

Corticotropin-releasing factor signaling and visceral response to stress

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Abstract

Stress may cause behavioral and/or psychiatric manifestations such as anxiety and depression and also impact on the function of different visceral organs, namely the gastrointestinal and cardiovascular systems. During the past years substantial progress has been made in the understanding of the underlying mechanisms recruited by stressors. Activation of the corticotropin-releasing factor (CRF) signaling system is recognized to be involved in a large number of stress-related behavioral and somatic disorders. This review will outline the present knowledge on the distribution of the CRF system (ligands and receptors) expressed in the brain and peripheral viscera and its relevance in stress-induced alterations of gastrointestinal and cardiovascular functions and the therapeutic potential of CRF₁ receptor antagonists.

Keywords: cardiovascular system, corticotropin-releasing factor (CRF), irritable bowel syndrome (IBS), stress, urocortin (Ucn)

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Introduction

In 1936, Hans Selye pioneered the concept of biological stress based on macroscopic evidence that specific organs are commonly altered by various stressors. He showed that rats develop similar hypertrophy of the adrenals, involution of lymphatic organs and gastric erosions in response to exposure to noxious agents irrespectively of their chemical or physical nature.^{1,2} Thereafter, in the 1950s, Geoffrey Harris^{3,4} introduced the seminal concept of hypothalamic neurohumoral control of the pituitary adrenocorticotropin (ACTH) secretion that is activated by stress. Fifteen years later, the Nobel laureates, Drs Guillemin⁵ and Schally⁶ independently proved the existence of a corticotropin-releasing factor (CRF) in line with its stimulatory effect on ACTH release from the pituitary in rats. However, although CRF was one of the first hypothalamic releasing factors to be named, the elucidation of its structure lingered for nearly three decades until Vale *et al.*⁷ characterized CRF as a 41 amino acid (aa) peptide isolated from 490,000 ovine hypothalami. Subsequently, the same group cloned the CRF₁ and CRF₂ receptors, identified additional members of the CRF family^{8–12} and developed specific peptide CRF receptor antagonists^{13–15} providing relevant tools to identify the molecular mechanisms of the stress response.

With great foresight, Selye² had suspected early on that the yet to be chemically characterized CRF was ‘the first

mediator that integrates the adaptive bodily response to stress’. Soon after the availability of the synthetic peptide, compelling reports established that CRF exerts a number of biological actions independently of the HPA activation. Existing experimental evidence showed that CRF injected into the brain mimics the overall behavioral (anxiety/depression and alteration of food consumption), autonomic (sympathetic and sacral parasympathetic activation), immune, metabolic and visceral adaptive changes produced by exposure to various systemic or cognitive stressors.^{16–21} These initial findings built the foundation to explore the mechanisms of CRF’s actions in the brain and the role of CRF receptor activation in driving the stress-related visceral response. Consequently, curtailing the CRF signaling system was viewed as a potential pharmacological target in the drug treatment of various stress-related disorders.^{22–24} Among those, irritable bowel syndrome (IBS) is a highly prevalent functional bowel disorder that affects up to 20% of the North American population.²⁵ It is characterized by chronic abdominal pain or discomfort associated with changes in bowel habits (diarrhea, constipation or alternating pattern), generally in the absence of detectable organic alterations.^{25–27} Stressful life events and psychosocial trauma, alone or combined with previous episodes of bowel infection/inflammation, have been identified as important risk factors that may account for the

development, severity and/or maintenance of IBS symptoms.²⁸ Moreover, IBS patients often show somatic²⁹ as well as psychological comorbidities such as anxiety and depression reaching 40–90% in tertiary care centers.³⁰ Therefore, the understanding of stress signaling pathways seems to be relevant in unraveling the contributing mechanisms of IBS and to identify possible new drug targets.

In this review, we will outline the state-of-knowledge on the CRF signaling system in the brain and visceral organs, and experimental evidence for a role of CRF pathways in mediating stress-related changes in behavioral and visceral functions with an emphasis on gastrointestinal and cardiovascular systems. Potential intervention strategies to dampen CRF signaling in stress-related disorders will also be highlighted.

