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Special Review

Role of Corticotropin-releasing Factor in Gastrointestinal Permeability

Bruno K Rodiño-Janeiro, Carmen Alonso-Cotoner,* Marc Pigrau, Beatriz Lobo, María Vicario, and Javier Santos

Neuro-Immuno-Gastroenterology Group, Digestive Diseases Research Unit, Gastroenterology Department, Hospital Universitari Vall d'Hebron, Vall d' Hebron Research Institute; and Department of Medicine, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Barcelona, Spain

The interface between the intestinal lumen and the mucosa is the location where the majority of ingested immunogenic particles face the scrutiny of the vast gastrointestinal immune system. Upon regular physiological conditions, the intestinal microflora and the epithelial barrier are well prepared to process daily a huge amount of food-derived antigens and non-immunogenic particles. Similarly, they are ready to prevent environmental toxins and microbial antigens to penetrate further and interact with the mucosal-associated immune system. These functions promote the development of proper immune responses and oral tolerance and prevent disease and inflammation. Brain-gut axis structures participate in the processing and execution of response signals to external and internal stimuli. The brain-gut axis integrates local and distant regulatory networks and supersystems that serve key housekeeping physiological functions including the balanced functioning of the intestinal barrier. Disturbance of the brain-gut axis may induce intestinal barrier dysfunction, increasing the risk of uncontrolled immunological reactions, which may indeed trigger transient mucosal inflammation and gut disease. There is a large body of evidence indicating that stress, through the brain-gut axis, may cause intestinal barrier dysfunction, mainly via the systemic and peripheral release of corticotropin-releasing factor. In this review, we describe the role of stress and corticotropin-releasing factor in the regulation of gastrointestinal permeability, and discuss the link to both health and pathological conditions.
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Key Words
Corticotropin-releasing factor; Inflammation; Permeability; Stress

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*Correspondence: Carmen Alonso-Cotoner, MD, PhD
Neuro-Immuno-Gastroenterology Group, Digestive Diseases Research Unit, Gastroenterology Department, Hospital Universitari Vall d'Hebron, Vall d' Hebron Research Institute, Paseo Vall d' Hebron 119-129, 08035, Barcelona 08022, Spain
Tel: +34-93-4894035, Fax: +34-93-4894032, E-mail: caralons@vhebron.net
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ORCID: Bruno K Rodiño-Janeiro, http://orcid.org/0000-0002-0633-6774; Carmen Alonso-Cotoner, http://orcid.org/0000-0002-3483-2919; Marc Pigrau, http://orcid.org/0000-0001-6801-3017; Beatriz Lobo, http://orcid.org/0000-0003-3391-7125; María Vicario, http://orcid.org/0000-0001-9622-3185; Javier Santos, http://orcid.org/0000-0002-4798-5033.

Official abbreviations according to UPHARM guideline were used in this review series.

CRF, corticotrophin-releasing factor
CRF_1, corticotrophin-releasing factor receptor 1
CRF_2, corticotrophin-releasing factor receptor 2

Introduction

The epithelium that lines our digestive tract harbors 100 trillion bacteria[1] and mediates our relationship to the world outside. Our intestine is constantly exposed to a wide variety of immunogenic particles and microorganism-derived antigens. Its surface area is large enough to process over 50 tons of food in a lifetime. Intestinal and microbial components are strongly linked to each other and represent the most important barrier to limit luminal antigens from travelling across and reach the mucosal-associated immune system. They also play a critical role in training and modulating our immune system, helping it to distinguish between friend and foe.[2,3] The absence of constructive engagement between microbes and intestinal mucosa may render epithelial barrier more permeable, allowing it to be breached. When this happens, resident cells become over-activated, and a cascade of pro-inflammatory signals is initiated.[4,5] Altered intestinal permeability has large implications for human health, being involved in the origin and development of many digestive and non-digestive diseases. However, up to date, it is not clear whether intestinal barrier dysfunction is a primary event, an epiphenomenon or a consequence in the pathogenesis of these disorders.

The regulation of intestinal permeability involves terminals from ascending and descending pathways from the autonomic nervous system and the central nervous system, the hypothalamic pituitary-adrenal axis, and the enteric nervous system as well. The vast and versatile array of bidirectional and integrative communications between the brain and the gut allows the brain to respond to internal and external signals and, in return, to modify the autonomic and enteric nervous systems to keep intestinal permeability tight.

Physical and psychological stresses represent convincing evidence of the influence of the brain-gut axis on the intestinal barrier function. In fact, stress has been associated with reactivation of inflammatory and functional gastrointestinal disorders mainly through disruption of the intestinal barrier[6] in both human and animal models. The stress response is vehiculated via 2 main systems: the sympatho-adrenomedullary system and the hypothalamic pituitary-adrenal axis. Stress stimulates the parvocellular neurons in the paraventricular nucleus of the hypothalamus to secrete corticotropin-releasing factor (CRF), and arginine vasopressin. CRF promotes the synthesis and release of adenocorticotropic hormone in the anterior pituitary. The adenocorticotropic hormone, in turn, activates the adrenal cortex to induce a temporary rise in blood levels of cortisol and corticosterone[7] and also the release of catecholamines by the adrenal medulla. It has been believed for a long time that central secretion of CRF was the main and unique mediator of the majority of the endocrine, behavioral and gastrointestinal changes induced by stress.[8] However, we will show that peripheral release of CRF also plays a key role in the regulation of gastrointestinal permeability, and discuss the link to both health and pathological conditions.

