Anaphylaxis-induced alterations in intestinal motility: role of extrinsic neural pathways

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Scott, R. B., D. T. M. Tan, M. Miampamba, and K. A. Sharkey. Anaphylaxis-induced alterations in intestinal motility: role of extrinsic neural pathways. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G812–G821, 1998.—The roles of mast cells and extrinsic and vagal neural pathways in the anaphylaxis-induced alterations in motility observed at sites remote from antigen exposure were explored. Rats were sensitized to egg albumin (EA) and prepared with 1) electrodes to monitor intestinal myoelectric activity, 2) an isolated intestinal loop, and 3) either intact vagal innervation or a subdiaphragmatic vagotomy. Fasting myoelectric activity was recorded before and after challenge of the jejunum in continuity or the isolated loop with EA or BSA. Intestinal segments and the brain stems were processed for mast cell identification (intestine) or Fos immunoreactivity (brain stem). EA but not BSA challenge of the jejunum or the isolated loop induced altered motility at both sites and diarrhea. Granulated mast cells were significantly reduced at the site local to but not remote from challenge. Vagotomy did not inhibit antigen-induced alterations in motility or diarrhea. The number of Fos-immunoreactive nuclei in vagal sensory or motor nuclei was not significantly altered by vagotomy. Thus antigen challenge of sensitized animals causes mast cell degranulation only at the site of direct challenge but alters motility at sites local and remote from challenge. The remote response requires intact extrinsic but not necessarily vagal neural pathways.

intestine; mast cell; neural regulation; vagotomy

ADVERSE REACTIONS TO FOOD antigens that are the result of immunologic responses occur in 0.5–5% of the population (7). The majority of these food antigen-induced allergic reactions in the gastrointestinal tract appear to be mediated by a type 1 IgE and a mast cell-dependent hypersensitivity response, with clinical manifestations that may include abdominal cramping, emesis, and diarrhea. The pathophysiological basis of these symptoms remains to be fully clarified in humans, although from animal studies it appears likely that the mucosal immune system acts to integrate epithelial, smooth muscle, and neural and probably intramural circulatory responses of the gastrointestinal tract to foreign antigens in an organized and adaptive fashion (5, 7).

The potential of food protein-induced anaphylaxis to alter gastrointestinal motility has been explored in the Hooded-Lister rat model (3, 4, 6, 9, 11–14, 16, 22–24). In the small intestine, the anaphylactic response is characterized by IgE antibody-mediated mast cell degranulation and the release of preformed or newly generated mediators. The mast cell mediators that are released stimulate the contraction of circular and longitudinal smooth muscle in vitro (23) and altered myoelectric and motor activity in vivo (22, 24). The altered small intestinal myoelectric or motor activity is characterized by interruption of normal fasting or fed motility, initiation of a succession of aborally propagating bursts of spike activity or clusters of contractions that are associated with an increased rate of aboral transit, and diarrhea (9, 14, 22), geared to remove antigens from the gastrointestinal tract.

In this rat model, atropine and hexamethonium block antigen-induced alterations in motility and diarrhea, suggesting that these pathophysiological alterations are dependent on the mast cell-mediated activation of neuronal circuitry (12, 24). This is consistent with a report that the duration of the antigen-induced alterations in motility was shortened by systemic capsaicin pretreatment and substance P antagonists (11). This suggests that substance P and capsaicin-sensitive afferent nerves also play a role in anaphylaxis-induced disturbances of intestinal motility. Recently, Castex et al. (3) reported that pretreatment with the selective serotonin (5-HT3) antagonist ondansetron or perivagal capsaicin treatment blocked both brain stem Fos expression and the alteration in fasting motility in sensitized animals challenged with antigen. This suggests that vagal afferents at least monitor the intestinal response to food protein-induced intestinal anaphylaxis or might be the afferent limb of a centrally programmed intestinal motor response.

The primary purpose of this study was to determine in this model of intestinal anaphylaxis if the in vivo antigen challenge of an isolated segment of intestine in a sensitized animal utilizes extrinsic neural pathways to initiate an alteration in intestinal motility at both the site of direct exposure and the site excluded from direct antigen exposure. If intestinal anaphylaxis alters motility at both sites, does mast cell degranulation occur at both sites or only the site of direct antigen exposure? The second goal of this study was to determine if neuronal activation in the central nervous system (CNS) observed concurrently with the alterations in intestinal motility that occur after antigen challenge of sensitized animals is prevented by subdiaphragmatic vagotomy. Neuronal activation of brain stem nuclei was determined using the immunohistochemical identification of Fos, a well-characterized marker of neuronal activity in the CNS (8, 13).

