

Allergic Reactions of Rat Jejunal Mucosa

Ion Transport Responses to Luminal Antigen and Inflammatory Mediators

SHEILA E. CROWE, PIERSANTE SESTINI,
and MARY H. PERDUE

Intestinal Disease Research Unit and Departments of Medicine and Pathology, McMaster University, Hamilton, Ontario, Canada

This study examined the electrophysiological responses to antigen and to various stimuli in jejunal mucosa from rats sensitized to egg albumin with alum and pertussis adjuvants. Luminal antigen caused an immediate increase in short-circuit current, a measure of net ion transport, which was one of three different patterns. All were inhibited by the chloride channel blocker diphenyl-2-carboxylate, by chloride-free buffer, and by doxantrazole, a mast cell stabilizer. Depending on the pattern, the histamine-1 antagonist diphenhydramine, the 5-hydroxytryptamine-2 antagonist ketanserin, and the cyclooxygenase inhibitor piroxicam also reduced the responses. A neural component was indicated by inhibition of the responses to luminal antigen by the neurotoxin tetrodotoxin and by neonatal capsaicin treatment, which depletes substance P-containing nerves. In the absence of antigen, histamine and substance P caused increases in short-circuit current; the magnitude of these changes was significantly greater in tissues from sensitized animals than in controls. These data suggest that sensitization itself may result in hypersecretory responses to some inflammatory mediator and neurotransmitter substances.

Luminal antigens play a role in the pathogenesis of a number of gastrointestinal disorders including food allergy, eosinophilic gastroenteritis, parasitic infections, celiac disease, cow's-milk protein enteropathy, and possibly inflammatory bowel disease (IBD) and irritable bowel syndrome (1-5). Symptoms in these disorders include abdominal pain, diarrhea, nausea, and vomiting. Little is known about the mechanisms involved in intestinal reactions to foods. In some conditions it is assumed that immediate hypersensitivity reactions to ingested proteins occurring in the intestinal wall cause these symptoms.

To study these hypersensitivity reactions, a variety of animal models of intestinal anaphylaxis have been developed using enteric parasites or food proteins as sensitizing agents (6-12). Several groups have examined intestinal ion transport responses to these antigens. We have previously shown in vivo accumulation of water and electrolytes (13) and in vitro increases in short-circuit current (Isc) caused by Cl⁻ secretion in response to egg albumin (EA) (14). However, these and other in vitro experiments have mainly described Isc responses to antigens added to the serosal side of the intestine or delayed responses to luminal antigens.

In this paper we report results using a model in which jejunal mucosa from sensitized rats develops an immediate (within 3 minutes) increase in Isc in response to antigen added to the luminal side of the tissue. This response has been further characterized using pharmacological inhibitors. In addition, studies with secretagogues show that jejunum from sensitized rats demonstrates increased responsiveness when compared with tissues from control animals.

Methods

Animal Model

Sprague-Dawley rats (Charles River Breeding Laboratories, St. Constant, Quebec, Canada) were sensitized with 1.0 mg chicken EA (grade V, Sigma Chemical Co., St. Louis, MO) in 1.0 mL of alum solution injected SC and 1 mL of

Abbreviations used in this paper: β LG, β -lactoglobulin; BSA, bovine serum albumin; G, conductance; DMSO, dimethylsulfoxide; DPC, diphenylamine-2-carboxylate; DOX, doxantrazole; EA, egg albumin; 5-HT, 5-hydroxytryptamine; NDGA, nordihydroguaretic acid; Isc, short-circuit current; SCG, sodium cromoglycate; SP, substance P; TTX, tetrodotoxin; TS, transmural stimulation.

© 1990 by the American Gastroenterological Association

0016-5085/90/\$3.00

Bordetella pertussis vaccine (2×10^{10} organisms; Connaught Laboratories, Willowdale, Ontario, Canada) injected IP (15). The rats were studied 10–14 days after sensitization. Unsensitized littermates served as controls.

Specific immunoglobulin E (IgE) titers were determined by passive cutaneous anaphylaxis as previously described (13). Sensitized animals had serum titers of $1 \geq 64$ while control animals had no detectable anti-EA antibody. Heat treatment of serum from sensitized rats abolished reactivity by passive cutaneous anaphylaxis.

Each rat was anesthetized with IM urethane (5 mg/g; Aldrich Chemical Co. Inc., Milwaukee, WI), and the abdominal cavity was opened to remove a 10-cm segment of jejunum beginning 10 cm distal to the ligament of Treitz. This segment was stripped of serosa, longitudinal muscle, and myenteric plexus, leaving the submucosal plexus intact (14). The tissue was then opened along the mesenteric border and placed in 37°C oxygenated mannitol-Krebs buffer. After removal of intestine, rats were killed by cervical dislocation.