Biochemical coding of stress signaling: CRF ligands and receptors

CRF peptide family

In addition to the 41 aa peptide CRF, three additional members of the CRF signaling family have been identified more recently, namely urocortin 1 (Ucn 1), urocortin 2 (Ucn 2) and urocortin 3 (Ucn 3).^{8–11} CRF has a well conserved primary structure among mammalian species including humans, primates, dogs and rodents.³¹ The mammalian Ucn 1 (also known as urocortin) is a 40 aa peptide isolated from the rat midbrain that displays 45% sequence identity with rat/human (r/h) CRF. As observed for CRF, the primary structure of Ucn 1 is highly conserved across mammalian species, including rat, mouse and sheep.^{8,32,33} Mouse Ucn 2 (mUcn 2) is a 38 aa peptide that shares 34% homology with r/h CRF and 42% with r/m Ucn 1,¹⁰ whereas human Ucn 3 has only little overlap with the structure of r/h CRF and r/h Ucn 1 (18% and 21% homology, respectively).^{9,11}

CRF receptors

So far, two receptors, CRF₁ and/or CRF₂, have been cloned from two distinct genes that share 70% identity at the aa level.^{21,34} Both CRF receptor subtypes belong to the B1 subfamily of seven-transmembrane domain receptors that signal largely, but not exclusively, by coupling to G proteins resulting in the stimulation of adenylate cyclase.³⁴ CRF₁ and CRF₂ receptors are subject to extensive alternative splicing resulting in various isoforms.^{34–36} However, only the CRF_{1α} isoform is coupled directly to adenylate cyclase, whereas the majority of other variants are lacking the ligand binding and/or signaling domains.^{34,35,37} Nonetheless, alternative splicing variants of CRF₁ receptors are emerging to play an important role as modulators of downstream signaling, as recently thoroughly reviewed.³⁸

With regards to CRF₂ receptors, three functional splice variants differing in their N-terminal extracellular domains are known in humans (2α, 2β and 2γ) and two in rodents (2α and 2β).³⁹ The C-terminus is common to CRF_{2α}, CRF_{2β} and CRF_{2γ} receptor splice variants, while the N-terminal extracellular region, which interacts with the ligand, varies in length and is composed of 34, 61 and

20 aa, respectively.^{36,40,41} The sequence of the CRF_{2α} is highly conserved across mammalian species and also found in amphibians, indicating an early development of this isoform which points towards its physiological importance.⁴¹ In contrast, the CRF_{2β} is not as well conserved and only detected in mammals, indicating a more recent evolution.⁴² Four additional non-signaling CRF_{2α} splice variants have been identified in the mouse brain and rat esophagus and some of them can act as a soluble binding protein (sCRF_{2α})^{36,43} or alter the cellular levels of full-length CRF_{2α} mRNA and hence functional receptor levels.⁴⁴ Recently a novel CRF_{2β} isoform in the mouse heart has been found to act as a dominant-negative regulator of CRF_{2β} membrane expression.⁴⁵

The CRF₁ and CRF₂ receptors display different pharmacological binding characteristics. The CRF_{1α} receptor has high affinity to CRF and Ucn 1 and no affinity to Ucn 2 and Ucn 3. In contrast, the CRF_{2α/β} receptor displays high affinity to Ucn 1, Ucn 2 and Ucn 3 and lower affinity to CRF.^{8,10,39,41,46,47} While Ucn 2 and Ucn 3 have been identified as selective endogenous CRF₂ agonists, so far there is no endogenous ligand exclusively binding to the CRF₁ receptor. However, selective CRF₁ peptide agonists such as cortagine and stressin₁-A have been recently developed providing new tools to characterize the effects of selective activation of CRF₁ receptors.^{48,49}

