Colonic mucin release in response to immobilization stress is mast cell dependent

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Divisions of 1Gastroenterology and 2Experimental Pathology, Beth Israel Deaconess Medical Center, 3The Combined Program in Pediatric Gastroenterology and Nutrition, and 4Division of Endocrinology, Children's Hospital, Harvard Medical School, Boston 02215; and 5Department of Pathology, Boston University School of Medicine, Boston, Massachusetts 02118

Castagliuolo, Ignazio, Barry K. Wershil, Katia Karalis, Asiya Pasha, Sigfus T. Nikulasson, and Charalabos Pothoulakis. Colonic mucin release in response to immobilization stress is mast cell dependent. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1094–G1100, 1998.—We recently reported that immobilization stress increased colonic motility, mucin, and prostaglandin E2 (PGE2) release and mucosal mast cell degranulation in rat colon (Proc. Natl. Acad. Sci. USA 93: 12611–12615, 1996; Am. J. Physiol. 271 (Gastrointest. Liver Physiol. 34): G884–G892, 1996). To directly assess whether mast cells contribute to stress-induced colonic responses, we investigated the participation of mast cells in intestinal secretory responses to antigenic and electrical field stimulation (22) as well as to the neuropeptide substance P (36). In our studies, we compared colonic mucin and PGE2 secretion and colonic motility changes in response to immobilization stress in normal and mast-cell-deficient KitW/KitW− mice. Stress-mediated colonic responses were also examined in mast-cell-deficient KitW/KitW− mice that had undergone selective reconstitution of their mast cell populations by the systemic injection of bone marrow-derived mast cells from normal mice. Because immobilization stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased corticotropin-releasing factor (35), ACTH, and finally corticosterone release (1, 34), we also evaluated the corticosterone levels in these mice under basal and stress conditions. We found that despite corticosterone levels similar to their congenic normal controls, mast-cell-deficient mice exhibited reduced colonic mucin and PGE2 secretion in response to restraint stress.

MATERIALS AND METHODS

Immobilization stress model. Adult male mast cell-deficient WBB6F1-KitW/KitW− mice (referred to as KitW/KitW−) (Charles River Breeding Laboratories, Wilmington, MA) and male congenic normal WBB6F1-(+/+) mice (referred to as +/+ ) were used in all experiments. Mice were housed individually under controlled conditions on a 12:12-h light-dark cycle and provided with food and water ad libitum. Mice were handled daily for 7 days before stress experiments. Experiments were performed between 10:00 and 11:00 AM to minimize the influence of the circadian rhythm. Immobilization stress was applied by placing the mice in a restraint cage (Harvard Apparatus, Cambridge, MA); control mice walked freely. After 30 min, mice were killed with a bolus of pentobarbital sodium (120 mg/kg ip), their abdomens were opened, and colons were removed and cut longitudinally. Sections (1 × 1 mm) were cut and cultured for measurements of mucin and PGE2 as described below. Colonic mucin was estimated by counting the number of fecal pellets expelled during the immobilization period (19). This study was approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

Measurement of colonic mucin release. Colonic mucosal explants were cultured in 35-mm tissue culture dishes (Fisher, Springfield, NJ) in 1.5 ml Trowell’s medium (GIBCO BRL, Gaithersburg, MD) containing 10 μCi/ml [3H]glucosamine.

RECENT EVIDENCE SUPPORTS substantial “cross talk” between the central nervous system and the endocrine and immune systems in control of intestinal function. Several laboratories reported stress-mediated stimulation of ion secretion (16, 27), intestinal permeability (27), and increased colonic motility (19, 39) in experimental animal models of acute, nontraumatic stress such as immobilization. We have recently shown that immobilization stress in rats causes colonic mucin secretion from goblet cells and stimulated release of PGE2 from colonic explants (6, 7). We also found that immobilization stress in rats caused activation of colonic mucosal mast cells (6, 7) and that pretreatment of rats with the mast cell “stabilizer” lodoxamide reduced colonic mucin secretion and PGE2 release in response to immobilization stress (6). These results provided indirect evidence that mast cells are involved in stress-mediated secretion of colonic mucin and PGE2.

