Chronic peripheral administration of corticotropin-releasing factor causes colonic barrier dysfunction similar to psychological stress

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Teitelbaum AA, Gareau MG, Jury J, Yang PC, Perdue MH. Chronic peripheral administration of corticotropin-releasing factor causes colonic barrier dysfunction similar to psychological stress. Am J Physiol Gastrointest Liver Physiol 295: G452–G459, 2008. First published July 17, 2008; doi:10.1152/ajpgi.90210.2008.—Chronic psychological stress causes intestinal barrier dysfunction and impairs host defense mechanisms mediated by corticotrophin-releasing factor (CRF) and mast cells; however, the exact pathways involved are unclear. Here we investigated the effect of chronic CRF administration on colonic permeability and ion transport functions in rats and the role of mast cells in maintaining the abnormalities. CRF was delivered over 12 days via osmotic minipumps implanted subcutaneously in wild-type (+/+ ) and mast cell-deficient (Ws/Ws) rats. Colonic segments were excised for ex vivo functional studies in Ussing chambers [short-circuit current (Isc), conductance (G), and macromolecular permeability (horseradish peroxidase flux)], and analysis of morphological changes (mast cell numbers and bacterial host-interactions) was determined by light and electron microscopy. Chronic CRF treatment resulted in colonic mucosal dysfunction with increased Isc, G, and horseradish peroxidase flux in +/+ but not in Ws/Ws rats. Furthermore, CRF administration caused mast cell hyperplasia and abnormal bacterial attachment and/or penetration functions into the mucosa only in +/+ rats. Finally, selective CRF agonist/antagonist studies revealed that stimulation of CRF-R1 and CRF-R2 receptors induced the elevated secretory state and permeability dysfunction, respectively. Chronic CRF causes colonic barrier dysfunction in rats, which is mediated, at least in part, via mast cells. This information may be useful in designing novel treatment strategies for stress-related gastrointestinal disorders.

THE ROLE OF CHRONIC, PERSISTENT stress in mediating intestinal dysfunction has been established both in humans (44) and rodents (3, 8, 37, 39). Stressful life events have been associated with the onset of symptoms or relapse in patients with either inflammatory bowel disease or irritable bowel syndrome (IBS) (26). In support of this association, rats exposed to stress develop an increased severity to hapten-induced colitis (29), and stress triggers colonic changes in adult rats exposed to early life stress in a rodent model of IBS (11, 46).

Models of stress vary considerably depending on the nature of the stressor (psychological vs. physical) and the duration of the exposure (acute vs. chronic). We have focused our studies on models of psychological stress, which mimic the life stress facing humans on a daily basis. Water avoidance stress (WAS) was selected since the stressor is purely psychological with no physical component as in models involving restraint or cold stress.

In the intestinal tract, acute stress stimulates active ion secretion and increases paracellular permeability (passive ion transport) and macromolecular permeability via the transcellular pathway (20, 41, 46). More prolonged psychological stress (5 days of 1 h/day WAS) caused weight loss, mitochondrial swelling, and mast cell degranulation (37). After 10 days of WAS, additional abnormalities were evident including increased bacterial-epithelial adhesion and penetration accompanied by mucosal inflammation and goblet cell depletion (45). Mast cells were found to be responsible for maintaining the observed gastrointestinal dysfunctions but not whole body effects (e.g., weight loss) (37, 45) because these effects were not observed in mast cell-deficient rats.

The hypothalamus-pituitary-adrenal (HPA) axis has been extensively studied in the maintenance of behavioral and mood changes (anxiety and depression) associated with stress and, more recently, of gastrointestinal parameters. Corticotropin-releasing factor (CRF), a 41-amino acid peptide, has been identified as the primary neuroendocrine factor mediating the effects of stress. CRF binds to two G protein-coupled receptor subtypes, CRF-R1 and CRF-R2, located both centrally and peripherally (16). A single injection of CRF in the rodent brain (24) or periphery (38) mimicked the short-term effects of acute stress on gut function; the role of CRF receptors was confirmed since pretreatment of rats with the nonselective CRF antagonist α-helical CRF(9–41) inhibited the stress-induced intestinal abnormalities (38).

