Central and Peripheral Modulation of Visceral Pain and Visceral Hypersensitivity by the CRF-CRFR System

Abstract
Visceral pain refers to the pain originating from the internal organs and is the common symptom shared by many disorders. The corticotropin releasing factor (CRF) family encompasses CRF, urocortin (Ucn) 1, Ucn2, and Ucn3 (collectively termed as CRFs in this review), while CRF receptors (CRFR) include CRF receptor type 1 and type 2 (CRF1 and CRF2) and their different splice forms. CRFs and CRF receptors are extensively expressed in both the brain and the peripheral tissues including the gastrointestinal (GI) tract, the spinal cord and so on. The CRF-CRFR system has been shown to play key roles in modulating visceral pain and visceral hypersensitivity by multiple groups in the past two decades. However, a comprehensive review to summarize and integrate the different, even contradictory results is lacking. This review summarizes the role of the CRF-CRFR system in modulation of visceral pain and visceral hypersensitivity at the layers of the brain, the spinal cord and the GI tract.

Keywords: Visceral pain; Visceral hypersensitivity; Corticotropin releasing factor (Crf), Corticotropin releasing factor receptor type 1 and Type 2 (Crf1 and Crf2)

Introduction
Visceral pain is defined as the pain sensed as arising from the internal organs of the body. It has five clinical characteristics, including not being evoked from all viscera, not always being linked to injury, poor localization, referred pain and being accompanied with motor and autonomic reflexes [1]. Etiological factors are, but not limited to, inflammation, mechanical disruption, neoplasms, and alterations in neurotransmission from the viscera, and ischemia [2]. Visceral hypersensitivity refers to the altered pain sensation to physiological stimuli, consisting of allodynia and visceral hyperalgesia [3]. Visceral pain and visceral hypersensitivity is the common feature of many disorders, including inflammatory bowel disease (IBD), interstitial cystitis/painful bladder syndrome, pancreatitis, and many gastrointestinal functional diseases, especially irritable bowel syndrome (IBS).

Irritable bowel syndrome is a functional bowel disorder that is not associated with detectable structural and biochemical abnormalities [4]. The prevalence of IBS is 7%-21% of the general population [5], casting a huge economic burden [6,7]. In addition to visceral pain, IBS is characterized by stool irregularities and bloating. According to the predominant stool pattern, the Rome III criteria divide IBS into four subtypes, IBS with constipation, IBS with diarrhea, mixed IBS and un sub typed IBS. The pathological mechanisms of IBS remain largely unknown. Disruption of epithelia barrier, dysbiosis, low-grade inflammation, and altered brain-gut axis has been proposed to contribute to the pathogenesis and symptoms of IBS [4]. An interesting observation is that IBS is highly comorbid with stress-related psychiatric disorders [8]. Depression and anxiety accounts for 20%-60% of these comorbidities [9] and psychological stress contributes to the exaggeration of visceral pain. Therefore, elaborating the roles of stress in development of visceral pain may provide insights to the underlying mechanisms of IBS.

CRF is the key mediators of stress and visceral pain. CRF is commonly believed to promote the releasing of the ACTH by binding to its receptors. However, the role of CRF and its related peptides extends far beyond mediating the endocrine component of stress [10]. Intravenous administration of CRF induces visceral hypersensitivity after repetitive rectal distention in healthy humans and mimics an IBS-specific visceral response [11], indicating that the CRF-CRFR system contributes to the pathogenesis of visceral pain and hypersensitivity and IBS. Indeed, the role of the CRF-CRFR system in visceral pain modulation has been repetitively demonstrated in both the patients and animal models.

In this review, the relationship between CRF-CRFR system and visceral pain is summarized, especially focused on the expression pattern of CRFs and CRF receptors in the brain, the spinal cord and the peripheral tissues, and on the dynamic change of the CRF-CRFR system in different physiological conditions. Finally, it is analyzed how the CRF-CRFR system modulates visceral pain at both peripheral tissues and the brain.

Corticotropin-Releasing Factor Sand CRF Receptors
Corticotropin-releasing factor and its related peptides

The mammalian corticotropin releasing factor (CRF) family consists of four members, CRF, urocortin (Ucn) 1, urocortin 2 and urocortin 3 [12]. The first member of CRF family was a 44-amino acid peptide, first isolated from ovine brain and found to...
stimulate the secretion of adrenocorticotropic hormone (ACTH) and beta-endorphin [13]. Thereafter, it was named corticotropin-releasing factor (CRF). The CRF peptides are highly conserved across different species, with human, mouse and rat CRF identical (therefore termed h/m/r CRF) [14]. In addition to CRF, CRF like peptides were subsequently identified in mammals, amphibians and fish [15]. Vaughan et al. [16] first, in the rat midbrain, cloned urocortin (later normalized as Ucn1 by UPHARM guideline), which is a 40-amino acid peptide and shares 45% amino acid sequence identity with h/m/r CRF [16,17]. Reye et al. [18] isolated a new member of CRF family, Ucn2, which is a 38-amino acid peptide and shares 34% and 42% homology with rat CRF and rat Ucn1 at amino acid level, respectively [18]. Ucn3 is a 38-amino acid protein and was found to be relatively more closely related to Ucn2 than CRF and Ucn1 [19,20]. Similarly to CRF, Ucn peptides share high identity among mammals in terms of amino acid sequence of the mature peptides. Mouse Ucn1 and rat Ucn1 are identical, which share 95% identity with that of human [16]. Human Ucn3 and mouse Ucn3 are 90% identical with each other [19,20].

The biological activity of CRFs relies on the amino-terminals containing the first 21 amino acid residues, the carboxyl-terminals containing the last 5 amino acid residues, and their internal segments that have a high average probability of alpha helix formation [15,21,22].

**Corticotropin-releasing factor receptors (CRFR)**

The receptors for CRFs are CRF receptor type 1 and 2 (CRF<sub>1</sub> and CRF<sub>2</sub>, respectively), which belong to the secretin-like family B1 of G protein-coupled receptors (GPCR) [23]. The structure of CRF<sub>1</sub> and CRF<sub>2</sub> are typical of GPCR, consisting of 7 alpha-helical transmembrane segments, an extracellular amino-terminal and an intracellular carboxyl-terminal [15].