To directly assess whether mast cells contribute to stress-induced colonic responses, we investigated the effects of immobilization stress on colonic mucin and PGE2 release, colonic goblet cell depletion, and fecal pellet output in normal and mast cell-deficient mice. These mice have been used previously to delineate the participation of mast cells in intestinal secretory responses to antigenic and electrical field stimulation (22) as well as to the neuropeptide substance P (36). In our studies, we compared colonic mucin and PGE2 secretion and colonic motility changes in response to immobilization stress in normal and mast-cell-deficient KitW/KitW− mice. Stress-mediated colonic responses were also examined in mast-cell-deficient KitW/KitW− mice that had undergone selective reconstitution of their mast cell populations by the systemic injection of bone marrow-derived mast cells from congenic normal mice. Because immobilization stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased corticotropin-releasing factor (35), ACTH, and finally corticosterone release (1, 34), we also evaluated the corticosterone levels in these mice under basal and stress conditions. We found that despite corticosterone levels similar to their congenic normal controls, mast-cell-deficient mice exhibited reduced colonic mucin and PGE2 secretion in response to restraint stress.

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were incubated (37°C for 30 min) in 1 ml of modified Krebs in a blinded fashion, and results are expressed as the number examined by a gastrointestinal pathologist (S. T. Nikulasson) including 10 parallel colonic crypts. We only quantified the

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described by us (6, 7). Briefly, colonic samples were fixed in

mucin glycoprotein release was measured (28) and forskolin (9) or medium alone (control). After incubation at 37°C for 6 h, mucin glycoprotein release was measured as described above.

Mucin secretion was also examined histologically by counting the number of goblet cells depleted of mucin, as previously described by us (6, 7). Briefly, colonic samples were fixed in Formalin, embedded in paraffin, and stained with hematoxy-

in and eosin and Alcian blue. The number of goblet cells containing mucin was quantified in each sample in an area including 10 parallel colonic crypts. We only quantified the number of surface goblet cells, because our previous results showed that restraint stress caused mucus discharge from surface but not crypt goblet cells (6). Coded sections were examined by a gastrointestinal pathologist (S. T. Nikulasson) in a blinded fashion, and results are expressed as the number of goblet cells containing mucin per 100 colonic surface epithelial cells.

Colonic PGE2 release. Colonic explants (3 sections per dish) were incubated (37°C for 30 min) in 1 ml of modified Krebs buffer (15). After 30 min, the medium was replaced with fresh medium, and explants were incubated for 2 h at 37°C. PGE2 was measured in aliquots of supernatant by an immunoena-

Basic statistical analyses were performed with the use of SigmaStat version 1.00 (Jandel Scientific Software, San Rafael, CA). ANOVA was used for intergroup comparisons. All data are expressed as means ± SE, and probabilities are regarded as significant at 95% confidence level (P < 0.05), using Student’s t-test.

RESULTS

Mast cell-deficient Kitv/-/Kitw-/- mice exhibit normal colonic mucin glycoprotein release in response to mucin secretagogues. We compared mucin glycoprotein secretion in colonic explants of mast cell-deficient and control (+/+ ) mice in response to the known mucin secretagogues PGE2 and forskolin. Incubation of colonic explants of control (+/+ ) mice with PGE2 and forskolin showed increased mucin glycoprotein release compared with mucin release from explants exposed to medium alone (Table 1). Colonic explants from mast cell-

deficient Kitv/-/Kitw-/- mice also showed increased mucin release in response to PGE2 and forskolin (Table 1). Furthermore, the levels of colonic mucin after administration of both secretagogues were statistically indistin-

Table 1. Effect of secretagogues on mucin glycoprotein release from colonic explants of mast cell-deficient Kit1v/-/Kit1w-/- and congenic normal (+/+ ) mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mucin Glycoprotein Release, dpm/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4,840 ± 1,970</td>
</tr>
<tr>
<td>PGE2</td>
<td>11,240 ± 3,420*</td>
</tr>
<tr>
<td>Forskolin</td>
<td>8,310 ± 1,200*</td>
</tr>
<tr>
<td>Kitv/-/Kitw-/-</td>
<td>5,240 ± 1,400</td>
</tr>
<tr>
<td>Kitv/-/Kitw-/-</td>
<td>8,970 ± 1,860*</td>
</tr>
<tr>
<td>Kitv/-/Kitw-/-</td>
<td>8,270 ± 1,980*</td>
</tr>
</tbody>
</table>

Results are means ± SE; 4 rats were tested for each group. Colonic explants from either mast cell-deficient Kitv/-/Kitw-/- or congenic normal (+/+ ) mice were cultured at 37°C in medium containing [3H]glucosamine and either 10 M of PGE2 or forskolin or medium alone (control). After 6 h, mucin release was measured by incorporation of [3H]glucosamine into TCA-phosphotungstic acid precipitates of culture supernatants as described in MATERIALS AND METHODS. *P < 0.05 vs. the respective explants challenged with medium alone.
These data demonstrate that there is no difference in colonic mucin release between mast cell-deficient and normal mice in their ability to respond to direct stimulation with mucin secretagogues in vitro.

