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

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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 the contribution of mast cells, we compared colonic responses to stress in mast cell-deficient KitW/Citw– mice and normal (+/+) mice. Mucin and PGE2 release were measured in colonic explants cultured from KitW/Citw– and (+/+) mice 30 min after immobilization stress. We found that stress stimulated colonic mucin release (1.8-fold), goblet cell depletion (3-fold), and PGE2 (2.3-fold) release in (+/+) but not mast cell-deficient KitW/Citw– mice. However, mast cell-deficient mice that had their mast cell population reconstituted by injection of bone marrow-derived mast cells from (+/+) mice had colonic responses to stress similar to those of normal (+/+) mice. In contrast, colonic transit changes in response to stress, estimated by fecal output, were similar between KitW/Citw– and normal (+/+) mice. We conclude that mast cells regulate colonic mucin and PGE2 release but do not colonic transit changes in response to immobilization stress.

MATERIALS AND METHODS

Immobilization stress model. Adult male mast cell-deficient WBB6F1-KitW/Citw– mice (referred to as KitW/Citw–) (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 motility 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.
 ROLE OF MAST CELLS IN COLONIC RESPONSES TO STRESS

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(New England Nuclear, Boston, MA) and 1% penicillin-streptomycin as previously described (6, 7). After incubation (37°C for 18 h) in a 5% CO₂–95% O₂ atmosphere, the medium was aspirated and TCA and phosphotungstic acid (TCA/PTA; Sigma, St. Louis, MO) were added to a final concentration of 10% TCA and 1% PTA. The mixture was kept overnight at 4°C and then centrifuged (1,600 g for 30 min at 4°C). The pellets were resuspended in ice-cold TCA/PTA and centrifuged several times until unincorporated [³H]glucosamine was completely removed. Pellets were then dissolved in 1 ml of 0.3 M NaOH and neutralized with 0.1 ml of 0.6 M acetic acid, and radioactivity was measured. In some samples, to validate that [³H]glucosamine was incorporated into mucin, the TCA/PTA precipitate was examined by density-gradient ultracentrifugation as described previously (6). In keeping with our previous findings (6), our results here also showed that the majority (>75%) of the [³H]glucosamine migrated in fractions of density >1.45 g/ml, consistent with the density of mucin (data not shown).

In some experiments, colonic mucosal explants from nonimmobilized mast cell-deficient or control (+/+ ) mice were incubated in medium containing 10 µCi/ml [³H]glucosamine with 10⁻⁶ M of either the known mucin secretagogues PGE₂ (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 hematoxylin and eosin and Alcian blue. The number of goblet cells containing mucins 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 immunoenzymatic assay (PerSeptive Diagnostics) as previously described (6, 7). Results are expressed as picograms PGE₂ per milligram of tissue wet weight.

Selective mast cell reconstitution of mast cell-deficient Kitw⁻/Kitw⁻ mice. Mast cell-deficient Kitw⁻/Kitw⁻ mice exhibit several abnormalities as a result of the mutations in the W locus, including macrocyclic anemia, absence of melanocytes, sterility, diminished numbers of interstitial cells of Cajal (ICC), and age-dependent changes in intestinal intraepithelial lymphocytes, as well as profound mast cell deficiency (11, 14, 24). However, the expression of immunologic or inflammatory reactions that do not involve mast cells is generally unaffected in these mice, including mast cells. Nakano et al. (20) have shown that mast cell populations can be selectively reconstituted in Kitw⁻/Kitw⁻ mice by the adoptive transfer of immature mast cells derived from the bone marrow cells of congenic normal (+/+) mice. Briefly, femoral bone marrow cells from (+/+) mice were cultured for 4 wk in WEHI 3 medium until >99% of total cells assessed by neutral red staining were mast cells. Kitw⁻/Kitw⁻ mice were injected with Dulbecco’s modified Eagle’s medium (0.2 ml iv) containing 2 × 10⁶ bone marrow-derived mast cells or an equal volume of medium alone. Ten weeks later, the hematocrit was measured in all mice to establish that mice were still anemic, and then mice were used for immobilization stress experiments. Histological examination of colonic tissues obtained at the end of the immobilization experiments confirmed reconstitution of colonic mast cells. In these experiments, a full-thickness section of mouse colon was processed into Epon-embedded, Giemsa-stained sections (40). The sections were examined by a single observer, who was blinded as to the identity of the specimens.

