Corticotropin-releasing hormone mimics stress-induced colonic epithelial pathophysiology in the rat

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1Intestinal Disease Research Program, Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5; and
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Santos, Javier, Paul R. Saunders, Nico P. M. Hanssen, Ping-Chang Yang, Derrick Yates, Jack A. Groot, and Mary H. Perdue. Corticotropin-releasing hormone mimics stress-induced colonic epithelial pathophysiology in the rat. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G391–G399, 1999.—We examined the effect of stress on colonic epithelial physiology, the role of corticotropin-releasing hormone (CRH), and the pathways involved. Rats were restrained or injected intraperitoneally with CRH or saline. Colonic segments were mounted in Ussing chambers, in which ion secretion and permeability (conductance and probe fluxes) were measured. To test the pathways involved in CRH-induced changes, rats were pretreated with hexamethonium, atropine, bretylium, doxantrazole, α-helical CRH-(9–41) (all intraperitoneally), or aminoglutethimide (subcutaneously). Restraint stress increased colonic ion secretion and permeability to ions, the bacterial peptide FMLP, and horse-radish peroxidase (HRP). These changes were prevented by α-helical CRH-(9–41) and mimicked by CRH (50 μg/kg). CRH-induced changes in ion secretion were abolished by α-helical CRH-(9–41), hexamethonium, atropine, or doxantrazole. CRH-stimulated conductance was significantly inhibited by α-helical CRH-(9–41), hexamethonium, bretylium, or doxantrazole. CRH-induced enhancement of HRP flux was significantly reduced by all drugs but aminoglutethimide. Peripheral CRH reproduced stress-induced colonic epithelial pathophysiology via cholinergic and adrenergic nerves and mast cells. Modulation of stress responses may be relevant to the management of colonic disorders.

neuroimmune interactions; stress; colon; Wistar Kyoto rats

CLINICAL EVIDENCE INDICATES that life stress is associated with reactivation of colonic disorders (4, 8). However, the mechanism by which stress influences colonic pathophysiology remains obscure. Corticotropin-releasing hormone (CRH) has been implicated in various stress-induced abnormalities, including changes in both human and rat gastrointestinal functions such as motility and gastric acid secretion (19, 34).

Epithelial cells in the gastrointestinal tract have recently been recognized as critical cells in the regulation of immunoinflammatory disorders (10), with both an active role (production of cytokines and chemokines) and a passive role (acting as a barrier to limit the uptake of luminal antigens). However, relatively little information is available regarding the effects of stress and CRH on gastrointestinal epithelial physiology, particularly in the colon. It has been shown that physical and psychological stressors induce marked alterations in water and electrolyte transport across the epithelium in human and rat small intestine (2, 31, 32). In addition, epithelial permeability to small and large molecules has been reported to be enhanced by stress in the rat jejunum (17, 32). Although the colon is considered as a relatively tight epithelium for medium or large size molecules, permeation by the bacterial peptide FMLP (mol mass 438 kDa) and insulin (mol mass 5 kDa) does occur in the intact colon (21, 29). Factors that modulate tight junction resistance, such as prostaglandins, seem to be involved in this phenomenon, although the ultimate mechanism remains to be elucidated (21, 29). Increased transepithelial uptake of macromolecules, such as food or microbial-derived antigens, could provide a pathogenic link between intestinal barrier defects and immunologic abnormalities that may be involved in the initiation and/or relapses of mucosal inflammation in inflammatory bowel diseases, especially in stress-susceptible individuals (43).

On the basis of previous findings related to the role of CRH in stress-induced changes in gastrointestinal physiology (19, 34), we hypothesized that CRH might produce stress-like colonic epithelial abnormalities. The specific aims of the present study were to compare the effects of stress and peripheral CRH on colonic epithelial ion secretion and permeability to ions and macromolecules and to determine the mechanisms involved in the CRH-induced mucosal pathophysiology.

METHODS

Animals

Male Wistar Kyoto rats (200–250 g) from Charles River Laboratories, (St. Constant, PQ, Canada), housed two per cage, were maintained on a normal 12:12-h dark-light cycle and provided with food and water ad libitum. Rats were handled daily by the same investigator for 2 wk before the study. The experimental procedures were performed at the same time of the day, between 8:00 and 10:00 AM, to minimize the effect of circadian rhythm. Rats were euthanized by decapitation. All procedures were approved by the Animal Care Committee at McMaster University.