Despite these acute studies, the prolonged effects of CRF administration on gut mucosal barrier function have not been investigated. Therefore, the aims of the present study were to determine whether chronic administration of CRF to rats via a continuous osmotic minipump system would induce long-lasting colonic mucosal defects similar to those previously described for chronic psychological stress. We used mast cell-deficient rats and their wild-type controls to determine the role of mast cells in CRF-induced gut abnormalities.

MATERIALS AND METHODS

Animals

Mast cell-deficient (Ws/Ws) rats and their wild-type (+/+ ) littermates were obtained from our breeding colony at McMaster University. Original breeder pairs, deficient in the c-kit gene, were provided by Dr. Y. Kitamura (Osaka University Medical School, Japan) and have been previously characterized (32). Age-10 wk) and weight

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(200–250 g)-matched male rats were selected for this study. Animals were housed in pairs in cages lined with chip bedding while being maintained on a 12-h:12-h light/dark cycle (lights on at 8:00 AM) and with free access to food and water. Conscious rats were euthanized by decapitation, after which trunk blood was collected and colonic tissues were excised. All procedures were approved by the Animal Care Committee at McMaster University.

Administration of CRF

ALZET osmotic minipumps (DURECT, Cupertino, CA) were used to deliver a constant dose of CRF (or vehicle) over several days. Each pump contained 200 μl of CRF (or saline as vehicle) and was surgically implanted subcutaneously in +/+ or Ws/Ws rats. The effective dose (Table 1) was achieved 2 days after the surgical procedure, and rats were therefore euthanized on day 14 after 12 days of continuous exposure to CRF. Weight was recorded both before surgery and at euthanasia.

To assess the role of specific CRF receptors, minipumps containing selective CRF agonists and antagonists (soluble in saline) were used. Stressin1 was used as a selective CRF-R1 agonist, and urocortin III was used as a selective CRF-R2 agonist. The selective CRF-R2 antagonist (antisauvagine) in the presence of CRF was employed to confirm the role of the CRF-R2 in both +/+ and Ws/Ws rats. In this case, one minipump containing antisauvagine was implanted 2 days before the second minipump containing CRF, allowing time for the antagonist to take effect before addition of the factor. The doses of the neuropeptides were selected on the basis of those described to be effective in the literature and/or preliminary experiments that we conducted (Table 1). Selective agonists/antagonists were chosen on the basis of their solubility in saline, a requirement for the osmotic minipump to deliver the pharmacological agents appropriately. Pharmacological doses were selected on the basis of published studies examining colonic physiology following stress or as models to mimic acute stress.

Surgical Procedure

Rats were anesthetized by inhalation of a gaseous mixture of isoflurane (Pharmaceutical Partner of Canada, Richmond Hill, ON, Canada) and oxygen USP (Vital Aire, Mississauga, ON, Canada). Briefly, a paravertebral line incision was made in the back skin to form a subcutaneous pouch for minipump insertion. When two minipumps were used, they were separated by 2.3 cm. Stainless steel skin clips (MikRon Precision, Gardena, CA) were used for postoperative closure. Following minipump implantation, rats were housed individually.

Corticosterone

Truncal blood was collected as described above. Blood was centrifuged (3,000 revolution/min for 15 min at room temperature), and serum was obtained and stored at –70°C until further analysis. Serum corticosterone was quantified by radioimmunoassay (MP Biomedicals, Orangeburg, NY) according to the manufacturer’s instructions. Briefly, samples were thawed, diluted, and labeled with 125I isotope. Radioactivity was measured by a gamma counter (LKB Wallac, Turku, Finland), and the results were expressed in ng/ml.