Intestinal Permeability

The intestinal mucosal barrier includes several consecutive layers, from the outermost microbiota, through the external mucus, the epithelium, and down to the innermost lamina propria.[9] The epithelial layer is described as a continuous polarized monolayer of columnar cells that separates the intestinal lumen from the internal milieu. Aside from epithelial cells, a variety of different cell types are also intermingled with enterocytes, including goblet cells, Paneth cells, enteroendocrine cells, and M cells. Within the lamina propria we can find blood and lymph vessels, and a plethora of distinct immune cells such as plasma cells, lymphocytes, macrophages, eosinophils, mast cells, dendritic cells, and a significant number of intrinsic and extrinsic nerve terminals.[10] All of these components are exquisitely reactive and adaptive and display critical effector and modulatory functions. These functions are relevant for the control of inflammation, absorption and secretion, transport of macromolecules, and metabolic processes.[11] Moreover, most of the cellular components have been shown to express receptors for CRF.[12,13] But the job of the intestinal barrier now appears to be far more nuanced and complex as it communicates multidirectionally with the immune system and the microbes. Communication is developed through the release of an extensive array of chemical mediators, namely neuropeptides, neurohormones, neurotransmitters, cytokines, chemokines, growth factors, and other regulatory molecules.[14,15]

Enterocytes are tightly bonded to each other sealing the paracellular space through the apical junctional complex, composed of tight junctions (TJs), adherens junctions, and desmosomes.[16] The transmembrane TJ proteins occludin and claudins form complex protein systems, which interact with zonula occludens proteins that bind to the actin cytoskeleton. When actin contracts,

it leads to increased permeability to electrolytes and small molecules.[17] The paracellular space is not fully impermeable to molecules and antigens, allowing a controlled amount of small particles (less than 400 daltons) to penetrate across to reach the lamina propria,[9] a phenomenon that plays a key role in the induction of immune tolerance. This passage of molecules also takes place through the transcellular pathway via endocytosis or exocytosis.[18] Disruption of the intestinal barrier leads to uncontrolled flux of luminal antigens and possibly microbes across the epithelium, which may trigger immune activation and sepsis, and also lead to the development of chronic inflammation in the gut.[6,19,20] Therefore, the tight regulation of intestinal permeability emerges as a central mechanism to prevent inflammatory diseases. Numerous pathogens and toxins, hormones and neurotransmitters, and gastrointestinal and non-gastrointestinal diseases have been associated with an augmented intestinal permeability.[21] Among them, stress hormones and neurotransmitters have been consistently shown to modulate ion and water secretion, intestinal permeability, mucus secretion, and also intestinal flora.[22-25]

Stress-induced Intestinal Barrier Dysfunction: Role of Corticotropin-releasing Factor and Related Peptides

Stress represents a threat to the internal homeostasis that initiates a systemic coordinated response driven by the autonomic, endocrine, and immune systems to maintain stability. CRF and other members of the CRF signaling family, including urocortin (Ucn) 1, Ucn2, and Ucn3, are the best known and most important neuroendocrine mediators of the stress response.[26,27] Central release of CRF and urocortins mediates autonomic, hormonal, and behavioural responses to stress and at the gastrointestinal tract, stimulates the enteric nervous system to modulate gastrointestinal motility and secretion.[28-30] In addition, immune cells, regional sensory and sympathetic nerves, enterochromaffin cells, and enteric cells release CRF and urocortins within the gastrointestinal tract[28] to modulate mucosal function and gastrointestinal motility.[29]

Different type of stress, acute or chronic, physical or psycho-

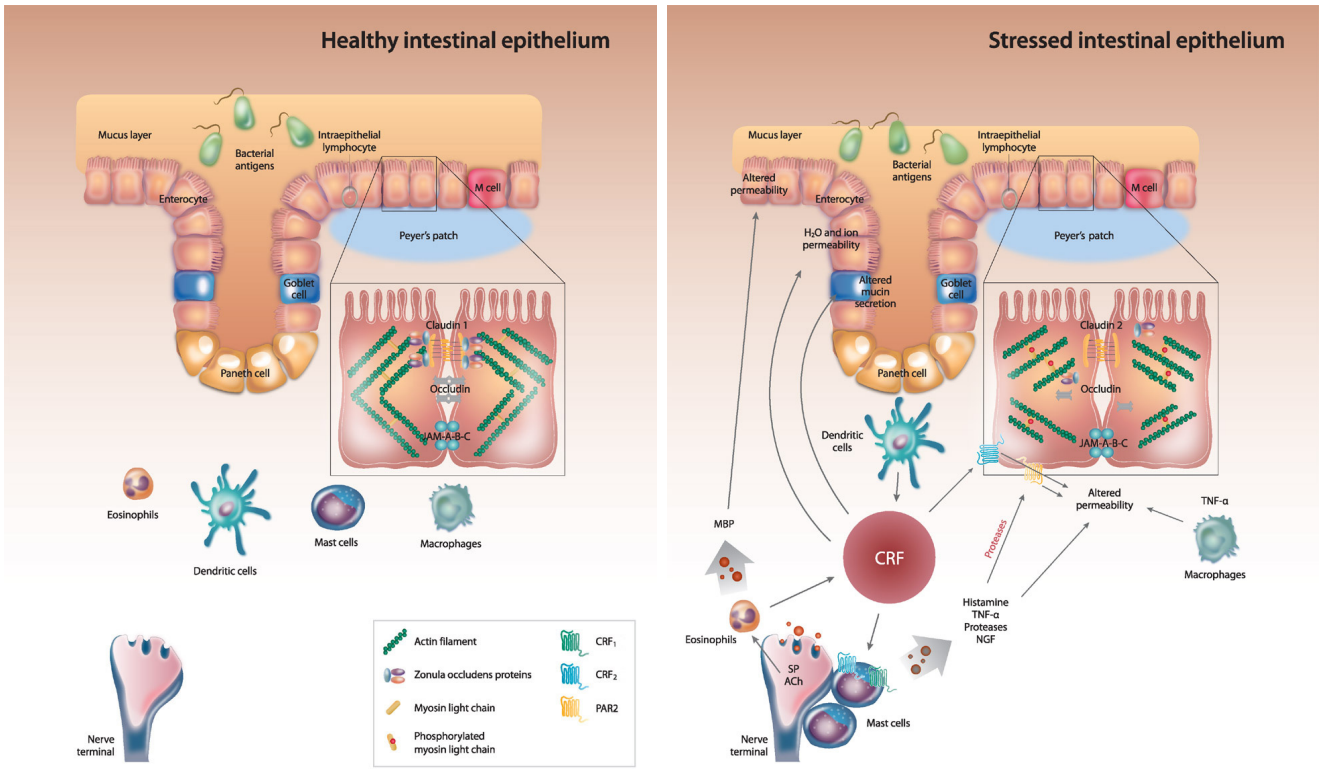

Figure. Corticotropin-releasing factor actions on gastrointestinal permeability under stress conditions. JAM-A-B-C, junctional adhesion molecule A-B-C; SP, substance P; ACh, acetylcholine; MBP, major basic protein; CRF, corticotropin- releasing factor; CRF_1, CRF receptor 1; CRF_2, CRF receptor 2; PAR2, protease activated receptor 2.

logical, have been shown to influence properties of the intestinal barrier function, including ion and water secretion, intestinal permeability, mucus secretion, and also intestinal flora in both human and animal models.[6,31-35] Similarly, both central and peripheral administration of exogenous CRF have been shown to mimic the effects of acute stress in the gastrointestinal tract inducing mucin release,[36] and increasing ion and water secretion and intestinal permeability.[37,38] Figure summarizes the main effects of stress on the intestinal barrier and the role of CRF in gastrointestinal permeability.