MATERIALS AND METHODS

Animal Model and Experimental Design

Experimental procedures were approved by the University of Calgary Animal Care Committee. Hooded-Lister rats weighing 150–200 g were sensitized by intraperitoneal injection of 10 μg of chicken egg albumin (EA; reagent grade V; Sigma, St.

G812
Louis, MO) and 10 mg of aluminum hydroxide (Sigma) as adjuvant in saline (22).

Protocol 1. Seven days after sensitization and following an overnight fast with free access to water, animals were anesthetized with halothane (3–4% in oxygen) and surgically prepared. With the use of a sterile technique, a midline laparotomy was performed and a modified Thiry-Vella loop was constructed from a 10-cm length of proximal ileum such that it was a self-emptying intestinal segment open only at its distal ostomy. A plastic cannula bearing an electrode assembly for recording intestinal myoelectric activity (6 pairs of Teflon-coated bipolar stainless steel electrodes) and a jejunostomy catheter to facilitate luminal antigen administration (polyvinyl chloride: 0.75 mm ID, 1.45 mm OD, distal end hole) were tunnelled subcutaneously from an interscapular exit site to the anterior abdominal wall and brought into the peritoneal cavity through a stab incision (22). The first three pairs of bipolar electrodes were fixed in the muscular wall of the jejunum at 2.5-cm intervals, the first being 2.5 cm distal to the ligament of Treitz. The electrodes of each pair were placed 3 mm apart for bipolar recording. The next three pairs of electrodes were fixed at 2.5-cm intervals in the muscular wall of the isolated intestinal segment. The jejunostomy catheter was introduced into the proximal jejunal lumen and secured with its distal end adjacent to the second electrode pair. Finally, a reference electrode was fixed in the muscle of the anterior abdominal wall. Animals were allowed oral fluids 24 h postoperatively and were returned to a full diet after 48 h.

On day 13, blood was obtained by cardiac puncture for determination of anti-EA IgE antibody titers by passive cutaneous anaphylaxis (2, 22). On day 14 after sensitization, recordings of fasting myoelectric activity were obtained in three groups of conscious animals before and after 1) a single challenge of the stomach (orogastric administration), jejunum (administration through the jejunal cather), or the isolated intestinal segment (administration through a catheter passed retrogradely through the external orifice of the loop) with either 10 mg EA or BSA (reagent grade V; Sigma) in 0.5 ml saline, 2) repeated challenge of the jejunum or isolated segment with EA (10 mg in 0.5 ml saline; 2-h interval between exposures), or 3) challenge of first the isolated segment and then the stomach or jejunum with EA (10 mg in 0.5 ml saline; 2-h interval between exposures).

In animals (from group 1, above) in which a single challenge of either the jejunum or isolated loop with EA or BSA was performed, blood was drawn and serum rat mast cell protease II (RMCPII) levels were measured 90 min after challenge, as previously described (19). Animals were euthanized with 100 mg/kg pentobarbital sodium (MTC Pharmaceuticals, Cambridge, ON, Canada). In these animals, tissues from both the proximal jejunal and the isolated segment were removed and processed for histological assessment of the number of granulated mucosal mast cells using standard procedures described in more detail in Histological Studies (22).

Protocol 2. From the time of initial sensitization, chow was removed from the cages and rats were adapted to a liquid diet (Isocal, Mead Johnson, Belleville, ON, Canada). The volume was advanced to ad libitum, and fresh formula was provided daily. Adapting rats to Isocal before the surgery ensured that the animals did not form a conditioned taste aversion to the diet after surgery. After vagotomy, gastric emptying of solids would be inhibited, which would result in poor feeding, malnutrition, and mortality if animals in this protocol were maintained on their normal chow diet.

Seven days after sensitization and following an overnight fast with free access to water, animals were anesthetized as described above and surgically prepared with 1) an isolated, self-emptying 10-cm length of proximal ileum and 2) a plastic cannula with three pairs of bipolar electrodes that were fixed in the muscular wall of the jejunum, at 2.5-cm intervals, with the first being 2.5 cm distal to the ligament of Treitz. In one-half of the animals in this group, a subdiaphragmatic vagotomy was performed, whereas the other half of the group served as controls with intact vagi. Animals were maintained on liquid diet from 24 h after surgery to the time of experimentation.

To perform the subdiaphragmatic vagotomy, the liver was gently retracted from the stomach to expose the esophagus. The branches of the anterior vagus were identified with a dissecting microscope; then one piece of surgical silk (5-0) was placed above the accessory branch of the vagus, ~0.5 cm below the diaphragm. The second suture was tied ~0.5 cm below the first suture. Next, a cautering iron was used to cut between the two sutures. With the use of a small brush, the cauterized ends were then treated with 5% phenol. The anterior vagus was reexamined with the dissecting microscope to ensure that all branches above the accessory branch were ligated, cauterized, and treated with phenol. A stay suture (7-0 surgical silk) was placed through the muscle layer of the esophagus and was used to turn the esophagus to reveal the posterior branches of the vagus. The same procedure was then repeated on the posterior aspect until all the posterior branches of the vagus from just above to 0.5 cm below the accessory branch had been ligated, cauterized, and treated with phenol.