Mast cell-deficient Kit^w/v/Kit^w^v mice exhibit reduced colonic mucin release in response to immobilization stress. The levels of colonic mucin release measured either biochemically or by counting the number of goblet cells containing mucin were similar in nonimmobilized Kit^w/Kit^w^v and normal (+/+) mice (Fig. 1A; Table 2). We next compared mucin release in colonic explants of normal mice and mast cell-deficient mice in response to 30 min of immobilization stress. As shown in Fig. 1A, colonic explants from stressed (+/+ ) mice released significantly more [3H]-glucosamine-labeled glycoproteins in vitro vs. nonimmobilized (+/+ ) mice (P < 0.05). Histological examination of colonic tissues also showed a significant reduction in the number of superficial goblet cells containing mucus in stressed vs. nonstressed (+/+ ) mice (Fig. 2; Table 2). In contrast, 30-min immobilization stress had no significant effect on colonic mucin glycoprotein release in mast cell-deficient Kit^w/Kit^w^v mice (Fig. 1A). Furthermore, there was no significant reduction in the number of superficial goblet cells containing mucin in immobilized vs. nonimmobilized Kit^w/Kit^w^v mice (Fig. 2; Table 2).

Mast cell-deficient Kit^w/v/Kit^w^v mice exhibit reduced colonic PGE_2 release in response to immobilization stress. There was no significant difference in basal colonic PGE_2 levels of nonimmobilized Kit^w/Kit^w^v and (+/+ ) mice (Fig. 1B). As observed in rats (6, 7), immobilization for 30 min resulted in a 2.3-fold increase in PGE_2 release in (+/+ ) mice compared with nonimmobilized (+/+ ) mice (P < 0.05, Fig. 1B). However, immobilization of Kit^w/Kit^w^v mice did not increase colonic PGE_2 release (Fig. 1B).

Effect of stress on colonic transit in mast cell-deficient Kit^w/v/Kit^w^v mice. As shown in Fig. 3, immobilization stress for 30 min resulted in an 18.5-fold increase in fecal pellet output in normal (+/+ ) mice compared with nonimmobilized mice (P < 0.01). Immobilization of mast cell-deficient mice also caused a 15.2-fold increase in fecal pellet output compared with nonstressed mast cell-deficient mice (P < 0.01). Furthermore, there was no significant difference in the number of fecal pellets after restraint stress between (+/+) and Kit^w/Kit^w^v mice (Fig. 3).

Mast cell reconstitution of Kit^w/Kit^w^v mice normalizes stress-induced colonic responses. To further elucidate the contribution of mast cells in colonic responses to immobilization stress, we selectively reconstituted mast cells in Kit^w/Kit^w^v mice. As previously reported (20), this procedure only corrects the mast cell defi-
ciency and does not affect the other abnormalities that result from mutations at the W locus. Quantitative analysis showed that the numbers of mast cells in the mucosa, submucosa, and muscularis propria of the colon of mast cell-reconstituted KitW/KitW-vmice 10–12 wk after injection of (+/+)-derived mast cells was similar to those of normal (+/+) mice (n = 5 per group, data not shown). Colonic mucin and PGE_2 release and colonic goblet cell depletion elicited by immobilization stress were not statistically different between mast cell-reconstituted KitW/KitW-vmice and age-matched normal (+/+) mice (Fig. 4; Table 2).

Mast cell-deficient KitW/KitW-vmice exhibit normal stress-induced corticosterone levels in response to immobilization stress. We examined the possibility that altered stress-induced corticosterone release in mast cell-deficient mice may account for their reduced colonic response to stress. We found that after 30-min restraint KitW/KitW-vmice and (+/+ ) mice achieved similar plasma corticosterone levels (53.8 ± 6.1 and 56.9 ± 6.2 µg corticosterone/dl of plasma, respectively; n = 5 per group). Also, basal corticosterone plasma levels in the two groups were statistically indistinguishable (12.2 ± 3.4 and 9.5 ± 3.2 µg corticosterone/dl of plasma in KitW/KitW-vmice and (+/+) mice, respectively; n = 5 per group).

