Stress stimulates transepithelial macromolecular uptake in rat jejunum

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1Intestinal Disease Research Program, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3S5; 2Institute of Neurobiology, University of Amsterdam, 1098 SM Amsterdam; and 3Department of Pediatric Gastroenterology and Nutrition, Academic Medical Centre, 1105 AZ Amsterdam, The Netherlands

Kiliaan, Amanda J., Paul R. Saunders, Pieter B. Bijlsma, M. Cecilia Berin, Jan A. Taminiau, Jack A. Groot, and Mary H. Perdue. Stress stimulates transepithelial macromolecular uptake in rat jejunum. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1037–G1044, 1998.—Evidence suggests that stress may be a contributing factor in intestinal inflammatory disease; however, the involved mechanisms have not been elucidated. We previously reported that acute stress alters epithelial physiology of rat intestine. In this study, we documented stress-induced macromolecular transport across intestinal epithelium. After exposure of Wistar-Kyoto rats to acute restraint stress, transport of a model protein, horseradish peroxidase (HRP), was assessed in isolated segments of jejunum. The flux of intact HRP was significantly enhanced across intestine from stressed rats compared with controls. Electron microscopy revealed HRP-containing endosomes within enterocytes, goblet cells, and Paneth cells of stressed rats. The number and area of HRP endosomes within enterocytes were found to be significantly increased by stress. HRP was also visualized in paracellular spaces between adjacent epithelial cells only in intestine from stressed rats compared with controls. Electron microscopy revealed HRP-containing endosomes within enterocytes, goblet cells, and Paneth cells of stressed rats. The number and area of HRP endosomes within enterocytes were found to be significantly increased by stress. HRP was also visualized in paracellular spaces between adjacent epithelial cells only in intestine from stressed rats. Atropine treatment of rats prevented the stress-induced abnormalities of protein transport. Our results suggest that stress, via a mechanism that involves release of acetylcholine, causes epithelial dysfunction that includes enhanced uptake of macromolecular protein antigens. We speculate that immune reactions to such foreign proteins may initiate or exacerbate inflammation.

enteric nerves; epithelium; permeability; endocytosis; tight junctions

For many years, stress and anxiety were thought to have an etiological role in chronic inflammatory diseases and gastrointestinal pathophysiology. It was suggested that patients with inflammatory bowel disease (IBD) and irritable bowel syndrome have an anxiety-prone personality profile (2, 35). Intestinal dysfunction was also correlated with depression (39). Others argued that psychological problems occur as a result rather than a cause of chronic health problems (18). A recent report confirmed that stressful life events frequently precede disease relapses in patients with IBD (12). However, the underlying mechanisms remain undefined.

Although numerous experimental studies have provided evidence that stress is involved in causing gastric ulceration (reviewed in Ref. 15) and altering intestinal motility (5a, 24, 25, 37), relatively few studies have examined the effects of stress on mucosal function (4, 7, 13). The mucosa of the gastrointestinal tract consists of the lamina propria, covered by a single cell layer of epithelial cells (mainly transporting enterocytes, but also goblet cells, enterendocrine cells, and Paneth cells) joined together by tight junctions that create a barrier restricting uptake of luminal material (21). The lamina propria contains various immunocytes (mast cells, eosinophils, macrophages, neutrophils, lymphocytes, etc.), and their numbers increase during inflammation. These cells react nonspecifically to certain bacterial products or specifically to foreign protein antigens. In addition, the mucosa is highly innervated: networks of nerves surround the crypts, and nerve fibers extend into the villi with varicosities in close proximity to the epithelium (14). Convincing evidence has been presented that enteric nerves regulate the transport function of the epithelium (8). Signals can be communicated from the central nervous system to the gut via extrinsic nerves or their connections to intrinsic nerves in the plexus regions of the gut wall (38). Although less is known about the nature of extracellular signals regulating epithelial barrier function, it is reasonable to hypothesize that similar neural circuits might be involved.

We previously reported (31) that stress causes intestinal mucosal pathophysiology in Wistar-Kyoto rats, a stress-susceptible strain (26). After exposure of rats to restraint stress, with or without cold at 8°C, jejunal preparations exhibited a secretory state, indicated by an increased baseline short-circuit current (Isc) that was due to net Cl− secretion. Stress also resulted in increased conductance and permeability to two small probes, mannitol and Cr-EDTA. Reduced temperature enhanced the magnitude of the intestinal responses to stress but did not alter them qualitatively. A subsequent study (30) demonstrated that although both Wistar-Kyoto rats and the parent Wistar strain responded to stress with intestinal epithelial transport abnormalities, the stress-induced changes were more profound in Wistar-Kyoto rats, apparently due to a defect of intestinal cholinesterase activity resulting in hyperresponsiveness to cholinergic stimulation. In confirmation of this hypothesis, atropine treatment of Wistar-Kyoto rats prevented the transport abnormalities.