Subdiaphragmatic vagotomy was chosen over cervical vagotomy because it blocks vagal sensory afferents from the gastrointestinal tract but leaves the supradiaphragmatic input intact, such as cardiorespiratory afferents, which may also be stimulated during anaphylaxis and which also terminate in the brain stem. Thus a reduction in anaphylaxis-induced input to the brain stem after cervical vagotomy, which has been previously reported (3), could represent blockade of afferent signals from the gut and/or supradiaphragmatic sources including the cardiorespiratory system, whereas a reduction after subdiaphragmatic vagotomy represents blockade of gut signals alone. To permit subsequent confirmation of intact vagal pathway (controls) or effective subdiaphragmatic vagotomy, rats received intraperitoneal injections of the retrograde fluorescent tracer fast blue (3 mg in 0.6 ml physiological saline) at two sites on day 11 after surgery (21).

On day 13 after sensitization, blood was obtained for determination of anti-EA IgE antibody titers by passive cutaneous anaphylaxis. On day 14, recordings of fasting motor activity were obtained during a control period and for 90 min after challenge of the isolated intestinal segment with saline and 20 mg BSA or EA in 0.5 ml saline. Only the isolated segment was challenged with antigen to ensure that the response seen in the gut in continuity could not be attributed to direct antigen exposure. Ninety minutes after antigen challenge, rats were euthanized with 100 mg/kg pentobarbital sodium (MTC Pharmaceuticals) and transcardially perfused with 300 ml PBS, followed by 200 ml of 4% parafomaldehyde. Brain stems were removed and postfixed overnight in 4% paraformaldehyde at 4°C. After specimens were rinsed in PBS, they were transferred to PBS containing 20% sucrose and left overnight to cryoprotect the tissue. They were then embedded in OCT, and 40-µm sections were made using a cryostat. Separate sections were processed for detection of fast blue or Fos immunoreactivity at three defined locations...
The presence of fast blue in the area postrema (AP; outside the blood-brain barrier) was used as an indicator of successful uptake of the tracer. The presence of fast blue-labeled cells in the dorsal motor nucleus of the vagus (DMNX) in control animals or in animals after subdiaphragmatic vagotomy was used to demonstrate vagal integrity or an incomplete subdiaphragmatic vagotomy, respectively. Data from animals in the subdiaphragmatic vagotomy group were included for analysis only when cells in the DMNX were not labeled with fast blue.

Recording of Intestinal Myoelectric Activity

The bipolar electrodes were connected to bioelectric amplifiers (model 8811A; Hewlett-Packard, Palo Alto, CA) with lower and upper cutoff frequencies of 0.05 and 300 Hz, respectively. The output signals were simultaneously recorded on an eight-channel chart recorder (model 7858A, Hewlett-Packard) and an eight-channel FM tape recorder (model 3968A, Hewlett-Packard) for later replay and visual analysis.

On the day of study, each animal was connected to the recording system and allowed 1 h to settle in an enclosure 28 cm long and 17 cm wide. Intestinal myoelectric activity was recorded until at least one complete cycle of the migrating motor or myoelectric complex (MMC) in the small intestine was observed. Myoelectric activity was then measured for 1 h before and after luminal antigen challenge.

Myoelectric and Clinical Parameters Analyzed

In the jejunum and isolated ileal segment of fasted animals, the slow wave frequency, presence and periodicity of the MMC and the nature and duration of disruption of the MMC by intestinal anaphylaxis were documented. The frequency (cycles/min) of the rhythmic variation in smooth muscle membrane potential known as electrical control activity or slow wave potential was measured directly from original recordings. The MMC was recognized as a band of intense electrical spike activity (action potentials); during MMC, spike activity occurred with each depolarization of the slow wave potential (phase II), followed by a period of quiescence during which electrical spike activity was absent (phase I) and preceded by a period during which electrical spike activity occurred irregularly in association with slow wave activity (phase III). It has been previously shown that jejunal antigen challenge of sensitized animals in this model results in a disruption of the MMC and initiation of a pattern of successive aborally propagating spike bursts in the small intestine (28), and all records were analyzed for the presence and length of this disturbance.

Anti-EA IgE Levels

Anti-EA IgE levels in serum samples were determined by passive cutaneous anaphylaxis (2, 22). In addition, sera from four experimental animals with titers of ≥1:64 were heated (56°C for 4 h), and determination of titer by passive cutaneous anaphylaxis was repeated to see if this abolished the passive cutaneous anaphylaxis response, consistent with the characteristic heat lability of IgE (2).