Distribution of CRF peptides and receptors

CRF expressing neurons are widely distributed in the rat brain with major sites of CRF mRNA and CRF immunoreactivity in the paraventricular nucleus (PVN) of the hypothalamus, cerebral cortex, amygdalar-hippocampal complex and pontine Barrington's nucleus.^{50,51} Of interest, the parvicellular part of the PVN contains a discrete group of CRF expressing neurons in the dorsal and ventral parts sending direct projections to spinal cord and brain stem nuclei, respectively, which regulate autonomic outflow to the viscera.^{52,53} Although being members of the same family, little neuroanatomical overlap exists between CRF and Ucn in the rat brain.^{8,10,53–56} The central distribution of Ucn 1 is very limited with the highest expression in the Edinger-Westphal nucleus (EWN).^{55,56} Ucn 2 gene expression is localized in the PVN, supraoptic nucleus (SON), arcuate nucleus of the hypothalamus (Arc) and locus coeruleus (LC) as well as in several cranial motor nuclei (trigeminal, facial and hypoglossal) and the ventral horn of the spinal cord.¹⁰ Due to the lack of specific Ucn 2 antibodies, little is known about the brain distribution of Ucn 2 peptide. Ucn 3 mRNA is detected in the PVN mainly in the dorsal and ventral aspects, amygdala (basomedial nucleus) and the basomedial nucleus of the stria terminalis.⁵⁴

With regards to CRF receptors, dense CRF₁ receptor expression is found in the forebrain, subcortical limbic structures such as the septal region and amygdala, whereas the expression in the hypothalamus is low under basal conditions but markedly up-regulated by stress.^{57–59} Moreover, CRF₁ receptors are prominently expressed in the anterior and intermediate lobe of the pituitary.⁶⁰ CRF₂ expression

in the forebrain is restricted to subfornical structures with high expression in the lateral septum, amygdala and hypothalamus, including the ventromedial hypothalamus and SON.⁶¹ In the hindbrain, CRF₂ receptors are expressed in the dorsal raphe nucleus, area postrema, nucleus of the solitary tract and choroid plexus.⁶¹

As for a variety of other neuropeptides, initially thought to be restricted to the brain and pituitary and later shown to be widely expressed in the periphery, CRF ligands and receptors are also detected in peripheral tissues including the gastrointestinal tract,^{62,63} heart, lung, spleen, testis and adipose tissue⁶⁴ in animals and humans. In the gastrointestinal tract, immunostaining studies detected CRF immunoreactive neurons in the rat⁶⁵ and guinea pig⁶⁶ enteric nervous system. Ucn 1 is expressed in the rat colonic enteric nervous system at the gene⁶⁷ and peptide⁶⁵ level. In rodents, CRF receptors have been identified throughout the gastrointestinal tract. Whereas CRF₁ receptor expression is localized in the myenteric and submucosal nervous plexus of the distal gut,^{68,69} CRF₂ receptor expression was mainly identified on the luminal surface of the crypts, on blood vessels of the submucosal layer⁶⁸ and also on myenteric neurons.⁷⁰ Importantly, CRF_{2α} is the main variant expressed in the rat brain, whereas CRF_{2β} is expressed in non-neuronal tissues centrally as well as peripherally.⁷¹ In contrast, in humans CRF_{2β} and CRF_{2γ} are expressed mainly in brain neurons whereas CRF_{2α} is found peripherally and centrally.^{72,73} In the human heart, Ucn 1 peptide has been shown by immunostaining⁷⁴ along with the expression of CRF_{2α} and CRF₁ gene and protein expression.⁷⁵ Whereas Ucn 1 concentrations were highest in the left ventricle as assessed by radioimmunoassay, CRF was very low or undetectable in the human heart.⁷⁵

CRF signaling and stress-related behavioral responses

The effects of CRF ligands on behavior have been extensively characterized. Injection of CRF and Ucn 1 directly into the brain induces a variety of behavioral changes. For instance, CRF injected at low doses into the cerebrospinal fluid of non-stressed animals activates locomotor activity as well as rearing and grooming behavior which are unrelated to the activation of the pituitary adrenal axis.^{17,76,77} Central administration of CRF also induces arousal as reflected in typical activation patterns assessed by electroencephalography.⁷⁸ Under basal conditions, central injection of CRF at low doses also stimulates memory and learning, whereas higher doses have an opposite effect.⁷⁹ This enhancement of learning induced by central administration of CRF is CRF₁ receptor-mediated.⁸⁰ Likewise, Ucn 1 increases memory retention at low doses but decreases learning at high doses.⁸¹ In several tests including open field or the elevated plus maze, CRF or Ucn 1 induce CRF₁-mediated, anxiogenic-like behavioral responses.^{76,82–84} Moreover, exogenous central injection of either CRF or Ucn 1 decreases food intake, which is mediated by both CRF₁ and CRF₂ receptors in the early and late phases of the anorexigenic response, respectively.^{85,86} CRF has also been implicated in drug dependence as reflected in CRF

dysregulation in abstinent alcohol dependent rats. Consistent reports showed that CRF₁ receptor antagonists decrease the amount of self-administered drugs such as ethanol or heroin during a period of stress-related escalation.⁸⁷

