Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells

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Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells. Am J Physiol Gastrointest Liver Physiol 278: G847–G854, 2000.—We examined the impact of chronic stress on rat growth rate and intestinal epithelial physiology and the role of mast cells in these responses. Mast cell-deficient (Ws/Ws) rats and +/− littermate controls were submitted to water avoidance stress or sham stress, 1 h/day, for 5 days. Seven hours after the last sham or stress session, jejunal segments were mounted in Ussing chambers, in which secretion and permeability were measured. Body weight (as a growth index) and food intake were determined daily. Stress increased baseline jejunal epithelial ion secretion (indicated by short-circuit current), ionic permeability (conductance), and macromolecular permeability (horseradish peroxidase flux) in +/− rats, but not in Ws/Ws rats, compared with nonstressed controls. Stress induced weight loss and reduced food intake similarly in the groups. In +/− rats, these parameters remained altered 24–72 h after the cessation of stress. Modulation of stress-induced mucosal mast cell activation may help in the management of certain intestinal conditions involving epithelial pathophysiology.

jejunum; epithelial transport

The impact of chronic life stress on human health and well-being remains a debated issue. It is generally believed that chronic exposure to stress may lead to the development of a variety of human pathologies (20). In particular, the influence of psychosocial factors on gastrointestinal diseases, despite some controversy, is being increasingly recognized (12, 46). In fact, the clinical evolution and the intensity of symptoms in certain irritable bowel syndrome patients seems to depend, to a great extent, on the presence of stress as a comorbid factor (4, 17, 18). Although experimental evidence that stress alters gastrointestinal motility (40) and enhances visceral perception (7) supports this association, a physiological link to the underlying mechanisms is still lacking.

Epithelial cells are involved in the modulation of mucosal immune/inflammatory disorders by releasing a number of bioactive molecules (cytokines, chemokines, and growth factors) and by acting as a barrier to limit the uptake of luminal antigens. However, information on the effects of stress, other than chemical or metabolic stressors, on intestinal epithelial physiology is rather scarce. We and others have shown that acute stressors elicit epithelial secretory and transport abnormalities in human and rat small intestine (3, 19).

Although increased transport of glucose in rat jejunum after prolonged stress has been reported (43), the effect of chronic stress on small intestinal epithelial physiology remains largely unexplored.

Mast cells are key cells in the regulation of gut epithelial transport function. It has been established that mast cell activation is associated with ion and macromolecular epithelial transport changes in human and rat intestine (5, 9).

Furthermore, some reports suggest that mast cells play an important role in human and rat intestinal epithelial responses to acute stressors (8, 33). Chronic stress is known to induce weight loss and anorexia (22). Weight loss may be a reflection of chronic psychological distress in patients with functional gastrointestinal disorders (32). However, the participation of mast cells in epithelial and biological responses to chronic stress has not been addressed.

On the basis of our previous findings, we hypothesized that chronic stress might produce intestinal epithelial abnormalities. Therefore, we developed a model of chronic mild stress suitable for evaluation of the effects of stress on gut function. We chose a model of repetitive application of a homotypic stressor rather than an acute stressor because it better reflects the pattern of daily stress experienced by humans. The specific aims of the present study were 1) to investigate the effects of chronic stress on intestinal epithelial physiology and animal growth; 2) to determine the role of mast cells in stress-induced abnormalities, and 3) to establish the duration of stress-induced effects.

Methods

Animals

Ws/Ws (homozygous at “white spotting” locus) rats and their +/- littermate controls were obtained by breeding male Ws/+ heterozygous rats (from the original colony developed by Y. Kitamura in Japan [28]) in our colony at McMaster University. Ws/Ws rats have a 12-base deletion in the tyrosine kinase domain of the c-kit gene (44) that results in a lack of melanocytes and mast cells and a reduced number...
of erythrocytes. By 10 wk of age, no mast cells can be detected in the intestine of Ws/Ws rats, whereas +/+ rats have normal numbers of mast cells (1) that are entirely functional, as shown by normal responses to antigen challenge (5). Rats, individually housed, were maintained on a 12:12-h dark-light cycle and provided with food and water ad libitum. On the day of the experiments, rats were euthanized by decapitation immediately after the final sham or stress procedure. All procedures were approved by the Animal Care Committee at McMaster University.

