurotransmitter. However, because it required high concentrations of ATR (50 μM) to significantly inhibit these responses, it is possible that the atropine effect was the result of nicotinic blockade of the enteric nervous system rather than muscarinic blockade of ACh receptors on the colonocyte.

Two different responses were observed: a brief *Isc* response to immune cell stimulants and a prolonged, biphasic response to immune cell products. The brief response to anti-IgE or FMLP resembles that elicited in the colon by ACh or ACh analogues (46, 47). However, it could represent a response to some other released agonist (e.g., serotonin) or, conceivably, the brief response could signify tachyphylaxis to the released prostaglandin (48). The biphasic and sustained response to PAF and ROS would be the result of release of long-acting neurotransmitters such as vasoactive intestinal polypeptide, or due to a direct effect of the released prostaglandins or of PAF and ROS themselves on the epithelium. In that regard, we have been able to demonstrate a stimulation of *Isc* in the T84 colon carcinoma cell line with PAF and H_2O_2, but not with FMLP or anti-IgE (Berschneider, H., unpublished observations). Thus, the varied responses could be due to neurotransmitter-epithelial cell interactions of varying proportions and timing. Furthermore, the responses might be different in the small and large intestine and in various animal species.

The electrolyte transport responses reported here have been noted to some degree with other immune cell stimulants such as leukotrienes (6), phorbol esters (49, 50) and interleukins (51). The responses to these agonists are all partially inhibited by cyclooxygenase blockers. Furthermore, models of intestinal anaphylaxis created by sensitization to various proteins respond to specific antigen and inhibitors in the Ussing chamber in a manner similar to that reported here (52–56).

Fig. 9 represents a current working model for immune system control of intestinal electrolyte transport. This model is in keeping with the growing body of knowledge suggesting close interactions between the immune system and the neuro-

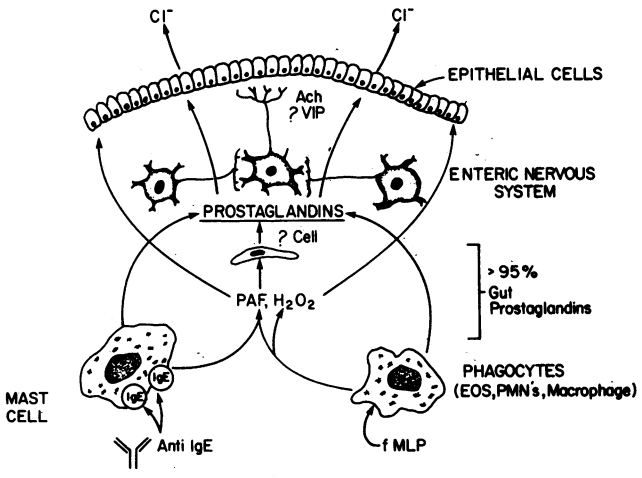

Figure 9. Proposed model for immune system–mediated alterations in colonic electrolyte transport. In this model, stimulants of mast cells (anti-IgE) or of phagocytes (FMLP) and immune cell products (PAF or H_2O_2) release prostaglandins from immune cells and mesenchymal cells in the lamina propria of the intestine. The prostaglandins (or other novel cyclooxygenase metabolites) may directly stimulate the epithelial cells to secrete Cl. However, at least 50% of rat colonic Cl secretion appears to be mediated by activation of the enteric nervous system by these cyclooxygenase products. The final neurotransmitters responsible for secretion are unclear, but ACh appears to play a role.

endocrine system (57–60). Immune cells are known to have receptors for several neurotransmitters and it appears that immune system mediators can activate the enteric nervous system. Thus, it is probable that the immune system interacts with the enteric nervous system to regulate intestinal water and electrolyte transport in both health and disease.

Acknowledgments

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