The current investigation was designed to determine whether the stress-induced epithelial barrier defect, previously identified for small inert probes, extends to biologically relevant macromolecules such as protein antigens that might trigger an inflammatory/immune response. We examined protein uptake across isolated
segments of intestine in Ussing chambers to eliminate any possible indirect effects of stress (e.g., changes in blood flow, motility, or mucus secretion) that might affect protein transport in vivo. The study was focused at the cellular level, on ultrastructural visualization of the transepithelial transport pathway. Under carefully controlled conditions, we found that stress stimulated protein uptake via both the transcellular and paracellular pathways. Our studies also showed that acetylcholine released during the stress response was critical in enhancing uptake of macromolecules across the epithelium.

**METHODS**

**Animals**

The experiments were approved by the Animal Care Committee at McMaster University. Adult male rats (mean weight 150 g) of the Wistar-Kyoto strain were purchased from Charles River (St. Constant, PQ, Canada). They were maintained on a normal 12:12-h light-dark cycle and provided with food and water ad libitum. Rats were handled daily by the same investigator for 2 wk before study to prevent any uncontrolled effects of stress. Thirty minutes before the experiment, rats were injected intraperitoneally with saline, atropine sulfate, or atropine methyl nitrate (0.1–10 mg/kg) (Sigma Chemical, St. Louis, MO). A relatively high dose was used, justified by our results and also by findings that Wistar-Kyoto rats have abnormally low circulating and mucosal cholinesterase activity (19, 30), resulting in decreased degradation of acetylcholine and therefore higher tissue levels after cholinergic stimulation. Rats in the stress group were injected with atropine or saline but remained in their home cage before study. Rats were then anesthetized with intramuscular urethan and maintained at 37°C via a rectal probe and thermally controlled heating pad while a laparotomy was performed. A 15- to 20-cm segment of jejunum (beginning 5 cm distal to the ligament of Treitz) was removed and placed in 37°C oxygenated Krebs buffer.

**Ussing Chamber Studies**

The jejunal segment was carefully stripped of external muscle and cut into four to eight pieces, which were returned to a large volume of 37°C oxygenated buffer. This rinsing removed any mediators that may have been released during the stripping process. The pieces were then mounted in Ussing chambers (opening of 0.6 cm²), taking care to avoid Peyer’s patches. (This entire procedure was completed within 5 min and tissues were always kept under physiological conditions of temperature, pH, and oxygenation to avoid damage or deterioration that may influence macromolecular transport. With this experimental approach, tissues were viable for at least 3 h with consistent I ᵈ responses to added agonists or field stimulation.) In the chambers, tissues were bathed in 37°C oxygenated Krebs buffer (10 ml on each side) containing (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 K₂HPO₄, and 25 NaHCO₃, pH 7.35 ± 0.02. The serosal buffer included 10 mM glucose as an energy source, osmotically balanced by 10 mM mannitol in the mucosal (luminal) buffer. The chambers contained agar-salt bridges to monitor the potential difference (PD) across the tissues and inject I ᵈ to maintain zero PD as measured via an automatic voltage clamp (W-P Instruments, Narco Scientific, Downview, ON, Canada). Conductance was calculated from Ohm’s law.

**Permeability**

The inert probe, ⁵¹Cr-EDTA (6 µCi/ml) (Radiopharmacy, Chedoke-McMaster Hospital, Hamilton, ON, Canada), was added to the mucosal buffer of the Ussing chambers, balanced by an equivalent concentration of unlabeled Cr-EDTA in the serosal buffer. Samples, 1.0 ml from the serosal buffer and 0.05 ml from the mucosal buffer, were obtained at 30-min intervals or at the beginning and end of the experiment, respectively. Buffers were replaced as required to keep the volume constant. The radioactivity of ⁵¹Cr-EDTA was measured in a gamma-counter. Transepithelial fluxes were calculated by standard formulas and were expressed as nanomoles per hour per square centimeter.