Stress Protocol

Rats were handled daily by the same investigator for 2 wk before the study and then submitted to water avoidance stress daily for 5 days. The procedure involved placing the rat on a glass platform (8 × 6 cm) in the middle of a plastic container (56 × 50 cm) filled with warm water (25°C) to 1 cm below the height of the platform. Rats avoided the aversive stimulus (water) by remaining on the platform for 1 h. Control rats were placed on the same platform above a waterless container for 1 h. Body weight changes, as an index of growth, and food intake were measured (g/day) just before the stress or sham protocol. All experimental procedures were performed between 8:00 and 10:00 AM to minimize the effect of circadian rhythm.

Epithelial Measurements

Ussing chamber studies. The proximal jejunum was removed, placed in 37°C oxygenated Krebs, stripped of longitudinal muscle and myenteric plexus, and opened along the mesenteric border. Two to four adjacent pieces from each rat were mounted in Ussing chambers (World Precision Instruments, Sarasota, FL). The chamber opening exposed 0.6 cm² of tissue surface area to 8 ml of circulating oxygenated Krebs buffer at 37°C. The buffer contained (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 KH₂PO₄, and 25 NaHCO₃ (pH 7.35). In addition, the serosal buffer also contained 10 mM glucose as an energy source that was osmotically balanced by 10 mM mannitol in the mucosal buffer. The chambers contained agar-salt bridges to monitor the potential difference across the tissue and to inject the required short-circuit current (Isc) to maintain a zero potential difference as registered via an automated voltage clamp (World Precision Instruments). Isc (µA/cm²) was recorded by a computer connected to the voltage-clamp system. Tissue conductance was calculated according to Ohm’s law and expressed as millisiemens per square centimeter.

Mucosal-to-serosal transport of macromolecules was assessed by measuring transepithelial flux of horseradish peroxidase (HRP). HRP (type VI, Sigma Chemical, St. Louis, MO) was added to the luminal buffer 15 min after the tissues were mounted at a final concentration of 10⁻⁵ M and allowed to equilibrate for 30 min. Serosal samples (0.5 ml) were obtained at 30-min intervals for 2 h and were replaced with Krebs buffer to maintain a constant volume in the chambers. HRP activity was determined by a modified Worthington method (21) as previously described (19). Mucosal-to-serosal fluxes of HRP were calculated according to standard formulas (21) and expressed as picomoles per hour per square centimeter.

Histology. Full-thickness segments of jejunum, adjacent to the pieces of tissue mounted in Ussing chambers, were coded, processed for morphological microscopic analysis. Tissues were fixed in Carnoy’s solution for 24 h followed by 70% ethanol. Sections were cut and stained with toluidine blue (Sigma). Mast cells were counted at a ×400 magnification using a micrometer grid fitted in a eyepiece. At this magnification, the grid covered a 0.032-mm² area. For each tissue, six contiguous non-overlapping areas above the muscularis mucosae were counted for the estimation of mucosal mast cell numbers expressed as cells per square millimeter.

Electron microscopy. Tissues, removed 2 h after the addition of HRP into the chambers or obtained after death, were immediately fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at 22°C, rinsed for 18 h (4°C) with 0.05 Tris buffer (pH 7.6), washed three times for 5 min each, and processed for routine transmission electron microscopy.

Methods for HRP product identification were modified from Graham and Karnovsky (15) and are described in detail elsewhere (19). Quantitative analysis of HRP uptake was performed on randomly selected epithelial windows (area 300 µm²) on coded high-magnification photomicrographs (15/rat; 5–6 tissues/group). The area of HRP-containing endosomes within jejunal enterocytes was determined using a computerized image analysis system and reported as square micrometers per window. Tissues were also examined for evidence of paracellular HRP transport in coded photomicrographs that were randomly selected. HRP was identified as an electron-dense material within the paracellular spaces between enterocytes.

Mast cell activation was assessed on randomly selected sections (20 for each tissue) containing mast cells. For each mast cell, the total number of intracytoplasmatic granules was counted and each granule was analyzed for loss of density and perigranular vacuolization.