Rat Mast Cell Protease II

Blood taken from animals at the end of each experiment was assayed for RMCP II, a specific marker of mucosal mast cell degranulation (19), by ELISA (Moredun Animal Health, Edinburgh, UK).

Histological Studies

Mast cell identification. To define the extent and location of mast cell degranulation in response to EA challenge in protocol 1, full-thickness cross sections from the proximal jejunum and the isolated intestinal segment were removed and fixed in Carnoy’s fixative, embedded in paraffin, sectioned (7 µm), stained with Alcian blue, and counterstained with safranin to identify mucosal (blue staining) and connective tissue-type mast cells (10). Mast cells per 10 villus-crypt units were counted using a Leitz Wetzlar microscope. A decrease in the number of granulated mucosal mast cells indicates that mast cell activation and the release of mediators from mast cell granules have occurred.

Fast blue labeling. This was directly visualized in the brain stem using fluorescence microscopy after sections were mounted in bicarbonate-buffered glycerol (pH 8.6). Figure 1

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**Fig. 1.** A diagrammatic representation of cross sections of the rat brain stem at 3 defined bregma locations (−14.3, −13.8, and −13.3 mm from bregma). Area postrema, which is outside the blood-brain barrier, and the vagal sensory (nucleus tractus solitarius) and motor (dorsal motor nucleus vagus) nuclei, which are within the blood-brain barrier, are labeled.
shows a representation of cross sections of the rat brain stem at the three locations (~14.3, ~13.8, and ~13.3 mm from bregma; Ref. 20). The AP and the vagal sensory [nucleus tractus solitarius (nTS)] and motor nuclei (DMNX), which are within the blood-brain barrier, are labeled.

Fos immunoreactivity. Floating sections of brain stem were incubated in 10% normal goat serum (diluted in PBS containing 0.1% Triton X-100) for 30 min at room temperature and then overnight at 4°C in a primary anti-Fos antibody (Fos antibody-2, Oncogene Science, Cambridge, MA; 1:1,000). After incubation in the primary antibody, sections were incubated with biotinylated goat anti-rabbit IgG (Jackson Immuno Research, West Grove, PA; 1:200) for 1 h at room temperature. Finally, sections were incubated using Vectastain ABC reagents (Vector Laboratories, Burlingame, CA) at room temperature using nickel-intensified diaminobenzidine as the chromogen. After tissues were mounted, they were cleared and coverslipped using Entellan (Merck).

The AP, nTS, and the DMNX were identified at each level, and the total number of Fos-immunoreactive neuronal nuclei/side in the nTS and DMNX was counted.

Statistical Analysis

All data are presented as means ± SE. The number of animals (n) was ≥5 in all groups. Comparisons between two groups of data were made using the Student’s t-test with statistical significance being P < 0.05. In cases in which multiple comparisons were made, an ANOVA with a Tukey’s test for post hoc comparison was employed. The binomial probability distribution was utilized to determine the probability of a specific treatment (e.g., subdiaphragmatic vagotomy), reducing the frequency or preventing the clinical symptom of diarrhea in sensitized animals challenged with antigen (24).

RESULTS

Protocol 1

Intestinal anaphylaxis: local and remote motility responses. The primary objective of these experiments was to determine if, in a sensitized animal, antigen challenge of an isolated segment of intestine (one communicating with the remaining gut through only humoral or extrinsic neural pathways in the intact vascular pedicle) initiates an alteration in intestinal motility at sites both exposed to and excluded from direct antigen exposure. Table 1 and 2 summarize the data on the characteristics of fasting myoelectric activity in the proximal jejunum and the isolated ileal segment of sensitized animals before and after EA challenge of the stomach, jejunum, or isolated ileal segment. The frequency of electrical control activity or the intestinal slow wave was significantly lower in the isolated ileal segment compared with the jejunum, both before and after EA challenge of the stomach, jejunum, or isolated ileal segment (Table 1). MMCs were observed in both the jejunum and the isolated ileal segment of fasting sensitized animals before antigen challenge. The prechallenge MMC cycle period in the jejunum or the isolated segment was not significantly different between groups of animals subsequently challenged with BSA or EA (Fig. 1). After BSA challenge of the stomach, MMC in both the jejunum and the isolated ileal segment was significantly lower compared with the control value. In contrast, MMC in the jejunum was not significantly different from the control value after EA challenge of the isolated ileal segment (Table 1). MMC cycle period in the jejunum compared with the isolated segment within groups subsequently challenged with antigen in the stomach, jejunum, or isolated ileal segment (Table 2). Rats sensitized to EA passed a watery stool (diarrhea) after EA but did not defecate after BSA challenge of the stomach, jejunum, or isolated ileal segment (P < 0.001). Challenge of EA-sensitized rats with EA, but not BSA, caused disruption of the MMC and appearance of a succession of aborally migrating clusters of action potentials (see also Refs. 22 and 24) lasting between 25 and 40 min in both the jejunum and the isolated segment in each of the sensitized animals, regardless of whether the EA was administered orogastrically, through the jejuno-stomy catheter, or into the isolated segment. To determine the duration of disruption of the MMC, we measured the time from BSA or EA challenge to the end of phase III of the next MMC (Table 2). At any site of antigen exposure, this interval was not significantly different from the prechallenge MMC cycle period in animals challenged with EA but was significantly (P < 0.05) prolonged in comparison to the prechallenge MMC cycle period in animals challenged with EA. The duration of this anaphylaxis-induced alteration in motility was not significantly different in the jejunum compared with the isolated segment either within groups of animals challenged at a given site or between groups challenged at different sites.