Drugs and Treatments

Stress protocol. Rats were placed in a Plexiglas adjustable restraining device (6 × 21.5 cm) for 2 h at 8°C, followed by 2 h of free movement in their home cage at 22°C. Control rats were maintained in their home cage.

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Drugs. CRH and α-helical CRH-(9—41) (Peninsula Laboratories, Belmont, CA) were dissolved according to the manufacturer's instructions, aliquoted, and kept frozen at -80°C until used. Immediately before the experiments, the peptides were diluted in saline for intraperitoneal injections. Doxantrazole, a gift from Burroughs Wellcome (Research Triangle Park, NC), atropine sulfate, bretylium tosylate, and hexamethonium (all from Sigma, St. Louis, MO) were dissolved immediately before the experiments and administered intraperitoneally in a 1-ml/kg volume injection.

We used aminoglutethimide (Sigma) to assess the participation of the hypothalamic-pituitary-adrenal axis in the colonic epithelial response to CRH. Aminoglutethimide was dissolved in 50% DMSO and injected subcutaneously in a 1-ml/kg volume. To completely block the synthesis of adrenal steroids, rats were given aminoglutethimide injections at 25, 21, and 17 h (83 mg/kg) and at 0.5 h (50 mg/kg) before CRH or vehicle administration. Similar doses have been shown to induce prolonged suppression of adrenal steroidogenesis (11).

**Epithelial Colonic Measurements**

Using chamber studies. The distal colon was removed, placed in 37°C oxygenated Krebs buffer, stripped of longitudinal muscle and myenteric plexus, and opened along the mesenteric border. Four adjacent pieces from each rat were mounted in Ussing chambers (W-P Instruments, Narco Scientific, Mississauga, ON, Canada). The chamber opening exposed 0.6 cm² of tissue surface area to 8 ml of circulating K-rebs 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 (Iₛₛ) to maintain a zero potential difference as registered via an automated voltage clamp (W-P Instruments). Iₛₛ (µA/cm²) was recorded continuously by a computer connected to the voltage clamp system. Tissue conductance (G) was calculated according to Ohm's law and expressed as mS/cm².

Mucosal-to-serosal transport of probes was determined by measuring transepithelial flux of FMLP and horseradish peroxidase (HRP). In some studies, radiolabeled [³²P]FMLP (DuPont, Wilmington, DE) was added to the mucosal buffer (1 Ci/ml) and brought up to a concentration of 10⁻⁵ M with cold FMLP (Sigma) and was allowed to equilibrate for 25 min. Serosal samples (1 ml) were taken every 20 min for 2 h, and mucosal samples (0.05 ml) were taken at the beginning and end of the experiment. Buffers were replaced as required to keep the volume constant. The radioactivity of [³²P]FMLP was measured in a scintillation counter. Fluxes were calculated by standard formulas (22) and expressed as pmol·h⁻¹·cm⁻².

HRP was used as a model protein to examine macromolecular permeability because it can be quantitatively measured with an enzymatic assay and the reaction product visualized as electron-dense material in ultrastructural studies (17). Fifteen minutes after mounting the tissues, type VI HRP (Sigma) was added to the luminal buffer at a final concentration of 10⁻⁵ M and was allowed to equilibrate for 30 min. Serosal samples (0.5 ml) were obtained at 30-min intervals for 2 h and replaced with K-rebs buffer to maintain a constant volume in the chambers. HRP activity was determined by a modified Worthington method (23). Briefly, 0.15 ml of sample was mixed with 0.8 ml of phosphate buffer (pH 6.0) containing 0.003% H₂O₂ and 0.009% α-dianisidine (Sigma). Enzyme activity was determined from the increase in optical density at 460 nm during a 1.5-min period. Mucosal-to-serosal fluxes of HRP were calculated according to standard formulas and expressed as pmol·h⁻¹·cm⁻².