Mucus Production and Release

The intestinal mucosal surface is covered by a layer of mucus gel containing antibacterial peptides and digestive enzymes.[39,40] Mucus protects the epithelial lining from adhesion and invasion by attacking microorganisms, and other antigens present in the intestinal lumen.[41] Mucus is produced by goblet cells, which express the CRF receptor 1 (CRF_1).[42] Stress has been shown to increase mucin release in colonic explants from rats submitted to immobilization, and to enhance rat mast cell protease II and prostaglandin E2 secretion. Intravenous or intracerebral injection of CRF in non-stressed rats reproduced stress-associated changes whereas administration of the CRF antagonist α-helical-CRF_{9-41} or the mast cell stabilizer lodoxamide inhibited them.[36] In addition, those changes were abolished in mast-cell deficient mice, highlighting the key role of the CRF-mast cell axis in stress-mediated mucin release.[43] On the contrary, rats submitted to chronic stress display mucus depletion and reduced number of goblet cells in the distal colon in association with increased bacterial adhesion and penetration into enterocytes,[44,45] what could represent a step towards intestinal barrier dysfunction.

Ion and Water Secretion

Ion and water secretions also help to prevent the adhesion of pathogens and noxious substances to the mucosal surface, and dilute and flush out them down the gut to minimize their penetration to the lamina propria. Convergent evidences from studies in animal models indicate that both water and ion secretions increase in the intestinal tract in response to acute and chronic stress[24,46] and in response to peripheral administration of CRF[47] whereas selective CRF_1 receptor agonists prevent this response to stress.[48,49] Increased baseline short-circuit current (*Isc*), indicative of enhanced anion secretion, was found in the jejunum of Wistar-Kyoto rats submitted to restraint stress or cold restraint stress when compared to non-stressed rats. This increase in *Isc* was mediated by chloride anion secretion because replacement of the buffer by chloride free solution normalized *Isc* values.[50] Later, Santos et al[37,51] reproduced the results using a model of repetitive exposure to water avoidance stress (WAS) and found that peripheral CRF reproduced stress-induced rat jejunal and colonic epithelial barrier dysfunction via cholinergic and adrenergic nerves, and mast cells. More recently, long-term crowding exposure, a model of psychosocial stress, has been shown to increase baseline *Isc* in both the jejunum and the colon compared to non-stressed animals.[52] Interestingly, colonic tissues from stressed rats exhibited a reduced *Isc* response to the addition of cholinergic agonists and CRF, suggesting an impaired secretory response to incoming stressful stimuli. Mitochondrial activity was disturbed throughout the intestine, although mitochondrial response to CRF was preserved. These changes were associated with an increased expression of CRF_1 receptor in the colon of stressed rats and colonic hyperalgesia.[53] Several other studies using various stress models have reproduced the elevation of *Isc* in the colon and ileum of rodents and showed the involvement of CRF receptors.[24,33,45,50,54-56]

Both the acute intraperitoneal administration of CRF and chronic peripheral administration of CRF, the selective CRF_1 receptor agonist stressin-1 and the selective CRF_2 receptor ligand, Ucn3, increase the basal rat colonic *Isc*.[33,57] Therefore, a role for both CRF receptor subtypes in this alteration is suggested, although the response is not reduced by the selective CRF_2 receptor antagonist antisauvagine.[57] Furthermore, elevated baseline *Isc* has been also observed in the jejunum of early-weaned pigs, while activation of mast cells and CRF induced elevations in *Isc* in the pig jejunum mounted in Ussing chambers. This elevation in *Isc* was inhibited using mast cell protease inhibitors.[58] Experiments using selective CRF receptor antagonists reveal that CRF_1 receptor activation mediates barrier dysfunction and hypersecretion in the porcine intestine in response to early life stress.[58]

Apart from ex vivo and in vivo studies, convincing evidence for a direct effect of CRF peptides on intestinal epithelial secretory response comes from in vitro work showing a consistent increase in *Isc* induced by CRF in the rat colon.[33,37,59] Interestingly, maximal *Isc* increments were obtained with sauvagine, a CRF agonist with high affinity to both CRF receptors, at doses 200-1000 fold lower than CRF, suggesting a predominant CRF_2 receptor effect.[59] In addition, the effect of peripheral CRF on *Isc* increase in rat colonic tissue implicates mast cells, and nicotinic and muscarinic receptors, as established by the pharmacological blockade

of specific receptors and the use of mast cell deficient rats.[37,57] Other studies performed in the guinea-pig support the ample distribution of CRF_1 receptor, but not CRF_2 receptor throughout the myenteric and submucosal plexuses of the gastrointestinal tract.[60] However, all CRF and Ucn1, Ucn2, and Ucn3 were able to increase the excitability of neurons from the enteric nervous system in vitro.[61]

Experimental studies in humans reveal that acute physical or psychological stress modulate the intestinal barrier. More than 20 years ago, Barclay and Turnberg et al,[62,63] using segmental perfusion techniques in the human jejunum, showed that acute stress reduced net water absorption or increased secretion, supporting the role of the central and the autonomic nervous system in the control of intestinal function. In particular, a reduction in water absorption coupled with net sodium/chloride secretion was observed in healthy subjects subjected to psychological stress induced by dichotomous listening.[62,63] Later, Santos et al[64] showed that cold pain stress increased jejunal water secretion in healthy subjects and in patients with food allergy. These observations have been confirmed and extended recently: using the cold pressor test. The increase in intestinal water secretion during cold pain stress was higher in healthy female volunteers with lower background stress levels, yet blunted in those with elevated background stress.[65] These results suggest that chronic life stress could lead to the loss of regulatory mechanisms in healthy individuals, as previously described in the jejunum of chronic stressed rats after neural stimulation.[50] CRF_1 receptor is expressed throughout the gastrointestinal tract in healthy subjects, and located in the lamina propria, mostly in macrophages, epithelial cells and enteric neurons, with the highest levels in the ileum and rectum and the lowest level in the colon.[66] CRF_2 receptors are located in the lamina propria and in the epithelial cells of the distal/sigmoid biopsy samples in healthy subjects.[67] In contrast to animal studies, the addition of CRF to human colonic biopsies from healthy individuals mounted in Ussing chambers did not show any effect on *Isc*.[68]