CRF<sub>1</sub> and CRF<sub>2</sub> have different splice forms. CRF<sub>1a</sub> is the main receptor variant, which is composed of 415 amino acids [24]. CRF<sub>1b</sub>, CRF<sub>1c</sub>, CRF<sub>1d</sub>, CRF<sub>1e</sub>, CRF<sub>1f</sub>, and CRF<sub>1g</sub> have been identified and their functional roles remains elusive [25]. For simplicity, the CRF<sub>1</sub> variant, designated as CRF<sub>1</sub>, below in this paper, refers to CRF<sub>1a</sub>. CRF<sub>2</sub> splice variants are differentially localized, which is discussed later in this article. CRF<sub>2</sub> encompasses 3 main is of orms, a, b and c [26-29]. As CRF<sub>1</sub>, CRF<sub>2</sub> variants are expressed differentially in tissues. However, CRF<sub>2</sub> splice variants exert similar pharmacological properties as compared to those of CRF<sub>1</sub> [26-29]. CRF<sub>2</sub> are highly conserved across different species with human and mouse CRF<sub>2</sub> only differing in 10 amino acids [30]. The CRF<sub>2</sub> shares approximately 70% amino acid sequence identity with CRF<sub>1</sub> [26].

The primary cellular functions of CRF<sub>1</sub> and CRF<sub>2</sub> are mediated through G<sub>a</sub> proteins. Binding of CRFs to the receptors initiates a series of conformational change, which conveys extracellular stimuli into intracellular domains of CRF receptors, resulting in the activation of G<sub>a</sub> adenylatecyclase-cAMP-PKA pathway [31]. The signaling cascade manifests its diverse cellular effects by activating ERK1/2, p38 MAPK and so on [32-35]. Additionally, CRF<sub>1</sub> and CRF<sub>2</sub> may couple to different G proteins and regulate both cAMP-dependent and cAMP-independent signalings [36].

The natural ligands of CRF<sub>1</sub> and CRF<sub>2</sub> are CRF, Ucn1, Ucn<sub>2</sub> and Ucn3. OCRF is relatively selective for CRF<sub>1</sub>, while Ucn2 and Ucn3 are significantly selective for CRF<sub>2</sub>. The other natural ligands are lack of specificity for CRF receptors (Table 1).

Non-endogenous agonists and antagonists for CRF receptors are classified into peptidic and non-peptidic groups according to the chemical nature. The non-peptidic group distinguishes from the peptidic group by its ability to freely cross the blood-brain barrier [37]. The difference in crossing the blood-brain barrier enables their differential applications for studying the CRF-CRFR system at central and peripheral levels. Specifically, for the peptidic group of antagonists, it may be subdivided into three subgroups, CRF selective, CRF<sub>2</sub> selective and non-selective (Table 1). CRF<sub>2</sub> selective peptidic agonists include astressin (a), and anti-sauvagine 30. The representative peptides of non-selective group are helical CRF<sub>9-41</sub>, D-Phe<sub>12</sub> CRF<sub>12-41</sub>, a stressin in-B and astressin [38]. As shown in Table 1, the selective peptidic antagonists for CRF<sub>1</sub> and peptidic agonists are relatively underdeveloped. Non-peptidic agonists and antagonists for CRF receptors and their therapeutic utility have been extensively reviewed by John H Kehne & Christopher K Cain [37], which is not discussed elaborately in this article.

The K<sub>a</sub> value was determined by competitive radio ligand displacement as says by using radio labeledsauvagine(a, b, j), astressin (g) and h/CRF(h). According to this article, YM19 is a CRF<sub>2</sub> selective peptide. However, the supporting data is not shown. The EC<sub>50</sub> was determined by measuring alteration of CRF-induced release of ACTH by rat anterior pituitary cells in culture.

* Rivier et al. [38].
* Lewis et al. [20].
* Dautzenberg et al. [39].
* Mesleh et al. [40].
* Tache et al. [41].
* Rivier et al. [42].
* Rivier et al. [43].
* Tezval et al. [44].

**Central expression of CRFs and CRF receptors**

To investigate the role of the CRF-CRFR system in visceral pain modulation, characterization of the expression pattern is prerequisite. The pain sensation pathway provides insights to the sites and layers that we focus in this part. The gastrointestinal (GI) tract is diffusely sensed by the afferent fibers from the vagal, pelvic and splanchnic nerves, which ascend via the vagal nerves, sympathetic and parasympathetic pathways [45]. Secondary processing occurs at the spinal cord and at the brain stem. In the spinal cord, primary visceral afferent terminations in laminae I and V, constituting the splanchnal tract, or in laminae VII and X, constituting the dorsal column pathway [46,47]. Then secondary neurons diffusely project into the pain matrix, including the
prefrontal cortex (dorsolateral), the insula, the thalamus, the amygdala, and the anterior cingulated cortex (ACC) [2]. Thereafter, we focus the expression of CRF and CRF receptors at the levels of the GI tract, the spinal cord and the brain.

### Table 1: Pharmacological Properties of CRF Receptors.

| Ligand                        | CRF<sub>1</sub> Kön or EC<sub>50</sub> (nM) | CRF<sub>2</sub> Kön or EC<sub>50</sub> (nM) |
|-------------------------------|------------------------------------------|------------------------------------------|
| Natural Ligands               |                                          |                                          |
| CRF<sub>1</sub> selective     | oCRF<sup>a</sup> 1.2                     | 52                                      |
| CRF<sub>2</sub> selective     | mlUcn2<sup>b</sup> >100 0.66             |                                          |
|                              | hUcn2<sup>a</sup> >100 0.5               |                                          |
|                              | mlUcn3<sup>b</sup> >100 1.8             |                                          |
|                              | hUcn3<sup>a</sup> >100 13.5             |                                          |
| Non-selective                 |                                          |                                          |
|                              | r/hCRF<sup>a</sup> 1.0 6.2             |                                          |
|                              | rUcn1<sup>b</sup> 0.32 0.62             |                                          |
|                              | hUcn1<sup>c</sup> 0.1 0.5               |                                          |
|                              | Urotensin<sup>b</sup> 0.4 2.2           |                                          |
|                              | Sauvagine<sup>b</sup> 0.7 4.3           |                                          |
| Non-endogenous Antagonist (Peptide) |                                          |                                          |
| CRF<sub>1</sub> selective     | YAM19<sup>d,e</sup> 5.9 -               |                                          |
| CRF<sub>2</sub> selective     | astressin<sub>2</sub>-B<sup>f</sup> >500 1.3 |                                          |
|                              | anti-sauvagine 30<sup>f</sup> 400 1.1   |                                          |
| Non-selective                 |                                          |                                          |
|                              | α-helical CRF<sub>9-41</sub> 19 1.1     |                                          |
|                              | D-Phe<sup>12</sup>CRF<sub>9-41</sub> 19.2 4.4 |                                          |
|                              | astressin<sub>1</sub> 0.7 0.6            |                                          |
|                              | astressin-B<sup>g</sup> 0.3 1.2         |                                          |
| Non-endogenous Agonist (Peptide) |                                          |                                          |
| CRF<sub>1</sub> selective     | Cortagine<sup>h</sup> 2.6 540           |                                          |
|                              | stressin<sub>1</sub> 1.7 222            |                                          |