**Epithelial uptake of HRP.** Epithelial uptake of HRP was measured in electron photomicrographs. Tissues were removed 2 h after the addition of HRP to the chambers, were fixed in 2.5% glutaraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.4) for 2 h at 22°C, were rinsed for 18 h (4°C) with 0.05 Tris buffer (pH 7.6), and then were washed three times for 5 min. Methods for HRP product identification were modified from Graham and Karnovsky (12). 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 for 15 min in the same buffer containing 0.01% H₂O₂. Tissues were then processed for routine transmission electron microscopy. Quantitative analysis was performed on coded high-magnification photomicrographs, 60 per group (3–4 tissues from each of 4 rats/group). The area of HRP-containing endosomes within colonic enterocytes was determined (with a computerized image analysis system) in the apical region (between the microvilli and the nucleus), the midregion (adjacent to the nucleus), and the basal region (between the nucleus and the basal membrane) and was reported as µm²/window (window area 25 × 30 µm²). Tissues were also examined for evidence of paracellular HRP transport.

**Experimental Design**

In the first part of the study, we determined the effects of stress on colonic Iₛₛ, G, and fluxes of FMLP and HRP and compared results with the effects induced by different doses of CRH (5, 12.5, 25, and 50 µg/kg ip). Pilot experiments showed that epithelial transport abnormalities were maximal at 4 h after injecting CRH or beginning the restraint stress. Baseline values for Iₛₛ, as an indicator of ion secretion, and G, as an indicator of ion permeability, were calculated at equilibrium, 15 min after mounting the tissues, and then every 30 min for 2 h. Fluxes of FMLP and 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.

In the second part of the study, we evaluated whether peripheral CRH contributed to the colonic stress responses. Rats were injected intraperitoneally with either saline or α-helical CRH-(9—41) (250 µg/kg), 30 min before the stress protocol. Ussing chamber studies were conducted as described and colonic Iₛₛ, G, and HRP flux examined.

Finally, to characterize the mechanisms involved in the response to intraperitoneal CRH, the same experimental protocol was followed. Thus 30 min before the administration of the higher dose of CRH (50 µg/kg; maximal colonic responses) or its vehicle, different groups of rats were treated with α-helical CRH-(9—41) (250 µg/kg ip), doxantrazole (20 mg/kg), hexamethonium (15 mg/kg), atropine sulfate (1 mg/kg), bretylium tosylate (15.8 mg/kg), or aminoglutethimide (300 mg/kg). The doses of α-helical CRH-(9—41), doxantrazole, hexamethonium, atropine, and bretylium were based on previous studies showing effective blocking of CRH receptors, mucosal mast cell activation, or muscarinic, nicotinic, and adrenergic receptors, in comparable experimental conditions (7, 36). Ussing chamber studies were then conducted as previously described.

**Statistical Analysis**

Results are expressed as means ± SE. Multiple groups were compared by Dunnett's test and Fisher's exact test after a significant one-way ANOVA. Single comparisons were per-
formed by paired or unpaired Student's t-test when appropriate. P values of <0.05 were considered significant.

RESULTS

Comparison of Stress and CRH Effects on Colonic Epithelial Physiology

Stress induced a significant increase in both baseline \( I_{sc} \) and G compared with controls. CRH (50 \( \mu \)g/kg) also induced significant increases in both baseline \( I_{sc} \) and G compared with controls that were similar to those induced by stress (Table 1).

Both stress and CRH (50 \( \mu \)g/kg) induced a two- to threefold increase in HRP fluxes compared with their controls (Table 1). In addition, tissues from stressed rats were twice as permeable to FMLP (1.04 \( \pm \) 0.52 vs. 0.52 \( \pm \) 0.13 pmol·h\(^{-1} \)·cm\(^{-2} \), \( P < 0.05 \)) as tissues from control rats (6 rats/group).