Ussing Chamber Studies

Adjacent pieces of stripped jejunum were mounted in Ussing-type chambers that contained agar-salt bridges to monitor potential difference (PD) and inject Isc. The chambers included stimulating electrodes on opposite sides of the tissue (16). The chamber opening exposed 0.6 cm² of mucosal and serosal surfaces to 10 mL of oxygenated Krebs buffer that contained (in mmol/L) 115 NaCl, 8.0 KCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 KH₂PO₄, and 25 NaHCO₃, pH 7.35 at 37°C. In addition, the serosal buffer contained 10 mmol/L glucose and the mucosal buffer contained 10 mmol/L mannitol. In some experiments, chloride-free buffer was used instead of Krebs. This contained (in mmol/L) 116 Na isethionate, 4.6 K acetate, 1.5 Ca acetate, 1.2 Mg acetate, 22 NaHCO₃, and 1.2 NaH₂PO₄. The tissue was clamped at zero volts using a W-P Instruments automatic voltage clamp (Narco Scientific, Downsview, Ontario, Canada). The Isc (in μ A) was recorded continuously, and PD measurements (in mV) were taken at 10-minute intervals throughout the experiments. Conductance (G, in mS/cm²) was calculated using values of PD and Isc. The tissues were allowed to equilibrate for 20 minutes, reaching a stable Isc reading, before antigen or secretagogues were added. Electrical transmural stimulation (TS) was carried out at the start and the end of each experiment by passing rectangular current pulses from one side of the tissue to the other through stimulating electrodes. Stimulus parameters were 10 mA, 10 Hz in a 0.5-millisecond pulse width delivered for a total of 5 seconds. Change in Isc (Δ Isc) was calculated as the difference between the basal Isc and the maximal Isc seen in response to TS (17).

Responses to Antigen

Egg albumin was added to either the serosal or mucosal half of the chamber containing jejunum from sensitized or control rats. The response (Δ Isc) was determined as the difference between the basal Isc and the maximum increase in Isc seen after antigen was added. In

addition to calculating Δ Isc for the total duration of the luminal EA response, Δ Isc was calculated separately for the initial 5-minute period (early phase) and the period following for up to 20 minutes (late phase). Based on the pattern of increase in Isc and the magnitude of the early phase (Isc changes compared with late phase changes), responses were categorized into three types. In biphasic type I responses, an early increase in Isc was significantly greater in magnitude than the late phase increase. In type II responses, a rapid increase in Isc also occurred, but the magnitude of early and late phase changes were the same in this monophasic pattern. For type III responses (also monophasic), a maximum increase in Isc was achieved only during the late phase, usually 10–15 minutes after addition of EA.

Dose response studies showed a maximal luminal response with concentrations of 100 μ g/mL. This dose was used for all subsequent experiments. Bovine serum albumin (BSA, Sigma) was used to test the specificity of the antigen response.

Pharmacological Inhibition Studies

In inhibition experiments, adjacent pieces of jejunum from a sensitized rat were mounted in the chambers and paired with respect to Isc, G, and response to TS. Pairs did not differ with respect to these parameters by more than 25%. Preliminary experiments showed that tissues paired in such a manner gave similar antigen responses. For each pair, one tissue was pretreated with the inhibitor; the other received the vehicle only. All drugs were added to the chambers 15 minutes before EA challenge except doxantrazole, which was added to the chambers at the start of the experiment.

Capsaicin Treatment

Newborn rats were injected SC with 50 μ g/g capsaicin (Caledon Laboratories, Georgetown, Ontario, Canada) (18). Untreated littermates acted as controls. Both groups were sensitized (as described above) at 4 months of age and studied in Ussing chambers 10–14 days after sensitization. Capsaicin-treated rats failed to show paw withdrawal to heat or respiratory irritation in response to ether (19).

Responses to Stimuli

Responses to various stimuli were compared in jejunum from sensitized and unsensitized control animals. Responses (Δ Isc) were calculated as the difference between basal Isc and maximal Isc seen after secretagogue addition.

Materials

Diphenylamine-2-carboxylate (DPC; Aldrich Chemical Co., Milwaukee, WI) which inhibits chloride conductance at the apical membrane (20), was prepared in dimethylsulfoxide (DMSO). The DPC was added to the mucosal half of the chamber at a concentration of 2×10^{-4} mol/L, a dose previously shown to be nontoxic to tissue (21). The mast cell stabilizers doxantrazole, (DOX; Burroughs Wellcome,

Research Triangle Park, NC) solubilized in Krebs buffer and sodium cromoglycate (SCG; Fisons, Bedford, MA), dissolved in water, were added to both sides of the chambers at concentrations of 10^{-3} mol/L. These doses were previously shown to inhibit histamine release from isolated rat mast cells (22). Diphenhydramine, tetrodotoxin (TTX), atropine, theophylline, and nordihydroguaretic acid (NDGA) (all from Sigma) were prepared in water (pH adjusted to 12.0 with concentrated NaOH for NDGA). The cyclooxygenase inhibitors, indomethacin and piroxicam (Squibb, Princeton, NJ), were prepared in 95% ethanol and in DMSO respectively. The 5-HT₂ receptor antagonist, ketanserin (Janssen Pharmaceuticals, Belgium), and the 5-HT₃ antagonist, BRL 43694 (Beecham Pharmaceuticals, Betchworth, Surrey, England), were dissolved in water. For each (diphenhydramine, ketanserin, BRL 43694, indomethacin, piroxicam, NDGA, theophylline, and atropine) the inhibitor was added to the serosal side of the tissue at 10^{-5} mol/L. Similar concentrations of inhibitors have been used in other in vitro studies of intestinal anaphylaxis (23–27). Piroxicam was also used at 10^{-6} mol/L, a dose which reduced intestinal ion transport in response to anti-IgE and inflammatory mediators (28). Serosal 5×10^{-6} mol/L TTX was used, a dose which abolished the response to TS (17).

Acetylcholine, histamine, 5-hydroxytryptamine (5-HT) (Sigma), substance P (SP, Peninsula, CA) and prostaglandin E₂ (PGE₂, Cayman Chemical Co., Ann Arbor, MI) were dissolved in water. Histamine and 5-HT were used at concentrations of 10^{-4} mol/L, acetylcholine at 10^{-5} mol/L, SP at 10^{-6} mol/L, and PGE₂ at 10^{-7} mol/L.