By immunohistochemical staining, CRF was found to be densely localized, in rat, in the cell bodies of the extra-hypothalamic areas of nucleus accumbens septi, basal nucleus of the striaterminalis (BNST), the medial preoptic region, and the central amygdaloid nucleus, and of hypothalamic areas of paraventricular nucleus (PVN) [48]. For fibers, CRF is enriched in the lateral septal region and throughout the external layer of the median eminence [48]. The distribution pattern has later been repetitively confirmed by different methods, including in situ hybridization in voles [49], immunohistochemical labeling by CRF specific polyclonal antibody in mice, rats [50], and tree shrews [51], and immunofluorescence by anti-GFP antibody targeting CRF-Venus fusion protein in the knock-in mice [52]. These studies demonstrate that the expression pattern of CRFs is conserved among species. Compared with CRF, Ucn peptides are more restrictedly expressed. Ucn1 is primarily expressed in Edinger-Westphal nucleus (EWN) [49] and the lateral superior olive [53]. In rats, Ucn2 neurons are mainly present in PVN with a few existing in the supraoptic nucleus of the hypothalamus, and Ucn3 are similarly expressed in PVN and in the extra-hypothalamic areas of the medial nucleus of the amygdala [54]. Throughout the spinal cord, CRF fibers were found to predominate the laminae I, V-VII, and X of Rexed while Ucn1 fibers the laminae VII and X, by immunofluorescence and in situ hybridization [55]. As expected, the expression of CRF and its related peptides is a dynamic process in accordance with different physiological conditions, for example, diverse stressful states. Evidence includes that CRF and Ucn3 are differentially expressed in eusocial naked mole-rats and solitary cape mole-rats [56] and that Ucn1 expression in mouse brain is strain-dependent [53].
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CRF is expressed with different intensity throughout the whole brain, with enrichment in neo cortices, the olfactory bulb, the hippocampus and sub cortical limbic structures in the septal region and the amygdala of the forebrain, in certain relay nucleus of the brain stem, and in the cerebellum [57-59]. The expression of CRF isoforms is disparate. CRF₁ and CRF₂ₐ predominate the brain while CRF₂ isoforms mainly locates in the peripheral tissues [29,60]. In the brain, CRF₁ is enriched in the olfactory bulb, the lateral septum, BNST, the ventromedial hypothalamus, the raphe nuclei, the amygdala, the ventral hippocampus, the solitary tract, and the area postrema [58,60-63]. CRF₂a is primarily located in the choroid plexus [62,63] and CRF₂b in the septum and the hippocampus [29]. In the spinal cord, CRF₁ is relatively widespread with occurrence in laminae III-X compared to the restricted distribution of CRF₂a in laminae III-VIII [55]. Genetically or molecularly mimicking of stress does not change the general localization of CRF receptors but do influence the expression level [63]. The density of CRF receptors varies within different physiological conditions, for example, photoperiod and sociality [64] and within disease conditions like depression and gastrointestinal function disorders [65].

The amygdala is a key component of the pain matrix. It consists of several nuclei, of which, the lateral (LA), basolateral (BLA), and central nuclei (CeA) are particularly important for sensory processing. The polymodal sensory, including nociceptive, inputs from thalamic nuclei and cortical areas are transmitted into the LA, and through the LA-BLA circuitry, sensory information is added with affective contents [66]. The integrated information is finally relayed to the CeA, which is the major output nucleus for amygdala functions [67]. Additionally, the laterocapsular division of the CeA (CelC), through the spino-parabrachio-amgdaloid pathway, directly receives the nociceptive information [68].

CRFs are apparently localized in both cell bodies and the fibers of the central nuclei of the amygdala among different species [48-52]. In the CeA, CRF is co-localized with GABA [52]. Another study using a transgenic mouse line, in which hr GFP was expressed under the control of CRF promoter demonstrates that CRF is primarily located in the interstitial nucleus of the posterior limb of the anterior commissure in addition to the central amygdala [69]. This study suggests that amygdalar CRF neurons are activated in certain stress conditions like social defeat stress and lipopolysaccharide (LPS) administration but not in restraint stress and forced-swimming stress. Neither Ucn1 nor Ucn2 is evidently expressed in the amygdala. Ucn 3 neurons are found in the dorsolateral division of the medial nucleus of the amygdala [54].

CRF₁ and CRF₂a are differentially localized in the amygdala, with CRF₁ predominant in the basolateral and medial nuclei, and CRF₂b in the posterior aspect of the medial nuclei [59,61]. CRF₁ is weakly expressed in the amygdala [29].

Peripheral expression of CRFs and CRF receptors

CRFs and CRF receptors are widely expressed in the peripheral tissues, including the heart, the muscle and the skin, in which CRF₁ is the dominant form [29,62]. Here, we focus the expression of CRFs and CRF receptors in the gastrointestinal tract. The CRF-CRFR system is expressed throughout the GI tract in a slightly different manner at different segments of the GI tract and at different layers in the same segment. Emphasis is put on the lower gastrointestinal tract, for example, the colon, for the usual involvement in multiple diseases manifesting visceral pain and visceral hypersensitivity.