The stimulatory effects of intraperitoneal CRH on rat colonic \( I_{sc} \) and G were dose dependent (Table 2). The averaged percent increase in \( I_{sc} \) over vehicle values was 36, 61, 64, and 96% for 5, 12.5, 25, and 50 \( \mu \)g/kg of CRH, respectively. CRH also increased G over vehicle values by 23, 20, 30, and 35% for 5, 12.5, 25, and 50 \( \mu \)g/kg of CRH, respectively. HRP flux was also enhanced in a dose-dependent manner, ranging from a threefold increase at the highest dose of CRH (14.4 \( \pm \) 2.7 pmol·h\(^{-1} \)·cm\(^{-2} \)) to no significant increase at the lower doses (Fig. 1), compared with animals injected with vehicle (5.2 \( \pm \) 0.6 pmol·h\(^{-1} \)·cm\(^{-2} \)).

Stress and CRH Enhanced Transepithelial Uptake of HRP

HRP was evident within endosomes in colonocytes from stress and CRH rats as well as in their controls (Fig. 2, A–C). However, endosomes appeared significantly more dense and numerous in stressed and CRH groups compared with controls, and the area of HRP-containing endosomes was significantly increased in all of the apical, mid, and basal regions of colonic enterocytes (Fig. 3). In addition, HRP was observed within tight junctions and paracellular spaces but only in tissues from stressed and CRH groups (Fig. 2D). This finding was present in every rat but not in all tissue sections.

Pharmacological Modulation of Stress and CRH-Stimulated Colonic \( I_{sc} \), G, and HRP Flux

Treatment with \( \alpha \)-helical CRH-(9–41) (250 \( \mu \)g/kg ip) inhibited both the stress-stimulated (Fig. 4) and CRH-stimulated (Fig. 5) increases in baseline \( I_{sc} \) and G. HRP flux increase was abolished in both groups by \( \alpha \)-helical CRH-(9–41) (16.6 \( \pm \) 3.4 vs. 6.3 \( \pm \) 3.3 pmol·h\(^{-1} \)·cm\(^{-2} \), \( P < 0.05 \)), stress vs. \( \alpha \)-helical CRH-(9–41) + stress; 14.4 \( \pm \) 2.7 vs. 3.75 \( \pm \) 0.6 pmol·h\(^{-1} \)·cm\(^{-2} \), \( P < 0.0001 \), CRH vs. \( \alpha \)-helical CRH-(9–41) + CRH). The antagonist of CRH, \( \alpha \)-helical CRH-(9–41), did not modify epithelial parameters when administered alone to control rats.

Hexamethonium (15 mg/kg ip) or atropine (1 mg/kg ip) abolished the CRH-stimulated increase in baseline \( I_{sc} \), whereas bretylium (15.8 mg/kg ip) showed no effect (Fig. 6). The increase in G induced by CRH was reduced by hexamethonium or bretylium but was not altered by atropine (Fig. 6). The increase in HRP flux induced by CRH was reduced by hexamethonium, atropine, or bretylium, all to a similar extent (from 14.4 \( \pm \) 2.7 to 7.45 \( \pm \) 2.4, 7.9 \( \pm \) 1.8, and 8.0 \( \pm \) 3.4 pmol·h\(^{-1} \)·cm\(^{-2} \), respectively, \( P < 0.05 \) vs. CRH). No changes in epithelial parameters were observed when each drug was administered in control rats.

Table 1. Effect of restraint stress and CRH on colonic epithelial function

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( I_{sc} ), ( \mu )A/cm(^2 )</th>
<th>G, mS/cm(^2 )</th>
<th>HRP flux, pmol·h(^{-1} )·cm(^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham RS</td>
<td>38.8 ( \pm ) 2.6</td>
<td>17.3 ( \pm ) 2.5</td>
<td>6.6 ( \pm ) 1.4</td>
</tr>
<tr>
<td>RS</td>
<td>88.4 ( \pm ) 9.3†</td>
<td>26.7 ( \pm ) 1.5</td>
<td>16.8 ( \pm ) 4.7†</td>
</tr>
<tr>
<td>Saline</td>
<td>40.2 ( \pm ) 4.5</td>
<td>18.5 ( \pm ) 1.0</td>
<td>4.1 ( \pm ) 0.6</td>
</tr>
<tr>
<td>CRH 50</td>
<td>78.5 ( \pm ) 5.4†</td>
<td>25.3 ( \pm ) 1.1*</td>
<td>15.1 ( \pm ) 1.7†</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 6 \) rats with 2–4 tissues averaged for each rat. Rats were submitted to restraint stress (RS) or sham stress for 2 h or injected intraperitoneally with corticotropin-releasing hormone (CRH) (50 \( \mu \)g/kg) or saline. Four hours after the beginning of stress or injections, colonic segments were mounted in Ussing chambers. Short-circuit current (\( I_{sc} \)) and tissue conductance (G) values were taken at baseline. Horseradish peroxidase (HRP) flux is the averaged value of 2 consecutive stable flux periods. \( * P < 0.05 \) vs. corresponding control; \( † P < 0.005 \) vs. corresponding control.