Statistics

Data were compared using Student's *t* tests or paired *t* tests where appropriate. A value of $P < 0.05$ was considered significant.

Results

Responses to Antigen

The addition of EA to the serosal or mucosal side of jejunum from unsensitized control rats had no effect on Isc. In contrast, the addition of EA to jejunum from sensitized rats produced a rapid increase in Isc whether added to the serosal or mucosal surface of the tissue. The increase in Isc was paralleled by an increase in PD so that G did not change at any point during the reaction. Specificity of the EA response (Δ Isc) was demonstrated by the complete lack of response to BSA (100 μ g/mL) in jejunum from EA-sensitized rats.

Egg albumin added to the mucosal side of the chamber caused an increase in Isc (luminal response) that conformed to one of three patterns illustrated in Figure 1. All luminal responses were rapid in onset, with a change in Isc that began 1–2 minutes after antigen was added. In the biphasic pattern (type I responses), the initial peak occurred within 5 minutes

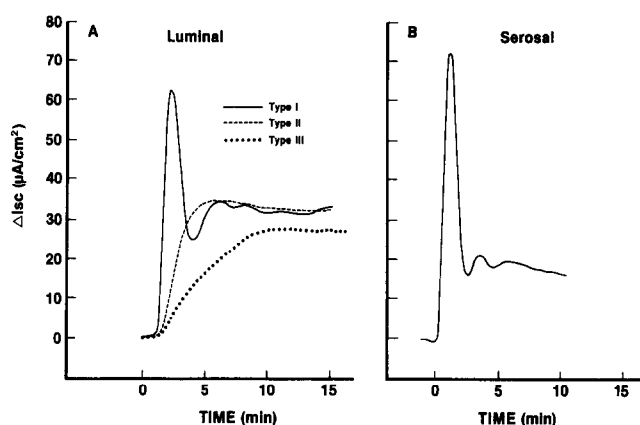


Figure 1. Representative tracings of Isc responses to EA (100 μ g/mL) added to the luminal (A) or serosal (B) side of the chamber. O represents the time at which antigen was added.

of antigen addition, followed by a decrease in Isc and then a second increase. Type II responses also included an early increase in Isc which reached a maximum within the first 5 minutes and remained elevated. The third pattern (type III) consisted of a more gradual increase in Isc which reached a maximum only after 5 minutes, usually 10–15 minutes after addition of antigen. In all three patterns, the Isc remained elevated above baseline for the remainder of the experiment. Changes occurring in the first 5 minutes after luminal EA addition were designated as the “early phase” and later changes as the “late phase” of the response. The three patterns occurred with the following frequencies: type I, 29.4%; type II, 32.5%; type III, 38.1% ($n = 126$). More than one type could be seen in adjacent tissues from a single rat. In table 1, Δ Isc is compared with luminal EA and TS in the three types of responses. For the early phase of the antigen response, Δ Isc differed significantly between

Table 1. Comparison of Types of Short-Circuit Current Responses to Luminal Antigen

	Type I	Type II	Type III
n	37	41	48
Total	53.7 ± 3.2	33.5 ± 1.5^a	29.5 ± 1.6^a
Early	53.7 ± 3.2	30.0 ± 1.7^a	$15.8 \pm 1.4^{a,b}$
Late	30.4 ± 2.5	31.6 ± 1.6	29.5 ± 1.6
TS	66.3 ± 5.1	57.0 ± 3.8	$42.6 \pm 2.4^{a,b}$

NOTE. Values are expressed as mean $\pm$ SEM Δ Isc values (in μ A/cm²). Total refers to the maximum increase in Isc occurring for the total duration of the Isc response to luminally added antigen, early refers to the maximum increase in Isc occurring during the first 5 minutes of the Isc response to antigen, late refers to the maximum increase in Isc occurring during the subsequent portion of the Isc response to luminal antigen, TS refers to the maximum increase in Isc after TS, before antigen was added, and n indicates the number of tissues in which the observed types of responses occurred and represent results from a total of 84 rats.

^a $P < 0.01$ compared with type I.

^b $P < 0.01$ compared with type II.

the three patterns, while no differences were seen for the late phase. Increased Isc responses to TS were seen in type I and II antigen responses compared with type III responses.

In contrast to the three patterns seen with luminal EA, serosal EA (100 $\mu\text{g/mL}$) always resulted in a single pattern that consisted of an initial large peak beginning 10–15 seconds after addition of antigen, followed by two smaller peaks (Figure 1). The Isc remained elevated above baseline throughout the remainder of the experiment. The magnitude of the maximal Isc response to serosal EA ($66.2 \pm 5.6 \mu\text{A/cm}^2$, mean $\pm$ SEM) was greater than mucosal EA ($37.6 \pm 1.5 \mu\text{A/cm}^2$) ($P < 0.05$).

Pharmacological Inhibition Studies

In these inhibitor studies, the response to luminal EA refers to the maximum increase in Isc seen for the total response (both early and late phases) unless otherwise specified.

In the presence of the Cl^- channel blocker DPC (20), the Isc response to luminal EA was significantly reduced ($14.3 \pm 3.9 \mu\text{A/cm}^2$, $n = 10$, compared with $32.9 \pm 4.3 \mu\text{A/cm}^2$, $n = 7$), indicating active anion secretion in response to antigen. This inhibition was seen for all types of responses. Marked reduction of responses occurred when chloride-free buffer was used ($4.2 \pm 0.7 \mu\text{A/cm}^2$, $n = 8$, compared with $26.6 \pm 1.6 \mu\text{A/cm}^2$, $n = 8$), also suggesting that the increased Isc was caused by chloride secretion.

