Effect of splanchnectomy on jejunal motility and Fos expression in brain stem after intestinal anaphylaxis in rat

R. BRENT SCOTT,1 DAIMEN T. M. TAN,1 AND KEITH A. SHARKEY1,2
1Gastrointestinal Research Group, Department of Pediatrics, and 2Neuroscience Research Group, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2T 5C7

Received 5 January 2000; accepted in final form 7 June 2000

Scott, R. Brent, Daimen T. M. Tan, and Keith A. Sharkey. Effect of splanchnectomy on jejunal motility and Fos expression in brain stem after intestinal anaphylaxis in rat. Am J Physiol Gastrointest Liver Physiol 279: G990–G997, 2000.—This study was to determine whether alterations in jejunal motility observed after antigen challenge of sensitized rats occurred after extirpation of the celiac-superior mesenteric ganglia. Hooded-Lister rats were prepared with an intact or extirpated celiac-superior mesenteric ganglion, an isolated Thiry-Vella loop of ileum for instillation of antigen, and jejunal electrodes for myoelectric recording. Animals were sensitized by injection of 10 μg egg albumin (EA, ip), and specific anti-EA IgE titers were determined to be >1:64. In both control and splanchnectomized rats, normal fasting migrating myoelectric complexes (MMC) were observed before challenge with EA. MMCs were disrupted, and diarrhea was observed immediately after EA challenge of control but not splanchnectomized animals. Brain stems were removed and processed for Fos immunoreactivity. The absence of perivascular neuropeptide Y immunoreactivity in the submucosa was used to confirm the success of splanchnectomy. The number of Fos-immunoreactive neuronal nuclei was significantly reduced in the brain stem after splanchnectomy. Thus the mesenteric sympathetic ganglia are an integral part of the extramural neuronal pathways required for altered motility in this model of intestinal anaphylaxis.

Address for reprint requests and other correspondence: R. B. Scott, Dept. of Pediatrics, Alberta Children’s Hospital, 1820 Richmond Road S.W., Calgary, AB, Canada T2T 5C7 (E-mail: Brent.Scott@CRHA-Health.Ab.Ca).

The majority of food antigen-induced allergic reactions in the gastrointestinal tract appear to be mediated by a type I IgE and a mast cell-dependent hypersensitivity response. Clinical manifestations of food allergy include abdominal cramping, emesis, and/or diarrhea (7). 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, neural, and probably intramural circulatory responses of the gastrointestinal tract to foreign antigen in an organized and adaptive fashion (5, 7).

The mechanisms whereby food protein-induced anaphylaxis is able to alter gastrointestinal motility have been explored in the Hooded-Lister rat model (3, 4, 9, 11, 12, 17, 20, 28–31). 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. These mediators stimulate the contraction of circular and longitudinal smooth muscle in vitro (29) and altered myoelectric and motor activity in vivo (28, 30). The altered small intestinal myoelectric/motor activity is characterized by interruption of normal fasting or fed motility, initiation of a succession of aborally propagating bursts of spike activity/clusters of contractions that are associated with an increased rate of aboral transit and diarrhea (9, 17, 28), with the capacity to effectively clear antigen from the gastrointestinal tract.

In the rat model, atropine and hexamethonium block the in vivo antigen-induced alterations in motor activity and the diarrhea, suggesting that these changes in motility are dependent on the mast cell-mediated activation of neuronal circuitry (12, 30). 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) and suggests that substance P and capsaicin-sensitive afferent nerves also play a role in anaphylaxis-induced disturbances of intestinal motility. Castex et al. (3) reported that pretreatment with the selective 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. Thus vagal afferents at least monitor the intestinal response to food protein-induced intestinal anaphylaxis, or they might be the afferent limb of a centrally programmed intestinal motor response. However, subdiaphragmatic vagotomy fails to block anaphylaxis-mediated alterations in jejunal motility or the extent of Fos expression in the brain stem (31). This suggests that spinal pathways are also important in mediating activation of brain stem nuclei responsible for jejunal motility.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

G990 0193-1857/00 $5.00 Copyright © 2000 the American Physiological Society http://www.ajpgi.org
The aim of these experiments was to determine whether, in the Hooded-Lister rat model of intestinal anaphylaxis, extirpation of the celiac-superior mesenteric ganglia will 1) diminish or prevent alterations in intestinal motility at sites excluded or remote from antigen challenge and 2) reduce the Fos expression in the brain stem compared with that which would normally be observed in intact animals.