**Protein Transport**

We used horseradish peroxidase (HRP) as a model protein because it has a molecular mass of 40 kDa, similar in size to antigenic proteins known to stimulate immune responses in sensitive individuals (9). Intact HRP can be quantitatively measured using an enzymatic assay, and the reaction product is easily visualized as electron-dense material in ultrastructural studies. HRP (10⁻⁵ M, type VI, Sigma) was added to the mucosal buffer. Samples, 0.5 ml from the serosal buffer and 0.05 ml from the mucosal buffer, were obtained at 30-min intervals or at the beginning and end of the experiment, respectively. Buffers were replaced as required to keep the volume constant. A modified kinetic assay (5) was used to measure intact HRP. Briefly, the reaction mixture (0.8 ml) contained 0.003% H₂O₂ and 0.009% o-dianisidine dihydrochloric acid (Sigma) with 0.15 ml of sample added. The rate of appearance of reaction product was used to calculate flux values expressed as picomoles per hour per square centimeter.

**Electron Microscopy**

Tissues were removed at 60 and 120 min, fixed, and processed for visualization of HRP reaction product by electron microscopy. Methods for HRP product identification were modified from Graham and Karnovsky (16). The tissues were 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 M Tris buffer (pH 7.6), and then washed three times, 5 min each time. Peroxidase activity was demonstrated by incubating the tissues for 15 min in 0.5 mg/ml diaminobenzidine in 0.05 M Tris buffer (pH 7.6, 22°C) and subsequently incubating them for 15 min in the same buffer containing 0.01% H₂O₂. Tissues were then processed for routine transverse electron microscopy. Quantitative analysis was performed on coded high-magnification photomicrographs; 80 micrographs (i.e., at least 80 enterocytes) were evaluated per rat group (20 per rat in 4 rats per group). The number and diameter of HRP-containing endosomes were determined in 4 × 6-µm windows in the apical region (between the microvilli and the nucleus) of villus enterocytes, and the area occupied by such endosomes was calculated. Preliminary studies determined that endogenous peroxidase was not evident in epithelial cells or lamina propria of tissues from control or stressed rats.

**Statistical Analysis**

ANOVA and subsequent Newman-Keuls analyses were used to compare groups; single comparisons were performed.
using Student's t-test. Pearson's coefficient was used to determine correlations. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Tissue Conductance and Permeability to Cr-EDTA

Compared with controls, jejunal tissues obtained from stressed rats exhibited a significantly increased ($P < 0.013$) baseline conductance (Table 1). This abnormality was inhibited by treating rats with atropine before exposing them to stress. Atropine had no significant effect on tissue conductance in control rats. The stability of tissue conductance over time in all groups (i.e., lack of significant difference between initial values and those at the end of the experiment) indicated a lack of significant deterioration of the tissues during the 2-h experimental period. The flux of Cr-EDTA was also significantly increased ($P = 0.023$) across tissues from stressed rats compared with controls (Fig. 1). Again, pretreatment of rats with atropine abolished these stress-induced changes. There was a highly significant coefficient of correlation ($r = 0.89, P < 0.001$) for conductance values of individual tissues and their permeability to Cr-EDTA, as would be expected for paracellular transport of this small (molecular weight of 340) probe.

Protein Transport

Figure 2A illustrates the difference in fluxes of HRP among stressed and control rats and stressed rats administered atropine, in either of two salt preparations. Transport of HRP was also dramatically enhanced by stress, with the flux value approximately fourfold that across control tissues ($43.6 \pm 7.1$ vs. $10.3 \pm 2.1$ pmol·h$^{-1}$·cm$^{-2}$, mean ± SE; $P = 0.003$). The stress-induced increase in protein transport was prevented by atropine sulfate, but atropine sulfate did not significantly affect protein transport in tissues from control rats (atropine sulfate-treated stressed rats, $11.1 \pm 3.0$ pmol·h$^{-1}$·cm$^{-2}$; atropine sulfate-treated control rats, $6.9 \pm 1.5$ pmol·h$^{-1}$·cm$^{-2}$). Similar results were obtained in rats treated with atropine methyl nitrate ($14.6 \pm 2.7$ pmol·h$^{-1}$·cm$^{-2}$). Figure 2B confirms the dose-dependent inhibitory effect of atropine sulfate and justifies the use of the relatively high dose (10 mg/kg).