Using Chambers

Segments of distal colon were excised and placed into 37°C oxygenated Krebs buffer. Two adjacent pieces from each segment were stripped of longitudinal muscle layer and myenteric plexus, opened along the mesentric border, and mounted in Ussing chambers (WP Instruments; Narco Scientific, Mississauga, ON, Canada). The chamber exposed 0.6 cm² of tissue surface area to 8 ml of circulating oxygenated Krebs buffer on both the mucosal and serosal sides. The buffer contained (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 KH₂PO₄, and 25 NaHCO₃ (pH 7.35). The serosal buffer also contained 10 mM glucose as an energy source, which was osmotically balanced by 10 mM mannitol in the mucosal buffer.

Following mounting, the tissues were allowed to equilibrate for 20 min. The electrical current was measured in the voltage-clamp mode at zero potential difference and expressed as short-circuit current (Isc; μA/cm²). The circuit was opened or unclamped at timed intervals to record potential difference. Tissue conductance (G, mS/cm²), which measures passive ion transport and represents paracellular permeability, was calculated according to Ohm’s law (V = IR). Tissue viability was assessed by monitoring Isc and G deviation over time. Segments with unstable parameters over the 2-h study period were excluded.

Macromolecular Permeability

Type VI horseradish peroxidase (HRP, 44 kDa) (Sigma-Aldrich, St. Louis, MO) was used as a model protein probe to study macromolecular permeability. Following equilibration, HRP was added to the luminal buffer (10⁻⁵ M). Serosal samples (500 μl) were taken at 30-min intervals over the course of 2 h and replaced with fresh buffer to maintain constant volume. Enzymatic activity of HRP was determined using a modified Worthington method as previously described (20) using a kinetic assay with 20 μl of samples run in duplicate on a 96-well microtiter plate. The mucosal-to-serosal flux was calculated as the average value of two consecutive stable flux periods (60–90 and 90–120 min). HRP flux was expressed as picomoles per centimeter squared per hour (pmol/cm² per h).

Morphological Studies

Light microscopy. To quantify mast cell numbers, tissue segments were fixed in Carnoy’s fixative for 2 h and then transferred to 70% alcohol for 48–72 h. Fixed samples were embedded in paraffin, and 4-μm circular sections were cut onto coated slides for immunohistochemistry. Briefly, slides were deparaffinized, treated with graded alcohols, rehydrated, and stained with toluidine blue. Positive cells were quantified using ten randomly chosen fields per slide using coded slides. Image analysis, including semi-automatic cell counts, was performed using ImagePro Plus 5.1 Image Processing & Analysis Software (Media Cybernetics, Silver Spring, MD).

Electron microscopy. Tissue segments were immediately fixed in 2.5% glutaraldehyde for 2 h, then transferred to 0.1 M sodium cacodylate buffer (pH 7.4), and stored at 4°C until processing for transmission electron microscopy (EM). Epithelial damage was stud-

### Table 1. CRF analogs used in the studies

<table>
<thead>
<tr>
<th>CRF Receptor Specificity</th>
<th>Dose, μg/kg per day</th>
<th>References</th>
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<tr>
<td>CRF</td>
<td>-R1; -R2 agonist</td>
<td>50</td>
</tr>
<tr>
<td>Urocortin III, r/h UrcIII</td>
<td>-R2 agonist</td>
<td>25</td>
</tr>
<tr>
<td>Stressin1</td>
<td>-R1 agonist</td>
<td>50</td>
</tr>
<tr>
<td>Antisauvagine-30</td>
<td>-R2 antagonist</td>
<td>50</td>
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</table>

Corticotropin-releasing factor (CRF) and selective CRF receptor (CRF-R) agonists/antagonists were administered via osmotic minipump for 12–14 days depending on the treatment. Effective doses were selected on the basis of published values or preliminary studies.
Cortisol levels were similarly increased in Ws/Ws rats treated with CRF compared with vehicle-treated controls (Fig. 1A). Serum corticosterone levels were similarly increased in Ws/Ws rats treated with CRF but not vehicle controls (Fig. 1B).