MATERIALS AND METHODS
Animal model and experimental design. Experimental procedures were approved by the University of Calgary Animal Care Committee and conform to the guidelines of the Canadian Council for Animal Care. Hooded-Lister rats weighing 150–200 g were sensitized by intraperitoneal injection of 10 μg of chicken egg albumin (EA, reagent grade; Sigma, St. Louis, MO) and 10 mg of aluminum hydroxide (Sigma) as adjuvant in saline (28).

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. Using sterile techniques, a midline laparotomy was performed and a modified Thirty-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 (three pairs of Teflon-coated bipolar stainless steel electrodes, each Teflon-coated wire being 0.009 in. in external diameter and the electrode contact point being 1 mm in length) was tunneled subcutaneously from an interscapular exit site to the anterior abdominal wall and brought into the peritoneal cavity through a stab incision (28). The 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. A reference electrode was fixed in the muscle of the anterior abdominal wall. In 9 animals, the celiac-superior mesenteric ganglia was extirpated, and in 14 it was left intact and the animals served as controls. Celiac-superior mesenteric ganglionectomy was performed using previously described methods (32). Briefly, the junction of the aorta with the gastric and superior mesenteric arteries was identified, and the ganglion, which lies at this junction, and its connecting nerves were removed, severing all connections. Animals were allowed oral fluids 24 h postoperatively and returned to a full diet after 48 h.

On day 13, blood was obtained for determination of anti-EA IgE antibody titers by passive cutaneous anaphylaxis (PCA) (2, 28). On day 14, recordings of fasting motor activity were obtained during a 60-min control period and then for 90 min after challenge of the isolated intestinal segment with 0.5 ml saline (n = 5 control rats) or 20 mg EA in 0.5 ml saline (n = 9 control rats; n = 9 splanchectomized rats). 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, Cambridge, ON, Canada), the jejunum was removed, and blood was drawn by cardiac puncture for rat mast cell protease II (RMCP II) levels [a specific marker of mucosal mast cell degranulation (24)]. Animals were then transcardially perfused with 300 ml PBS, followed by 300 ml 4% paraformaldehyde (pH 7.3). Brain stems were removed and postfixed overnight in 4% paraformaldehyde at 4°C. After washing in PBS, brain stems were transferred to PBS containing 20% sucrose overnight to cryoprotect the tissue. The jejunum was opened along the mesenteric border, pinned flat on Sylgard (Dow Corning), and fixed by immersion overnight in 4% paraformaldehyde at 4°C, and whole mount preparations of the submucosa were processed for the immunohistochemical detection of neuropeptide Y (NPY).

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 7855A; Hewlett Packard) and an eight-channel FM tape recorder (model 3968A; Hewlett Packard) for later replay and 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 typical migrating myoelectric complexes (MMC) in the small intestine were observed. Myoelectric activity was then measured for 1 h before and 90 min after luminal antigen challenge.

Myoelectric and clinical parameters analyzed. In the small intestine of fasted animals, the MMC was recognized as a band of intense electrical spike activity (phase III), followed by a period of quiescence (phase I) and preceded by irregular spike activity (phase II). 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. The presence or absence of diarrhea, defined as the passage of a soft, watery stool rather than a dry fecal pellet, during the recording periods was also noted.

Anti-EA IgE levels. Anti-EA IgE levels were determined by PCA (2, 28). Briefly, duplicate dilutions of serum (1:8 to 1:64) were injected intradermally in Sprague-Dawley rats weighing 200–300 g. Seventy-two hours later, 2.5 mg of EA and 0.5 ml of 1% Evans blue were injected intravenously, and skin reactions were read after 60 min. Titers were recorded as the greatest dilution of serum producing a colored reaction measuring 5 mm or more in diameter. Only data from animals with a specific titer of ≥1:64 were taken into account in this work.

Sera from four experimental animals with titers of ≥1:64 were heated (56°C for 4 h), and determination of titer by PCA was repeated. This treatment abolished the PCA response, thus demonstrating the characteristic heat lability of IgE (2).

