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Potentiation of anaphylaxis in guinea pig ileal mucosa by a selective δ-opioid receptor agonist

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Abstract

Immediate hypersensitivity reactions in the intestinal mucosa evoke active chloride secretion which enhances the elimination of luminal antigens. The prosecretory actions of histamine and other soluble mediators of anaphylaxis are mediated by submucosal neurons, as are the antisecretory actions of opioid antidiarrheal medications. We tested the hypothesis that the selective δ-opioid receptor agonist [D-Pen^2, D-Pen^5]enkephalin (DPDPE) alters anaphylaxis-associated ileal anion secretion in vitro. Sheets of ileal mucosa with attached submucosa from guinea pigs sensitized to cow's milk were mounted in Ussing chambers under short-circuit conditions. Mucosal sheets responded to the serosal application of the milk protein, β-lactoglobulin, with a rapid rise in transepithelial short-circuit current (Isc); in contrast, the egg protein, ovalbumin, was without effect. Pretreatment of tissues with the neuronal conduction blocker, saxitoxin, or the H_1 histamine receptor antagonist, diphenhydramine, but not the opioid receptor antagonist, naloxone, significantly reduced mucosal responses to antigen. [D-Pen^2, D-Pen^5]enkephalin (0.1 μM, serosal addition) decreased baseline Isc, but potentiated mucosal responses to antigen; its effects were abolished in tissues pretreated with naloxone. These results suggest that immediate hypersensitivity reactions in the guinea pig ileal mucosa are mediated by submucosal neural circuits that are phasically modulated by both mast cell products and opioids.

Keywords: Pharmaconeuroimmunology; Food allergy; Intestinal host defense; Enkephalin

1. Introduction

As an extensive interface between the external environment and the internal milieu, the gastrointestinal tract has evolved a number of potent defense mechanisms to exclude pathogens and other foreign antigens. One of these initial host responses is the release of histamine and other inflammatory mediators from antigen-sensitized mucosal mast cells. Histamine elicits active anion secretion in ileal mucosa from guinea pigs and other species, an effect that is primarily mediated through H_1 histamine receptors located on submucosal neurons (Linaker et al., 1981; Cooke et al., 1984; Perdue and Gall, 1986a). Anion secretion and the accompanying water flux dilute pathogens and promote the luminal extrusion of immunoglobulin A, mucus, antimicrobial defensins and other protective substances (Perdue and McKay, 1994). In the intestines of animals sensitized to milk or egg proteins, these food antigens produce an immediate hypersensitivity reaction, manifested by the release of histamine and other mast cell products, which in turn promote mucosal anion secretion (Cuthbert et al., 1983; Perdue et al., 1991). Like mucosal responses to histamine, a preponderance of evidence indicates that antigen-induced increases in epithelial secretion are mediated by submucosal neurons (Perdue and Gall, 1986b; Baird and Cuthbert, 1987; Crowe et al., 1990; Javed et al., 1992).

Morphine and other opiates are known to promote the release of histamine from mast cells, an effect that is not mediated by opioid receptors (Barke and Hough, 1993). On the other hand, the opioid receptor antagonist, naloxone, has been found to augment ovalbumin-induced increases in short-circuit current (Isc), an electrical measure of active ion transport, across the small intestinal mucosa of rats previously sensitized to this egg protein (Djuric et al., 1995). These latter results suggest that naloxone interrupts endogenous opioid activity in the small intestine which normally functions to dampen anaphylaxis-evoked anion secretion. Highly-selective δ-opioid receptor agonists, such as [D-Pen^2, D-Pen^5]enkephalin (DPDPE), re-

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duce Isc and active chloride secretion through interactions with δ-opioid receptors in the guinea pig ileum (Kachur et al., 1980; Kachur and Miller, 1982; Schulzke et al., 1990). These receptors are localized on submucosal neurons and probably mediate the antisecretory actions of opioids by altering the release of enteric neurotransmitters at neuroepithelial synapses (Surprenant et al., 1990; Tatsumi et al., 1990).

In the present investigation, we addressed the hypothesis that immediate hypersensitivity reactions in the intestinal mucosa are modulated by submucosal neurons that express δ-opioid receptors. The guinea pig is a common animal model for studies of immediate hypersensitivity reactions and has been employed in previous studies of intestinal anaphylaxis to cows' milk proteins (Baird et al., 1984; Verdier et al., 1994). Therefore, we induced anaphylaxis by exposing isolated sheets of ileal mucosa from guinea pigs sensitized to cow's milk to the antigenic milk protein, β-lactoglobulin. Our results suggest indicate that antigen-induced increases in Isc are mediated by neural pathways in the ileal submucosa that are sensitive to histamine and positively modulated by δ-opioid receptors.