Chronic CRF treatment causes colonic mucosal barrier dysfunction in +/+ but not in Ws/Ws rats. Ussing chamber studies used to monitor colonic mucosal barrier function ex vivo have shown that chronic stress leads to altered secretory state and increased permeability (45). Similarly, following chronic administration of CRF for 12 days in +/+ rats, a threefold increase in baseline $I_{sc}$ was observed compared with vehicle-treated controls (Fig. 2A). Treatment with CRF also significantly affected both paracellular and transcellular permeability, as assessed by increased baseline tissue conductance (Fig. 2B) and HRP flux (Fig. 2C) in +/+ rats compared with vehicle-treated controls.

In contrast, colonic mucosal barrier dysfunction was completely absent in CRF-treated Ws/Ws rats, with similar baseline $I_{sc}$, G, and HRP flux values in rats treated with either CRF or vehicle (Fig. 2, A–C).

Chronic CRF treatment induced mucosal mast cell hyperplasia in +/+ rats. Chronic stress has been shown to induce mast cell hyperplasia in the colon (45). Therefore, total mast cell counts were evaluated using toluidine blue-stained sections. The total number of mast cells in the colonic mucosa was significantly increased in +/+ rats following chronic exposure to CRF compared with vehicle-treated controls (Fig. 3). As expected, no mast cells were present in the colon of Ws/Ws rats.

Bacterial-epithelial interactions were altered in CRF-treated +/+ but not Ws/Ws rats. Bacterial-epithelial cell interactions were evaluated using EM. The presence of commensal bacteria in close proximity or directly associated with the mucosa were observed in all CRF-treated +/+ rats. Photomicrographs revealed bacteria adhering to the surface of colonic epithelial cells with microbes penetrating into or through the epithelium in treated rats [16.0 ± 1.0 bacteria/100 grids (100 μm²); n = 4 rats per group] (Fig. 4, B and C). These bacterial-host interactions were not observed in Ws/Ws rats treated chronically with CRF or vehicle (control shown in Fig. 4A).

Colonic physiology recovered 10 days after CRF treatment. Since the effects of 5 days of chronic stress on gut physiology have been shown to last for at least 3 days following stress exposure (39) and the effects of 10 days of chronic stress to last for at least 7 days (M. Perdue, unpublished observations), we assessed recovery from prolonged exposure to CRF. Rats (only +/+ rats used since Ws/Ws did not develop abnormalities) were therefore housed for an additional 5 and 10 days following emptying of the minipump, on the basis of the volume of CRF or vehicle added, and barrier function was assessed. Tissue was collected at postsurgical days 19 and 24 and compared with those collected previously on postoperative day 14. After 5 days of recovery (postsurgical day 19), the colonic mucosal barrier abnormalities were still present, although reduced (Table 2). After 10 days (postsurgical day 24), ion transport and conductance values were similar to those in control rats (Table 2). However, HRP flux was still

Results are expressed as means ± SE. Differences between experimental groups were compared by one-way ANOVA with nonparametric Kruskal-Wallis test, or unpaired $t$-test with Welch’s correction where appropriate. Differences were considered significant at $P < 0.05$.

**RESULTS**

**Chronic CRF administration caused systemic changes in both +/+ and Ws/Ws rats.** As exposure to chronic psychological stress has been shown to significantly inhibit weight gain in rats (45), changes in body weight were assessed following CRF treatment. A significant reduction in weight gain was observed over the 14-day period in both +/+ and Ws/Ws rats treated with CRF, compared with vehicle-treated rats (Fig. 1A). Circulating levels of serum corticosterone were used to confirm activity of CRF. A significant increase in the concentration of serum corticosterone was found in +/+ rats following chronic exposure to CRF compared with those that received vehicle for 12 days (Fig. 1B). Serum corticosterone levels were similarly increased in Ws/Ws rats treated with CRF but not vehicle controls (Fig. 1B).