Experimental Design

In the first part of the study, rats (n = 10/group) were submitted to the sham/stress protocol for five consecutive days, an approach similar to that used by other groups to obtain a chronic stressful condition (23, 26, 29). We previously determined that jejunal epithelial responses were maximal 5–7 h after acute stress (unpublished observations). On the basis of these findings, 7 h after the last sham or stress session, tissues from +/+ and Ws/Ws rats were mounted in Ussing chambers. Baseline values for Isc, as an indicator of ion secretion, and conductance, as an indicator of ion permeability, were calculated at equilibrium, 15 min after the tissues were mounted, and then every 30 min for 2 h. Fluxes of HRP were determined at 30-min intervals over the course of the study and expressed as the average value of two consecutive stable flux periods. Although body weight and food intake were determined daily in all rats (10/group), as described, only six rats/group were killed for Ussing chamber studies.

Additional experiments were performed to assess the duration of stress-induced effects on epithelial physiology, body weight, and food intake. Because only +/+ rats showed abnormal stress-related epithelial responses and body weight and food intake were mast cell independent, a different group of +/+ rats (n = 24) was submitted to the sham or stress protocol (5 days) and then returned individually to their home cage, where they remained undisturbed except for daily measurements of body weight and food intake. Rats were euthanized at days 1, 3, and 7 (n = 8 for each time) after the end of sham or stress procedures, and tissues were mounted in Ussing chambers as described.

In each experimental group, rats were matched by initial body weight (250–300g) and age (14–20 wk) to avoid potential differences in the measured responses related to these factors (41).

Statistical Analysis

Results are expressed as means ± SE unless otherwise stated. The results were analyzed by a two-way ANOVA with
contrasts to show the simple effects of the main factors of treatment group (stress vs. sham) and strain (Ws/Ws vs. +/±). A between-within univariate ANOVA, in which the third factor was a repeated-measures factor (time), was used to compare means at different days in strain and treatment groups. Unpaired Student’s t-test was used to compare post-treatment differences (at each time) on epithelial parameters in sham and stress groups. Treatment (stress or sham) vs. posttreatment (day 1, day 3, day 7) within- and between-groups differences in body weight and food intake were compared by Tukey’s test after a one-way ANOVA. For other data, unpaired Student’s t-test and Fisher’s exact test were also used when appropriate. P values < 0.05 were considered significant.

RESULTS

Effect of Stress on Jejunal Epithelial Physiology in +/+ and Ws/Ws Rats

After sham stress, baseline $I_{Sc}$ was similar in +/+ (41.2 ± 6.5 µA/cm²) and Ws/Ws (37.4 ± 3.4 µA/cm²) rats. Stress induced a marked increase in baseline $I_{Sc}$ (61.0 ± 11%) in +/+ rats but not in Ws/Ws rats (5.0 ± 7.3%) compared with their corresponding controls (Fig. 1). This increase was significant, as shown by the treatment-by-strain interaction ($P < 0.05$).

![Fig. 1. Effect of chronic stress on jejunal electrophysiological values. Mast cell-deficient rats (Ws/Ws) and their normal mast cell-containing littermates (+/+) were submitted (1 h/day) to sham stress (open bars) or water avoidance (filled bars) for 5 consecutive days. Seven hours after last session, tissues were mounted in Ussing chambers, in which baseline short-circuit current ($I_{sc}$, A) and conductance (G, B) were measured. Bars represent means ± SE; n = 6 rats/group, with 2–4 tissues averaged per rat. *P < 0.05, †P < 0.005 vs. control.](http://ajpgi.physiology.org/)

After sham stress, baseline conductance was not different in +/+ (32.4 ± 1.3 mS/cm²) and Ws/Ws (29.0 ± 2.0 mS/cm²) rats. In contrast, stress markedly increased colonic conductance in +/+ rats (44.0 ± 8.7%, $P < 0.01$) but not in Ws/Ws rats (11.2 ± 7.9%) compared with their controls (Fig. 1).