Effect of mast cell stabilizing agents. Doxantrazole, which has been shown to stabilize both mucosal and connective tissue-type rat mast cells [22], significantly inhibited the response to luminal antigen ($17.0 \pm 2.5 \mu\text{A/cm}^2$, $n = 18$, compared with $31.8 \pm 5.6 \mu\text{A/cm}^2$, $n = 11$) while SCG, which only stabilizes connective tissue-type rat mast cells [22], did not ($25.6 \pm 4.2 \mu\text{A/cm}^2$, $n = 8$, compared with $30.3 \pm 4.7 \mu\text{A/cm}^2$, $n = 6$). The inhibition produced by DOX was seen in all types of responses. These results suggest a

Table 2. Effect of Mast Cell Mediator Inhibitors on Short-Circuit Current Responses to Luminal Antigen

Inhibitor	Control	Experiment
Diphenhydramine (10^{-5} mol/L)	53.6 ± 7.2	31.7 ± 3.6^b
Indomethacin (10^{-5} mol/L)	32.2 ± 3.8	33.6 ± 4.0
Piroxicam (10^{-6} mol/L)	31.7 ± 3.5	23.9 ± 4.2
Piroxicam (10^{-5} mol/L)	35.8 ± 3.5	17.2 ± 5.2^b
Diphenhydramine and piroxicam (10^{-5} mol/L)	33.3 ± 4.5	5.8 ± 2.1^c
NDGA (10^{-5} mol/L)	37.9 ± 7.0	44.2 ± 8.1
Ketanserin (10^{-5} mol/L)	39.4 ± 5.4	27.7 ± 3.2^a
BRL 43694 (10^{-5} mol/L)	36.8 ± 3.9	32.6 ± 2.8

NOTE. Results are expressed as mean $\pm$ SEM ΔIsc (in $\mu\text{A/cm}^2$) and represent the maximum increase in Isc for the total duration of the Isc response to luminal antigen in the presence (experiment) or absence (control) of inhibitors. Inhibitors were added to the serosal side of the chambers at the concentration shown. Six to 12 tissues were tested for each group.

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.0005$ compared with controls.

role for mucosal mast cells in the Isc responses to luminal EA.

Effect of inhibitors of mast cell degranulation products. Results of these inhibitor studies are presented in Tables 2 and 3. In Table 2, ΔIsc was calculated using the maximum increase in Isc seen for the total response to EA, while in Table 3 ΔIsc was calculated separately for both early and late phases of the response.

Diphenhydramine (10^{-5} mol/L), a histamine-1 antagonist, reduced the increase in Isc seen with luminal antigen to 60% of the control response. This inhibition was seen for both phases of the response and in all types of responses. Theophylline (10^{-5} mol/L), an adenosine antagonist, had no significant effect on responses ($26.9 \pm 5.0 \mu\text{A/cm}^2$, $n = 8$, compared with $30.1 \pm 6.4 \mu\text{A/cm}^2$, $n = 8$).

The cyclooxygenase inhibitors indomethacin (10^{-5} mol/L) and piroxicam (10^{-6} mol/L) had no significant effect. However, at 10^{-5} mol/L, piroxicam produced inhibition of the total response ($P < 0.01$), particularly of the late phase ($P < 0.0005$). Higher concentrations of

Table 3. Effect of Mast Cell Mediator Inhibitors on Early and Late Phases of Short-Circuit Responses to Luminal Antigen

Inhibitor (10^{-5} mol/L)	Early		Late	
	Control	Experiment	Control	Experiment
Diphenhydramine	52.5 ± 7.8	26.7 ± 4.4^b	46.9 ± 4.9	31.7 ± 3.6^a
Piroxicam	27.8 ± 6.6	16.9 ± 5.4	30.3 ± 2.8	6.2 ± 2.5^c
Diphenhydramine and piroxicam	28.1 ± 6.4	5.0 ± 2.1^b	33.3 ± 4.5	5.0 ± 2.0^c
Ketanserin	38.3 ± 5.2	15.4 ± 3.6^b	32.2 ± 4.7	27.7 ± 3.2

NOTE. Values are expressed as mean $\pm$ SEM ΔIsc (in $\mu\text{A/cm}^2$) and represent the maximum increase in Isc occurring during the first 5 minutes of the Isc response to antigen (early) and the subsequent portion of the Isc response to antigen (late) in the presence (experiment) or absence (control) of inhibitors. Inhibitors were added at the concentrations shown. Only inhibitor experiments showing significant differences between control and experimental groups are shown here. Six to eight tissues were tested for each group.

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.0005$ compared with controls.

Table 4. Effect of Tetrodotoxin, Atropine, and Neonatal Capsaicin Treatment on Short-Circuit Current Responses to Luminal Antigen and Transmural Stimulation

	TTX (5×10^{-6} mol/L)		Atropine (10^{-5} mol/L)		Neonatal capsaicin	
	Control	Experiment	Control	Experiment	Control	Experiment
Total	54.4 ± 6.3	34.7 ± 5.4 ^a	43.1 ± 5.1	40.0 ± 2.9	41.7 ± 7.6	19.6 ± 3.6 ^a
Early	54.0 ± 6.5	27.4 ± 5.6 ^b	29.8 ± 7.0	21.1 ± 5.7	41.7 ± 7.6	17.3 ± 3.6 ^b
Late	34.0 ± 3.0	26.0 ± 3.3 ^a	43.1 ± 5.1	38.1 ± 3.7	21.1 ± 4.1	9.4 ± 3.3 ^a
TS	53.1 ± 3.4	0.0 ^c	51.0 ± 7.2	39.4 ± 5.0	94.3 ± 12.6	56.7 ± 12.2 ^a

NOTE. Values are expressed as mean ± SEM Δ Isc (in μ A/cm²) and represent the maximum increase in Isc occurring during the total duration (total), the first 5 minutes (early), and the remaining portion (late) of the Isc response to luminal antigen or to TS in the presence (experiment) or absence (control) of inhibitors or neonatal capsaicin treatment. Six to 13 tissues were tested in each group.