**Chronic CRF treatment causes colonic mucosal barrier dysfunction in +/+ but not in Ws/Ws rats.** Since the effects of 5 days of chronic stress on gut physiology have been shown to last for at least 3 days following stress exposure (39) and the effects of 10 days of chronic stress to last for at least 7 days (M. Perdue, unpublished observations), we assessed recovery from prolonged exposure to CRF. Rats (only +/+ rats used since Ws/Ws did not develop abnormalities) were therefore housed for an additional 5 and 10 days following emptying of the minipump, on the basis of the volume of CRF or vehicle added, and barrier function was assessed. Tissue was collected at postsurgical days 19 and 24 and compared with those collected previously on postoperative day 14. After 5 days of recovery (postsurgical day 19), the colonic mucosal barrier abnormalities were still present, although reduced (Table 2). After 10 days (postsurgical day 24), ion transport and conductance values were similar to those in control rats (Table 2). However, HRP flux was still
impaired although significantly improved compared with CRF-treated rats. This indicates that macromolecular transport requires a longer time for normalization than the other parameters of barrier function.

Administration of a selective CRF-R1 agonist increased secretory state but not permeability in the colon of +/- rats. Stressin1, a selective CRF-R1 agonist (33, 34, 51), was administered to investigate the contribution of CRF-R1 in the CRF-induced colonic mucosal dysfunction. In +/- rats, prolonged treatment with stressin1 significantly increased (P < 0.05) baseline secretory state (\(I_{sc}\): 40.4 ± 2.6 vs. 16.4 ± 1.6 \(\mu\)A/cm²), but did not alter tissue conductance (\(G\): 13.8 ± 1.7 vs. 13.0 ± 1.7) or HRP flux (8.9 ± 1.1 vs. 8.1 ± 1.1 pmol/cm² per h). In all cases the number of rats per group was 7–9.

Increased secretory state resulting from chronic administration of the CRF-R1 agonist appears to be mediated by mast cells because no change in baseline secretory state was observed in Ws/Ws rats (\(I_{sc}\): 19.1 ± 1.8 vs. 22.5 ± 6.4 \(\mu\)A/cm²). Tissue conductance (\(G\): 16.6 ± 1.5 vs. 12.6 ± 1.4 mS/cm²) and HRP flux (6.7 ± 0.8 vs. 9.5 ± 1.1 pmol/cm² per h) were also unaffected in Ws/Ws rats (\(n = 5–7\)).

Administration of a selective CRF-R2 agonist increased both secretory state and permeability in the colon of +/- rats. Urocortin III, a selective CRF-R2 agonist (21), was used to examine the role of the CRF-R2 receptor. Administration of urocortin III for 12 days significantly increased (P < 0.05) baseline \(I_{sc}\) (49.7 ± 6.9 vs. 16.4 ± 1.6 \(\mu\)A/cm²), \(G\) (46.2 ± 6.7 vs. 13.0 ± 1.7 mS/cm²), and HRP flux (42.7 ± 5.0 vs. 13.6 ± 3.2 pmol/cm² per h) in +/- rats (\(n = 5\)). In contrast, no significant changes in mucosal function were observed in Ws/Ws rats following treatment with urocortin III: \(I_{sc}\) (16.8 ± 1.8 vs. 20.7 ± 2.7 \(\mu\)A/cm²), \(G\) (18.8 ± 0.6 vs. 16.7 ± 2.8 mS/cm²), and HRP flux (11.1 ± 1.3 vs. 16.7 ± 1.0 pmol/cm² per h) (\(n = 5\)).

Administration of a selective CRF-R2 antagonist before CRF inhibited permeability but not ion transport abnormalities in +/- rats. Administration of antisauvagine in the presence of CRF to +/- rats significantly inhibited (P < 0.05) the CRF-enhanced values for both conductance (19.9 ± 2.3 vs. 39.8 ± 4.5 mS/cm²) and HRP flux (14.7 ± 7.6 vs. 32.4 ± 3.4 pmol/cm² per h) (\(n = 6–9\)). With respect to secretory state, there was no significant difference between values in the antisauvagine group (34.7 ± 7.4 \(\mu\)A/cm²) compared with CRF-treated rats (45.6 ± 2.1 \(\mu\)A/cm²). Since CRF treatment did not produce colonic mucosal pathophysiology in Ws/Ws rats, we did not administer antisauvagine to this group.