Table 1. Conductance values for jejunal tissues

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<th>Rat Group</th>
<th>20 min</th>
<th>120 min</th>
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<tr>
<td>Control</td>
<td>39.8±1.9</td>
<td>38.7±2.1</td>
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<td>Control (atropine sulfate)</td>
<td>40.4±2.9</td>
<td>43.3±3.6</td>
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<tr>
<td>Stress</td>
<td>50.1±3.1*</td>
<td>51.5±3.0†</td>
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<tr>
<td>Stress (atropine sulfate)</td>
<td>45.2±2.8</td>
<td>42.0±3.6</td>
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<tr>
<td>Stress (atropine methyl nitrate)</td>
<td>38.9±0.9</td>
<td>43.4±1.0</td>
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Data are means ± SE of conductance values (expressed as mS/cm$^2$) of jejunal tissues at beginning (20 min) and end (120 min) of experiment; 4 tissues were studied from each of 6 or 7 rats per group. Conductance was significantly increased (*$P = 0.011$; †$P = 0.013$) in tissues from stressed rats compared with controls.

Fig. 1. Effect of stress in atropine- or saline-treated rats on intestinal transport of Cr-EDTA across jejunal tissues mounted in Ussing chambers. Bars indicate mucosal-to-serosal flux values expressed as means ± SE; 3 or 4 tissues were studied from each of 7 rats per group. Fluxes of Cr-EDTA were significantly increased across tissues from stressed rats compared with other groups: *$P = 0.023$, stress compared with controls; †$P = 0.002$, stress + atropine sulfate (Atr-S) compared with stress.

Fig. 2. Intestinal transport of horseradish peroxidase (HRP) across jejunal tissues mounted in Ussing chambers. Bars indicate mucosal-to-serosal flux values expressed as means ± SE; 3 or 4 tissues were studied from each of 6 or 7 rats per group. A: effect of stress in atropine- or saline-treated rats. Overall significance is $P = 0.006$, with responses in stress group being significantly different from controls (*$P < 0.05$), and responses in stressed rats treated with 10 mg/kg of either atropine sulfate or atropine methyl nitrate (Atr-MN) are significantly different from responses in saline-treated stressed rats (#$P < 0.05$). B: inhibitory dose responses of atropine sulfate on intestinal responses to stress. Only 10 mg/kg atropine sulfate dose significantly reduced intestinal responses to stress (*$P = 0.003$).
Electron Microscopy

Transcellular pathway. Eighty high-power electron photomicrographs were evaluated per rat group to obtain qualitative and quantitative information on the uptake and pathway of HRP transport across the epithelium. No epithelial damage was observed in tissues from any rat group. HRP was clearly evident within endosomes in villus enterocytes of rats in all groups (Fig. 3, A–C). Endosomes appeared more dense and numerous in enterocytes from stressed rats (Fig. 3B) compared with controls (Fig. 3A) and atropine sulfate-treated stressed rats (Fig. 3C). A lower-power photomicrograph from a stressed rat (Fig. 4C) shows a large number of HRP-containing endosomes located throughout enterocytes and goblet cells. In the crypts, HRP was observed in endosomes within enterocytes (not shown) and Paneth cells and also aggregated on the microvilli of these cells (Fig. 3D).

The numbers of HRP-containing endosomes within 4 × 6-µm windows in the apical region of villus enterocytes are shown in Fig. 5A. A significantly greater (P = 0.013) number of HRP-positive endosomes were present in enterocytes of stressed rats (2.9 ± 0.3) compared with controls (1.4 ± 0.3). In addition, the area occupied by HRP was increased approximately fourfold (P = 0.015) in stressed rats compared with controls (Fig. 5B). Atropine sulfate treatment of rats before stress prevented the increased uptake of HRP. The amount of HRP in goblet cells was not quantified because the mucin content within the cells made this technically impossible, although our impression was that the pattern of transport was similar to that in enterocytes.

Paracellular pathway. In tissues from control rats, HRP was not demonstrated in the paracellular spaces between adjacent epithelial cells (Fig. 4A). In contrast, photomicrographs from 75% of stressed rats revealed HRP within paracellular spaces and tight junctions (Fig. 4B). This finding was most evident at the 120-min time point. Paracellular transport was apparent both in the villus region, where HRP penetrated the entire length of the lateral space (Fig. 6A), and in the crypts (Fig. 6B), where the depth of HRP penetration was incomplete but clearly past the tight junctional region in many instances. HRP was never observed in the paracellular spaces in stressed rats treated with atro-
pine sulfate. HRP fluxes were higher in tissues from rats where paracellular HRP was demonstrated; a significant correlation ($r = 0.79$, $P = 0.002$) was found between conductance values of tissues from rats in all groups and HRP fluxes. These findings suggested that the paracellular route contributed to the higher protein transport induced by stress.