Intestinal anaphylaxis: motility in response to a second local or remote challenge. Groups of sensitized rats initially challenged with EA in the jejunum or isolated ileal segment were, after a 2-h interval, subjected to a second EA challenge of the stomach, jejunum, or isolated ileal segment. If the site of the second antigen challenge was the same as the first (i.e., repeated challenge of the jejunum or isolated segment; n = 3 animals in each case), animals that had already exhibited an alteration in motility in both the jejunum and isolated ileal segment in response to the first exposure failed to experience a second disruption of the MMC at either the local or remote site with the second exposure.
Table 2. Motility in the proximal jejunum and isolated ileal segment of rats

<table>
<thead>
<tr>
<th>Site of Direct Antigen Exposure</th>
<th>Stomach</th>
<th>Jejunum</th>
<th>Isolated ileal segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC cycle period pre-challenge, min</td>
<td>J jejunum</td>
<td>11.3 ± 1.1</td>
<td>11.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Isolated segment</td>
<td>12.6 ± 2.2</td>
<td>11.8 ± 1.5</td>
</tr>
<tr>
<td>Diarrhea postchallenge, no. of rats</td>
<td>BSA challenge</td>
<td>0 of 6</td>
<td>0 of 6</td>
</tr>
<tr>
<td></td>
<td>EA challenge</td>
<td>6 of 6</td>
<td>6 of 6</td>
</tr>
<tr>
<td>MMC disrupted postchallenge, no. of rats</td>
<td>After BSA challenge</td>
<td>0 of 6</td>
<td>0 of 6</td>
</tr>
<tr>
<td></td>
<td>After EA challenge</td>
<td>6 of 6</td>
<td>6 of 6</td>
</tr>
<tr>
<td>Time to end phase III of the next MMC, min</td>
<td>J jejunum</td>
<td>10.2 ± 0.6</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Isolated segment</td>
<td>11.0 ± 1.1</td>
<td>12.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>After BSA challenge</td>
<td>6 of 6</td>
<td>6 of 6</td>
</tr>
<tr>
<td></td>
<td>After EA challenge</td>
<td>6 of 6</td>
<td>6 of 6</td>
</tr>
<tr>
<td></td>
<td>J jejunum</td>
<td>33.8 ± 1.0</td>
<td>38.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Isolated segment</td>
<td>37.5 ± 2.5</td>
<td>34.2 ± 5.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats for each site. Motilities in proximal jejunum and isolated ileal segment of animals sensitized to EA before and after BSA or EA challenge of the stomach, jejunum, or isolated segment are given. Migrating motor complex (MMC) cycle period prechallenge was not significantly different between animals subsequently challenged with BSA or EA, and data have been pooled to provide baseline normative values. Rats passed a watery stool after EA but did not defecate after BSA challenge (P < 0.05). MMCs were replaced by a continuous pattern of aborally propagating clusters of myoelectrical spike activity after EA but not after BSA challenge (P < 0.05). To determine the duration of disruption of MMC, we measured the time from BSA or EA challenge to the end of phase III (see text) of the next MMC. This interval was not significantly different from the prechallenge MMC cycle period in animals challenged with BSA and was significantly (P < 0.05) prolonged in comparison to the prechallenge MMC cycle period in animals challenged with EA.

However, if the site of the second antigen challenge was anatomically isolated from that of the initial antigen exposure (i.e., gastric or jejunal challenge of rats in which the isolated ileal segment had been the site of initial exposure; n = 3 animals in each case), disruption of the MMC was observed at both the local and remote sites after each exposure (P < 0.001). Figure 2 is a typical tracing showing jejunal myoelectric activity from an EA-sensitized animal before and after initial EA challenge of the isolated intestinal segment (Fig. 2A) as well as the response obtained 2 h later before and after gastric EA challenge (Fig. 2B). Note that EA challenge of this sensitized animal was followed by disruption of the jejunal MMC after both the initial challenge of an isolated intestinal segment and subsequent EA challenge of the stomach.