^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.0005$ compared with controls.

indomethacin were not tested because they interfere with calcium uptake and release by endoplasmic reticulum (29). Combination of diphenhydramine and piroxicam (both 10^{-5} mol/L) reduced the luminal EA response to 17.5% of control values. The lipooxygenase blocker NDGA did not alter responses to antigen.

Significant inhibition was produced by ketanserin (10^{-5} mol/L), but when assessed in terms of early and late phases, ketanserin only had a significant inhibitory effect on the early phase (Table 3). In tissues showing only a monophasic late response (type III), no inhibition was seen with ketanserin. The 5-HT₃ antagonist BRL 43694 had no effect on luminal EA responses.

These results suggest that mast cell degranulation products, histamine, 5-HT, and products of the cyclooxygenase pathway of arachidonic acid metabolism are mediators of the response to luminal antigen with 5-HT involved in the early phase, PGs in the late phase, and histamine in both phases.

Effect of modulators of neural components. The effect of TTX, atropine, and capsaicin on the response to luminal antigen (total response as well as early and late phases) and to TS are presented in Table 4. The neurotoxin TTX significantly inhibited the response to EA, reducing it to 64% of the response in control tissues. This inhibitory effect was greater for the early phase ($P < 0.005$) than the late phase ($P < 0.05$). Tetrodotoxin completely abolished the response to TS. The muscarinic antagonist atropine did not affect the Isc responses to luminal EA or TS. In jejunum from EA-sensitized rats treated with capsaicin as neonates, the Isc responses to mucosal antigen and to TS were markedly reduced compared with controls. When responses were assessed in terms of early and late phases, the inhibitory effect of capsaicin was greater for the early phase. Based on these results, it appears that nerves mediate Isc responses to luminal antigen and that peptidergic but not cholinergic nerves are involved.

Comparison of Jejunum From Sensitized and Control Rats

Basal electrical parameters and response to transmittal stimulation. There were no differences in the basal measurements of Isc, PD, or G in jejunum from EA-sensitized or control animals (Table 5). The magnitude of the response to TS was similar in both groups.

Intestinal response to secretagogues. In this series of experiments, responses to secretagogues were compared in jejunum from EA-sensitized and unsensitized control rats. Compared with controls, tissues from sensitized animals showed a significant increase in the magnitude of the increase in Isc when histamine or SP were added. No differences were seen between jejunum from sensitized or control rats with 5-HT, acetylcholine, or PGE₂. These results are shown in Figure 2.

Discussion

These studies describe a model of food allergy in which immediate changes in ion transport occur in response to luminal antigen added in vitro to jejunum from sensitized rats. Most previous reports of in vitro intestinal anaphylaxis have focused on the ion transport response to serosal antigen. Although an immediate Isc response to luminal antigen has been described in β -lactoglobulin (β LG)-sensitized guinea pigs (27),

Table 5. Comparison of Basal Electrical Parameters in Jejunum From Control and Sensitized Rats

	Control	Sensitized
n	30	30
Isc (μ A/cm ²)	41.3 ± 3.1	39.1 ± 2.2
PD (mV)	1.3 ± 0.1	1.2 ± 0.1
G (mS/cm ²)	54.0 ± 2.8	54.5 ± 2.5
Δ Isc to TS (μ A/cm ²)	48.4 ± 4.5	46.8 ± 4.6

NOTE. Results are given as mean ± SEM.

n, Number of tissues (results from a total of 24 animals).

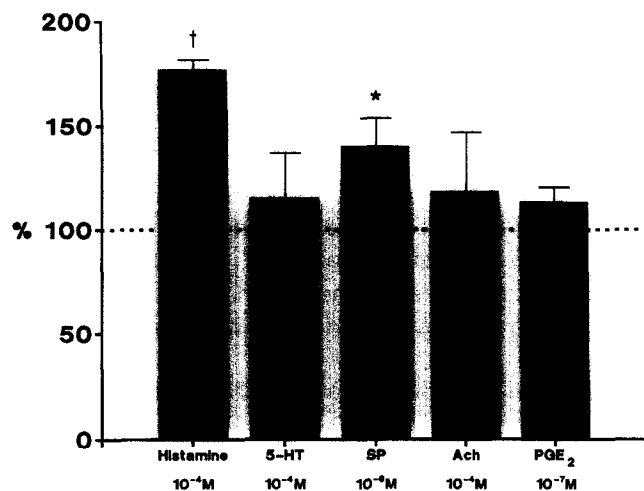


Figure 2. The mean Isc responses to secretagogues in jejunum from sensitized rats are depicted as a percentage of the mean Isc responses to these substances in controls. The SEMs are also shown as a percentage of control results. The dotted line indicates the control values (100%). Six to 10 tissues were tested for each secretagogue in both control and sensitized groups; * $P < 0.05$, † $P < 0.01$ compared with controls.

luminal antigen produced only a gradual increase in Isc after a lag of 25 minutes in jejunum from EA-sensitized Hooded Lister rats (14). Mucosal responses do not occur in most models, and only serosal responses have been examined in detail (9,24,25,30).