CRF is expressed in the colonic mucosal epithelia, lamina propria, crypts, and the enteric neurons (both bodies and fibers) in the rat colon [70], in healthy people and in patients with ulcerative colitis (UC) [71]. In the mice ileum, the expression pattern is slightly different with CRF₁ primarily expressed in stromal cells and nerve fibers [72]. Specifically, enterochromaffin cells (EC) that synthesize serotonin (5-HT) constitute a large proportion of CRF positive cells in the epithelia [70]. Cells expressing CRF in the lamina propria includes macrophages [71] and eosinophils [73], indicating roles of the CRF-CRFR system in the gut immunity. In the enteric neurons, CRF expressing bodies and fibers are more abundant in the sub mucosal plexus than in the myenteric plexus in the guinea pig [74] and in the rat [75], and almost all the CRF expressing neurons co-express vasoactive intestinal peptide (VIP), suggesting the VIP and CRF interaction in intestinal regulation [75]. Additionally, CRF is not co-expressed with CRF₁ in the same neurons and CRF positive neurons are usually in proximal with CRF₂b neurons [74], suggesting that CRF-CRFR system modulates the neuronal activity enteric in a local circuit. Ucn1 is expressed primarily in the macrophages of the mucosa in the colon of healthy human [76] and in the plasma cells and the EC of the mucosa in the patients with UC [77], and both experiments detect weak signals of Ucn1 in the epithelial cells and in the enteric neurons. Ucn2 is expressed in the leukocytes and the external muscular layer in the wall of rat ileum [72]. Ucn3 is expressed ubiquitously in the epithelial cells and the myenteric neurons through the GI tract in rats [78].

In the rat colon, CRF₁ and CRF₂ are expressed differentially in the crypts. CRF₁ is also expressed in the lamina propria and in the enteric plexus, while CRF₂ is expressed in the blood vessels of the sub mucosa [79]. Similar results were obtained in healthy human [80,81]. Specifically, macrophages account for 79% of the cells that express CRF₁ in the lamina propria with the rest being mast cells and other cells [80].

The expression level and sites of CRFs and CRF receptors are dynamically regulated in different diseases. CRF expression is up-regulated especially in the sub mucosa plus muscular layers in response to LPS administration in a corticosteroid-independent manner in the rat colon [70] and the number of CRF expressing eosinophils is increased in the UC rat [73]. In the human UC patients, CRF is only slightly increased in the mucosal epithelia but considerably increased in the mucosal macrophages [71]. The number of Ucn1 expressing cells increases in proportion to the severity of inflammation in the UC patients [77], however, Ucn3 is down-regulated in UC [78]. CRF₁ expressing cells with macrophages in particularly increase in mucosa of UC patients [80]. In contrast, CRF₂ is down-regulated in the epithelial cells, but not the lamina propria, of mild-moderately active UC and of recovery from UC [81]. In general, CRF₁ and its natural ligands, including CRF and Ucn1, are up-regulated, while CRF₂ and its natural ligand, Ucn3 are down-regulated in the inflamed state. However, in the patients of functional bowel disease, the expression CRF₂ is similar to that in healthy people [81].

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Modulation of Visceral Pain by the CRF-CRFR System

Central modulation of visceral pain by CRF-CRFR system

Intracerebroventricular (icv) injection of α-helical CRF$_{1}$ was found to block the visceral pain induced by colorectal distention or by icv injection of CRF, indicating that visceral hypersensitivity is modulated by CRF-CRFR systems in the brain [82]. Consecutive experiments has shown that visceral pain is ameliorated by specifically blocking CRF, using small molecule antagonists including NBI-35965 [83], CP154265 [84,85], NGD 98-2, NGD 9002 [86], antalarin in [87], E2508 [88] in multiple stress models of maternal separation, acute water avoidance stress [83,86], repeated psychological stress [84,86], repeated stress in combination of colitis [85] and genetically anxious WKY rats [87]. These data demonstrate that CRF-CRF, is the common modulator of visceral pain and visceral hypersensitivity in multiple stress settings and that CRF$_{1}$ plays key roles in both the development and the maintenance of visceral hyper sensitivity [84]. In these experiments, the small molecule CRF$_{1}$ antagonists are administrated orally, intravenously or subcutaneously. Due to the ability of small molecules to freely cross the blood brain barrier, the peripheral roles of the CRF-CRF, in modulation of visceral pain cannot be excluded. The experiments of brain sites specific injection of CRF$_{1}$ antagonists further consolidated the brain as the sites of pain modulation, as shown below.

Next questions are what parts of the brain are involved in pain modulation by the CRF-CRF, system. As mentioned above, the amygdala is the site playing key roles in visceral pain and visceral hypersensitivity. Initial data includes that microinjection of α-helical CRF$_{1}$ into the CeA significantly reduces anxiety [89]. To further characterize the role of the CeA in visceral pain modulation, either the CRF, specific antagonist CP-37.6395 was administrated into the CeA [90], or CRF was site-specifically knocked down in the CeA [91]. In both experiments, rats showed decreased visceral pain in response to colorectal distension (CRD). Similarly, site-specific injection of CRF into the CeA evokes visceral hypersensitivity [92,93]. Additionally, expression of CRF and CRF$_{1}$ is up-regulated in the CeA in the visceral hypersensitivity models of cystitis [94] and in models of comorbid depression and functional gastrointestinal disorders [65]. Taken together, these experiments demonstrate that amygdala is the site of visceral pain modulation by CRF-CRF$_{1}$.

In addition to amygdala, CRF-CRF, may modulate visceral pain in PVN, BNST, EWN, the hippocampus, and locus coeruleus (LC). Under acute pain stress, expression of CRF and Ucn1 peaks at different time point in PVN, BNST and EWN, suggesting that CRF in the PVN acts in the initiation phase, while Ucn1 in the EWN acts in termination of the adaptation response to APS [95]. Intra hippocampal administration of α-helical CRF or intra peritoneal administration of JTC-0-17, a CRF, specific antagonist, significantly attenuates hippocampal noradrenaline release and visceral hypersensitivity induced by acute distention [96]. In the anterior lateral BNST, micro infusion of CP-376395, a CRF, specific antagonist, reduces visceral pain in response to CRD under non-stressful baseline conditions or following water avoidance stress [97]. In the rats that experienced CRD in the neonatal phase, CRD during adulthood induces visceral pain by activating the microglial cells and CRF expressing neurons in the PVN, which are prevented by intra-PVN infusion of small interference mRNA that targets CRF [98]. Injection of D-Phe$_{1}$-CRF$_{12-41}$ into LC attenuates the activation of noradrenergic LC neurons in response to CRD [99,100] and similar results were obtained by LC-specific administration of CRF, antagonist, NB-35965 in response to CRD and intracisternal injection of CRF [100]. Noradrenergic LC neurons also project into the CRF neurons of the CeA [101]. And injection of CRF into the CeA increases noradrenalin release in the CeA and evokes visceral hypersensitivity, which is blocked by CRF, antagonists [92]. The CRF expressing neurons in the CeA and the noradrenergic neurons in the LC form a circuit that may underlie the mechanisms of visceral pain modulation.