CRF signaling and stress-related changes in the gastrointestinal tract

Brain CRF receptors are involved in stress-related inhibition of gastric motor function

Several stressors are known to delay gastric emptying in animals as well as in healthy humans.²⁰ Likewise, it is well established that CRF, Ucn 1 and Ucn 2 injected into the cerebrospinal fluid inhibit gastric emptying of a non-caloric or caloric liquid or solid meal and suppress propagative contractions through activation of CRF₂ receptors.⁶³ Moreover, pretreatment with peptide CRF receptor antagonists administered into the brain ventricle blocked the inhibition of gastric motor function induced by various stressors.^{20,88} However, under conditions of surgical stress (abdominal surgery with cecal palpation), central CRF₁ receptors play a major role as indicated by the absence of the postoperative delay of gastric emptying 2 h after surgery in CRF₁ knockout mice.⁸⁹ The central action of CRF to delay gastric transit is not mediated by the associated stimulation of the HPA axis but by the autonomic nervous system since the gastric inhibitory motor response can still be observed in hypophysectomized or adrenalectomized rats and no longer in vagotomized rats.^{90,91} Key structures that influence autonomic outflow to the stomach, namely the PVN and the dorsal vagal complex in the brainstem, have been identified as the brain nuclei mediating the CRF-induced inhibition of gastric emptying and motility in rats.^{63,92,93}

CRF injection into the brain and stress inhibit small intestinal motor function

Much less is known about the effects of stress on small intestinal motor function compared with the large amount of data on the other segments of the gut.⁸⁸ Nonetheless, as observed in the stomach, acute stress as well as central injection of CRF or Ucn 1 inhibits duodenal and small intestinal transit and motility.^{94,95} However, the slowing of CRF-induced small intestinal transit is less pronounced compared with the stomach, which could be attributed to the more prominent enteric than autonomic control of the small intestinal motor function compared with the stomach.⁹⁶ So far, the central CRF receptor subtype involved in the mediation of the inhibitory response in the small intestine remains to be described.

Brain CRF receptors are involved in stress-related stimulation of colonic motor function

Whereas several stressors delay gastric emptying and small intestinal transit, colonic motility is increased in stressed experimental animals and healthy subjects.²⁰ The stimulatory response on colonic transit and defecation is mimicked by

central injection of CRF or Ucn 1 primarily through activation of brain CRF₁ receptors in rodents.^{63,97,98} In contrast to gastric motor functions, the CRF₂ antagonist, astressin₂-B, did not prevent the colonic response to central injection of CRF in rodents.^{20,63} As observed in the stomach, the CRF- and stress-induced stimulation of colonic motor function occurs independently from the activation of the HPA axis.⁹¹ The peripheral pathways involve increased parasympathetic outflow to the colon via vagal celiac branches innervating the proximal colon and more prominently the sacral parasympathetic fibers innervating the distal colon and rectum.⁹⁹

Neuroanatomical and functional evidence support that activation of the CRF₁ signaling pathway in the PVN and LC integrates the behavioral and autonomic-colonic responses to stress.^{100,101} CRF neurons are localized in the dorsal cap of the parvocellular PVN known to have transsynaptic connections to the colon.¹⁰² Moreover, CRF-containing neurons in the Barrington's nucleus project to the LC and to the intermediolateral column of the sacral spinal cord, thereby providing input to catecholamine neurons projecting to the forebrain and sacral parasympathetic nervous system innervating the descending colon.^{96,102} Other studies established that water avoidance stress induces CRF gene transcription in the PVN and activates neurons in the PVN and LC/Barrington's nuclei as assessed by the neuronal marker Fos and the response is blunted by the CRF receptor antagonist, α -helical CRF₍₉₋₄₁₎ injected into the lateral brain ventricle.^{103,104} Moreover, microinjection of CRF into the PVN or LC complex results in a stimulation of colonic motor function and α -helical CRF₍₉₋₄₁₎ microinjected directly into the PVN prevents the stimulated colonic transit and defecation induced by water avoidance or restraint stress,⁹³ indicating the importance of the PVN in orchestrating these responses. Furthermore, CRF injected intracerebroventricularly (icv) increases the activity of noradrenergic neurons in the LC, resulting in the release of noradrenalin into the brain cortex mediating arousal and anxiogenic behavior.^{101,105}