Permeability

Numerous studies demonstrate the enhancement of intestinal permeability by acute and chronic stressors. In rodents, acute or subchronic stressors, including restraint stress, cold restraint stress, WAS, mild noise stress, and mixed restraint and acoustic stress, increased tissue conductance, and the fluxes of ^3H mannitol,[51] Cr-labeled EDTA, horseradish peroxidase, in the jejunum, ileum, and colon.[32,50,54,69-72] These results indicate the ability of stress to modulate paracellular and transcellular transport of ions, and small and large macromolecules. Multiple mechanisms have been invocated to explain stress-mediated transport abnormalities. The list includes acetylcholine release, histamine, glucagon-like peptide-2, myosin light chain kinase, cytokines such as interferon-γ, interleukin (IL)-4 and IL-13, and mast cell activation, among the most relevant.[32,61,71,73-75] Moreover, increased intestinal permeability, measured by lactulose, mannitol and sucralose ratios, was found in rats' small intestine, following acute swimming stress.[23] This increase was glucocorticoid-mediated because adrenalectomy and pharmacologic blockade of glucocorticoid receptors inhibited the response, and dexamethasone increased gastrointestinal permeability in control rats.[23]

Similarly, rats models of chronic stress (maternal deprivation,[55,77-79] WAS,[45] and crowding stress[52]) also show increased ionic permeability, and macromolecular permeability throughout the intestine. The increase was mediated partly by mechanisms including, muscarinic and nicotinic pathways, activation and number of mast cells and the release of nerve growth factor.[79] Stress-induced enhancement of colonic permeability was mimicked by exogenous administration of CRF,[33] and abolished by pretreatment with the peripheral administration of the non-selective CRF antagonists astressin or α-helical CRF_{9-41}.[37,55,80] Likely, the selective CRF_1 receptor agonist, cortagine,[48] the selective CRF_1 receptor antagonist, SSR-125543,[80] and the selective CRF_2 receptor antagonist, antisauvagine-30,[78] reduced the response, supporting the participation of both CRF receptors in the modulation of colonic permeability.

More recently, studies in several cultured epithelial cell lines, including intestinal human HT-29 cells, showed that CRF induced the upregulation of the endotoxin receptor, toll-like receptor 4 (TLR4), and that pretreatment with the CRF_2 receptor antagonist, antalarmin, abrogated the response. The concomitant presence of CRF and lipopolysaccharide increased permeability to horseradish peroxidase and decreased transepithelial resistance. This effect was abolished by the CRF_2 receptor antagonist, $astressin_2$-B, and was mediated through the upregulation of claudin-2. Similarly, the expression of TLR4 and claudin-2 increased in the small and large intestine of pregnant mice submitted to 10 days of WAS, as was enhanced the permeability to horseradish peroxidase, and the effect abolished by α-helical CRF_{9-41}[81] and anti-claudin antibodies. Interestingly, rats submitted to WAS for 10 days, showed higher sensitization to orally-delivered horseradish peroxidase, as shown by enhanced intestinal permeability upon antigen re-exposure. This effect was prevented by CRF

blockade with by α-helical CRF_{9-41}.[82]

Another line of evidence in animal models highlights the relevance of stress-CRF-mast cell axis in the regulation of intestinal permeability. Elevation of intestinal permeability, in response to restraint stress or intraperitoneal administration of CRF, was reduced or abolished after pretreatment with doxantrazole.[59] Similarly, pharmacological inhibition of mast cell activation has been shown to inhibit stress-induced increased intestinal permeability in different animal models.[37,58,59,83] Stress/CRF-induced changes in the rat intestinal permeability were also significantly reduced in tissues from mast-cell deficient rats.[45,51,59,84] Teitelbaum et al[57] also found CRF to cause mast cell hyperplasia and abnormal bacterial attachment and/or penetration into the rat mucosa except in mast-cell deficient rats. The authors revealed that stimulation of CRF_1 receptor induced an elevated secretory state, while CRF_2 receptor stimulation was associated with permeability dysfunction.[57] Although it is clear that mast cell activation disrupts intestinal barrier and increases intestinal permeability, the ultimate mechanisms remain to be elucidated. In this sense, several mast cell mediators have been involved. Tumor necrosis factor-α (TNF-α) has been shown to increase intestinal permeability through nuclear factor-kappa B activation in association with nuclear factor-kappa B-dependent downregulation of zona occludens protein-1 expression and alteration in junctional localization in Caco-2 cells,[85] and through myosin light chain kinase phosphorylation.[86] More recently, using a porcine ex vivo intestinal model, Overman et al[87] found that CRF increased intestinal paracellular permeability via mast cell dependent release of TNF-α and proteases. On the other hand, the tryptase released by mast cells upon activation has been shown to induce TJs disassembly through the activation of proteinase-activated receptor-2 of the epithelial cells.[88-90] These receptors can modulate enteric neurotransmission, secretion, motility, epithelial permeability, and visceral sensitivity, and are also known to regulate intestinal inflammation.[91] Anatomical contacts between mast cells and enteric nerve fibers have been demonstrated in the human gastrointestinal mucosa and inflammation multiplies these contacts.[92,93] Mast cells communicate, bidirectionally, with both the enteric, autonomic and central nervous system through mast cell mediators and neuropeptides.[94] In fact, human mast cells synthesize and secrete both CRF and Ucn in response to immunoglobulin E receptor activation.[95] Mast cells also express CRF receptors, whose activation leads to the release of cytokines and other pro-inflammatory mediators. CRF enhances the transcellular uptake of macromolecules in the human colonic mucosa in vitro, via CRF receptor subtypes CRF_1 and CRF_2 expressed on subepithelial mast cells.[58] CRF_1 and CRF_2 receptors have been described in human umbilical cord blood-derived mast cells, while only CRF_1 receptor has been found in the human leukemic mast cell HMC-1 line. CRF stimulation induces the secretion of vascular endothelial growth factor without tryptase, histamine, IL-6, IL-8, or TNF-α release through CRF_1 receptor.[96] More recently, acute stress- induced bladder vascular permeability and vascular endothelial growth factor release have been shown to be dependent on CRF_2 receptor.[97]