RMCP II. Blood taken from animals at the end of each experiment was assayed for RMCP II, a specific marker of mucosal mast cell degranulation (24), by ELISA (Moredun Animal Health, Edinburgh, UK).

Immunohistochemistry. Brain stem tissues were embedded in OCT, and 40-μm sections were made using a cryostat and processed for detection of Fos immunoreactivity at three defined bregma locations (14.3, −13.8, and −13.3 mm). The gene c-fos is an immediate-early gene, and its protein product Fos is localized exclusively to the cell nucleus (14, 19). Detection of Fos protein following neuronal stimulation permits identification of functional polysynaptic pathways. Fos was used to assess the activation of brain stem nuclei involved in the response to antigen challenge as previously described (3). Figure 1 shows a diagrammatic representation of cross-sections of the rat brain stem at the three defined bregma locations (−14.3, −13.8, and −13.3 mm, 25)]. The area
The number of animals \((n)\) was included for analysis only if known to be exclusively of sympathetic origin (10). Data for the purpose of subsequent data analysis, it is important to note here that NPY localized around submucosal blood vessels is shown the characteristic heat lability of IgE (2). The antigen specificity of mucosal mast cell degranulation in the above experiments was demonstrated by a significant \((P < 0.01)\) increase of RMCP II above baseline levels after EA challenge of the isolated intestinal segment (control before EA, 248 ± 17 ng/ml; after EA, 4,318 ± 739 ng/ml and splanchnecotomized before EA, 288 ± 25 ng/ml; after EA, 4,651 ± 1,153 ng/ml; \(n = 9\) group).

Effect of splanchnectomy on anaphylaxis-induced alterations in motility. The effect of splanchnectomy on fasting jejunal myoelectric activity in a sensitized rat before and after EA challenge of the isolated intestinal segment is illustrated in Fig. 2. In both the control and splanchnectomy rat, normal MMCs were observed in the jejunum before antigen challenge. Antigen challenge of animals with an intact celiac-superior mesenteric ganglia provoked disruption of the MMC and a pattern of successive aborally propagating spike bursts that was accompanied by passage of a watery stool. In splanchnectomized animals, antigen challenge failed to alter intestinal myoelectric activity, and the normal pattern of MMCs persisted.

The histograms shown in Fig. 3 present quantitative data for the duration of the MMC cycle period before antigen challenge and the duration of the disruption of jejunal motility after antigen challenge in the control and splanchnectomy treatment groups. The duration of the MMC cycle period before antigen challenge was not significantly different in control or splanchnectomy treatment groups, averaging ~10 min in either group. Antigen challenge of sensitized animals was followed by disruption of fasting jejunal motility and passage of a diarrheal stool in, respectively, 9 of 9 and 7 of 9 rats with an intact celiac-superior mesenteric ganglion. However, after splanchnectomy, antigen challenge of sensitized animals disrupted fasting jejunal motility in these experiments. Sera from four animals with titers of ≥1:64 against EA were used in this experiments.
only 1 of 9 animals ($P < 0.001$ compared with the response in the intact group), and passage of a diarrheal stool was observed in 0 rats ($P < 0.001$ compared with the response in the intact group).

Confirmation of splanchectomy. Figure 4 shows representative whole mount preparations from rats processed for the immunohistochemical demonstration of NPY. Dense NPY immunoreactivity was localized around submucosal blood vessels in the controls as previously described (10). However, in successfully splanchectomized animals there was a very marked reduction or the complete absence of perivascular NPY immunoreactivity.

Fos expression in the brain stem. Brain stem sections were processed for the detection of Fos immunoreactivity. After saline challenge of sensitized rats, there were relatively few Fos-immunoreactive neuronal nuclei in

Fig. 2. Jejunal myoelectric activity from egg albumin (EA)-sensitized animals with intact celiac-superior mesenteric ganglion (control) and after surgical extirpation of the celiac-superior mesenteric ganglion (splanchnectomy) before and after EA challenge of the isolated intestinal segment. Recording electrodes are labeled J1 to J3 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 the arrows. A recurring band of higher-amplitude myoelectric spike activity is seen at ~10-min intervals in the tracing from the control animal before antigen challenge and in the splanchectomized animal both before and after antigen challenge. There is only continuous irregular myoelectric activity, without the cyclical increase in amplitude, in the control animal after antigen challenge.