CRF system in the gut and stress-related alteration of gut motor function

The expression of both CRF receptors and ligands in the gastrointestinal tract points towards a role of a local CRF signaling system in the gut.^{68,70,106-108} Peripheral injection of CRF or Ucn 1 in rats inhibits gastric emptying, delays small intestinal transit and stimulates colonic transit resulting in increased defecation.¹⁰⁹⁻¹¹¹ The mediation of the delay in gastric emptying involves CRF₂ receptors, whereas the stimulation of colonic motility is CRF₁ receptor-mediated.⁶² Therefore, peripheral injection of Ucn 1 or CRF, which interact with both CRF₁ and CRF₂ receptors, simultaneously inhibits gastric motor function while increasing colonic motility in rodents.^{95,111,112} Consistent with a differential inhibitory and stimulatory effect of CRF₂ and CRF₁ on gastric and colonic motor function, respectively, peripherally injected Ucn 2 selectively delays gastric emptying without altering colonic motility,^{111,112} whereas the selective CRF₁ agonists, stressin₁-A and cortagine, stimulate defecation and have no effect on gastric emptying.^{49,113}

Moreover, upon peripheral injection, the selective CRF₂ antagonists, astressin₂-B and antisauvagine-30, selectively block CRF- and Ucn 1-induced delay of gastric emptying,^{111,112} whereas the selective CRF₁ antagonists, CP-154,526 and NBI 27914, selectively abolish CRF- or Ucn 1-induced stimulation of colonic motor function in rodents.¹¹⁰⁻¹¹⁴ Likewise, the wrap restraint-induced delay of gastric emptying is blocked by peripheral injection of peptide non-selective CRF₁/CRF₂ antagonists¹¹⁵⁻¹¹⁷ and selective CRF₂ antagonist.¹¹² Moreover, the restraint- or water avoidance stress-induced stimulation of colonic transit and fecal pellet output can be abolished by peripherally administered antagonists, α -helical CRF₍₉₋₄₁₎ or astressin, which are peptides not crossing the blood-brain barrier.^{109,110,118,119} These observations highlight the involvement of the peripheral CRF signaling system in mediating the gut motor response to stress. Collectively these data support the concept that activation of the CRF signaling system in the gut serves as the local efferent effector of the brain-gut interaction during stress.¹²⁰ There is also evidence that the gut CRF system locally modulates gut inflammatory processes as recently reviewed.¹²¹

Implication of CRF₁ receptors in the pathophysiology of IBS symptoms

Exogenous administration of CRF or Ucn 1 can reproduce cardinal features of diarrhea predominant IBS including anxiogenic behavior, enhanced visceral pain to colorectal distention, increased colonic mucus secretion, propulsive motility, development of watery stool/diarrhea and increased colonic mucosal permeability facilitating bacterial translocation into colonic tissue (Figure 1).^{62,98,113,120,122} Colonic mast cells activated by peripheral CRF in response to stress are involved in the enhanced permeability, bacterial uptake and pain response as shown by the mast cell stabilizer, doxantrazole, that prevents the central CRF- and stress-induced colonic hypersensitivity to colorectal distention in experimental animals and humans (Figure 1).^{119,123,124} Acute or chronic stress-induced visceral hyperalgesia is also mediated by the activation of CRF₁ receptors.^{24,98} This is supported by pharmacological studies showing that central injection of CRF mimics stress-induced colonic hyperalgesia after colorectal distention²² and that CRF₁ antagonists block colonic hypersensitivity to colorectal distention induced by various stressors in different rodent models of visceral hypersensitivity.^{22,24,98} In contrast, activation of the CRF₂ receptor induced by peripheral injection of Ucn 2 or the CRF₂ preferring agonist, sauvagine, reduces visceral pain following colorectal distention in rats.^{125,126} These data indicate that CRF₁ and CRF₂ receptors exert counteracting effects as shown by activation of CRF₁ receptors facilitating visceral sensitization and activation of CRF₂ receptors preventing visceral hyperalgesia.

