Activation of Corticotropin-Releasing Factor Receptor 2 Mediates the Colonic Motor Coping Response to Acute Stress in Rodents

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BACKGROUND & AIMS: Corticotropin-releasing factor receptor-1 (CRF_1) mediates the stress-induced colonic motor activity. Less is known about the role of CRF_2 in the colonic response to stress. **METHODS:** We studied colonic contractile activity in rats and *CRF_2–/–*, CRF-overexpressing, and wild-type mice using still manometry; we analyzed defecation induced by acute partial-restraint stress (PRS), and/or intraperitoneal injection of CRF ligands. In rats, we monitored activation of the colonic longitudinal muscle myenteric plexus (LMMP) neurons and localization of CRF_1 and CRF_2 using immunohistochemical and immunoblot analyses. We measured phosphorylation of extracellular signal-regulated kinase 1/2 by CRF ligands in primary cultures of LMMP neurons (PC-LMMPn) and cyclic adenosine monophosphate (cAMP) production in human embryonic kidney-293 cells transfected with CRF_1 and/or CRF_2. **RESULTS:** In rats, a selective agonist of CRF_2 (urocortin 2) reduced CRF-induced defecation (>50%), colonic contractile activity, and Fos expression in the colonic LMMP. A selective antagonist of CRF_2 (astressin$_2$-B) increased these responses. Urocortin 2 reduced PRS-induced colonic contractile activity in wild-type and CRF-overexpressing mice, whereas disruption of *CRF_2* increased PRS-induced colonic contractile activity and CRF-induced defecation. CRF_2 colocalized with CRF_1 and neuronal nitric oxide synthase in the rat colon, LMMP, and PC-LMMPn. CRF-induced phosphorylation of extracellular signal-regulated kinase in PC-LMMPn; this was inhibited or increased by a selective antagonist of CRF_1 (NBI35965) or astressin$_2$-B, respectively. The half maximal effective concentration, EC_{50}, for the CRF-induced cAMP response was 8.6 nmol/L in human embryonic kidney-293 cells that express only CRF_1; this response was suppressed 10-fold in cells that express CRF_1 and CRF_2. **CONCLUSIONS: In colon tissues of rodents, CRF_2 activation inhibits CRF_1 signaling in myenteric neurons and the stress-induced colonic motor responses. Disruption of CRF_2 function impairs colonic coping responses to stress.**

Keywords: Colonic Contraction; Myenteric Neurons; nNOS; Stress Response.

BASIC–ALIMENTARY TRACT

Clinical and experimental studies show that chronic or uncontrolled stress triggers or exacerbates a number of pathologies including gastrointestinal diseases.[1–4] Corticotropin-releasing factor (CRF) is the primary hypothalamic mediator of the mammalian neuroendocrine and behavioral responses to stress.[5] The CRF signaling system, in addition to CRF, encompasses 3 CRF-related peptides, urocortins (Ucns), Ucn 1, Ucn 2, and Ucn 3, and 2 receptor subtypes, CRF_1 and CRF_2.[6,7] CRF and Ucn 1 bind to both CRF_1 and CRF_2 receptors, although with different affinities.[5–7] On the other hand, Ucn 2 and Ucn 3 bind selectively to CRF_2 receptors.[7] CRF_1 is found abundantly in the central nervous system with limited expression in peripheral tissues, whereas CRF_2 is widespread in the periphery and is confined in discrete brain nuclei.[8–12] Multiple alternatively spliced transcripts of CRF_1 have been identified (CRF_{1a}–CRF_{1i}), with only a few of them being functional. CRF_2 is expressed in 3 major functional isoforms in human beings (CRF_{2a}, CRF_{2b}, and CRF_{2c}), 2 in rodents (CRF_{2a} and CRF_{2b}), with 5 additional

Abbreviations used in this paper: AUC, area under the curve; CRF, corticotropin releasing factor; CRF_1, corticotropin releasing factor receptor 1; CRF_2, corticotropin releasing factor receptor 2; CRF_2 –/–, CRF_2-deficient mice; CRF-OE, corticotropin releasing factor overexpressing; ERK1/2, extracellular signal-regulated kinase 1/2; GMCs, giant migrating contractions; HEK, human embryonic kidney cells; hUcn 2, human urocortin 2; IR, immunoreactivity; LMMP, longitudinal muscle myenteric plexus; mUcn 2, mouse urocortin 2; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; PC-LMMPn, primary culture longitudinal muscle myenteric plexus neurons; PCR, polymerase chain reaction; PRS, partial-restraint stress; Ucn 1, urocortin 1; WAS, water avoidance stress; WTL, wild-type littermate.