Fig. 3. Quantitative data for the duration of the migrating myoelectric complex (MMC) cycle period (CP) before antigen challenge (left) and the duration of the disruption of jejunal motility after antigen challenge (right) in the control and splanchectomy treatment groups. There was a significant reduction in the duration of disruption of the MMC after splanchectomy (*$P < 0.001$).

Fig. 4. Fluorescence micrographs of rat jejunal whole mounts of the submucosa prepared for the immunohistochemical detection of neuropeptide Y (NPY) and obtained after EA challenge of sensitized control (intact celiac-superior mesenteric ganglion) and surgically prepared (splanchnectomy 1 wk previously) animals. Scale bar, 100 μm.
the brain stem (saline, 1.5 ± 0.3 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 (33.1 ± 8.1 neuronal nuclei/side at −13.8 mm from bregma; n = 9). Figure 5 shows representative photomicrographs of brain stem sections obtained after EA challenge of sensitized control (intact celiac-superior mesenteric ganglion) and surgically prepared (splanchnectomized 1 wk previously) rats. In the control animal with an intact celiac-superior mesenteric ganglion, the total number of Fos-positive neuronal nuclei is subjectively much greater in the NTS than in the DMNX. More importantly, there appear to be more Fos-immunoreactive neuronal nuclei in the NTS in the control animal with the intact celiac-superior mesenteric ganglion compared with the splanchnectomized animal.

A quantitative analysis of Fos immunoreactivity in the brain stem is shown in Fig. 6. The number of Fos-immunoreactive neuronal nuclei per side in response to intestinal anaphylaxis is shown for both control and splanchnectomized animals for the NTS and the DMNX. In the NTS, the total number of Fos-immunoreactive neuronal nuclei per side was significantly greater in the intact control animals compared with the splanchnectomized animals. In the DMNX, significantly fewer neuronal nuclei were Fos positive in either treatment group, and there were no significant differences between the control and splanchnectomy treatment groups.

DISCUSSION

These experiments confirm that in the Hooded-Lister rat model of intestinal anaphylaxis, luminal antigen challenge of fasted, sensitized animals provokes an immediate prolonged alteration in myoelectric activity and diarrhea. In addition, they demonstrate that effective splanchnectomy blocks anaphylaxis-induced alterations in jejunal motility at a site anatomically isolated from direct antigen challenge and significantly reduces Fos expression in the brain stem. We interpret this to indicate that the mesenteric
sympathetic ganglia are an integral part of the extramural neuronal pathways required for food protein-induced alterations in motility in this model of intestinal anaphylaxis and that the NTS receives afferent neural input through spinal pathways.

The role of specific anti-EA IgE in the anaphylaxis-induced alteration of motility has been previously documented in this model by 1) the antigen specificity of the response, 2) the fact that it occurs only in EA-sensitized animals, 3) demonstration of the characteristic heat lability of sera utilized for determination of the PCA response, and 4) demonstration of passive transfer and the heat lability of the transfer of the capacity for EA-induced, anaphylaxis-mediated alterations in intestinal motility in this model (17, 20). The involvement of mast cells was demonstrated by the elevation of RMCP II (24) in response to challenge of sensitized rats with EA, suggesting that mast cell degranulation occurred.

In the Hooded-Lister rat, just as in humans, small intestinal motility is characterized by MMCs during fasting (28) and a continuous irregular pattern of myoelectric spike and associated contractile activity (the fed pattern) postprandially (9). Exposure of sensitized jejunum to EA in vivo alters both fasting (28) and postprandial (9) small intestinal myoelectric and motor activity and is associated with an increased rate of aboral transit and diarrhea (17). 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 comprised of a succession of aborally migrating clusters of action potentials and their motor correlate, which resembles the giant migrating contractions (30). These alterations in motility are observed along the entire length of the small intestinal tract almost immediately after proximal jejunal antigen exposure (17). They are dependent on mast cell degranulation, which occurs only at the site of direct exposure (31), and the release of mediators, which activate intrinsic and extrinsic neuronal circuitry (11, 12, 18, 30, 31). The antigen-induced excitation of neuronal circuitry initiates disruption of the MMC by aborally migrating clusters of myoelectric spike activity that occur at sites both local to and isolated from (communicating with the remaining gut through only humoral or extrinsic neural pathways in the intact vascular pedicle) antigen exposure (31).