It is important to note here the ability of eosinophils to alter intestinal permeability and the mechanisms and mediators involved.[98] Barrier function has been shown to be affected by eosinophil-derived major basic protein (MBP) through downregulation of occludin in a mast cell independent fashion.[98] The neuropeptide substance P (SP) has been found to induce the release of vasoactive mediators from mast cells, macrophages, and T cells, contributing to chloride secretion, enhanced intestinal permeability, and vascular leakiness.[99,100] SP increases the expression of CRF_1 receptor in mast cells, and in turn, CRF induces the expression of the specific receptor neurokinin (NK)-1.[101] More recently, psychological stress, through SP release, mediated stress-induced CRF expression in mice eosinophils and eosinophil-derived CRF was responsible for mast cell activation and epithelial barrier dysfunction. In this work, a cell line of eosinophils was treated with a number of stress mediators, but only SP induced CRF release via NK-1 and NK-2. Moreover, priming of eosinophils with SP resulted in mast cell activation through eosinophil-derived CRF that in turn induced intestinal barrier dysfunction.[102] Wallon et al[83] examined non-inflamed colonic mucosal biopses from patients with ulcerative colitis and found that eosinophils displayed immunoreactivity to CRF. In addition, in co-culture studies, carbachol activation of eosinophils induced CRF release and subsequent activation of mast cells, which increased permeability of epithelial cells to macromolecules.[83] Similar to mast cells, eosinophils have also been shown to localize close to airway nerve terminals in patients with asthma as well as in animal models of bronchial hyperreactivity.[103,104] This proximity to nerve terminals is described in certain digestive disorders,[105,106] providing the anatomical substrate to understand the relevance of the mast cell-eosinophil-stress/CRF axis in the regulation of epithelial permeability and the initiation of immune and inflammatory diseases.

Stress-induced increase in gut paracellular permeability has also been shown to depend on CRF_1 receptor-mediated mast cell

release of nerve growth factor (NGF). Maternal deprivation has been shown to enhance colon permeability in association with elevated NGF expression.[76] A subsequent study from the same group showed that CRF, acting through its receptor CRF_1 receptor, stimulated NGF release from mast cells, which in turn increased gut paracellular permeability.[80] Dendritic cells are relevant for the regulation of intestinal immune function and permeability through CRF production, a process augmented by commensal bacteria.[107] Similarly, CRF_1 and CRF_2 receptor agonists exert a biphasic effect on macrophages. During the early stages of the inflammatory response, they suppress TNF-α release via induction of cyclooxygenase-2/prostaglandin E2 while later on they induce TNF-α transcription.[108] Unfortunately, the role of CRF-mediated activation of macrophages and dendritic cells in stress-related intestinal barrier dysfunction is largely ignored.

Vanuytsel et al[38] have recently shown that, both psychological stress (public speech) and a single intravenous bolus of CRF (100 μg) increased small intestinal permeability in healthy humans, measured by the lactulose/mannitol ratio. Two weeks pretreatment with 800 mg/day of the mast cell stabilizer, disodium cromoglycate, blocked the effect of both stress and CRF, invoking the participation of the CRF-mast cell axis in this response.[38] We have also observed in healthy volunteers that cold pain stress enhanced both the blood-to-lumen albumin[65] ratio and the blood-to-lumen mannitol and xylose permeability.[109] This response was mainly observed in females with higher background stress levels, suggesting an impaired epithelial response to incoming stressful stimuli in this group. In addition, acute cold stress was shown to induce a significant release of α-defensin in the jejunum in this study, supporting the possibility that stress might affect this protective pathway in the gut, as have been shown in the skin.[110] Furthermore, we have also found that cold stress evoked a differential gender-determined increase in human intestinal macromolecular permeability.[111] This enhanced permeability could lead to excessive uptake of luminal antigens and bacterial products that may initiate an inflammatory response in the mucosa.[112] Other studies showing the effect of stress and CRF on intestinal and extraintestinal permeability are shown in Tables 1 and 2, respectively.

Clinical Consequences of Stress/Corticotropin-releasing Factor-mediated Dysregulation of Gastrointestinal Permeability

The growing acknowledgment of the scientific community to the role of intestinal permeability in keeping health and well-being and its relation to the origin of digestive and extradigestive disorders is becoming more and more universal for clinicians. Increased permeability and breakdown of intestinal barrier have been implicated in the origin of gastrointestinal and liver disorders, including celiac disease,[113] inflammatory bowel disease,[114-118] food allergy,[119] acute pancreatitis,[120] irritable bowel syndrome (IBS),[121,122] functional dyspepsia,[123] infectious diarrheal syndromes,[124] primary biliary cirrhosis, and primary sclerosing cholangitis,[125] liver cirrhosis,[126] alcoholic liver disease,[127] liver encephalopathy,[128] and gastroesophageal reflux disease,[129-131] among others. However, whether enhanced gut permeability is an early manifestation of disease, a central step in disease pathogenesis, or a simple epiphenomenon, and its relationship with life stress in the clinical setting, remains to be elucidated. A few examples can illustrate this controversy.