The studies of the roles of CRF-CRF, in modulation of visceral pain are relatively scant and remain controversial. Ideas are borrowed from the somatic pain hypersensitivity, which may share similar mechanisms with visceral hypersensitivity. CeA-specific blockade of CRF, inhibits evoked responses and background activity in arthritis but not in healthy controls, while blocking CRF$_{2}$ in the CeA only increases neurons’ response in healthy controls but has no effect in arthritis [66,102]. This indicates that in the CeA, CRF, is activated under arthritis but not normal conditions and contributes to pain sensation while CRF$_{2}$ is inhibitory to pain sensation under normal conditions but the inhibitory effect is lost in arthritis. This concept is in consistent with the observation that in stress models of WKY rats or maternal separation, CRF$_{1}$ mRNA was higher in the amygdala, PVN and dorsal raphe nucleus (DRN) while CRF$_{2}$ mRNA is lower in the dorsal raphe nucleus in comparison with control rats [65]. In BNST, the opposing roles of CRF, and CRF$_{2}$ holds true under different conditions. Specifically, under baseline conditions, blocking of CRF$_{2}$ reduces anxiety, somatic pain and visceral pain in response to high CRD pressure, but the ameliorating effect is lost under water avoidance stress [97]. Confusingly, in the normal rats in the absence of tissue damage, CRF increases somatic pain behavior which is blocked by CRF, antagonists but not by CRF$_{2}$ antagonists [103]. The complexity of the CRF-CRF, in modulating visceral and somatic pain may be attributed to the differential regulation of CRF$_{2}$ under different stress conditions and in different strains, to the differential localization of CRF$_{2}$ in the brains and to the different behaviors under investigation.

Concerning the upstream mediators that may modulate the CRF-CRF, system in the modulating visceral pain and visceral hypersensitivity, corticosterone (CORT) is of great interest, which is commonly considered as the stress hormone in the hypothalamic-pituitary-adrenal gland (HPA) axis. Stereotaxic delivery of CORT into the amygdala induced anxiety-like behaviors and colonic hypersensitivity in rats [104,105], which are mediated via glucocorticoid receptors (GR) or mineralocorticoid receptors (MR) [106]. Decreased expression of GR and increased expression of CRF and HCN1 channel with no change of CRF, and CRF, expression was observed in the CeA in exposure to elevated CORT [107]. Visceral hypersensitivity was triggered by knocking down of steroid receptors (either GR or MR) in the CeA [108,109] and was blocked by knocking down of CRF in the CeA [91]. One of the interesting observations is that transient exposure of CeA to...
CORT resulted in long lasting increases of visceral sensitivity and anxieties [110]. The maintenance of chronic anxiety and visceral hypersensitivity is mediated by epigenetically deacetylation of his tone 3 at lysine 9 (H3K9), which sequesters the GR expression, leading to disinhibition of CRF [111,112]. However, contradictory results were found in WKY rats, a high anxiety strain, compared to the results from the low anxiety F344 rat strain. In WKY, visceral hypersensitivity is mediated by CRF \(_2\) instead of steroid receptors [90]. The difference indicates the heterogeneous nature of visceral hypersensitivity in terms of strains [113] and stresses.

Above all, the central mechanisms of visceral pain modulation by CRF-CRFR systems include, but are not limited to, dysregulation of CRF and CRFR expression, loss of balance between CRF \(_1\) and CRF \(_2\) altered circuits plasticity in the brain pain matrix and dysregulation of noradrenergic release in different brain sites, in response to different stress and in different strains.

### Peripheral modulation of visceral pain by CRF-CRFR system

Administration of small molecule CRF\(_{\text{antagonists}}\) ameliorates visceral pain and visceral hypersensitivity [83-88], as mentioned above. The pharmacological effects are likely to happen at both central and peripheral layers. The major peripheral organ we focus on is the GI tract. The peripheral role of the CRF-CRFR system has been corroborated by subcutaneous administration of astressin, a peptidic nonselective CRF \(_{1}\) and CRF \(_{2}\) antagonist, which blocks the visceral hypersensitivity induced by repeated water avoidance stress [84]. Both CRF, and CRF \(_{2}\) participate in the peripheral modulation of visceral pain and visceral hypersensitivity. Intraperitoneal injection of cortagine, a CRF, specific peptidic antagonist exacerbates the visceral pain in response to CRD [114]. On the other hand, subcutaneous injection of astressin2-B, a CRF\(_{2}\) specific peptidicantagonist, blocks visceral hyposensitivity [115].

Sensitization of peripheral afferent nerves is the major mechanisms accounting for the visceral pain and hypersensitivity of peripheral origin. The nerves may be sensitized by lipids, hormone, cytokines, immune mediators and substances from the GI lumen [8]. These sensitizers activate different neuronal receptors and ion channels that associates with pain signaling, such as transient receptor potential cation channel subfamily V type 1 (TRPV1), protease-activated receptors (PAR), cholecystokinin receptors, serotonin receptors, cannabinoid receptors, ATP gated ion channels, sodium channels, calcium channels, and acid-sensing ion channel [116]. The interaction between epithelia cells, EC, mast cells and other inflammatory cells, and neurons determines the synthesis and release of these sensitizers. The CRF-CRFR system regulates this interaction, therefore modulates visceral pain peripherally.

Increased permeability has been shown to induce visceral pain in response to acute CRD stress [117]. Increased permeability results in the translocation of gut microbota, antigens and other luminal contents, which activate gut immunity and facilitates nerve sensitization. Cortagine administered intraperitoneally induces visceral hypersensitivity, which is blocked by the CRF-specific antagonist, astressin [114]. In vitro experiments using healthy human colon tissues demonstrate that CRF increases mucosal permeability, which is inhibited by α-helical CRF\(_{24-41}\) and mast cell stabilizer, suggesting mast cells mediates the effect of increased permeability [118]. Interestingly, administration of astressin or antalarmin, which are CRF, and CRF\(_{2}\) antagonists respectively, only induces partial inhibition, indicating both receptors on the mast cells regulates the permeability. Mast cells activated by CRF release proteases and TNF-α, and these molecules facilitate intestinal epithelia barrier injury, increased permeability and nerve sensitization [119]. The increased permeability is facilitated via either the paracellular [117] or the transcellular [118] pathway according to two different contradictory experiments. However, roles of different pathways in increased permeability under different conditions await clarification, as different pathways determine differential gut immune reactions in response to different substances leaked in.