Role of mast cell degranulation. The secondary objective of the first protocol was to define the role of mast cells in the local (directly exposed) and the remote (occurring at sites anatomically isolated from direct antigen exposure) alterations in fasting intestinal motility in sensitized animals challenged with EA. The antigen specificity of mucosal mast cell degranulation in the above experiments is demonstrated by the significant (P < 0.05) increase above baseline (<0.25 µg/ml) of RMCPII after EA challenge of the jejunum (28.3 ± 3.1 µg/ml, n = 7) or isolated intestinal segment (25.2 ± 2.5 µg/ml, n = 6) of sensitized animals and the absence of any change relative to baseline after BSA challenge of sensitized animals (n = 6). The antigen-induced activation of mast cells in this model was shown to be dependent on heat labile antibody.

The following histological findings support the role of mast cells local to the site of antigen exposure as the source of mediators contributing to the anaphylactic response. In tissues from both the jejunum and the isolated ileal segment, numerous mucosal-type mast cells were observed in the mucosa and submucosa, with very rare peritoneal-type mast cells visible in the muscularis propria. Preliminary experiments performed in six animals not challenged with antigen showed no significant difference in the number of granulated mucosal mast cells in the jejunum of sensitized control animals (68 ± 5 mucosal mast cells/10 villus crypt units, n = 6) compared with the jejunum of those that were surgically prepared with an isolated ileal segment (55 ± 7, n = 6). However, the manipulation required for the surgical preparation of the isolated ileal segment was associated with a significant

Fig. 2. A: jejunal myoelectric activity from an egg albumin (EA)-sensitized animal before and after initial EA challenge of the isolated intestinal segment. B: jejunal myoelectric response obtained 2 h later before and after gastric EA challenge. Recording electrodes are labeled as electromyograms (EMGs), a time bar is shown on the x-axis, and the time of antigen challenge is shown by arrows.
increase in the number of mucosal mast cells at that site (135 ± 27, n = 6, P < 0.05 compared with jejunum). In subsequent experiments in which animals were evaluated after a single antigen exposure, jejunal EA challenge of sensitized animals was associated with a significant reduction in the number of mucosal mast cells compared with the jejunum of sensitized controls (47 ± 5 vs. 68 ± 5, respectively, n = 8, P < 0.05). Similarly, EA challenge of the isolated ileal segment of sensitized animals was associated with a significant reduction in the number of mucosal mast cells compared with the isolated ileal segment of sensitized controls (51 ± 8 vs. 99 ± 4, respectively, n = 8, P < 0.05). In all experiments, the number of granulated mucosal mast cells was significantly decreased at the site of challenge (indicative of mast cell activation and degranulation) compared with the same site in the absence of challenge. BSA challenge of the jejunum or isolated segment was not associated with any reduction in the number of mucosal mast cells (n = 8).

Protocol 2

Effect of subdiaphragmatic vagotomy on anaphylaxis-induced alterations in motility. The effect of subdiaphragmatic vagotomy on fasting jejunal myoelectric activity in a sensitized rat before and after EA challenge of the isolated intestinal segment is illustrated in Fig. 3. In both the control and vagotomized rats, normal MMCs were observed in the jejunum before antigen challenge. Antigen challenge provoked disruption of the MMC and a pattern of successive aborally propagating spike bursts that was accompanied by passage of a watery stool in both the control and vagotomized animal.

The histograms shown in Fig. 4 present quantitative data for the duration of the MMC cycle period before and after EA challenge of sensitized rats, with or without vagotomy.

Fig. 3. Effect of subdiaphragmatic vagotomy on fasting jejunal myoelectric activity in sensitized rats before and after EA challenge of the isolated intestinal segment. For the control (A) and vagotomized (B) animals, recording electrodes are labeled J1 to J2 in a proximal-to-distal orientation. A time bar is shown on the x-axis, and the time of antigen (Ag) challenge is shown by arrows.

Fig. 4. Quantitative data for the duration of the migrating motor complex (MMC) cycle period (CP) before antigen challenge (A) and duration of the disruption of jejunal motility after antigen challenge (B) in the control and vagotomy treatment groups. There were no significant differences between any of the groups.
antigen challenge (Fig. 4A) and the duration of the disruption of jejunal motility after antigen challenge (Fig. 4B) in the control and vagotomy treatment groups. The duration of the MMC cycle period before antigen challenge was not significantly different in the control or vagotomy treatment groups, averaging 10 min in either group. Antigen challenge disrupted fasting jejunal motility in every animal in both treatment groups and provoked diarrhea in six of seven animals in the control group and five of seven in the vagotomy group (not significantly different). The duration of the antigen-induced disruption of fasting jejunal motility was not significantly different between control and vagotimized animals.