2. Materials and methods

2.1. Animals and antigen sensitization

Male, Hartley guinea pigs (6–10 weeks old) were obtained from Charles River Laboratories (Wilmington, MA) and housed in the University of Minnesota Animal Care facility. They were initially treated with 100 μg/ml of cholera toxin (Sigma, St. Louis, MO) that was diluted in phosphate buffer and administered intragastrically with a feeding tube. After administration of this permissive adjuvant, animals were given free access to standard chow and pasteurized cow's milk, which was substituted for water for a period of 2 weeks. Milk was withdrawn and water was reintroduced at least 3 days before all experiments.

2.2. Materials

DPDPE was obtained from Peninsula Laboratories (Belmont, CA), dissolved in 0.01 M acetic acid with 0.1% bovine serum albumin at a stock concentration of 10 mM, and stored until use at −20°C. Naloxone and saxitoxin were purchased from Research Biochemicals (Natick, MA), and β-lactoglobulin, ovalbumin, diphenhydramine and other substances were obtained from Sigma; stock solutions of these substances were made in water.

2.3. Measurement of mucosal ion transport

Guinea pigs were anesthetized with isoflurane and subsequently euthanized with Beuthanasia-D® (30 mg/lb, i.v.; Schering-Plough Animal Health, Kenilworth, NJ) in accordance with the approved University of Minnesota Animal Care Committee protocol number 9505006. A midline laparotomy incision was made and the ileum was quickly removed. Ileal segments were placed in ice-cold, oxygenated Ringer's–HCO_3 solution (ionic composition in mM: Na^+, 148.5; K^+, 6.3; Cl^-, 139.7; Mg^{2+}, 0.7; Ca^{2+}, 3.0; HCO_3^-, 19.6; HPO_4^-, 1.3; $H_2PO_4^-$, 0.3) which was maintained at pH 7.4. They were stripped of serosa and underlying smooth muscle layers by blunt dissection and the remaining mucosa–submucosa preparation was mounted between two lucite Ussing half chambers (Jim's Instrument, Iowa City, IA) having a flux area of 0.64 cm^2. Mucosal sheets were bathed on the luminal and serosal aspects with Ringer–HCO_3 solution, maintained at pH 7.4 and 37°C and oxygenated continuously with 5% CO_2 in O_2. In addition, 10 mM D-glucose and mannitol were added, respectively, to the serosal and luminal bathing media.

The Isc ($\mu A/cm^2$), a measure of net transepithelial ion transport, was monitored continuously by an automatic voltage clamp (JWT, Overland Park, KS). After the basal Isc stabilized, the circuit was opened at 10-min intervals throughout each experiment to measure the transepithelial potential difference (mV). Tissue conductance (G_t, mS/cm^2) was calculated from potential difference and Isc by Ohm's law. Ovalbumin and β-lactoglobulin were added to either the luminal or serosal bathing media; all other substances were added to the serosal aspect of mucosal sheets 5 min (or 10 min in the case of naloxone) prior to β-lactoglobulin challenge.

2.4. Data analysis

All changes in Isc and G_t were determined as the mean ± S.E.M. of peak changes in these parameters relative to their baseline values in n tissues from N pigs. Comparisons between paired control and treatment means were made by a two-tailed paired t-test. Comparisons between multiple control and treatment means were made by one-way analysis of variance (ANOVA) followed by Dunnett's t-test or Tukey's test where appropriate. A P value of 0.05 or less was chosen as the limit for statistical significance.

3. Results

3.1. Effect of β-lactoglobulin on basal Isc

The baseline Isc and G_t in mucosal sheets were 35 ± 20 $\mu A/cm^2$ and 22 ± 2 mS/cm^2, respectively (37 tissues from five guinea pigs). When added to either side of the mucosa, β-lactoglobulin (300 μg/ml = 16.5 μM) produced a transient increase in Isc which was larger in magnitude after its serosal addition (Fig. 1). Serosal β-lactoglobulin also slightly reduced G_t by 1.0 ± 0.3