Enter chromaffin cells are an important source of CRFs and express CRF receptors. Two independent studies using different BON cell lines, which share functional similarities with intestinal EC cells, demonstrate that CRF stimulates serotonin release and synthesis from BON cells in a cAMP-dependent manner [120,121]. In the experiment using BON-1N cell line, only activation of CRF\(_{1}\) but not CRF\(_{2}\) leads to increased release of 5-HT [121]. In the experiment using another BON cell line, increased CRF release is not inhibited by CRF\(_{2}\) specific antagonists [120]. The discrepancy indicates differential involvement of CRF, and CRF in BON cells, which is partially explained by differential expression of CRF receptors on different BON cell lines. Serotonin, as an enteric neurotransmitter, induces excitatory postsynaptic potentials (EPSPs) participating in mucosal sensory transduction and stimulates vagal and intrinsic afferent nerve fibers, contributing to the visceral pain and visceral hypersensitivity [122].

The CRF-CRFR system also modulates visceral pain peripherally by regulating the local immune response. In a post-infectious IBS model, infection induces increased number of EC cells, mastocytosis and visceral hypersensitivity, which is blocked by T cell receptor knockout cells [123], indicating the involvement of immune cells and immune mediators in modulating visceral hypersensitivity. This is supported by the expression of CRF and CRF receptors in the immune cells including eosinophils, mast cells, macrophages, and plasma cells. Mast cells activated by CRF releases tryptase and histamine, directly sensitizing the nerve ends and contributing to visceral pain in IBS patients, as mast cells are in proximity with mucosal innervations [124]. In addition to directly regulate the activity of immune cells, CRF increases the permeability and exposes the luminal contents to these cells. CRF also promotes the release of immune mediators, such as TNFα, IL-1β and IL-6, from two lines of macrophages in vitro [125]. Antalarmin, a CRF, specific antagonist, administered before injection of LPS reduces TNFα, IL-1β and IL-6 production in the endo toxic shock mice model [125]. Blocking of IL-1, IL-6 or CRF ameliorates the LPS induced visceral hypersensitivity in rats [126]. In the model of visceral hypersensitivity induced by repeated CRD, CRD induces acute and chronic pain sensitization through CRF and IL-1, respectively [127]. These two experiments indicate in the presence and absence of inflammation, the roles of the CRF-CRFR system are different as different immune cells
are involved and the expression of CRF receptors is differentially regulated.

The specific roles of CRF₁ and CRF₂ remain controversial. Activation of CRF₁ by intravenously injection of Ucn2 induces visceral hyperalgesia in response to colorectal distention, which is reversed by the CRF₂ specific antagonist, astressin-B [128]. This is believed to involve the peripheral neurons and the spinal cord as increased activity of inferior splanchnic nerve and phosphorylation of Erk in laminae II and I were recorded. Another mechanism that may contribute to the inhibitory role of CRF₁ is by inducing colonic hyperemia through nitric oxide pathway upon activation5 [129]. However, other studies indicate that both CRF receptors contribute to the visceral pain by increasing the epithelial permeability, as mentioned above.

Summary

Visceral pain and visceral hypersensitivity is a common feature of many disorders including IBS. However, the underlying mechanisms are not fully understood. The CRF-CRFR system plays critical roles in modulating visceral pain and visceral hypersensitivity. CRFs including CRF, Ucn1, Ucn2 and Ucn3 initiate differential regulations of visceral pain and visceral hypersensitivity by binding selectively to CRF₁ or CRF₂.

In the brain, the amygdala integrates the stress and pain information and is a key component of the pain matrix. Under stressful conditions, increased level of CORT leads to down-regulation of GR and MR but increased expression of CRF in the amygdala. In the pain matrix of the amygdala, the hippocampus, EWN, BNST, PVN and LC, activation of CRF-CRFR system leads to visceral pain and visceral hypersensitivity. Additionally, dysregulation of CRFs and CRFR expression, loss of balance between CRF₁ and CRF₂, altered circuit plasticity in the brain pain matrix and dysregulation of noradrenergic release in different brain sites all contribute to the visceral pain and visceral hypersensitivity.

In the GI tract, activation of CRF-CRFR results in epithelia injury and increased permeability, which is facilitated by the immune cells. Increased permeability exposes luminal contents to the local immune cells, including eosinophils, macrophages, mast cells, T cells and plasma cells. Activation of these cells results in increased synthesis and release of cytokines and other immune mediators. CRFs, luminal contents and the immune mediators sensitize the nerve ends, contributing to the visceral pain and hypersensitivity. Although the role of CRF-CRFR remains controversial in different experimental settings, it is generally accepted that in the normal conditions, CRF activation inhibits visceral pain and hypersensitivity, and that the inhibitory roles are lost under pathological conditions.

References

1. Cervero F, Laird JM (1999) Visceral pain. Lancet 353(9170): 2145-2148.
2. Moloney RD, Siobhain MOM, Timothy GD, John FC (2015) Stress-induced visceral pain: toward animal models of irritable-bowel syndrome and associated comorbidities. Front Psychiatry 6:15.
3. Farzaei MH, Bahrami R, Abdollahi M, Rahimi R (2016) The Role of Visceral Hypersensitivity in Irritable Bowel Syndrome: Pharmacological Targets and Novel Treatments. J Neurogastroenterol Motil 22(4): 558-574.
4. Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, et al. (2016) Irritable bowel syndrome. Nature Reviews Disease Primers 2: pp. 1604.1
5. Chey WD, J Kurlander, S Esawaran (2015) Irritable bowel syndrome: a clinical review. JAMA 313(9): 949-958.
6. Zhang F, Wei Xiang, Chun-Yan Li, Shu-Chuen Li (2016) Economic burden of irritable bowel syndrome in China. World J Gastroenterol 22(47): 10450-10460.
7. Fortea J, M Prior (2013) Irritable bowel syndrome with constipation: a European-focused systematic literature review of disease burden. J Med Econ 16(3): 329-341.
8. Greenwood-Van MB, RD Moloney, AC Johnson, M Vicario (2016) Mechanisms of Stress-Induced Visceral Pain: Implications in Irritable Bowel Syndrome. J Neuroendocrinol 28(8): 8.
9. Moloney RD, Johnson AC, O’Mahony SM, Dinan TG, Greenwood-VMB, et al. (2016) Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS Neurosci Ther 22(2): 102-117.
10. Tache Y (2015) Corticotropin-releasing factor 1 activation in the central amygdala and visceral hyperalgesia. Neurogastroenterol Motil 27(1): 1-6.
11. Nuzzo T, M Kudaira (2006) Corticotropin-releasing factor induces rectal hypersensitivity after repetitive painful rectal distention in healthy humans. J Gastroenterol 41(8): 740-744.
12. Perrin MH, WW Vale (1999) Corticotropin releasing factor receptors and their ligand family. Ann N Y Acad Sci 885: 312-328.
13. Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213(4514): 1394-1397.
14. Rivier J, J Spiess, W Vale (1983) Characterization of rat hypothalamic corticotropin-releasing factor. Proc Natl Acad Sci U S A 80(15): 4851-4855.
15. Liapakis G, (2011) Members of CRF family and their receptors: from past to future. Curr Med Chem. 18(17): 2583-2600.
16. Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, et al. (1995) Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378(6554): pp. 287-292.
17. Hauger RL, Grigoriadis DE, Dallman ME, Plotsky PM, Vale WW, et al. (2003) International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. Pharmacol Rev 55(1): 21-26.
18. Reyes TM, Lewis K, Perrin MH, Kunikata KS, Vaughan J, et al. (2001) Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A 98 (5): 2843-2848.
19. Hsu SY, AJ Hsueh (2001) Human stresscopin and stresscopin-
related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med 7(5): 605-611.