Effect of immobilization stress on blood corticosterone levels of normal (+/+) and mast cell-deficient Kitw⁻/Kitw⁻ mice. Blood samples were collected in heparinized capillary tubes from the retroorbital plexus from five mice of each genotype before and after 30 min of immobilization stress. Corticosterone levels (expressed as µg/dl) in unextracted plasma samples were measured by a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA).

Statistical analyses. 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 Kitw⁻/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 Kitw⁻/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 indistinguishable between (+/+) and Kitw⁻/Kitw⁻ mice (Table 1).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal (+/+)</th>
<th>Mast cell-deficient Kitw⁻/Kitw⁻</th>
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<tbody>
<tr>
<td>Control</td>
<td>4,840 ± 1,970</td>
<td>5,240 ± 1,400</td>
</tr>
<tr>
<td>PGE₂</td>
<td>11,240 ± 3,420</td>
<td>8,970 ± 1,860</td>
</tr>
<tr>
<td>Forskolin</td>
<td>8,310 ± 1,200</td>
<td>8,270 ± 1,980</td>
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Results are means ± SE; 4 rats were tested for each group. Colonic explants from either mast cell-deficient Kitw⁻/Kitw⁻ or congenic normal (+/+) mice were cultured at 37°C in medium containing [³H]glucosamine and either 10⁻⁶ 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 KitW/KitW-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 KitW/KitW-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 KitW/KitW-v mice (Fig. 1A). Furthermore, there was no significant reduction in the number of superficial goblet cells containing mucus in immobilized vs. nonimmobilized KitW/KitW-v mice (Fig. 2; Table 2).

Mast cell-deficient KitW/KitW-v mice exhibit reduced colonic PGE2 release in response to immobilization stress. There was no significant difference in basal colonic PGE2 levels of nonimmobilized KitW/KitW-v and (+/+) mice (Fig. 1B). As observed in rats (6, 7), immobilization for 30 min resulted in a 2.3-fold increase in PGE2 release in (+/+) mice compared with nonimmobilized (+/+) mice (P < 0.05, Fig. 1B). However, immobilization of KitW/KitW-v mice did not increase colonic PGE2 release (Fig. 1B).

Effect of stress on colonic transit in mast cell-deficient KitW/KitW-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 in normal KitW/KitW-v mice (P < 0.01). Furthermore, there was no significant difference in the number of fecal pellets after restraint stress between (+/+) and KitW/KitW-v mice (Fig. 3).

Mast cell reconstitution of KitW/KitW-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 KitW/KitW-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−/− mice 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 PGE2 release and colonic goblet cell depletion elicited by immobilization stress were not statistically different between mast cell-reconstituted KitW/KitW−/− mice and age-matched normal (+/+) mice (Fig. 4; Table 2).

Mast cell-deficient KitW/KitW−/− mice 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−/− 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−/− and (+/+) mice, respectively; n = 5 per group].

**DISCUSSION**

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

Fig. 2. Effect of immobilization stress on goblet cell depletion in mast cell-deficient KitW/KitW−/− and mast cell-reconstituted KitW/KitW−/− mice. Mice were immobilized as described in MATERIALS AND METHODS; control rats moved freely in their cages. After 30 min, rats were killed and full-thickness samples of colon were fixed in Formalin and stained with hematoxylin and eosin and Alcian blue. Colon from a nonimmobilized normal (+/−) mouse (A), a mast cell-deficient mouse (C), and a mast cell-deficient mouse that had been selectively reconstituted with bone marrow-cultured mast cells obtained from congenic (+/+) mice (E) show normal mucosal architecture, with many goblet cells containing mucin in the crypts and the superficial epithelium. Colon obtained from an immobilized normal (+/+) mouse (B) shows disappearance of superficial mucin-containing goblet cells, in contrast to colon obtained from an immobilized mast cell-deficient mouse showing normal colonic architecture (D). Colon from an immobilized mast cell-reconstituted mast cell-deficient mouse (F) shows goblet cell degranulation comparable to colon of an immobilized normal (+/+) mouse (B). Original magnification, ×140.

Fig. 3. Mast cell-deficient KitW/KitW−/− mice exhibit normal fecal pellet output in response to immobilization stress. After 30-min immobilization, mice were killed, and the number of fecal pellets produced during immobilization was quantified. Bars are means ± SE of 7–12 animals per group. **P < 0.01 vs. respective controls.
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 levels 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 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 lodoxamide 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 Hinson 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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