**DISCUSSION**

We report here that genetically mast cell-deficient KitW/KitW-vmice have diminished colonic mucin release from goblet cells and colonic PGE_2 release, but not fecal pellet output, in response to acute restraint stress. We also show that mast cell reconstitution of KitW/KitW-vmice completely normalized stress-induced mucin and PGE_2 release. These findings provide the first direct evidence that release of colonic mucin and PGE_2 in response to immobilization stress is mast cell dependent. These results are consistent with our previous studies, which provided indirect evidence that colonic
mast cells participate in colonic mucin secretion and PGE2 release after immobilization stress (6).

Mast cells have been implicated in the pathogenesis of several gastrointestinal conditions, including acute (5, 23) and chronic colonic inflammation (see Ref. 37 for review) and functional bowel disorders (17, 38). Earlier studies indicated that events in the central nervous system may activate intestinal mast cells (18, 25) and that mucosal mast cells reside in close anatomic proximity to intestinal nerves (30, 31). Several studies have also shown that stress activates mast cells in various organs. For example, Theoharides et al. (32) reported that immobilization stress induces degranulation of mast cells in the dura matter of rats, and Spanos et al. (29) showed activation of bladder mast cells in response to restraint stress. Along the same lines, immobilization and cold stress in rats caused proliferation and degranulation of mast cells in the testis (33), isolation stress increased hypothalamic histamine content (2), and Pavlovian conditioning in rats caused activation of mucosal mast cells (18). We have previously reported that immobilization stress in rats stimulated colonic mucin and PGE2 release and caused degranulation of mucosal mast cells (6). Interestingly, in a preliminary report, Santos et al. (26) showed that exposing human volunteers to 30-min cold stress caused activation of jejunal mast cells, as evidenced by increased luminal contents of the mast cell mediators tryptase, histamine, and PGD2.

Although the results presented here indicate that mast cells play a significant role in the observed colonic responses to stress, the pathways that cause mast cell activation during stress remain to be elucidated. Previous results from our laboratory suggested an interaction between nerves and mast cells in mediating goblet cell mucin secretion in response to restraint stress (6). For example, pretreatment of rats with hexamethonium, atropine, or bretylium not only inhibited stress-induced mucosal mast cell activation but also reduced colonic mucin and PGE2 release caused by restraint stress (6), indicating an interaction between parasympathetic and sympathetic nerves and mast cells in the mediation of colonic goblet cell secretion. Neurotensin (NT), a peptide that can be released from intestinal and nonintestinal sources (4), may also interact with mast cells in the colon and participate in mast cell activation during stress. Castagliuolo et al. (7) showed that pretreatment of rats with the nonpeptide NT receptor antagonist SR-48,692 inhibited colonic mucin and PGE2 release as well as colonic mast cell activation caused by restraint stress, in agreement with studies indicating a functional interaction between NT and mast cells (3).

Similar to (+/+) mice, mast cell-deficient mice have increased colonic motility in response to restraint stress (Fig. 3), indicating that mast cells are not involved in stress-mediated motility changes. This is particularly interesting since mast cell-deficient KitW/v mice have a diminished number of ICC and abnormal intestinal pacemaker function (14). Thus our finding would suggest that ICC are not involved in stress-related motility changes. Our results are quite consistent with our previous data showing that pretreatment of rats with the mast cell stabilizer loxodamide inhibited colonic mucin and PGE2 release but did not affect colonic motility in stressed rats (6). Thus immobilization stress induces mucin secretion, which involves mast cell participation, whereas colonic motility changes are mediated through a mast cell-independent pathway. Previous studies suggested that colonic motility changes in response to immobilization stress most likely involve parasympathetic nerves and the neuropeptide substance P (6).

Mast cell-deficient mice had decreased colonic responses during stress, although their basal and stress-induced corticosterone levels were similar to those of normal (+/+) mice. This indicates that their HPA axis response to immobilization stress is not impaired and suggests that mast cells are not required for stress-induced HPA activation, although they mediate stress-induced colonic responses. Our findings are different...
from previous studies in rats, showing a requirement of adrenal mast cells for ACTH-induced corticosterone release (12, 13). Differences in the species (mice vs. rats) and/or methodological approaches (in vivo vs. in vitro) used in our study and the studies of Hinon et al. (12, 13) may account for these discrepancies.

In summary, our results directly demonstrate participation of mast cells in colonic goblet cell discharge and PGE2 secretion caused by restraint of mice. These findings provide direct evidence for a link between mast cells and the intestinal epithelium in the pathogenesis of stress-related responses. Our findings could be of importance for understanding the pathophysiology of irritable bowel syndrome, in which intestinal mast cell activation (17, 21, 38) and mucus discharge (8) have been reported.

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