**DISCUSSION**

Psychological stress, in the form of WAS, causes colonic barrier dysfunction (8, 45), activates enteric neurons (27), and causes visceral hypersensitivity (4, 5, 28, 43) in rodents. It is clear that the effects of acute stress can be mimicked by a single injection of CRF (38); however, the consequences of chronic CRF administration have not, to our knowledge, been reported. Since our interest has focused on mucosal pathophysiology induced by chronic stress, we examined the colonic mucosal changes following chronic CRF delivered by an osmotic minipump. In addition to documenting ion transport and permeability changes in the gut, we also identified that both CRF receptors are involved in the mucosal abnormalities.

Systemic effects of chronic stress including weight loss and increases in stress glucocorticoid hormones are well-established (18, 45). Exposure to WAS for 10 days leads to significantly reduced weight gain in wild-type (+/-) rats, a feature
not mediated by mast cells because it was also observed in mast cell-deficient Ws/Ws rats (45). Similarly, in our study, continuous exposure to CRF delivered by minipump resulted in reduced weight gain over a 12-day period. Elevated circulating corticosterone in rats indicates HPA axis activation downstream from release of CRF (12, 13, 15, 45). In this study, we found that chronic administration of CRF increased basal serum corticosterone, a phenomenon observed in both +/+ and Ws/Ws rats. These results indicate that chronic CRF can mimic the systemic effects of chronic stress and, as in previous studies (45), that mast cells are not important in the maintenance of these systemic effects.

Colonic mucosal barrier function is sensitive to stress, with dysfunctions observed following exposure to acute (20, 40), chronic (37, 45), metabolic (31), and early life stressors (15, 46). Stress can alter many elements of barrier function, elevating ion secretion, increasing tight junction permeability, enhancing macromolecular protein flux across the epithelial layer, and altering bacterial-host interactions, resulting in mucosal inflammation (39, 45). A single peripheral injection of CRF has been shown to mimic the effects of acute stress on mucosal barrier function (38), but the consequence of chronic CRF administration was unknown. In the present study, we found that 12 days of continuous CRF treatment via osmotic minipump increased baseline active ion transport, passive ion transport, and elevated macromolecular permeability similar to that observed following exposure to chronic stress in +/+ but not in Ws/Ws rats. Therefore, these findings suggest that chronic CRF administration provides an alternative model to chronic exposure to WAS to study both systemic effects and colonic mucosal barrier function in rats.

Altered bacterial epithelial cell interactions are known to occur following prolonged exposure to stressors, including chronic WAS (45), early life stress (15), and metabolic stress.
ies, baseline ion secretion, permeability, and macromolecular transport had partially improved by day 5, with complete normalization of secretory state and paracellular permeability observed by day 10 and significantly improved macromolecular transport. This suggests that, similar to chronic stress, CRF administration causes reversible abnormalities in colonic physiology, which recover over time.

The localization of the CRF receptor subtypes within the gastrointestinal tract (10, 22) and the recent development of specific receptor subtype agonists and antagonists have further improved our knowledge of the peripheral actions of this factor. Teasing out the individual components of the CRF response have demonstrated that each receptor subtype maintains different physiological functions in the colon (47, 48). For example, CRF-induced increases in colonic transit (24, 25, 36) and stress-induced visceral pain (28) are mediated by CRF-R1, whereas CRF-R2 mediates delayed gastric emptying (25) and opposes stress-induced visceral pain (30). To determine the specific receptor subtype involved in mediating the altered secretory state and permeability in the colon, we used a selection of selective agonists and antagonists, depending on the availability of water soluble compounds (a requirement for delivery via the minipump system). We found that CRF-R2 mediates both paracellular and transcellular permeability in the colon, a phenomenon that was also observed in neonatal rats exposed to early life trauma (14). In contrast to permeability, altered secretory state was found to be maintained by CRF-R1 and possibly also by CRF-R2. The CRF-R2 agonist stimulated ion transport changes, but, in response to CRF, the CRF-R2 antagonist produced no significant inhibition. This discrepancy may be due to some overlap in the effects of the agonist or incomplete effectiveness of the antagonist. Because of the laborious nature of the studies, it was not possible to carry out complete dose-response curves. Arguably, the systemic effects of CRF-R2 stimulation, which results in decreased systemic blood pressure (23) [a phenomenon not observed with CRF-R1 stimulation (34)], may be interfering with colonic mucosal barrier function and thus providing mixed results. Therefore, at this time, the role of CRF-R2 in the regulation of ion transport has not been resolved by these studies.