20. Lewis K, C Li, MH Perrin, A Blount, K Kunitake, et al. (2001) Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci U S A 98 (13): 7570-7575.

21. Dathe M, Heinz F, Klaus G, Dietrich Z, Rudiger W (1996) Conformational differences of ovine and human corticotropin releasing hormone. A CD, IR, NMR and dynamic light scattering study. Int J Pept Protein Res 47 (5): 383-393.

22. Pallai PV, M Mabilia, M Goodman, W Vale, J Rivier (1983) Structural homology of corticotropin-releasing factor, sauvagine, and urotensin I: circular dichroism and prediction studies. Proc Natl Acad Sci U S A 80 (22): 6770-6774.

23. Harmar AJ (2001) Family-B G-protein-coupled receptors. Genome Biol 2(12): 1-10.

24. Chen R, KA Lewis, MH Perrin, WW Vale (1993) Expression cloning of a human corticotropin-releasing factor receptor. Proc Natl Acad Sci U S A 90 (19): 8967-8971.

25. Hillhouse EW, DK Grammatopoulos (2006) The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr Rev 27 (3): 260-286.

26. Lovenberg TW, CW Liaw, DE Grigoriadis, W Cleveger, DT Chalmers, et al. (1995) Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A 92 (3): 836-840.

27. Stenzel P, R Kesterson, Yeung W, R D Cone, M B Rittenberg (1995) Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Mol Endocrinol 9 (5): 637-645.

28. Kishimoto T, RV Pearse, CR Lin, MG Rosenfeld (1995) A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc Natl Acad Sci U S A 92 (4): 1108-1112.

29. Kostich WA, Chen A, Sperle K, Largent BL (1998) Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. Mol Endocrinol 12 (8): 1077-1085.

30. Vita N, Laurent P, Lefort S, Chalon P, Lelias JM (1993) Primary structure and functional expression of mouse pituitary and human brain corticotropin releasing factor receptors. FEBS Lett 335 (1): 1-5.

31. Grammatopoulos DK (2012) Insights into mechanisms of corticotropin-releasing hormone receptor signal transduction. Br J Pharmacol 166 (1): 85-97.

32. Kovalovsky D, Rejofo D, Liberman AC, Hochbaum D, Pereda MP (2002) Activation and induction of NUR77/NURR1 in corticotrophs by CRH/cAMP: involvement of calcium, protein kinase A, and MAPK pathways. Mol Endocrinol 16 (7): 1638-1651.

33. Brar BK, Chen A, Perrin MH, Vale W (2004) Specificity and regulation of extracellularly regulated kinase1/2 phosphorylation through corticotropin-releasing factor (CRF) receptors 1 and 2beta by the CRF1/urocortin family of peptides. Endocrinology 145 (4): 1718-1729.

34. Dermitzaki E, Tsatsanis C, Gravanis A, Margioris AN (2002) Corticotropin-releasing hormone induces Fas ligand production and apoptosis in PC12 cells via activation of p38 mitogen-activated protein kinase. J Biol Chem 277 (14): 12280-12287.

35. Markovic D, Punn A, Lehnhert H, Grammatopoulos DK (2008) Intracellular mechanisms regulating corticotropin-releasing hormone receptor-2beta endocytosis and interaction with extracellularly regulated kinase 1/2 and p38 mitogen-activated protein kinase signaling cascades. Mol Endocrinol 22 (3): 689-706.

36. Berger H, Heinrich N, Wietfeld D, Bienert M, Beyermann M (2006) Evidence that corticotropin-releasing factor receptor type 1 couples to Gs- and Gi-proteins through different conformations of its J-domain. Br J Pharmacol 149 (7): 942-947.

37. Kehele JH, CK Cain (2010) Therapeutic utility of non-peptidic CRF1 receptor antagonists in anxiety, depression, and stress-related disorders: evidence from animal models. Pharmacol Ther 128 (3): 460-487.

38. Rivier J, Gulyas J, Kunitake K, DiGrucchio M, Canfile J, et al. (2007) Stressin1-A, a potent corticotropin releasing factor receptor 1 (CRF1) selective peptide agonist. J Med Chem 50 (7): 1668-1674.

39. Daunzenberg FM, Py-Lang G, Higelin J, Fischer C, Wright MB, et al. (2001) Different binding modes of amphibian and human corticotropin-releasing factor type 1 and type 2 receptors. Evidence for evolutionary differences. J Pharmacol Exp Ther 296 (1): 113-120.

40. Mesleh MF (2007) NMR structural characterization of a minimal peptide antagonist bound to the extracellular domain of the corticotropin-releasing factor1 receptor. J Biol Chem 282 (9): 6338-6346.

41. Tache Y, M Million (2015) Role of Corticotropin-releasing Factor Signalling in Stress-related Alterations of Colonic Motility and Hyperalgesia. J Neurogastroenterol Motil 21 (1): 8-24.

42. Rivier J, Gulyas J, Kirby D, Low W, Perrin MH, et al. (2002) Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. J Med Chem 45 (21): 4737-4747.

43. Rivier JE, CL Rivier (2014) Corticotropin-releasing factor peptide antagonists: design, characterization and potential clinical relevance. Front Neuroendocrinol 35 (2): 161-170.