In the lamina propria, HRP was observed inside phagocytes, particularly in eosinophils that appeared to be activated by this process because their granules were hypodense (Fig. 6C). Some mast cells also appeared activated, and released mast cell granules were found in the tissue (Fig. 6D). Such observations were frequent in tissues from stressed rats but rare in tissues from control rats.

**DISCUSSION**

Two previous studies have reported that severe physical stress (surgical trauma or burns) perturbs the intestinal barrier (6, 29). To our knowledge, our study is the first to document that a short period of relatively mild stress enhances intestinal epithelial permeability to macromolecules. Overall transport of HRP was increased by stress, and HRP was demonstrated to penetrate jejunal epithelium via both the transcellular and paracellular pathways. The number of HRP endosomes and their relative area inside enterocytes were significantly increased by restraint stress. HRP-containing endosomes were also observed in goblet cells and Paneth cells of stressed rats. HRP was visualized in the paracellular spaces between adjacent epithelial cells in stressed but not in control rats. In addition, our study implicated cholinergic mechanisms in the stress-induced epithelial abnormalities because treatment of rats with the muscarinic antagonist atropine prevented the changes in barrier function.

Our earlier studies (31) documented that Wistar-Kyoto rats react to acute stress with alterations in intestinal epithelial physiology. Ion secretion was stimulated and permeability to ions and the small inert probes, mannitol and Cr-EDTA, was enhanced. The transport abnormalities were maintained over the 3 h of the experiment and did not return to normal values until 24 h later. We also recorded a reduced $I_{sc}$ response to electric transmural stimulation of nerves in tissues from stressed rats. However, normal $I_{sc}$ responses to...
and atropine methyl nitrate treatment of rats before stress inhibited the fivefold increase in HRP flux caused by acute restraint stress. Ultrastructural analysis showed that the number and size of endosomes in a fixed region of enterocytes were significantly increased after HRP addition to the mucosal surface of tissue from stressed rats. Again, muscarinic blockade with atropine prevented the stress-induced increases. These findings are compatible with those from previous studies that have implicated nerves in the regulation of epithelial barrier function. General and specific neural blockade have been demonstrated to inhibit transepithelial transport of proteins such as bovine serum albumin and ovalbumin (11, 17). Other studies (5, 28) have shown that the cholinergic agonist carbachol increased transepithelial transport of HRP via transcellular and paracellular pathways in rat ileum. Carbachol also stimulated secretion of mucin from goblet cells (27), and stress has also been shown to induce mucus secretion (7). Therefore, cholinergic stimulation may be involved in the uptake of HRP into these cells. We found HRP bound to the apical membrane and within another secretory cell type in crypt epithelium, the Paneth cell. Our studies did not provide information on the possible mechanisms that might account for this effect.

HRP within tight junctions and paracellular spaces was observed in photomicrographs of intestinal tissues obtained from stressed rats but never in photomicrographs of tissues from control rats or stressed rats treated with atropine. The protein was visible along the entire length of the intercellular spaces between villus cells, but penetration appeared to be more limited between crypt cells, possibly due to the reduced ability of the macromolecule to enter the crypts. The presence of HRP in the paracellular regions was more evident in sections fixed at later times, suggesting gradual accumulation of HRP in this pathway. Protein in the paracellular spaces of specific tissues was associated with increased conductance, and the conductance values also correlated with HRP fluxes. Taken together, these findings suggest that stress enhances the permeability of the paracellular pathway, not only to Cr-EDTA but also to macromolecular proteins.

Tight junctions are impermeable to proteins under normal circumstances (21), although physiological regulation of tight junctional permeability to ions and small molecules is recognized. However, under certain conditions, including cholinergic stimulation with carbachol, HRP penetration of the tight junctions has been reported (5). Carbachol causes release of Ca$^{2+}$ from intracellular stores and activation of protein kinase C (PKC) in cultured epithelial cells (20); activation of PKC has been implicated in loosening of tight junctions (34). Decreased resistance of tight junctions in epithelial monolayers was shown to be associated with F-actin rearrangements and phosphorylation of myosin light chain in response to T cell activation or bacterial attachment (23, 33). Stress may be an extreme situation where cholinergic stimulation in combination with