Interestingly, life events may favor the clinical appearance of celiac disease,[132] and cellular stress, through MHC class I chain related genes A and B and endoplasmic reticulum stress pathways, is linked to the disregulation of mucosal homeostasis.[133,134] Early in the 80s, celiac patients were shown to display increased intestinal permeability that normalized after several months on a gluten-free diet.[113] More precise in vitro investigations revealed that although strict gluten withdrawal restored intestinal histology, a subjacent defect in mucosal permeability, measured by cellobiose/mannitol ratio, was transiently induced by short exposure to gluten, suggesting that increased intestinal permeability in celiac disease could be a primary defect.[135] Recently, this defect in intestinal permeability has been related to altered expression of TJ genes related to permeability, polarity, and cell proliferation in active celiac disease,[136] partly through the activation of the zonulin pathway in a MyD88-dependent fashion.[137,138] Again, the majority of genes returned to normal after 2 years of gluten eviction with the exception of PPP2R3A, possibly indicating a constitutive defect in these patients.[136] Moreover, unlike celiac disease, gluten sensitivity is not associated with increased intestinal permeability.[139]

Increased intestinal permeability has also been described in patients with inflammatory bowel disease. Enhanced intestinal permeability in this population is considered an initial event because it is increased in first-degree relatives of Crohn's disease patients.[140,141] Several observations link clinical relapses to the increase in intestinal permeability[142,143] and to life stress,[144,145] partly through mast cell activation[146] and the release of CRF from eosinophils[83] and neighboring nerves.[68] Moreover, inflammatory

Table 1. Studies Showing the Effect of Stress/Corticotropin-releasing Factor on Intestinal Permeability

Author	Permeability assessment methods	Stress model	Results
Santos et al,[37] 1999	Ussing chambers measuring conductance (*G*), short-current circuit (*Isc*) and horseradish peroxidase (HRP) flux in rat colon	Restraint stress and corticotrophin-releasing factor (CRF) administration	Restraint stress increased colonic ion secretion and permeability to ions, bacterial peptide peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP), and HRP. These changes were prevented by alpha-helical CRF_{9-41} and mimicked by CRF administration. Pre-treatment with hexamethonium, bretylium and doxantrazole also prevented CRF-induced changes in ion secretion and G.
Saunders et al,[33] 2002	Ussing chambers measuring G, and HRP flux in rat colon	Cold-restraint and water avoidance stress (WAS), and CRF administration	Cold-restraint stress, and WAS significantly elevated G and HRP flux. CRF mimicked the stress responses. Alpha-helical CRF_{9-41} inhibited the stress-induced abnormalities.
Guilarte et al,[164] 2004	Albumin release to the intestinal lumen in healthy volunteers and irritable bowel syndrome	CRF administration	CRF induced a significant increase in albumin release to the intestinal lumen.
Gareau et al,[180] 2006	Ussing chambers measuring G, and *Isc* in rat colon	Neonatal maternal separation.	Neonatal maternal separation stress increased plasmatic corticosterone, enhanced ion secretion, macromolecular permeability, bacteria adhering, and penetration into the colonic epithelium. Alpha-helical CRF_{9-41} reversed stress-induced effects.
Yang et al,[82] 2006	Ussing chambers measuring G, *Isc* and HRP flux in rat jejunum	WAS and oral HRP sensitization	Antigen challenge induced a rapid ion secretory response and an increase in G only in rats submitted to WAS. These effects were reversed by alpha-helical CRF_{9-41}.
Gareau et al,[78] 2007	Ussing chambers measuring HRP flux in rat colon	Neonatal maternal separation	Neonatal maternal separation stress increased HRP flux. The enhanced flux was inhibited by atropine and hexamethonium. Alpha-helical CRF_{9-41} and antisauvagine-30 inhibited stress-induced increase in HRP flux.
Santos et al,[59] 2008	Ussing chambers measuring *Isc* and HRP flux in rat colon	CRF and sauvagine exposure	Sauvagine and CRF induced a dose-dependent increase in *Isc* and HRP flux and an enhancement in protease II pre-treatment with astressin, and doxantrozole inhibited this response. Mast-cell deficient mice displayed a reduced epithelial response to stress peptides.
Teitelbaum et al,[57] 2008	Ussing chambers measuring G, *Isc* and HRP flux in rat colon	CRF administration	Chronic CRF administration increased *Isc*, G, and HRP flux, but not in mast-cell deficient rats. CRF administration induced mast cell hyperplasia and abnormal bacterial attachment into the mucosa that was absent in mast-cell deficient rats.
Alonso et al,[65] 2008	Albumin release to the intestinal lumen in healthy volunteers	Cold Pain Stress	Cold pain stress induced a significant increase in albumin release to the intestinal lumen.
Wallon et al,[68] 2008	Ussing chambers measuring *Isc*, HRP flux, ^{51}Cr-EDTA, and transepithelial resistance (TER) in human colon	CRF administration	CRF increased permeability to HRP. The increased permeability to HRP was abolished by alpha-helical CRF_{9-41}, and lodoxamide pre-treatment.
Larauche et al,[48] 2009	Evans blue extravasation in rat colon	Cortagine administration	Cortagine induced a significantly increased intestinal permeability. Astressin-B abolished the cortagine-induced increase in intestinal permeability.
Zheng et al,[102] 2009	Ussing chambers measuring *Isc*, HRP flux, and TER in mouse jejunum	Restraint stress and substance P (SP) exposure	SP stimulation induced a significant increase in *Isc* and HRP flux in stressed mice. Those changes were lower in mast cell-deficient mice. Alpha-helical CRF_{9-41}, inhibited SP-induced intestinal barrier dysfunction.