Effect of Stress on HRP Flux and Uptake in Jejunum

The effect of sham stress on HRP flux was not different in +/+ (5.7 ± 0.7 pmol·h⁻¹·cm⁻²) and Ws/Ws (5.3 ± 0.6 pmol·h⁻¹·cm⁻²) rats. Stress enhanced HRP flux across the jejunum in +/+ rats (219 ± 21.7%) but not in Ws/Ws rats (12 ± 14.8%) compared with their corresponding controls (Fig. 2). This increase was significant as shown by the treatment-by-strain interaction ($P < 0.01$).

HRP was evident within endosomes in enterocytes from sham-stressed and stressed rats. However, HRP-containing endosomes were larger and more numerous in stressed +/+ rats compared with the other three groups, as indicated by the HRP-endosome area within enterocytes (Fig. 3). In addition, HRP was observed within tight junctions and paracellular spaces, but only in tissues from stressed +/+ rats.

Effect of Stress on Body Weight and Food Intake

Throughout the sham/stress protocol, a weight gain was observed in both +/+ (2.0 ± 0.65 g/day) and Ws/Ws (2.4 ± 0.9 g/day) rats. In contrast, stress was associated with a marked weight loss in both +/+ (~1.7 ± 0.9 g/day) and Ws/Ws (~0.65 ± 0.4 g/day) rats in the same period ($P < 0.001$ vs. respective controls). The effect of sham stress or stress on body weight was not different between the two strains.

Food intake was significantly reduced in +/+ (13.6 ± 0.8 g/day) and Ws/Ws (14.2 ± 0.4 g/day) stressed rats compared with their controls (+/+: 17.0 ± 0.6 g/day, $P < 0.0003$; Ws/Ws: 17.6 ± 0.6 g/day, $P < 0.0003$). The effect of sham stress or stress on food intake was not different between the two strains. The effect of stress remained significant compared with controls in both strains when a day-by-day comparison was done throughout the experimental protocol, and no significant differences between Ws/Ws and +/+ rats were observed (Fig. 4).

Effect of Stress on Mast Cells

We confirmed that mast cells were absent in the intestine of Ws/Ws rats. As assessed by light microscopy, stress exposure did not change the number of mast cells in the jejunal mucosa of +/+ rats (245 ± 13 cells/mm²) compared with their controls (223 ± 22 cells/mm²). As assessed by electron microscopy, intragranular morphological changes were observed in a minority of mast cells in the mucosa of control rats (5.9%, n = 4 rats, 591 granules counted), whereas a higher proportion of mast cell granules from stressed +/+ rats showed signs of activation such as loss of density and perigranular vacuolization (26.1%, n = 4 rats, 643 granules counted) ($P < 0.0001$).
Duration of Stress Effects

One day after the cessation of stress, significantly increased baseline $I_{sc}$ (58.9 ± 3.7 µA/cm²) and conductance (39.7 ± 1.0 mS/cm²) were still observed in the jejunum of stressed $+/+$ rats in comparison with sham-treated animals ($I_{sc}$: 36.1 ± 2.1 µA/cm²; conductance: 28.4 ± 4.3 mS/cm²; P < 0.05 vs. stress for both). The stress-induced changes returned to control values at poststress day 3 and remained so at poststress day 7 (Fig. 5).

However, the stress-induced enhancement in HRP flux remained significantly elevated on days 1 and 3 after the cessation of stress compared with tissues from sham-treated rats (Fig. 5). By day 7 after stress, the HRP flux across the jejunum was not different between the two groups.

When data from the 5 days of sham stress were compared with data from postsham days 1, 3, and 7, no changes in body weight and food intake were observed (Fig. 6). We confirmed that during the 5 days of stress rats lost weight and ate less than their controls. These effects of stress were still significant at days 1 and 3 after cessation of stress (Fig. 6). When the group that remained in the home cage for 7 days after sham or stress was analyzed, it became apparent that at day 7, stressed and sham-treated rats showed similar weight gain and feeding patterns, although the mean body weight in the stressed rats (282 ± 35 g) was lower than their initial weight (287 ± 33 g) (Fig. 7).