Fig. 1. Dose effect of intraperitoneal corticotropin-releasing hormone (CRH) on colonic horseradish peroxidase (HRP) flux. HRP (10\(^{-5} \) M) was added to luminal side of colonic tissues from rats treated with CRH (5–50 \( \mu \)g/kg ip) 4 h previously. HRP flux is average value of 2 consecutive 30-min stable flux periods. All values are expressed as means \( \pm \) SE; \( n = 4–7 \) rats/group, with 2–4 tissues averaged for each rat. \( * P < 0.05 \) vs. vehicle; \( † P < 0.005 \) vs. vehicle.

Table 2. Effect of intraperitoneal CRH on colonic baseline electrophysiological values

<table>
<thead>
<tr>
<th>CRH Dose, ( \mu )g/kg</th>
<th>( I_{sc} ), ( \mu )A/cm(^2 )</th>
<th>G, mS/cm(^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (vehicle)</td>
<td>38.8 ( \pm ) 4.6</td>
<td>19.8 ( \pm ) 1.4</td>
</tr>
<tr>
<td>5</td>
<td>52.8 ( \pm ) 7.0</td>
<td>24.4 ( \pm ) 1.9</td>
</tr>
<tr>
<td>12.5</td>
<td>62.4 ( \pm ) 9.3*</td>
<td>23.8 ( \pm ) 1.8</td>
</tr>
<tr>
<td>25</td>
<td>63.7 ( \pm ) 7.0*</td>
<td>25.8 ( \pm ) 1.9*</td>
</tr>
<tr>
<td>50</td>
<td>76.1 ( \pm ) 6.6†</td>
<td>26.7 ( \pm ) 1.4†</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 4–7 \) rats with 2–4 tissues averaged for each rat. \( * P < 0.05 \) vs. vehicle; \( † P < 0.005 \) vs. vehicle.
Treatment with doxantrazole (20 mg/kg) reduced the CRH-stimulated increases in baseline $I_{sc}$, $G$, and HRP flux by 74, 73, and 96%, respectively (Fig. 7). In contrast, aminoglutethimide did not change these parameters. Neither doxantrazole nor aminoglutethimide changed $I_{sc}$, $G$, and HRP when administered to control rats.

**DISCUSSION**

Our results show that stress stimulated distal colonic secretion, indicated by increased $I_{sc}$, $G$, and HRP flux by 74, 73, and 96%, respectively (Fig. 7). In contrast, aminoglutethimide did not change these parameters. Neither doxantrazole nor aminoglutethimide changed $I_{sc}$, $G$, and HRP when administered to control rats.

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In this study, we have extended our previous findings (17, 32) to show that stress-induced pathophysiology occurs not only in the jejunum but also in the distal colon. Studies from other laboratories have documented stress-induced increases in mucus production (5), transit (34), and fluid secretion in rat colon (9). These responses, along with the stress-induced increases in ion secretion reported in this study, indicate