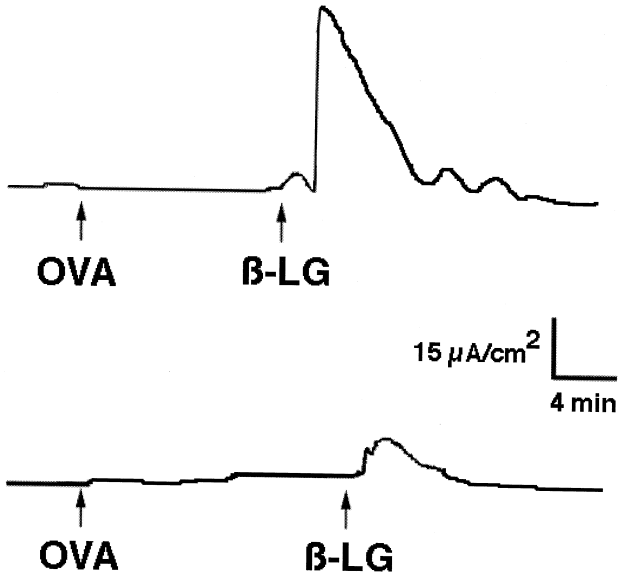

Fig. 1. Chart tracings of Isc responses to 300 μg/ml of either β-lactoglobulin (β-LG) or chicken ovalbumin (OVA) in ileal mucosa of milk-sensitized guinea pigs. Antigens were applied to either the serosal (top) or luminal (bottom) bathing media. Tracings are representative of mucosal responses to β-LG or OVA in five (luminal) or 10 (serosal) tissues from five milk-fed guinea pigs. Mean changes in Isc produced by serosal and luminal β-LG challenge were 22 ± 4 and 10 ± 2 $\mu A/cm^2$, respectively; OVA produced no mucosal response when added to either side of ileal sheets.

mS/cm^2 ($n = 10$ tissues). The specificity of β-lactoglobulin action was verified by the lack of mucosal responses to the luminal or serosal addition of ovalbumin at 300 μg/ml (Fig. 1).

3.2. Effect of inhibitors

To examine the mechanisms underlying anaphylaxis-induced increases in Isc, some tissues were pretreated seros-

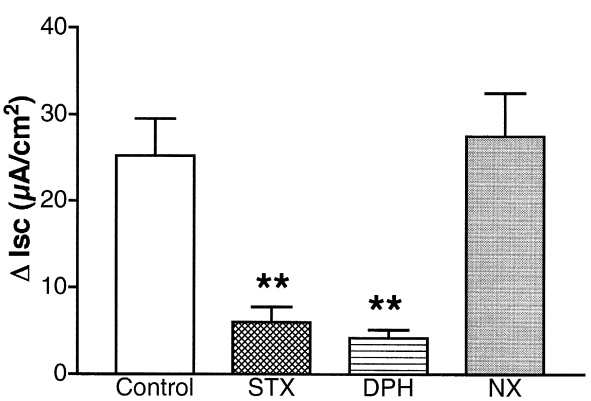

Fig. 2. Histogram illustrating mucosal responses to serosal β-lactoglobulin (300 μg/ml) in the absence (Control) and presence of saxitoxin (STX; 0.1 μM), diphenhydramine (DPH; 10 μM) or naloxone (NX; 0.1 μM). Data represent the mean ± S.E.M. of antigen-induced changes in Isc obtained in four to five tissues from five guinea pigs. Significant differences between mucosal responses to antigen in the absence and presence of each inhibitor are indicated as $^{**}P < 0.01$, Dunnett's test.

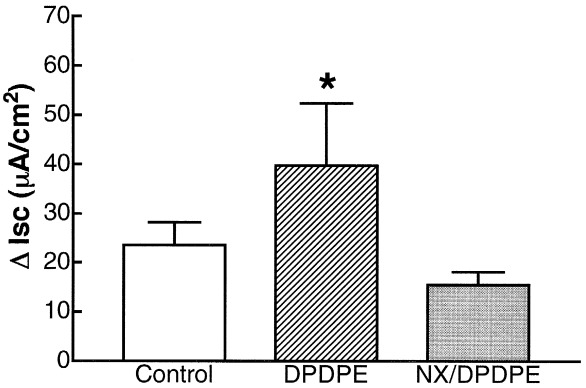

Fig. 3. Histogram illustrating mucosal responses to serosal β-lactoglobulin in the absence (Control) and presence (DPDPE) of the selective δ-opioid agonist, DPDPE (0.1 μM, serosal addition). In some experiments, tissues were treated with 0.1 μM naloxone (NX, serosal addition) prior to DPDPE addition and antigen challenge (NX/DPDPE). Data represent the mean ± S.E.M. of antigen-induced changes in Isc obtained in four to five tissues from five guinea pigs. Significant differences between mucosal responses to antigen challenge in the absence and presence of DPDPE or NX plus DPDPE are indicated as $^{*}P < 0.05$, Tukey's test.

ally with either the neuronal conduction inhibitor, saxitoxin (0.1 μM), the H_1 histamine receptor antagonist, diphenhydramine (10 μM), or the opioid receptor antagonist, naloxone (0.1 μM), prior to challenge with β-lactoglobulin. Saxitoxin did not significantly alter baseline Isc or G_t, but decreased mucosal Isc responses to antigen by $72 \pm 7\%$. Diphenhydramine had no significant effects on G_t alone or in the presence of antigen, but significantly reduced Isc responses to β-lactoglobulin by $82 \pm 2\%$. Naloxone did not alter antigen-induced elevations in Isc (Fig. 2).