In our study, the inhibition of the Isc response to EA seen in the presence of DPC or chloride-free buffer suggests that chloride secretion mediates much of the Isc response to luminal antigen seen in our model. Chloride appeared to be involved in all types of Isc responses we reported because inhibition was seen in both phases of the response and all three patterns of response. In experiments examining effects of platelet-activating factor on intestinal ion transport, results with DPC correlated with those in experiments using chloride-free buffer or ion fluxes (21). A similar role for chloride has been shown for antigen-induced Isc responses in other animal models (9–11,14,30).

Mast cells are known to mediate allergic reactions through release of histamine, 5-HT, PG, and other substances (31). Evidence that mast cells were involved in the luminal response to antigen in our model comes from the observation that DOX, a mast cell stabilizing agent, was capable of inhibiting the response. This inhibition was seen for all types of response. The failure of SCG to inhibit the response suggests that these mast cells were of the mucosal type because SCG has been shown to inhibit rat peritoneal mast cells only (22). Our results are in keeping with previous *in vivo* studies using a different model of intestinal anaphylaxis in which DOX, but not SCG, reduced secretion of fluid and release of histamine

(32). Doxantrazole also reduced the Isc response to serosal EA in Ussing chambers, but its effect on the small delayed luminal Isc response was not tested (14).

Further evidence implicating mast cells in the luminal response comes from the use of pharmacological inhibitors of mast cell degranulation products. Antagonists of two preformed mast cell mediators, histamine and 5-HT, reduced the response to luminal antigen seen in our model. A histamine-1 antagonist, DPH, was used because previous studies have shown histamine-2 antagonists to have no effect on histamine responses in rat intestinal mucosa (23). The DPH produced inhibition of both phases of the response. In contrast, ketanserin, a 5-HT₂ antagonist, inhibited the early phase only, while the 5-HT₃ inhibitor had no effect on either phase. These results suggest that 5-HT mediated part of the response to luminal antigen and that 5-HT₂ receptors were involved. Studies of serosal Isc responses to antigen have demonstrated involvement of histamine and 5-HT using pharmacological antagonists and direct measurement of these mediators (23–25,30). Castro et al. have shown inhibition with 5-HT₂ antagonists in their *Trichinella spiralis*-infected rat model (25), while 5-HT₃ receptors appear to be involved in BLG-sensitized guinea pigs (26).

Our results suggest that at least some products of arachidonic acid metabolism were involved in causing secretion following administration of luminal antigen. The inhibitory action of piroxicam suggests that PG was responsible for alterations in ion transport, particularly during the late phase of the response. Products of the lipoxygenase pathway did not appear to be involved because NDGA had no effect on the response. In other ion transport studies, similar use of cyclooxygenase inhibitors in addition to direct measurements of PG have provided evidence of PG-mediated ion transport responses to serosal antigen (24,25,30).

Adenosine, another mast cell mediator, did not appear to be involved in our model because theophylline had no inhibitory effect. A role for adenosine has not been examined in other animal models of intestinal anaphylaxis. However, Barrett (33) has demonstrated theophylline inhibition of Isc responses to rat basophilic leukemia cell lysates on cultured human colonic epithelial cells suggesting that adenosine is involved in that system.

In our model, 5-HT inhibition had a greater effect on the early phase of the luminal response while cyclooxygenase inhibition had a greater effect on the late phase, suggesting that different mediators are involved in the two phases of response. This conclusion is supported by the observation that 5-HT produced a rapid increase in Isc of short duration, while PGE produced a sustained increase. The release of

different mediators by antigen could account for the three different patterns of response observed. Our findings have similarities to those reported by Castro et al. in which inhibitors of histamine and 5-HT had the greatest effect on the first phase, while indomethacin inhibited the second phase of a biphasic response to serosal antigen in jejunum from *T. spiralis*-infected rats (25).

We have demonstrated inhibition of the luminal response with the neurotoxin TTX. The neural component did not appear to be cholinergic because atropine had no effect on the response. Other ion transport studies of intestinal anaphylaxis have shown neural involvement in the response to serosal antigen using atropine or TTX (23,25,26), although in some models no inhibition was seen (27). Baird et al. have provided evidence that the inhibitory effect of TTX is mediated by nerves rather than nonspecific effects on epithelial or mast cells. This study showed that antigen responses in "reconstructed" intestinal tissue were not inhibited by TTX while responses in intact tissue were (34).

Further evidence of neural involvement in our model comes from the capsaicin experiments, which suggest that the response to luminal antigen was mediated by peptidergic nerves. Neonatal capsaicin treatment is known to permanently destroy sensory fibers containing SP or other nonadrenergic, noncholinergic neurotransmitters (18,35), although one report showed no effect of capsaicin pretreatment on SP concentrations in rat intestine (36). Comparisons to other ion transport models of intestinal anaphylaxis cannot be made because capsaicin has not been used in those models. Consistent with our findings is a study in which capsaicin desensitization reduced the increased esophageal vascular permeability seen in response to IV antigen challenge in EA-sensitized guinea pigs (37). We have previously shown inhibition of antigen-induced Isc responses in tracheas from EA-sensitized rats by capsaicin treatment (38).