DISCUSSION

We have shown that chronic stress, induced by repetitive exposure to water avoidance for 5 days, stimulated secretion ($I_{sc}$) and permeability (conductance and HRP flux) in rat jejunal epithelium. These effects were mast cell dependent, because Ws/Ws rats showed no changes after stress whereas in $+/+$ rats these parameters were significantly increased in comparison with controls. Chronic stress also induced weight loss and reduced food intake. These changes, in contrast to the effects on jejunal epithelial physiology, were not mast cell dependent, because comparable alterations were observed in both strains. Our study also shows that the effect of stress on jejunal epithelial physiology, body weight, and food intake in $+/+$ rats was still observed up to 72 h after the cessation of stress.

In the normal, uninflamed small intestine, acute stress has been shown to increase water transport and epithelial ion secretion and permeability in both rats (35) and humans (3, 33). More importantly, we showed previously (19) that acute stress also enhances transepithelial transport of macromolecules in the rat jejunum. However, other than studies showing that prolonged exposure of rats to restraint or vibration increases intestinal glucose transport (43), decreases epithelial cell proliferation in the small intestine (16), and alters colonic epithelial secretory responsiveness to 5-hydroxytryptamine (14), the information on the effects of chronic stress on epithelial function is almost negligible. In the present study, we have extended our previous observations (19, 35) by showing for the first time that chronic stress (as opposed to acute stress) markedly stimulated $I_{sc}$ (indicating net ion secretion), conductance (indicating ion permeability), and HRP flux (indicating macromolecular permeability) in the rat jejunum. These data indicate that chronic stress may contribute to host defense by flushing noxious material out of the lumen but also may be detrimental by enhancing the absorption of antigenic molecules. A novel finding of our study is that the stress-induced...
changes in \( I_{\text{sc}} \), conductance were still present 24 h after the last session of stress. Moreover, changes in HRP flux lasted for up to 3 days. All epithelial abnormalities had normalized by day 7 after stress. The magnitude of these epithelial changes after stress cessation was similar to that induced by acute stress; however, the duration of these abnormalities was clearly longer in this model of chronic stress compared with acute stress, where \( I_{\text{sc}} \), conductance, and HRP flux had returned to control values 24 h after stress (P. R. Saunders, J. Santos, and M. H. Perdue, unpublished observations). The persistent elevation of macromolecular permeability could have clinical implications. It has been shown that intestinal permeability is increased in Crohn's disease patients (47). Increased uptake of food or microbial antigens may activate mucosa-associated immune cells, leading to an inflammatory response that may be involved in the pathogenesis of inflammatory bowel disease.

We also showed that during the exposure to chronic stress rats lost weight and reduced their food consump-

tion. It was previously noted that other chronic stressors also decrease body weight and food intake in the rat (23, 30). Stress-induced anorexia seems to be related mainly to the severity of the stressor rather than to the length of the exposure or the time of the day when the stressor is applied (23). Although several peptides and hormones have been implicated in the regulation of food intake and body weight, members of the corticotropin-releasing hormone (CRH) family, key mediators of stress responses, are suitable candidates to explain the anorexigenic effects of chronic stress. In this sense, it has been shown that CRH and urocortin, endogenous agonists of that family, induce anorexia and weight loss in rats when injected centrally (24, 37). Similar effects were reported in mice after peripheral injection of CRH and urocortin (2, 36). This effect could be due in part to the inhibition of gastric emptying (2, 24). However, other stress-mediated metabolic changes such as stimulation or inhibition of the release of catabolic (catecholamines, glucocorticoids) and anabolic (thyrotrophin and growth hormone) hormones, respectively, might also account for the stress-induced weight loss (22–23, 39). In support of this contention, it has been shown that the weight loss induced by chronic stress is only partially mediated by the reduction in food intake (23).

![Fig. 5. Duration of stress effects on jejunal \( I_{\text{sc}} \) (A), G (B), and HRP flux (C) in +/+ rats. After 5 days of sham stress (open bars) or stress (filled bars), rats were returned to their home cages, where they remained undisturbed for 1, 3, and 7 days. At these time points tissues were mounted in Ussing chambers, in which \( I_{\text{sc}} \), G, and HRP flux were determined. Bars represent means ± SE; n = 4 rats/group. *P < 0.05 vs control.