Table 1. Continued

Author	Permeability assessment methods	Stress model	Results
Smith et al,[58] 2010	Ussing chambers measuring *Isc*, ^{3}H mannitol flux, ^{14}C inulin flux, and TER in pig jejunum and colon	Early weaning	Early weaning reduced jejunal TER and enhanced *Isc* and mucosal-to-serosal flux of ^{3}H mannitol and ^{14}C inulin in association with increased lamina propria mast cell density. Sodium cromoglycolate ameliorated barrier dysfunction and hypersecretion in early-weaned pigs. C48/80 and CRF exposure increased *Isc* and induced intestinal barrier dysfunction that were inhibited with mast cell protease inhibitors.
Keita et al,[181] 2010	Ussing chambers measuring G, *Isc*, HRP flux, ^{51}Cr-EDTA, and *Escherichia coli* K-12 flux in rat follicle-associated epithelium (FAE) and villus epithelium (VE) from rat ileum	WAS	WAS increased G, *Isc*, HRP and *E. Coli* uptake in FAE and VE. SP increased bacterial and ^{51}Cr-EDTA intestinal permeability. These results were mimicked by CRF and carbachol and reduced by doxantrazole, CRF receptor antagonist and atropine.
Wallon et al,[83] 2011	Ussing chambers measuring *Isc*, HRP flux, ^{51}Cr-EDTA, fluorescein isothiocyanate (FITC)-Dextran 4000, and TER in non inflamed human colon biopsies from ulcerative colitis patients	None	HRP flux, TER, and *Isc* were increased in mucosa from patients with UC. Alpha-helical CRF_{9-41}, atropine and lodoxamide reversed the increase in intestinal permeability.
Alonso et al,[111] 2012	Albumin release to the intestinal lumen in healthy volunteers	Cold Pain Stress	Cold pain stress induced a significant increase in albumin release to the intestinal lumen.
Ait-Belgnaoui et al,[182] 2012	Ussing chambers measuring FITC-Dextran flux in rat colon	Partial restraint stress	Stress increased plasma ACTH and corticosterone, and hypothalamic CRF and enhanced colonic paracellular permeability. Probiotic treatment prevented stress-induced increased intestinal permeability.
Overman et al,[87] 2012	Ussing chambers measuring FITC-Dextran flux in porcine ileum	CRF exposure	CRF increased paracellular FITC-Dextran flux. Pre-treatment with astressin-B, sodium cromolyn, anti-TNF-α antibodies, protease inhibitors, and tetrodotoxin inhibited CRF-mediated intestinal barrier dysfunction.
Vicario et al,[53] 2012	Ussing chambers measuring *G* and *Isc* in rat colon	Crowding stress and CRF administration	Crowding stress significantly increased G and *Isc* and CRFR1 in the rat colon. CRF administration mimicked stress-induced epithelial dysfunction.
Hill LT et al,[183] 2013	Lactulose-mannitol urinary excretion test in shocked patients undergoing small bowel resection during emergency laparotomy and patients undergoing elective hepatobiliary surgery	Shock and abdominal surgery	Shock was associated with increased intestinal permeability. Plasma CRF was significantly increased in the shocked patients.
Yu et al,[81] 2013	HRP flux and TER in HT-29, T84, MDCK, and Caco2 monolayers Ussing chambers measuring G, *Isc*, and HRP flux in mouse colon	CRF exposure and WAS	WAS increased G and *Isc* and HRP flux, and this increase was higher after LPS stimulation. This response was abolished by pre-treatment with anti-claudin 2 (Cldn2) antibodies. Stress also increased the expression of Cldn2 and toll-like receptor-4 (TLR4) in mouse epithelium. Exposure to CRF induced Cldn2 and TLR4 expression in intestinal epithelial cells.
Vanuytsel et al,[38] 2014	Lactulose-mannitol urinary excretion test in healthy volunteers	Indomethacin administration, public speech, CRF administration, and electroshock anticipation	Public speech and CRF administration increased intestinal permeability and salivary cortisol. Increased permeability after public speech was only present in subjects with a significant elevation of cortisol. Pre-treatment with disodium cromoglycate inhibited stress and CRF-induced increased intestinal permeability.

Table 2. Studies Showing the Effect of Stress/Corticotropin-releasing Factor on Extraintestinal Permeability

Author	Permeability assessment methods	Stress model	Results
Wei et al,[184] 1986	Evans blue extravasation in rat paw	Antidromic stimulation of the saphenous nerve in innervated rat paw	Corticotropin-releasing factor (CRF) inhibited neurogenic plasma extravasation in the innervated rat paw. This effect was independent of the hypothalamus or the adrenal gland.
Wei and Kiang,[185] 1987	Evans-blue extravasation in rat trachea	Antidromic stimulation of the right vagus or exposure to dilute formalin vapors	CRF inhibited tracheal plasma protein extravasation.
Kiang et al,[186] 1987	Evans-blue extravasation in rat paw	Immersion of rat's paw in 48°C or 58°C water	CRF inhibited thermal injury-induced pasma extravasation and edema.
Wei et al,[187] 1988	Fluid displacement method in rat paw	Immersion of anesthetized rat paw in 58°C water	CRF inhibited the progressive development of swelling, and reduced edema, epidermal necrosis and the disruption of tissue architecture produced by thermal injury. CRF effects were reverted by alpha-helical CRF_{9-41}.
Tian and Wei,[188] 1989	Changes in skin weight and Evans blue extravasation in rat paw	Anesthetized rat paw immersion in 12 N hydrochloric, 18 N sulfuric, or 14 N hydrofluoric acids	CRF reduced the skin acid-induced skin injury.
Wei and Kiang,[189] 1989	Evans blue extravasation in rat paw	Anesthetized rat paw immersion in 48°C or 58°C and antidromic stimulation of the saphenous nerve	Sauvagine and CRF inhibited plasma extravasation induced by thermal and neurogenic injury.
Serda and Wei,[190] 1991	Evans blue and Monastral extravasation in rat paw	Anesthetized rat paw immersion in 22% NaCl solution maintained at −20 ± 0.5°C.	CRF inhibited the acute inflammatory response of rat paw skin to cold injury. The anti-inflammatory effects of CRF were blocked by alpha-helical CRF_{9-41}.
Wei and Gao,[191] 1991	Monastral blue extravasation in rat paw	Mechanical injury to muscle produced by a midline surgical incision in the rectus abdominis or freeze injury to the cortex produced by applying a cold probe (−50°C) to the skull	CRF inhibited the leakage of small blood vessel due to muscle and brain injury.
Gao et al,[192] 1991	Monastral blue extravasation in rat skin, muscle, trachea and esophagus	Substance P (SP) administration	SP induces plasma protein leakage in skin, muscle, trachea and esophagus. This effect was reverted by the treatment with CRF.
Kelley et al,[193] 1994	Measurement of lung wet-to-dry ratios to asses mice pulmonary vascular leak	Lipopolysaccharide (LPS) administration	Pulmonary vascular leak, and leukocyte infiltration were significantly depressed by CRF treatment.
Yoshihara et al,[194] 1995	Evans blue extravasation in Guinea pig trachea and main bronchi	Antigen challenge through inhalation of 5% ovalbumin in the presence of phosphoramidon	CRF reduced ovalbumin-induced plasma extravasation in guinea pig airways by inhibiting the release of tachykinins from primary sensory nerves.
Whitney et al,[195] 1997	Comparison of rat preischemic amputated limb weight with weight after ischemia and reperfusion	Hind limb replantation	CRF inhibited the gain of weight by ischemia-reperfusion and alpha-helical CRF_{9-41} administration partially reversed this effect.
Theoharide et al,[196] 1998	Evans blue vascular extravasation in the rat skin	CRF administration	CRF induced mast cell degranulation and increased capillary permeability, and the antalarmin blocked this effect.
Whitney et al,[197] 1998	Comparision of rat pedicled hind limb weight with ischemic pedicled hind limb weight	Pedicled hind limb	CRF administration decreased limb weight gain.