The findings that CRF-R1 and R2 mediate separate mucosal functions are not surprising because altered secretory state and permeability do not always correlate in all animal models, suggesting in part a different activation pathway associated with each CRF receptor. Neither receptor agonist had any effect on colonic barrier function in Ws/Ws rats, further supporting a role for CRF receptors in the periphery signaling via mast cells to induce colonic pathophysiology. Mast cells themselves exhibit differential release of mediators depending on the stimulus (17) and receptor being activated. Also some mast cell mediators such as histamine, serotonin, PGE₂, etc. stimulate ion secretion (35, 42), whereas others such as TNF-α, IL-4, IFN-γ, etc. increase permeability (6), and certain proteases have dual effects (7, 19).

In conclusion, our findings are the first to demonstrate that chronic administration of CRF evokes long-term changes in mucosal barrier function similar to those reported in models of chronic psychological stress, which can be reversed once the stimulus is removed. Furthermore, we have shown that CRF acts in conjunction with mucosal mast cells in maintaining colonic barrier dysfunction as reported previously with WAS. Finally, both CRF-R1 and CRF-R2 are involved in mediating

Table 2. Recovery of colonic physiology following CRF treatment

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<th>CRF</th>
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<tr>
<td><strong>Iₑ, μA/cm²</strong></td>
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<tr>
<td><strong>Gₑ, mS/cm²</strong></td>
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<td>39.8±4.5*</td>
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<tr>
<td><strong>HRP flux, pmol/cm² per h</strong></td>
<td>8.2±1.1</td>
<td>33.9±3.4*</td>
<td>27.4±3.6*</td>
<td>16.5±2.2*</td>
</tr>
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</table>

Rats were allowed to recover for 5 or 10 days following depletion of CRF from the minipump. Colonic physiology was compared in vehicle controls and CRF-treated rats. Values indicate the mean ± SE; *P < 0.05 compared to vehicle; n = 7–9 rats per group. HRP, horseradish peroxidase.

(31). EM was used to assess the ultrastructure of colonic epithelial cells and the presence of microorganisms following chronic CRF exposure. The CRF-treated +/+ rats had bacteria adhering to the surface of colonocytes and penetrating into the epithelium, whereas this was not the case in vehicle controls or mast cell-deficient Ws/Ws rats. Altered bacterial-host interactions are similar to those observed in rats exposed to WAS chronic stress, suggesting that CRF can adversely affect the relationship between the host and resident microflora in both models.

Mast cells are key cells in the gut that mediate secretory state and macromolecular permeability following exposure to stress (39, 45) and in models of food allergy (2). In this study, we found that chronic administration of CRF did not cause colonic defects in Ws/Ws rats, suggesting that CRF-altered mucosal barrier function is mediated by mast cells. Furthermore, increased numbers of mucosal mast cells were found in the colonic mucosa of +/+ rats compared with vehicle controls, similar to that observed following 10 days of WAS (45). The presence of CRF receptors on mast cells (9, 49, 50) supports a role for mast cells in maintaining the CRF-induced mucosal barrier dysfunction in treated rats. Therefore, we hypothesize that the CRF-induced colonic dysfunctions occur in part by CRF binding to its receptors, located on mast cells, with degranulation of these cells releasing mediators that stimulate ion secretion and enhance permeability in the colon. Although Ws/Ws rats provide an excellent model to study the role of mast cells in vivo, one cannot overlook other aspects the c-kit mutation may have on physiology. In the intestine, the absence of interstitial cells of Cajal in these animals results in abnormalities in colonic motility (1). Skin mast cells have been shown to respond to stress, resulting in exacerbation of conditions including atopic dermatitis and psoriasis (49). Exposure of rats to chronic CRF resulted in the development of skin lesions at the site of surgical implantation but only in wild-type rats. Ws/Ws rats displayed normal skin around the minipump site, suggesting that chronic administration of CRF resulted in activation of mast cells in the skin and inflammation.