In addition to convergent preclinical evidence, data obtained in human studies indicate that systemic injection of CRF induces similar changes in the colon as observed in experimental animals. The peripheral administration of CRF decreases the threshold for visceral pain to colorectal distention in healthy human subjects.^{127,128} Other studies

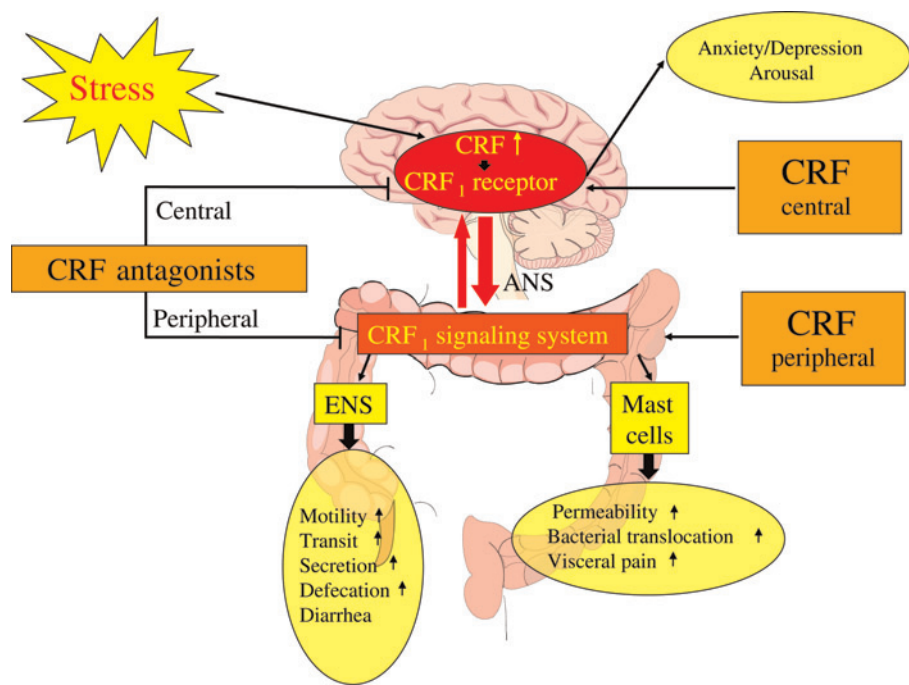


Figure 1 Activation of CRF₁ signaling pathways and IBS-like manifestations, which can be blocked by CRF₁ receptor antagonists. ANS, autonomic nervous system; ENS, enteric nervous system; IBS, irritable bowel syndrome (A color version of this figure is available in the online journal)

also showed that CRF activates subepithelial mast cells and stimulates transcellular uptake of protein antigens in colonic mucosal biopsies of healthy volunteers.¹²⁴ In addition, systemic injection of CRF induces a colonic motility response more prominently in subjects with IBS compared with healthy controls along with the induction of abdominal pain and discomfort in IBS patients but not in healthy controls (Table 1).¹²⁹ Conversely, peripheral injection of α -helical CRF₍₉₋₄₁₎ prevents rectal electrical stimulation-induced enhanced colonic motility, visceral perception and anxiety in IBS patients compared with healthy subjects without altering the HPA axis (Table 1).¹³⁰ Moreover, peptide CRF antagonists also result in near normalization of the altered electroencephalogram activities in IBS patients under basal conditions and in response to colorectal distention.¹³¹ Taken together, these clinical reports support that the activation of the CRF pathway with preferential CRF₁ agonists recaptures cardinal features of IBS symptoms which can be dampened by systemic injection

of peptide CRF receptor antagonists. Preclinical studies clearly demonstrated that the blockade of CRF₁ receptors can prevent/attenuate the development of those stress-related colonic functional or cellular alterations. Therefore, the conceptual framework supports that sustained activation of the CRF₁ system at central and/or peripheral sites may be one component underlying stress-related manifestations of IBS symptoms. However, two recent double-blind randomized clinical trials failed to show efficacy of CRF₁ antagonist treatment on colonic motility in patients with diarrhea-predominant IBS (Table 1)¹³² or on visceral pain in male and female IBS patients.¹³³ As several other CRF₁ candidates are being tested, results from these clinical trials should provide additional insight regarding the potential therapeutic benefit of CRF₁ receptor antagonists for anxiety, depression and IBS as established in a large array of preclinical *in vivo* studies.¹³⁴