The neural component appeared to be more important for the early phase of the luminal Isc response because both TTX and capsaicin had a greater inhibitory effect on the early phase when the two phases were assessed separately. Responses in which early increases in Isc were seen (types I and II) were associated with greater Isc responses to TS. This observation supports the hypothesis that neural elements of the sensitized intestinal mucosa are involved in the early phase of the luminal Isc response. Variation in the innervation of jejunum from sensitized rats may explain the different patterns of response to luminal antigen observed in our study.

It is difficult to explain the variation in Isc response to luminal antigen in different models; responses

ranged from none (9) to small and delayed (14) to large and immediate (our model), while a consistent serosal response is seen in all models of intestinal anaphylaxis. Clearly the models differ in terms of animal type, species, and intestinal region. The route of sensitization, dose of antigen, and use of adjuvants may also contribute to the variation in luminal antigen response. Further studies are needed to better understand these factors and other mechanisms that may be involved in producing luminal responses seen in our model.

The onset of responses to serosal antigen seen in our model occurred within 10–15 seconds of addition of EA to the chambers. Previous reports (9–11,14,25,27,30) have described similar rapid-onset responses to serosal antigen. Because fixed rather than continuous Isc readings were measured in those studies, the earliest time at which an increase was noted was 30 seconds after addition of antigen. The rapid serosal responses found in our model and others are a testimony to the sensitivity of the intestinal immune system. In less than 30 seconds, sufficient antigen must reach effector mast cells to induce degranulation. The electrophysiological consequences of this reaction are mediated through the action of released substances on epithelia and nerves (25). We believe similar sensitive mechanisms take place in luminal responses, although it is less clear where antigen-effector cell interactions occur and how luminally administered antigen reaches these cells within the short time (2–3 minutes) described.

Our data demonstrating increased Isc responses to histamine and SP in sensitized vs. control rat jejunum suggest that intrinsic properties of the intestinal mucosa were altered with sensitization. It is not clear what elements are responsible for the increased responses, but possibilities would include mast cells, nerves, and epithelial cells. Although other groups studying intestinal anaphylaxis have looked at Isc responses to a variety of secretagogues, including histamine (23,25,27), 5-HT (25,26), carbachol (27), and PG (25,27), none have compared these responses in intestines from sensitized and control animals. Increased responsiveness to mediators released through mast cell degranulation could act to amplify the secretion seen in response to antigen. These hypersecretory responses of sensitized intestine certainly warrant further investigation. These results, as well as those demonstrating a role for mast cells, their mediators, and neural factors in the Isc response to luminal antigen, agree with a general model of immune system regulation of water and electrolyte transport proposed by Powell (39). In this system, effector cells such as mast cells elaborate soluble mediators of inflammation that augment the signal by stimulating other

leukocytes, epithelial cells, endothelial cells, smooth muscle, and the enteric nervous system.

In conclusion, we have examined a model of intestinal hypersensitivity in which sensitization altered the intestinal mucosa to produce an immediate secretory response to luminally as well as serosally administered food protein. This luminal response appeared to be mediated by mast cells and enteric nerves, with different mediators accounting for the variation in patterns of response. Some aspects of this luminal antigen Isc response have similarities to previously reported models of serosal antigen response. However, a role for peptidergic nerves has been more clearly demonstrated in this model. In addition, this in vitro luminal response has relevance to the clinical situation of intestinal allergy, in which antigens are encountered intraluminally. As such, the model may provide insight into pathogenetic mechanisms of intestinal food allergy.