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**Fig. 2.** Effect of stress and CRH on transepithelial transport of HRP. Representative electron photomicrographs showing HRP product-containing endosomes (arrowheads) could be identified in colonocytes from (A) stressed rat, (B) control rat, and (C) rat injected with CRH (50 µg/kg ip). Number of HRP-containing endosomes was greater in colonocytes in stressed or CRH-treated rats than in controls. Distribution of HRP endosomes in control rats was mainly in apical region, whereas in stressed or CRH-treated rats, HRP endosomes were also observed throughout cell. D: paracellular spaces between colonocytes also contained HRP (arrows) in colonic tissue from a stressed rat. Scale bar = 2 µm.
that stress may contribute to host defense by flushing noxious material out of the lumen. However, other effects of stress may be detrimental, and, in particular, stress-induced epithelial responses may be involved in the initiation or potentiation of relapses of inflammatory bowel disease. Several reports suggest that intestinal permeability is increased in inflammatory bowel disease patients (25, 43); increased uptake of food or microbial antigens may activate resident immune cells, leading to an inflammatory response. Our study shows that colonic epithelium from stressed rats was significantly more permeable to bioactive molecules such as the bacterial proinflammatory peptide FMLP. Moreover, the stress-induced barrier defect extended to macromolecular proteins with antigenic properties such as HRP (mol mass 44 kDa). We previously showed that stress caused a dramatic increase in the flux of HRP across jejunal epithelium by stimulating transport via both intracellular and paracellular pathways (17). Here, we showed that the colon also responds to stress with enhanced transepithelial protein transport via transcellular and paracellular routes through tight junctions.

Peripheral administration of CRH has been shown to mediate or induce stresslike responses in the gastrointestinal tract, including decreased gastric acid secretion (19), increased duodenal bicarbonate secretion (19), and motility changes (34). The direct effects of CRH are mediated by the activation of specific receptors on the target cell population (37). In our study, intraperitoneal CRH increased $I_{sc}$, $G$, and HRP permeability, and these responses were inhibited by intraperitoneal $\alpha$-helical CRH-(9—41). Because both $\alpha$-helical CRH-(9—41) and CRH are believed not to cross the blood-brain barrier from the periphery to the brain (24), our findings indicate that colonic abnormalities induced by CRH were mediated by peripheral CRH receptors. At least two different subtypes of CRH...
receptors have been described in the periphery, CRH-R1 and CRH-R2β (37). Our work did not identify the population of receptors involved. More specific agonists and/or antagonists for CRH receptor subtypes will be required to address this particular issue. The importance of peripheral CRH receptors in stress responses in the intestinal tract is highlighted in our study because the effects of stress on colonic physiology were also inhibited by intraperitoneal α-helical CRH-(9—41).

Colonic secretion and permeability are regulated by neural and immune factors. In the present study, the increase in I_sc induced by CRH was abolished by both the muscarinic antagonist atropine and by the nicotinic

Fig. 5. Effect of α-helical CRH-(9—41) on CRH-stimulated increases in colonic I_sc (A), G (B), and HRP flux (C). Rats were injected with α-helical CRH-(9—41) (250 µg/kg ip) 30 min before CRH (50 µg/kg ip). Bars represent means ± SE; n = 4–7 rats/group, with 3–4 tissues averaged per rat. Open bars represent vehicle; closed bars represent CRH. †P < 0.005 vs. corresponding control.

Fig. 6. Effect of hexamethonium (HEX), atropine sulfate (ATR), and bretylium tosylate (BRT) on CRH-stimulated increases in colonic I_sc (A), G (B), and HRP flux (C). Rats were injected with HEX (15 mg/kg ip), ATR (1 mg/kg ip), or BRT (15.8 mg/kg ip) 30 min before CRH (50 µg/kg ip). Bars represent means ± SE; n = 4–7 rats/group, with 2–4 tissues averaged per rat. Open bars represent vehicle; closed bars represent CRH. *P < 0.05 vs. corresponding control; †P < 0.005 vs. corresponding control.
antagonist hexamethonium but was not affected by the adrenergic blocker bretylium. Previous studies have shown that muscarinic receptors on epithelial cells and enteric neurons in rat colon accounted for 70% of the increase in response to cholinergic receptors, whereas the remaining 30% was mediated by nicotinic receptors on enteric neurons (7, 27). These studies, in conjunction with the present report, suggest that the CRH-induced stimulation of colonic I$_{sc}$ may have been mediated by the release of acetylcholine.