Implication of CRF₁ receptors in the pathophysiology of inflammatory bowel disease

Although stress does not cause inflammatory bowel disease (IBD) encompassing ulcerative colitis as well as Crohn’s disease, it does aggravate the condition. Particularly, psychological stress increases the risk to exacerbate existing IBD symptoms and/or to induce the flare-up of symptoms.¹³⁵ Consequently, owing to its characterized role in stress-response, the CRF system has been investigated in relation with the pathophysiology of IBD in experimental models and clinical settings.¹³⁶

The various modalities through which the local CRF system is regulated in the gut during inflammation and conversely influences inflammatory processes have been recently reviewed in detail.¹²¹ CRF increases the release of

Table 1 Overlap of symptoms/parameters induced by CRF and dampened by CRF₁ antagonists

Symptoms/parameters	Effects of systemic CRF in healthy subjects	Effects of systemic CRF ₁ antagonists in IBS patients
Anxiety	↑ ¹⁶⁸	↓ ^{130,169}
Colonic motility	↑ ¹²²	↓, = ^{122,132}
Colonic permeability	↑ ¹²⁴	↓ ¹²⁴
Gut inflammation	↑ ¹⁷⁰	↓ ¹⁴⁰
Visceral sensitivity to colorectal distention	↑ ¹²⁸	↓, = ^{122,133}

↑, increase; =, no change; ↓, decrease
CRF, corticotropin-releasing factor; IBS, irritable bowel syndrome

proinflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-6 from macrophages *in vitro*.¹³⁷ *In vivo* studies showed that the CRF₁ receptor antagonist, antalarmin, inhibits the endotoxin-induced inflammatory response as reflected by the suppression of elevated cytokines, namely TNF- α , IL-1 β , and IL-6, resulting in prolonged survival after endotoxin treatment in BALB/c mice.¹³⁷ In the ileum, the inflammatory response to intraluminal perfusion of *Clostridium difficile* toxin is markedly reduced in mice with deletion of the CRF gene¹³⁸ or after treatment with double-stranded RNA locally silencing the CRF expression in the ileum.¹³⁹ Clinical observations showed that the expression of CRF is increased in enterochromaffin cells and macrophages in mucosal biopsies of patients with IBD and may exert a proinflammatory action.¹⁴⁰ Although the existing experimental and clinical data are encouraging, clinical trials are still to be performed to assess whether the modulation of CRF signaling impacts on IBD symptoms.

CRF signaling and the cardiovascular system

CRF administered into the lateral brain ventricle in rats elicits widespread effects on the cardiovascular system, namely increasing heart rate, mean arterial pressure and cardiac output that are similar to those induced by various stressors (Table 2).¹⁴¹ In addition, the icv administration of the CRF₁/CRF₂ antagonist, α -helical CRF₍₉₋₄₁₎ attenuates icv IL-1 β -induced tachycardia and hypertension.¹⁴² In contrast, intravenous injection of CRF, and more so Ucn, causes vasodilation and concomitantly decreases blood pressure accompanied by compensatory tachycardia.^{143,144} Under *in vitro* conditions using an isolated preparation of rat heart, CRF increases coronary blood flow, releases atrial natriuretic peptide and exerts a positive inotropic effect (Table 2).^{145,146} These findings suggest that CRF influences cardiovascular functions not only centrally but also via a local action. In line with this assumption, CRF and more abundantly Ucn along with CRF₂ receptors are expressed in the heart of rodents and humans.^{74,75,147,148}