Confirmation of subdiaphragmatic vagotomy. Figure 5 shows representative sections of rat brain stem obtained after EA challenge of sensitized control (intact vagi) and surgically prepared (subdiaphragmatic vagotomy 1 wk previously) that had received intraperitoneal injections of fast blue 3 days previously. Fast blue was detected in both treatment groups in six of seven animals in the control group and five of seven in the vagotomy group (not significantly different). The duration of the antigen-induced disruption of fasting jejunal motility was not significantly different between control and vagotimized animals.

Fos Immunoreactivity in the Brain Stem

After saline or BSA challenge of sensitized rats, there were relatively few Fos-immunoreactive neuronal nuclei in the brain stem (saline: 1.5 ± 0.3 neuronal nuclei/side at −13.8 mm from bregma, n = 5; BSA: 3.9 ± 0.5 neuronal nuclei/side at −13.8 mm from bregma, n = 5). These basal values were significantly (P < 0.05) less than the number of Fos-immunoreactive nuclei in the nTS of sensitized rats challenged with EA (61.5 ± 9.4 neuronal nuclei/side at −13.8 mm from bregma, n = 8). Figure 6 shows representative photomicrographs of brain stem sections obtained after EA challenge of sensitized control (intact vagi) and surgically prepared (subdiaphragmatic vagotomy 1 wk previously) rats. In the control animal, the total number of Fos-immunoreactive nuclei is greater in the nTS than in the DMNX. There appears to be no difference in the number of Fos-immunoreactive nuclei in the nTS or in the DMNX in the control compared with the vagotomized animal. A quantitative analysis of Fos immunoreactivity in the brain stem is shown in Fig. 7. In the nTS, the total number of Fos-immunoreactive nuclei/side was not significantly different between control and

Fig. 6. Representative photomicrographs of brain stem sections obtained after EA challenge of sensitized control (intact vagi, A) and surgically prepared (subdiaphragmatic vagotomy 1 wk previously, B) rats processed for the microscopic detection of Fos immunoreactivity in the vagal nuclei. Photomicrographs are from a point −13.8 mm from bregma.
vagotomy treatment groups at any of the three levels examined. Compared with the nTS, significantly ($P < 0.05$) fewer Fos-immunoreactive neuronal nuclei were observed in the DMNX in either treatment group. However, within the DMNX, there were also no significant differences between the control and vagotomy treatment groups (Fig. 7).

**DISCUSSION**

These experiments demonstrate that the antigen-induced disruption of the MMC by aborally migrating clusters of myoelectric spike activity occurs at sites directly exposed to antigen and at sites isolated from direct exposure that communicate with the remaining gut through humoral or extrinsic neural pathways in the intact vascular pedicle. The involvement of mast cells was demonstrated by an elevation of RMCPII in response to challenge of sensitized rats with EA but not BSA and the significant decrease in the number of granulated mucosal mast cells at only those intestinal locations directly exposed to the sensitizing antigen. The induction of altered motility at locations isolated from direct antigen exposure was not associated with a reduction in the numbers of mast cells at the isolated site. Repeated luminal antigen challenge in a sensitized animal only provoked a second generalized antigen-induced alteration in intestinal motility if that challenge occurred at a location isolated from recent exposure to the sensitizing antigen. The induction of altered motility at locations isolated from direct antigen exposure was not associated with a reduction in the numbers of mast cells at the isolated site. Repeated luminal antigen challenge in a sensitized animal only provoked a second generalized antigen-induced alteration in intestinal motility if that challenge occurred at a location isolated from recent exposure to the sensitizing antigen. We interpret these data to suggest that extrinsic neural pathways, stimulated by the antigen-induced release of mast cell mediators, in the challenged segment are required for the generalized motility response. Last, effective subdiaphragmatic vagotomy failed to block anaphylaxis-induced alterations in jejunal motility at sites anatomically isolated from antigen challenge and did not alter the degree of Fos expression in the nTS or DMNX. Thus vagal afferents monitor intestinal anaphylaxis, but vagal integrity is not required for the altered motility observed in response to food protein-induced intestinal anaphylaxis.