References

1. Saavedra-Delgado AM, Metcalfe DD. Interactions between food antigens and the immune system in the pathogenesis of gastrointestinal diseases. *Ann Allergy* 1985;55:694-702.
2. Smart HL, Mayberry JF. Atopy, food and ulcerative colitis. *Hepatogastroenterology* 1986;33:47-48.
3. Levo Y, Shalit M, Wollner S, Fich A. Serum IgE levels in patients with inflammatory bowel disease. *Ann Allergy* 1986;56:85-87.
4. Jones VA, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982;1:1115-1117.
5. Zwetchkenbaum JF, Burakoff R. Food allergy and the irritable bowel syndrome. *Am J Gastroenterol* 1988;83:901-904.
6. Byars NE, Ferraresi RW. Intestinal anaphylaxis in the rat as a model of food allergy. *Clin Exp Immunol* 1976;24:352-356.
7. Lake AM. Experimental models for the study of gastrointestinal food allergy. *Ann Allergy* 1983;51:226-228.
8. Perdue MH, Forstner JF, Roomi NW, Gall DG. Epithelial response to intestinal anaphylaxis in rats: goblet cell secretion and enterocyte damage. *Am J Physiol* 1984;247:G632-G637.
9. Baird AW, Cuthbert AW, Pierce FL. Immediate hypersensitivity reactions in the epithelia from rats infected with *Nippostrongylus brasiliensis*. *Br J Pharmacol* 1985;85:787-795.
10. Russell DA, Castro GA. Anaphylactic-like reaction of small intestinal epithelium in parasitized guinea-pigs. *Immunology* 1985;54:573-579.
11. Harari Y, Russell DA, Castro GA. Anaphylaxis-mediated epithelial Cl^- secretion and parasite rejection in rat intestine. *J Immunol* 1987;138:1250-1255.
12. Coombs RRA, Devey ME, Anderson KJ. Refractoriness to anaphylactic shock after continuous feeding of cows milk to guinea-pigs. *Clin Exp Immunol* 1978;32:263-271.
13. Perdue MH, Chung M, Gall DG. The effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984;86:391-397.
14. Perdue MH, Gall DG. Intestinal anaphylaxis in the rat: jejunal response to in vitro antigen exposure. *Am J Physiol* 1986;250:G427-G431.
15. Sestini P, Dolovich M, Vancheri C, Stead RH, Marshall JS, Perdue MH, Gaudie J, Bienenstock J. Evidence for mast cell-nerve interactions: antigen-induced lung epithelial permeability in rats is dependent on capsaicin-sensitive nerves. *Am Rev Respir Dis* 1989;139:401-406.
16. Perdue MH, Davison JS. Response of jejunal mucosa to electrical transmural stimulation and two neurotoxins. *Am J Physiol* 1986;251:G642-G644.
17. Perdue MH, Galbraith R, Davison JS. Altered regulation of intestinal ion transport by enteric nerves in diabetic rats. *Am J Physiol* 1988;254:G444-G449.
18. Jancso N, Jancso-Gabor A, Szolcsasanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br J Pharmacol Chemother* 1967;31:138-151.
19. Lundberg JM, Saria A. Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* 1983;302:251-253.
20. DiStefano A, Wittner M, Schlatter E, Lang HJ, Englert H, Greger R. Diphenylamine-2-carboxylate, a blocker of the Cl^- conductive pathway in Cl^- -transporting epithelia. *Eur J Pharmacol* 1985;405:S95-S100.
21. Hanglow AC, Bienenstock J, Perdue MH. Effect of platelet-activating factor on ion transport in isolated rat jejunum. *Am J Physiol* 1989;257:G845-G850.
22. Pearce FL, Befus AD, Gaudie J, Bienenstock J. Mucosal mast cells II. Effects of anti-allergic compounds on histamine secretion by isolated intestinal mast cells. *J Immunol* 1982;128:2481-2485.
23. Perdue MH, Gall DG. Rat jejunal mucosal response to histamine and anti-histamine in vitro. Comparison with antigen-induced changes during intestinal anaphylaxis. *Agents Actions* 1986;19:5-9.
24. Russell DA. Mast cells in the regulation of intestinal electrolyte transport. *Am J Physiol* 1986;251:G253-G262.
25. Castro GA, Harari Y, Russell DA. Mediators of anaphylaxis-induced ion transport changes in small intestine. *Am J Physiol* 1987;253:G540-G548.
26. Baird AW, Cuthbert AW. Neuronal involvement in type I hypersensitivity reactions in gut epithelia. *Br J Pharmacol* 1987;92:647-655.
27. Baird AW, Coombs RRA, McLaughlan P, Cuthbert AW. Immediate hypersensitivity reactions to cow milk proteins in isolated epithelium from ileum of milk drinking guinea pigs. Comparison with colonic epithelia. *Int Arch Allergy Appl Immunol* 1984;75:255-263.
28. Bern MJ, Sturbaum CW, Karaylacin SS, Berschneider HM, Wachsmann JT, Powell DW. Immune system control of rat and rabbit colonic electrolyte transport: role of prostaglandins and enteric nervous system. *J Clin Invest* 1989;83:1810-1820.
29. Burch RM, Wise WC, Halushka N. Prostaglandin-independent inhibition of calcium transport by nonsteroidal anti-inflammatory drugs: differential effects of carboxylic acids and piroxicam. *J Pharmacol Exp Ther* 1983;227:84-91.
30. Cuthbert AW, McLaughlan P, Coombs RRA. Immediate hypersensitivity reaction to B-lactoglobulin in the epithelium lining the colon of guinea pigs fed cows' milk. *Int Arch Allergy Appl Immunol* 1983;72:34-40.
31. Metcalfe DD. Mast cell mediators with emphasis on intestinal mast cells. *Ann Allergy* 1984;53:563-575.
32. Perdue MH, Gall DG. Transport abnormalities during intestinal anaphylaxis in the rat. Effect of anti-allergic agents. *J Allergy Clin Immunol* 1985;76:498-503.
33. Barrett KE. Immune-related intestinal secretion: control of colonic chloride secretion by inflammatory mediators. In: MacDermott RP, ed. *Inflammatory bowel disease. Current status and future approach*. Amsterdam: Elsevier, 1988:377-382.

34. Baird AW, Cuthbert AW, McVinish LJ. Type I hypersensitivity reactions in reconstructed tissues using syngeneic cell types. *Br J Pharmacol* 1987;91:857-869.
35. Hua XY. Tachykinins and calcitonin gene-related peptide in relation to peripheral functions of capsaicisensitive sensory neurons. *Acta Physiol Scand* 1986;551(suppl):1-45.
36. Holzer P, Gamse R, Lembeck F. Distribution of substance P in the rat gastrointestinal tract—lack of effect of capsaicin pretreatment. *Eur J Pharmacol* 1980;61:303-307.
37. Saria A, Lundberg JM, Skofitsch G, Lembeck F. Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. *Arch Pharmacol* 1983;324:212-218.
38. Sestini P, Bienenstock J, Crowe SE, Marshall JS, Stead RH, Perdue MH. Ion transport in rat tracheal epithelium in vitro: role of capsaicin-sensitive nerves in allergic reactions. *Am Rev Resp Dis* 1990;141:393-397.
39. Powell DW. The immunophysiology of intestinal electrolyte transport. In: Schultz SG, ed. *Handbook of physiology. The gastrointestinal system*. 1990 (in press).

Received June 5, 1989. Accepted December 20, 1989.

Address requests for reprints to: Dr. Sheila Crowe, Division of Gastroenterology, 4W8, McMaster University Health Sciences Center, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5, Canada.

This work was presented in part at the 1988 Annual Meeting of the American Gastroenterological Association and has been published in abstract form (*Gastroenterology* 1988;94:A81).