![Fig. 6. Duration of stress effects on body weight (A) and food intake (B) in +/+ rats. Rats were submitted to sham stress (open bars) or stress (filled bars) for 5 consecutive days (treatment period, Tx) and then returned to their home cages, where they remained undisturbed for 1, 3, and 7 days. Body weight and food intake were measured daily just before sham or stress procedures. Bars represent means ± SE; n = 12 rats/group for treatment period and 4 rats/group for days 1, 3, and 7 after treatment.*P < 0.05, ‡P < 0.005 vs. control.](http://ajpgi.physiology.org/)
Another finding of the present study is that the alterations in body weight and food intake were still apparent 3 days after the final session of stress. Moreover, by day 7 after stress the rats showed weight gain and ate increasing amounts of food but had not recovered their initial body weight. Similar persistent effects on body weight were shown by Ottenweller et al. (29, 30) in rats submitted to a chronic regimen (3 or 10 days) of inescapable tail shock and in mice after a single intraperitoneal injection of urocortin (2).

A large body of evidence indicates that mast cells, by releasing a vast array of biologically active mediators, regulate epithelial ion and macromolecular transport in the gut (5, 10). In the present study, the chronic stress-induced increases in jejunal $I_{sc}$ conductance, and HRP flux were documented in +/+ but not in Ws/Ws rats, suggesting that mast cells are required for the expression of these transport abnormalities. Others have shown that the stress-induced stimulation of colonic mucin release, another epithelial response, was absent in mast cell-deficient mice (8). Furthermore, in agreement with observations showing that acute stress resulted in mast cell activation in tissues such as the human jejunum (33) or the rat brain (42) and heart (31), our results also show that stress increased almost fivefold the number of activated mast cells in the jejunal mucosa. Together, these findings support the relevance of mast cell activation in stress-induced epithelial responses in the gut.

Although some studies indicate that mast cell granular content, as well as the number and morphology of the cells, may be affected by animal weight and feeding process (13, 41), the opposite, i.e., the effect of mast cell activation on these factors, has not been established. One study indicated that ongoing mast cell activation was not the main determinant of the reduced weight gain observed in sensitized rats submitted to chronic antigen challenge because pair-fed but nonsensitized rats showed a greater reduction in weight gain (11) than controls. In another study, mast cells were reported to be involved in the regulation of food intake in response to the intraperitoneal administration of lipopolysaccharide (27). Our results show that mast cells did not participate in the weight loss and food intake reduction induced by chronic stress, as indicated by the lack of differences between +/+ and Ws/Ws rats in the magnitude of these responses.

We did not look at the mechanisms and pathways involved in the stress-stimulated epithelial responses. Water avoidance is known to release CRH in the brain (6), and this neuropeptide is able to cross the blood-brain barrier unidirectionally from the brain to the periphery, resulting in physiological changes in peripheral organs (25). We have previously shown that peripheral injection of CRH mimicked colonic epithelial responses induced by acute stress and that these responses involved mast cells (34). Mast cells express CRH receptors and degranulate after exogenous administration of CRH (42). In addition, mast cells are in close association with neural varicosities in the gastrointestinal tract (38). Therefore, we speculate that mucosal mast cells could have been activated either directly by CRH or indirectly by neuropeptides released from CRH-stimulated neural processes.

In summary, we have developed a model of chronic stress that is suitable for study of the changes in gut epithelial physiology. We have shown that this regimen of repetitive application of water avoidance stress is associated with marked, persistent, and reversible alterations of the barrier and transport properties of the small intestine along with changes in growth rate and feeding patterns. We have also determined the critical role of mast cells in the stress-induced epithelial abnormalities. The clinical relevance of these findings remains to be established. However, there is a strong association between life stress and the clinical evolution of irritable bowel syndrome (4, 17, 18). In addition, increased numbers of mast cells have been reported in the small intestine of patients with irritable bowel syndrome (45). Although it has been recently reported that some patients with irritable bowel syndrome showed a high prevalence of eating disorders (32), weight loss and eating disorders are not recognized clinical features of this disorder. However, it is
conceivable that in a selected subgroup of irritable bowel syndrome patients, especially those affected by high levels of life stress, these factors could be temporarily present and could perhaps be useful as clinical markers of poor prognosis. In conclusion, therapeutic and preventive interventions to neutralize the undesirable effects of stress in the gastrointestinal tract might be beneficial in some patients with functional gut disorders.

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