Convincing evidence established that the CRF₂ receptor mediates the peripheral effects of the CRF signaling

system on the heart and blood vessels.^{149–151} CRF receptors, particularly CRF_{2 β} , are densely expressed in the heart and in blood vessels.^{152–154} Ucn 1 and CRF₂ receptor expression are modulated by stress as shown by systemic endotoxin administration and restraint that increase Ucn 1 mRNA expression but decrease CRF₂ mRNA in the rat atria and ventricles.^{155,156} The predominant role of peripheral CRF₂ receptors in cardiovascular functions is further underlined by the observation that CRF₂ KO mice display an elevated basal systolic and mean arterial pressure.¹⁵⁷ Moreover, CRF₂ KO mice do not respond to peripheral injection of Ucn 1, whereas their wild-type littermates show a pronounced decrease in blood pressure following peripheral injection of the peptide.¹⁵⁷ Due to the high binding affinity of Ucn for CRF₂ compared with CRF, the vasodilatory and inotropic effects of Ucn are more pronounced than those induced by CRF.^{158,159} These observations led to the assumption of a cardioprotective role of peripheral Ucn and their potential therapeutic use under conditions of heart failure. In preclinical studies, systemic injection of Ucn 1, Ucn 2 or Ucn 3 decreases peripheral resistance and atrial pressure and concomitantly increases cardiac output in animals with heart failure.^{160–162} Moreover, exogenous Ucn 1 reduced the number of cell deaths following hypoxia¹⁶³ resulting in decreased infarction size.^{164,165} Likewise, intravenous infusion of Ucn 2 decreased systemic vascular resistance while increasing cardiac output, heart rate and left ventricular ejection fraction in healthy human subjects.¹⁶⁶ Other clinical studies indicate that patients with systolic heart failure have elevated plasma Ucn 1 levels in the early stage of the disease (New York Heart Association, NYHA 1–2), whereas with progression (NYHA 3–4), the levels decrease¹⁶⁷ suggesting a possible predictive value of circulating Ucn 1 as a biomarker. Collectively, existing evidence supports the potential clinical relevance of using Ucn as a new therapeutic intervention for heart failure or following hypoxic stress such as myocardial infarction. However, clinical trials are needed to corroborate the preclinical and proof of concept clinical findings.

Summary

The CRF signaling system composed of CRF, Ucn 1, Ucn 2 and Ucn 3 along with CRF₁ and CRF₂ receptors is largely expressed in the brain and visceral organs such as the gut and cardiovascular system, all known to be affected by stress. Pharmacological approaches using selective CRF antagonists in experimental animals have demonstrated that the activation of CRF₁ receptors plays a major role in the development of anxiogenic and colonic propulsive responses to stress independently from the HPA axis. In the brain and gut, the activation of CRF₁ receptors recapitulates key features of IBS-diarrhea predominant patients such as watery stool, visceral hyperalgesia, an increase in colonic motility, mucus secretion and also induces hypervigilance. Moreover, these alterations can be abolished by CRF₁ antagonists in preclinical IBS-like models suggesting therapeutic potential for CRF₁ antagonists. However, two recent clinical trials failed to demonstrate efficacy of CRF₁ antagonists in

Table 2 Effects of activation of CRF₂ receptors on cardiovascular functions in animal models

Site of injection	Species	Effect	Reference
icv	Rat	↑ Heart Rate	141
	Rat	↑ Mean arterial pressure	141
	Rat	↑ Cardiac output	141
iv	Dog	Vasodilation	171
	Dog	↓ Blood pressure	171
	Dog	↑ Heart Rate	171
<i>In vitro</i>	Rat	↑ Coronary blood flow	146
	Rat	Vasodilation	146
	Rat	↑ Aortic pressure	146
	Rat	↑ Oxygen consumption	146
	Rat	Positive inotropic effect	146
	Rat	↑ Atrial natriuretic peptide	145

↑, increase; ↓, decrease; icv, intracerebroventricular; iv, intravenous

the treatment of IBS symptoms. Additional clinical studies are still ongoing with new CRF₁ antagonist candidates to establish their potential therapeutic value in the drug treatment of IBS. Another feature of the CRF system is its proinflammatory action in the gut that may have a bearing with the underlying mechanism of stress-related exacerbation of IBDs. Lastly, central activation of CRF receptors results in a hypertensive response that may contribute to the development of stress-related hypertension. By contrast, in the periphery the activation of Ucn-3/CRF₂ signaling exerts potent vasodilatory effects. Preclinical and clinical studies point towards the use of CRF family members, especially Ucn 1, as possible biomarkers in the diagnosis and progress monitoring of systolic heart failure as well as in the drug treatment of heart failure or following hypoxic stress such as myocardial infarction.

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