Following exposure to chronic WAS stress for 5 days, recovery of intestinal parameters occurs ~3 days following the last session of stress (37). Therefore, we assessed whether mucosal barrier function would return to baseline following completion of the CRF minipump treatment. We maintained the rats in their cages for an additional 5 and 10 days after the CRF-containing minipump was depleted and studied colonic physiology. Complementing the previous chronic stress studies, baseline ion secretion, permeability, and macromolecular transport had partially improved by day 5, with complete normalization of secretory state and paracellular permeability observed by day 10 and significantly improved macromolecular transport. This suggests that, similar to chronic stress, CRF administration causes reversible abnormalities in colonic physiology, which recover over time.

The localization of the CRF receptor subtypes within the gastrointestinal tract (10, 22) and the recent development of specific receptor subtype agonists and antagonists have further improved our knowledge of the peripheral actions of this factor. Teasing out the individual components of the CRF response have demonstrated that each receptor subtype maintains different physiological functions in the colon (47, 48). For example, CRF-induced increases in colonic transit (24, 25, 36) and stress-induced visceral pain (28) are mediated by CRF-R1, whereas CRF-R2 mediates delayed gastric emptying (25) and opposes stress-induced visceral pain (30). To determine the specific receptor subtype involved in mediating the altered secretory state and permeability in the colon, we used a selection of selective agonists and antagonists, depending on the availability of water soluble compounds (a requirement for delivery via the minipump system). We found that CRF-R2 mediates both paracellular and transcellular permeability in the colon, a phenomenon that was also observed in neonatal rats exposed to early life trauma (14). In contrast to permeability, altered secretory state was found to be maintained by CRF-R1 and possibly also by CRF-R2. The CRF-R2 agonist stimulated ion transport changes, but, in response to CRF, the CRF-R2 antagonist produced no significant inhibition. This discrepancy may be due to some overlap in the effects of the agonist or incomplete effectiveness of the antagonist. Because of the laborious nature of the studies, it was not possible to carry out complete dose-response curves. Arguably, the systemic effects of CRF-R2 stimulation, which results in decreased systemic blood pressure (23) [a phenomenon not observed with CRF-R1 stimulation (34)], may be interfering with colonic mucosal barrier function and thus providing mixed results. Therefore, at this time, the role of CRF-R2 in the regulation of ion transport has not been resolved by these studies.

The findings that CRF-R1 and R2 mediate separate mucosal functions are not surprising because altered secretory state and permeability do not always correlate in all animal models, suggesting in part a different activation pathway associated with each CRF receptor. Neither receptor agonist had any effect on colonic barrier function in Ws/Ws rats, further supporting a role for CRF receptors in the periphery signaling via mast cells to induce colonic pathophysiology. Mast cells themselves exhibit differential release of mediators depending on the stimulus (17) and receptor being activated. Also some mast cell mediators such as histamine, serotonin, PGE₂, etc. stimulate ion secretion (35, 42), whereas others such as TNF-α, IL-4, IFN-γ, etc. increase permeability (6), and certain proteases have dual effects (7, 19).

In conclusion, our findings are the first to demonstrate that chronic administration of CRF evokes long-term changes in mucosal barrier function similar to those reported in models of chronic psychological stress, which can be reversed once the stimulus is removed. Furthermore, we have shown that CRF acts in conjunction with mucosal mast cells in maintaining colonic barrier dysfunction as reported previously with WAS. Finally, both CRF-R1 and CRF-R2 are involved in mediating...
altered secretory state and permeability, respectively. These findings have importance in the understanding of the colonic physiology in response to stress and provide a new model to study the effects of psychological stress. The use of the minipump avoids daily interactions with the animals, possibly causing less variability in the results. CRF administered by osmotic minipump is an appropriate model for use in a germ-free facility to confirm the role of microbes in mediating the effects of stress with respect to colonic mucosal barrier function.

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