Table 2. Continued

Author	Permeability assessment methods	Stress model	Results
Singh et al,[198] 1999	Evans blue vascular extravasation in rat skin	Urocortin (Ucn) administration	Ucn induced rat skin mast cell degranulation and increased vascular permeability. Alpha-helical CRF_{9-41}, antalarmin and astressin reverted this effect.
Rapallino et al,[199] 2001	Ionic permeability of rabbit vestibular Deiters neurons membrane	Rotation platform	CRF blocked basal chloride permeation across the Deiters' membranes and this effect was partially reversed by alpha-helical CRF_{9-41}.
Esposito et al,[200] 2002	^{99}Tc gluceptate extravasation in rat brain	Restraint stress	Acute stress and CRF paraventricular nucleus injection increased blood-brain-barrier ^{99}Tc gluceptate extravasation. Antalarmin and cromolyn inhibited this effect.
Hendryk et al,[201] 2002	Evans blue vascular extravasation in rat brain	Closing of both internal carotid arteries	CRF decreased the endothelial damage in the acute phase of the ischemia.
Huang et al,[202] 2002	^{99}Tc gluceptate extravasation in mice skin and knee joints	Restraint stress	Vascular permeability to ^{99}Tc, as well as local CRF levels, were increased by stress, but not in mast-cell deficient mice.
Lytinas et al,[203] 2003	^{99}Tc gluceptate and Evans blue extravasation in rat skin	Restraint stress	Acute stress increased skin CRF and vascular permeability. These effects were inhibited by histamine-1 receptor antagonists.
Donelan et al,[204] 2006	Evans blue extravasation in mice skin	CRF administration	CRF increased vascular permeability. The neurotensin blocker (SR48692) inhibited CRF effects. CRF-induced increased vascular permeability was absent in mast cell deficient mice.
Wu et al,[205] 2006	Evans blue extravasation in the rat lung	Ucn aerosol inhalation	Ucn inhalation increased lung vascular permeability. Enhanced pulmonary vascular permeability induced by Ucn was markedly inhibited by pretreatment with cromolyn, and azelastine.
Cureton et al,[206] 2009	Measurement o hydraulic and macromolecule permeability in rat mesenteric venules	LPS-induced systemic inflammation	LPS and Ucn incremented hydraulic permeability. CRF_2 receptor blockade decreased the LPS-induced increase in hydraulic permeability.
Boucher et al,[97] 2010	Evans blue extravasation in mice bladder	Restraint stress	Acute stress increased bladder vascular permeability. CRF_2 receptor and $astressin_2$-B inhibited this effect.
Song et al,[207] 2013	Transepithelial resistance (TER) and permeability to horseradish peroxidase (HRP) in human endothelial cell monolayers (Hmvec)	CRF treatment	Exposure to CRF induced TNF-alpha release by CD14 effector cells, decreased TER and increased permeability to HRP in co-cultured Hmvec monolayers.
Wan et al,[208] 2013	Crystal violet, FITC-Dextran and resistance in human umbilical vein endothelial cells (HUVEC)	Lipopolysaccharide treatment	Pretreatment of HUVECs with urocortin increased LPS-induced endothelial permeability by regulating the cadherin-catenin complex via CRF_2 receptor.

bowel disease patients show associations with genes involved in the regulation of intestinal barrier and various alterations of the transmembrane and intracytoplasmic proteins.[147,148] In addition, increased CRF_1 receptor[66] and Ucn expression, but decreased CRF_2 receptor[67] expression have been shown in the colonic mucosa of active ulcerative colitis patients.[149,150]

Accumulating evidence also indicates that IBS is linked to abnormal intestinal permeability,[151-153] CRF release and life stress[154-157] in close association with low-grade mucosal inflammation and immune activation.[158] Several groups have provided preliminary evidence linking clinical manifestations of IBS to structural abnormalities of the apical junctional complex in both the small[159,160] and large bowel mucosa.[81,161-163] Furthermore, unpublished observations from our group indicate that a single intravenous bolus of CRF (100 μg) increased intestinal permeability, measured as the blood-to-lumen albumin ratio, in healthy subjects and in IBS patients through mast cell activation.[164]

In the same line, increased intestinal permeability and stress/

CRF axis have also been involved in the pathophysiology of food allergy, as these patients display an enhancement of intestinal permeability even in the absence of food allergens[165] and stress facilitates sensitization to luminal antigens.[82] In fact, patients following immunosuppressive treatment have been shown to develop new-onset food allergies that may be related to the increase in intestinal permeability induced by treatment.[166]

Many other stress-related conditions such as severe burn,[167,168] hemorragic shock,[169] chronic kidney disease,[170] type 1 diabetes and the metabolic syndrome,[171,172] neuropsychiatric disorders,[173] autism,[174] autoimmune thyroiditis,[175] IgA nephropathy,[176] patients with primary immunodeficiencies,[177] and sepsis[178] have been associated with increased intestinal permeability.

Conclusion

The ability of stress and peripheral CRF to affect intestinal epithelial function and, particularly, intestinal permeability, is well documented in human and several animal species. Interestingly, apart from the direct effects on the enterocytes, the stress-induced increase of intestinal permeability is mediated via recruitment and activation of mast cells, eosinophils, macrophages and other mononuclear cells, and implicates both CRFR1 and CRF_2 receptors.[28]

There is abundant literature supporting the link between life stress and the origin and clinical course of several human disorders,[179] however, the ultimate clinical relevance of stress-CRF-mediated dysregulation of gastrointestinal permeability, remains at present mostly intuitive, with few exceptions. More work in this area is needed to confirm the findings. In this sense, studies with CRF and related peptides and the development of new antagonists for human use, will offer the opportunity to test this hypoyhesis.

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