3.3. Effect of DPDPE on β-lactoglobulin-induced secretion

After its serosal addition at 0.1 μM, DPDPE decreased baseline Isc by 16 ± 3 $\mu A/cm^2$ and increased G_t by 7.6 ± 2.4 mS/cm^2 ($P < 0.05$ for each parameter, paired *t*-tests). The ability of β-lactoglobulin to increase Isc was significantly augmented by $76 \pm 17\%$ in tissues pretreated with DPDPE (Fig. 3). The actions of DPDPE on baseline electrical parameters and on mucosal responses to antigen challenge were absent in tissues pretreated with 0.1 μM naloxone.

4. Discussion

The ileal mucosa from milk-fed guinea pigs responded to β-lactoglobulin challenge with a rapid rise in Isc, which was attributable to inappropriate chloride secretion and was similar to that of an earlier study employing this tissue preparation (Baird et al., 1984). Unlike the previous study,

β-lactoglobulin-induced Isc elevations were somewhat smaller in overall magnitude and greater after serosal than luminal application of the antigen. Moreover, mucosal responses to β-lactoglobulin in the present study were markedly inhibited by the H_1 histamine receptor antagonist, diphenhydramine, a result indicating that they were mediated primarily by histamine. Histamine has previously been shown to evoke active anion secretion in the guinea pig ileal mucosa by activating submucosal cholinergic neurons (Cooke et al., 1984). In the study by Baird and Cuthbert (1987), responses to β-lactoglobulin were less sensitive to the H_1 histamine receptor antagonist, mepyramine, at a 10-fold lower concentration. Indeed, Baird et al. (1984)(1987) reported that β-lactoglobulin effects were mediated by serotonin. Aside from the lower concentration of antihistamine employed, the major differences between the present investigation and those of Baird et al. (1984) were in the time interval of milk-feeding (2 vs. 3 weeks) and the β-lactoglobulin concentration employed (30-fold higher in the present study). It is difficult to determine how these latter variables could contribute to the functional differences observed in the two studies. Nevertheless, mucosal responses to β-lactoglobulin were sensitive to neuronal Na^+ channel blockers in both the previous and present studies. These concordant results confirm that enteric neurons mediate the effects of β-lactoglobulin on Isc in the milk-sensitized guinea pig ileum.

DPDPE decreased baseline Isc and increased G_t, actions which were similar to those reported for this compound and other enkephalin derivatives acting selectively at δ-opioid receptors in earlier studies (Kachur et al., 1980; Kachur and Miller, 1982; Schulzke et al., 1990). Furthermore, this agonist markedly enhanced mucosal secretory responses to β-lactoglobulin in the milk-sensitized ileum. The actions of DPDPE on mucosal function under basal conditions and after β-lactoglobulin challenge were inhibited by naloxone, a classical antagonist at μ-, δ- and κ-opioid receptors. This result indicates that the effects of DPDPE are mediated by opioid receptors. Furthermore, naloxone failed to alter mucosal responses to β-lactoglobulin, a finding in contrast with that of a previous study employing the rat small intestine (Djuric et al., 1995). By blocking opioid receptors and thereby interrupting endogenous opioid pathways, naloxone would be expected to disrupt opioid circuits that tonically potentiate active secretion associated with anaphylaxis. The finding that naloxone was without effect suggests that mucosal hypersensitivity responses are not under tonic modulation by endogenous opioids in the guinea pig ileum. It does not rule out the possibility that opioid peptides may participate in the phasic regulation of anaphylaxis-associated secretion. There is growing appreciation that pronounced species differences in neural circuitry and chemical coding exist in the small intestine, and the contrasting results obtained in studies utilizing intestinal preparations from rats and guinea pigs may be due to differences in the characteristics of the submucosal opioid pathways that modulate intestinal anaphylaxis (Timmermans et al., 1997).

The present results suggest that enteric neural circuits exist in the guinea pig ileal submucosa which dampen active anion secretion associated with immediate hypersensitivity reactions. The stimulation of δ-opioid receptors by DPDPE may suppress activity in these inhibitory neural pathways and consequently enhance anaphylaxis-associated secretion. Indeed, neural pathways sensitive to opioids and mast cell products may converge in regulating mucosal ion transport across the guinea pig ileum. In future studies, it will be important to compare the distribution of δ-opioid receptors and receptors for histamine and other mediators of anaphylaxis on enteric neurons in the small intestine.

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