CRH also increased colonic G, indicating enhanced permeability to ions. This increase was blocked by hexamethonium and bretylium but not affected by atropine, suggesting that the effects of CRH on colonic G are mediated by both nicotinic and adrenergic receptors. We showed that the CRH-stimulated increase of HRP flux was reduced by muscarinic, nicotinic, and adrenergic antagonists. Cholinergic mechanisms have been implicated previously in macromolecular transport because stress-enhanced permeability to HRP in the rat jejunum was reduced by atropine (17), and other studies have shown that cholinergic agonists and vagal stimulation increased intestinal epithelial permeability (28, 30). A recent report indicated that paracellular permeability to Evans blue (mol mass 961 kDa) was regulated by adrenergic mechanisms (18). Our results, along with those from previous studies, suggest a complex interplay between sympathetic and parasympathetic nerves in the regulation of colonic permeability.

We also showed that the CRH-stimulated responses in colonic epithelium were dependent on mast cell activation because they were abolished by the mast cell stabilizer doxantrazole. Mast cells contain mediators, such as prostaglandins, histamine, and serotonin, that directly alter epithelial transport properties. Moreover, other stress-induced epithelial responses, such as colonic mucin release, were absent in mast cell-deficient mice, whereas reconstitution of bone marrow with mast cells reversed that response to normal stress values (6). Previous reports showing mast cell proximity to CRH-like nerves in the brain (36), expression of CRH-R$_1$ on cultured human leukemic mast cells (35), and degranulation of mast cells in the skin and colon with parallel increases in vascular permeability and mucin secretion, respectively, after peripheral administration of CRH (5, 35) support the hypothesis that mast cells could have been activated directly by CRH. However, colonic mast cells could have been activated indirectly by neuropeptides released from CRH-stimulated neural processes. This view is supported by studies showing close proximity between peptidergic terminals and mast cells in the rat intestinal mucosa (33), degranulation of mast cells by substance P in the intestine (14), and a functional interaction between intestinal mast cells and neuropeptide-containing nerves reflected by a mast cell-mediated increase in intestinal ion secretion on substance P administration (41). Despite the nature of the mechanism of mast cell activation, our study illustrates that cross talk between neural and immune cells is involved in the regulation of epithelial transport functions in the colon.

Peripheral CRH stimulates corticotropin release that ultimately causes adrenal steroid secretion (37). Because both mineral corticoids and glucocorticoids participate in the regulation of ion transport in the rat distal colon (3), we blocked their synthesis with aminoglutethimide, to test the role of CRH-induced steroid release in the observed colonic abnormalities. Aminoglutethimide did not alter basal or CRH-stimulated colonic responses, making it unlikely that steroids contributed to these effects.
Our study raises some interesting questions that remain to be answered, including the cellular origin of CRH in the periphery and its site of action. In the colon, CRH mRNA has been detected in the vicinity of the base of the crypts, probably being produced by enterochromaffin cells (16). CRH-like immunoreactivity has also been described in myofibroblasts, autonomic ganglia, and intrinsic and extrinsic neural cells (20, 26, 33, 40, 42). Furthermore, CRH is synthetized by several immunocytes on appropriate stimulation (15). Finally, colonic entocytes themselves may produce CRH, because their ability to secrete CRH and other regulatory molecules has been reported (39). Because these elements are represented in the intestinal wall, this group of studies, in conjunction with ours, suggests that local release of CRH in the gastrointestinal tract could play an important role in the modulation of epithelial physiology.

Other than mast cells, potential targets for CRH in the periphery may include other immune cells, as well as neural, epithelial, or endothelial cells. These cells might be partially responsible for some of the CRH-induced changes in epithelial physiology. Receptors for CRH have been demonstrated by molecular analysis in the intestinal wall (20). Binding and pharmacological studies have also provided evidence for CRH receptors on several immune cells and intrinsic and extrinsic neurons (1, 13, 15, 36, 38). The presence of CRH receptors on colonocytes is presently unresolved, although the requirement of mast cell activation for the full expression of CRH-induced changes in colonic physiology in our study suggests that these receptors are unlikely in colonic epithelium.

In conclusion, we have demonstrated that stress induced marked alterations in colonic epithelial physiology via CRH-mediated pathways involving mast cells and nerves. Recognition of the potentially adverse effects of stress in the gastrointestinal tract may lead to novel preventive and therapeutic approaches in colonic functional and inflammatory disorders.

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