The demonstration that the antigen-induced alteration in motility occurs almost immediately at sites both local to and isolated from antigen challenge supports the presence of extrinsic neural pathways that can either stimulate the necessary hard-wired programs throughout the enteric nervous system or are the afferent limb of a centrally programmed intestinal motor response. Which of these neuronal pathways is mediating the response is controversial.

Although the antigen-induced intestinal motor response may not be mediated by the identical neural pathway as the antigen-induced secretory response, neural involvement in intestinal anaphylaxis is supported by an extensive literature on anaphylaxis-induced alterations of mucosal ion transport. In food protein-sensitized rat models (8), the antigen-induced secretory response is significantly inhibited by tetrodotoxin and the muscarinic receptor antagonist atropine has no effect, whereas in the parasite-sensitized rat or the parasite or food protein-sensitized guinea pig, cholinergic nerves have been shown to be involved in the secretory response (6, 15, 33). Central neural output can degranulate mast cells [Pavlovian conditioning of sensitized rats to psychological cues (16)], and mast cell mediators appear to modulate neural activity controlling intestinal ion transport (13, 26). Thus intestinal mast cells can be activated by nerve stimulation, and mast cell stimulation of intestinal nerves is an intrinsic part of the effector response. Since the mucosa is the closest proximity to luminal antigen exposure, contains the greatest concentration of mast cells, and in sensitized animals reacts to antigen challenge with chloride secretion that is at least partially neurally mediated (6, 8, 15, 33), it is possible that it is the submucosal ganglia that both regulate secretory activity of the epithelium and recruit neuronal circuits within the myenteric plexus to influence motility.

Anaphylaxis-induced alterations in intestinal motility have also been shown to be part of an anamnestic, stimulus-specific, secondary rejection of live _Trichinella spiralis_ in immunized rats (22). 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 enteric nervous system 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 (21, 23). Data have also been accumulated in the Hooded-Lister rat model of intestinal anaphylaxis. 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 in vitro and in vivo (18). 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 central nervous system, findings that further support a role for the myenteric plexus in intestinal anaphylaxis (18). 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 (11). Castex et al. (3) reported that pretreatment with the selective 5-HT3 antagonist ondansetron or perivagal capsaicin treatment blocked both brain stem Fos expression (in the NTS, lateral parabrachial nucleus, and paraventricular nucleus) and MMC disruption in sensitized animals challenged with antigen, once again suggesting 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 latter possibility is not wholly 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 (18) or with the observation that animals systemically treated with capsaicin had Fos expression in myenteric neurons similar to that in untreated controls (18).

Exirpation of the celiac-superior mesenteric ganglia disrupts not only sympathetic nerve fibers but also the celiac branches of the vagus and visceral afferents. The ganglia are meshed in the plexus with vagal, splanchnic, and primary visceral afferent fibers. The celiac branch of the vagus joins the celiac-superior mesenteric plexus before the sympathetic postganglionic fibers go to their targets. However, not only does subdiaphragmatic vagotomy fail to block anaphylaxis-induced alterations in motility in intestine anatomically isolated from the site of antigen challenge, it fails to alter the degree of Fos expression in the NTS or DMNX. We interpret this to mean that although vagal afferents may monitor intestinal anaphylaxis, vagal integrity is not required for the altered motility observed in food protein-induced intestinal anaphylaxis and that 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. This hypothesis is supported by the data in this study showing that splanchnectomy prevents the disruption of the MMC and diarrhea observed after EA challenge of an isolated ileal loop in sensitized animals. Furthermore, the number of Fos-immunoreactive neuronal nuclei per side was significantly reduced in the NTS in the splanchnecotomized animals compared with the control group, although no difference was observed in the DMNX.

In summary, these data suggest that the mesenteric sympathetic ganglia are an integral part of the extramural neuronal pathways required for altered motility in this model of intestinal anaphylaxis and that the NTS receives neural input from spinal sensory pathways.

We thank Winnie Ho for technical assistance.

This work was supported by the Medical Research Council of Canada (Grant MT 10014 (R. B. Scott) and Grant MT 11366 (K. A. Sharkey)).

REFERENCES