In the Hooded-Lister rat, just as in humans, small intestinal motility is characterized by MMCs during fasting (22) and a continuous irregular pattern of myoelectric spike and associated contractile activity (the fed pattern) postprandially (9). Exposure of sensitized jejenum to EA in vivo alters both fasting (22) and postprandial (9) small intestinal myoelectric and motor activity and is associated with an increased rate of aboral transit and diarrhea (14). In fasting rats, the MMC is temporarily abolished by antigen challenge of sensitized animals and replaced by a continuous, irregular myoelectric and motor activity, which is composed of a succession of aborally migrating clusters of action potentials and their motor correlate, which resembles the giant migrating contraction (24). These alterations in motility are observed along the entire length of the small intestinal tract almost immediately after proximal jejunal antigen exposure (14). They are dependent on mast cell degranulation, which this study shows occurs only at the site of direct exposure, and the release of mediators that activate neuronal circuitry (11, 12, 24).

The dispersion of antigen along the length of the gut lumen cannot account for the almost immediate generalized anaphylaxis-induced alterations in motility at sites anatomically excluded from challenge. The demonstration that the antigen-induced alteration in motility occurs at sites isolated from, as well as local to, direct antigen challenge supports the presence of extrinsic neural pathways that can stimulate the necessary hard-wired programs throughout the enteric nervous system (ENS) or are the afferent limb of a centrally programmed intestinal motor response. Which of these neuronal pathways is mediating the response is controversial.

Anaphylaxis-induced alterations in intestinal motility have also been shown to be part of an anamnestic, stimulus-specific, secondary rejection of T. spiralis in immunized rats (18). Demonstration of immediate alterations in the intrinsic propulsive state of jejunal segments from sensitized guinea pigs exposed to T. spiralis antigen in vitro suggests that the anaphylaxis-induced propulsive motor response is mediated by the ENS of the guinea pig jejunum and does not require extrinsic neural or hormonal control (1). Electrophysiological
data support the potential for antigen-induced IgE and mast cell-mediated activation of myenteric neurons involved in the control of intestinal motor activity (17). Data on the Hooded-Lister rat model of intestinal anaphylaxis have also accumulated. Although we have been unable to induce propulsive motor activity in response to antigen challenge of jejunal segments from sensitized animals in vitro (unpublished data), it is clear that there is extensive activation of myenteric neurons in response to intestinal anaphylaxis and mast cell degranulation both in vitro and in vivo (15). Because activation of myenteric neurons occurs even after capsaicin pretreatment, it seems unlikely that myenteric Fos expression is solely the result of reflex activation of myenteric neurons via the CNS, findings that further support a role for the myenteric plexus in intestinal anaphylaxis (15). Systemic capsaicin pretreatment and substance P antagonists shorten the duration of the antigen-induced alterations in motility observed in this model, suggesting that substance P and capsaicin-sensitive afferent nerves are involved (12). Castex et al. (3) suggested that vagal afferents monitor the intestinal response to food protein-induced intestinal anaphylaxis and are the afferent limb of a centrally programmed intestinal motor response. The latter possibility is not consistent with our demonstration that, in the sensitized animal, anaphylaxis-induced alterations in motility are observed immediately in small intestine anatomically isolated from the site of antigen challenge, even after effective subdiaphragmatic vagotomy, or the observation that animals systemically treated with capsaicin had Fos expression in myenteric neurons similar to that of untreated controls (15).

In this study, subdiaphragmatic vagotomy not only failed to block anaphylaxis-induced alterations in motility in intestine anatomically isolated from the site of antigen challenge but did not alter the degree of Fos expression in the nTS or DMNX. We interpret this to mean that, whereas vagal afferents may monitor intestinal anaphylaxis, vagal integrity is not required for the altered motility observed in food protein-induced intestinal anaphylaxis and the nTS receives neural input from other neural pathways after vagotomy. The most likely route would be through mesenteric ganglia and spinal neural pathways still accessible through the intact mesenteric/vascular pedicle of the isolated intestinal segments in this model.

Our findings and interpretation of the data differ somewhat from those of Castex et al. (3) who argued that the brain controls the antigen-induced disturbances in intestinal motility, since perivagal capsaicin treatment almost completely abolished Fos expression in the nTS. The discrepancy between the findings of Castex et al. (3) and ourselves can be explained in part by the differences in the animal model. We used a subdiaphragmatic vagotomy, which leaves intact the cardiorespiratory afferents that also terminate in the nTS and which will likely be activated during anaphylaxis, whereas Castex et al. (3) used perivagal capsaicin applied at the level of the carotid arteries in the neck, which effectively blocks all afferent activity due to the vagal afferents. Both experimental approaches fail to assess the polysynaptic activation of neurons in the nTS from spinal pathways that we propose mediate the neuronal activation seen in the brain stem after vagotomy.

In conclusion, our data support a role for extrinsic neural pathways in response to food protein-induced anaphylaxis. Although it seems likely that the vagus plays a role in monitoring activity from the gastrointestinal tract during an anaphylactic response, our data suggest that vagal integrity is not an absolute requirement for the full manifestation of the motor disturbances observed in this model.

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