Fig. 5. Effect of stress on uptake of HRP into villus enterocytes. Number (A) and area (B) of HRP product-filled endosomes in enterocytes of tissues fixed 60 min after delivery of HRP to mucosal buffer in Ussing chambers are shown. Bars indicate values (expressed as means ± SE) for 4 × 6-µm windows in apical region (between the microvilli and the nucleus) of villus enterocytes; 20 such windows were evaluated for each rat, 4 rats per group (i.e., 80 windows per group); values per rat were averaged to obtain values shown (n = 4). Endosome number and area were significantly increased in tissues from stressed rats compared with other groups. A: *P = 0.013, stress compared with control; **P = 0.002, stress + atropine sulfate compared with stress. B: *P = 0.015, stress compared with control; **P = 0.005, stress + atropine sulfate compared with stress.

several secretagogues were not affected by stress, suggesting that stress induced neurotransmitter release from intestinal mucosal neurons.

In a subsequent study (30), we determined that Wistar-Kyoto rats respond to stress with epithelial abnormalities several times greater than those in Wistar rats (the parent strain). Our finding that Wistar-Kyoto rats have reduced activity of cholinesterase in intestinal mucosa suggested a role for cholinergic mechanisms in the gut abnormalities. Both atropine sulfate and atropine methyl nitrate, a quaternary salt that does not cross the blood-brain barrier, inhibited the stress-induced intestinal pathophysiology, suggesting a peripheral rather than a central location for muscarinic receptors. In contrast, hexamethonium had no effect. Those studies implicated acetylcholine released by stress in the changes in intestinal mucosal function.

In this study, we demonstrated that stress resulted in an impairment of the epithelial barrier to macromolecules and that cholinergic mechanisms were also involved in mediating this effect. Both atropine sulfate

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**Fig. 5.** Effect of stress on uptake of HRP into villus enterocytes. Number (A) and area (B) of HRP product-filled endosomes in enterocytes of tissues fixed 60 min after delivery of HRP to mucosal buffer in Ussing chambers are shown. Bars indicate values (expressed as means ± SE) for 4 × 6-µm windows in apical region (between the microvilli and the nucleus) of villus enterocytes; 20 such windows were evaluated for each rat, 4 rats per group (i.e., 80 windows per group); values per rat were averaged to obtain values shown (n = 4). Endosome number and area were significantly increased in tissues from stressed rats compared with other groups. A: *P = 0.013, stress compared with control; **P = 0.002, stress + atropine sulfate compared with stress. B: *P = 0.015, stress compared with control; **P = 0.005, stress + atropine sulfate compared with stress.
other factors may result in an increase in tight junctional permeability that extends to macromolecules.

Our studies implicate cholinergic nerves in the stressed-induced epithelial pathophysiology but do not rule out other mediators or mechanisms. Evidence indicates there may also be effects via mucosal immune cells such as mast cells (7). Studies have reported that vagal stimulation can cause histamine release from mast cells in rat ileum (3). In addition, acute stress resulted in release of substance P from guinea pig airways and chronic stress caused reduced tissue levels of substance P (1), a neuropeptide that can activate immune cells, including mast cells (32). The electron photomicrographs from this study showed eosinophils and other phagocytes in the lamina propria containing HRP. Reduced density and numbers of eosinophil granules and the presence of free mast cell granules in the lamina propria suggested activation of these cells. However, it is clear that cholinergic nerves are critical in the pathway because atropine treatment of rats prevented the stress-induced epithelial barrier defect.

Our findings may be important for understanding the role of stress in allergic and inflammatory conditions. Stress may increase epithelial permeability facilitating passage of protein antigens, including food allergens and microbial toxins and products, from the gut lumen. The excessive uptake of antigens may trigger an immune response. For example, stress in sensitive individuals may increase allergen uptake and result in a local anaphylactic reaction due to mast cell activation. Release of mast cell mediators, such as histamine, may also be enhanced by cholinergic mechanisms (36) and would further increase epithelial permeability (11) and stimulate secretory activity (10). In addition, there is evidence that intracellular processing of antigens by enterocytes is necessary for T cell suppression of immune responses resulting in oral tolerance (22). Epithelial penetration of proteins via the paracellular route would avoid such protective mechanisms. The consequence of transcellular passage of proteins through cells in the epithelium, such as goblet cells, remains to be determined. Our previous finding that the degree of epithelial dysfunction induced by stress varies in different rat strains together with the results of this study suggest that genetic factors relating to cholinergic sensitivity may be important in regulating the intestinal epithelial barrier and thus determining the predisposition of an individual to stress-induced intestinal inflammation.
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