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noncoding CRF_{2a} variants.[7,11,13–15] Rodent CRF_{2a} is expressed primarily in the brain, whereas CRF_{2b} is found mainly in the periphery, including the gastrointestinal tract.[11,14,16,17] CRF_{2a} and CRF_{2b} isoforms display similar pharmacologic profiles.[18] The dominant mode of signaling for both CRF_1 and CRF_2 is the Gs-coupled adenylate cyclase–phosphokinase cascade, although phospholipase C-protein kinase C and extracellular signal-regulated kinase (ERK)–mitogen activated protein kinase cascades are reported in different cell types.[13,19]

In rodents stress or CRF injected into the brain or periphery induces colonic secretomotor and pain sensation alterations that are blocked by CRF_1 antagonists.[20–25] These findings have led to the consensus that CRF_1 receptor is the primary receptor involved in the stress-induced alteration of lower-gut secretomotor and pain sensation. However, except the preliminary data on the inhibitory actions of Ucn 2 in mice defecation,[26] the mechanisms of action and role of peripheral CRF_2 in the colonic response to CRF or stress are largely unknown.

In the present study, we investigated whether peripheral CRF_2 activation or blockade modulates the colonic motor activity to peripheral injection of CRF or stress in rats and mice and assessed the underlying mechanisms involved in the CRF_2-mediated inhibitory actions. We show that CRF_2 activation plays a critical role in harnessing the CRF_1-mediated stimulation of colonic motor function induced by acute partial restraint stress (PRS) or peripheral injection of CRF by modulating CRF_1 signaling and/or recruiting inhibitory pathways such as nitric oxide. Such modulation is essential to establish homeostasis and it is likely that alteration of CRF_2 signaling impairs the normal stress-coping mechanisms and may contribute to the development of stress-related gut diseases.

Materials and Methods

Animals

Adult male Sprague–Dawley rats (280–300 g; Harlan, Indianapolis, IN), male and female CRF_2–/– (32.7 ± 3.8 g) and their wild-type littermates (WTL) (C57BL/6J, 31.7 ± 0.3 g), CRF-overexpressing (CRF-OE, 30.9 ± 2.1 g) and their WTL (C57BL/6, 28.8 ± 0.6 g), from the Oregon Health and Science University (Portland, OR),[27] and the University of California Los Angeles (Los Angeles, CA) were used. Animals were maintained under a temperature-controlled (20°C–24°C) and light-controlled (12/12 hours light/dark) environment and fed ad libitum with standard rodent chow (Prolab RMH 2500-5P14; Purina LabDiet, St. Louis, MO) and tap water. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Protocols (9906-820, 08047-05 and 06014-08) were approved by the Veteran's Affairs Institutional Animal Care and Use Committee.

Substances

Human/rat/mice Ucn 1, human Ucn 2 (hUcn 2), mouse Ucn 2 (mUcn 2), human/rat CRF, and astressin$_2$-B (Clayton Foundation Laboratories, The Salk Institute, La Jolla, CA) were synthesized and purified as previously described.[28] NBI-35965 was obtained from Neurocrine Biosciences, Inc (San Diego, CA).

Stress Models

Water avoidance stress (WAS) in rats and PRS in rats and mice for 1 hour were used as acute stressors[29,30] and CRF-OE mice were used as a genetic model of chronic stress.[26,27,30]

Measurements of Colonic Motor Function in Rats and Mice

Colonic contractions. Contraction was recorded in conscious nonfasted rats and mice using a newly developed, minimally invasive, solid-state manometry catheter.[22,30] Pressure sensors were positioned at 8 and 4 cm (rats) and at 2 cm (mice) past the anus. The 8-cm site (rats) corresponds to proximal–transverse whereas the 4-cm (rats) and 2-cm (mice) sites correspond to the distal colon. The phasic component of the area under the curve (AUC) was extracted from the original intraluminal colonic pressure. Colonic contractions were quantified by measuring, every minute, the phasic component AUC.[31] Because acute PRS-induced activation of colonic contractions primarily occur during the first 20 minutes,[30] the frequency, amplitude, duration, and propagation of contractions were determined for the 0–20 minute and 20–60 minute time periods (see Supplementary method, colonic contraction response, for additional information).

Fecal pellet output (defecation) and diarrhea. In nonfasted conscious rats and mice, defecation was monitored as number of fecal pellets output for 1 or 2 hours.[29,30] The percentage of rats with diarrhea was calculated.

Immunohistochemistry: Rat Colon Longitudinal Muscle Myenteric Plexus–Whole-Mount Preparation

Neuronal Fos. Proximal and distal colonic longitudinal muscle myenteric plexus (LMMP) whole-mount preparations were dissected and Fos immunohistochemistry was assessed as in our previous studies.[32,33] The mean number of Fos immunoreactivity (IR) nuclei/myenteric-ganglion from each rat was used to generate a mean number (see Supplementary method, neuronal Fos immunostaining, for additional information).

Double labeling of CRF_2 with CRF_1 or neuronal NO synthase. The proximal and distal segments of colon collected from 2 naive adult male Sprague–Dawley rats were processed for LMMP whole-mount preparations as described earlier[33] and processed for CRF_1, CRF_2, and neuronal NO synthase (nNOS) immunostaining as de-