44. Tzerval H, Jahn O, Todorovic C, Sasse A, Eckart K, et al. (2004) Cortagine, a specific agonist of corticotropin-releasing factor subtype 1, is anxiogenic and antidepressive in the mouse model. Proc Natl Acad Sci U S A 101 (25): 9468-9473.

45. Grundy D (2002) Neuroanatomy of visceral nociception: vagal and splanchnic afferent. Gut 51 (Suppl I): i2-i5.

46. Rustioni, A, NL Hayes, S O’Neill (1979) Dorsal column nuclei and ascending spinal afferents in macaques. Brain 102 (1): 95-125.

47. Bennett GJ, Seltzer Z, Lu GW, Nishikawa N, Dubner R (1983) The cells of origin of the dorsal column postsynaptic projection in the lumbosacral enlargements of cats and monkeys. Somatosens Res 1 (2): 131-149.

48. Joseph SA, KM Knigge (1983) Corticotropin-releasing factor: immunocytochemical localization in rat brain. Neurosci Lett 35 (2): 135-141.
Central and Peripheral Modulation of Visceral Pain and Visceral Hypersensitivity by the CRF-CRFR System

Chatzaki E, Crowe PD, Wang L, Million M, Tache Y, et al. (2004) CRF receptors mediate the transition from nociceptive to pro-nociceptive responses in the substantia nigra pars compacta in vivo. J Neurosci 24(46): 10124-10133.

50. Wang L, Goebel-Stengel M, Stengel A, Wu SY, Ohning G, et al. (2011) Comparison of CRF-immunoreactive neurons distribution in mouse and rat brains and selective induction of Fos in rat hypothalamic CRF neurons by abdominal surgery. Brain Res 1415: 34-46.

51. Shu YM, Ni RJ, Sun YJ, Fang H, Zhou JN (2015) Distribution of corticotropin-releasing factor in the tree shrew brain. Brain Res 1618: 270-285.

52. Kono J, Konno K, Talukder AH, Fuse T, Abe M (2016) Distribution of corticotropin-releasing factor neurons in the mouse brain: a study using corticotropin-releasing factor-modified yellow fluorescent protein knock-in mouse. Brain Struct Funct.

53. Weitemier AZ, Tsivkovskaia NO, Ryabinin AE (2005) Urocortin 1 distribution in mouse brain is strain-dependent. Neuroscience 122(3): 729-740.

54. Mano-Otagiri A, Shibusaki T (2004) Distribution of urocortin 2 and urocortin 3 in rat brain. J Nippon Med Sch 71(6): 358-359.

55. Korosi A, Kozicz T, Richter J, Veening JG, Olivier B, et al. (2007) Corticotropin-releasing factor mRNA expression in the mouse spinal cord. J Comp Neurol 502(6): 973-989.

56. Goen GW, Kalamianos T, Oosthuisen MK, Pourown R, Faulkes CG, et al. (2015) Sociality and the telencephalic distribution of corticotropin-releasing factor; urocortin 3, and binding sites for CRF type 1 and type 2 receptors: A comparative study of eusocial naked mole-rats and solitary Cape mole-rats. J Comp Neurol 523(16): 2344-2371.

57. Potter E, Sutton S, Donaldson C, Chen R, Perrin M, et al. (1994) Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci USA 91(19): 8777-8781.

58. Primus R, Yevich E, Baltazar C, Gallager DW (1997) Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain. Neuropharmacology 17(5): 308-316.

59. Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HV, et al. (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 428(2): 191-212.

60. Lovenberg TW, Chalmers DT, Liu C, De Souza EB (1995) CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136(9): 4139-4142.

61. Chalmers DT, Lovenberg TW, De Souza EB (1995) Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J Neurosci 15(10): 6340-6350.

62. Chen A, Perrin M, Brar B, Li C, Jamieson P, et al. (2005) Mouse corticotropin-releasing factor receptor type 2 alpha gene: isolation, distribution, pharmacological characterization and regulation by stress and glucocorticoids. Mol Endocrinol 19(2): 441-458.

63. Korosi A, Veening JG, Kozicz T, Henckens M, Dederen J, et al. (2006) Distribution and expression of CRF receptor 1 and 2 mRNAs in the CRF over-expressing mouse brain. Brain Res 1072(1): 46-54.

64. Beery AK, Vahaba DM, Grunberg DM (2014) Corticotropin-releasing factor receptor densities vary with photoperiod and sociality. Horm Behav 66(5): 779-786.

65. Bravo JA, Dinan TG, Cryan JF (2011) Alterations in the central CRF system of two different rat models of comorbid depression and functional gastrointestinal disorders. Int J Neuropsychopharmacol 14(5): 666-683.

66. Ji G, Neugebauer V (2008) Pro- and anti-nociceptive effects of corticotropin-releasing factor (CRF) in central amygdala neurons are mediated through different receptors. J Neurophysiol 99(3): 1201-1212.

67. Maren S (2005) Synaptic mechanisms of associative memory in the amygdala. Neuron 47(6): 783-786.

68. Gauriau C, JF Bernard (2004) A comparative reappraisal of projections from the superficial laminae of the dorsal horn in the rat: the forebrain. J Comp Neurol 468(1): 24-56.

69. De Francesco PN, Valdivia S, Cabral A, Reynaldo M, Raising J, et al. (2015) Neuroanatomical and functional characterization of CRF neurons of the amygdala using a novel transgenic mouse model. Neuroscience 289: 153-165.

70. Yuan P, Wu SY, Wang L, Taché Y (2010) Corticotropin releasing factor in the rat colon: expression, localization and upregulation by endotoxin. Peptides 31(2): 3232-31.

71. Kawahito Y, Sano H, Muki S, Asai K, Kimura S, et al. (1995) Corticotropin releasing hormone in colonic mucosa in patients with ulcerative colitis. Gut 37(4): 544-551.

72. Buckins R, Bagyanszki M, Avula LR, Adriaensen D, Van Nassauw L, et al. (2015) Expression of corticotropin-releasing factor and urocortins in the normal and Schistosoma mansoni-infected mouse ileum. Cell Tissue Res 359(2): 453-463.

73. Wallon C, Persborn M, Jönsson M, Wang A, Phan V, et al. (2011) Eosinophils express muscarinic receptors and corticotropin-releasing factor to disrupt the mucosal barrier in ulcerative colitis. Gastroenterology 140(5): 1597-1607.

74. Liu S, Gao N, Hu HZ, Wang X, Wang GD, et al. (2006) Distribution and chemical coding of corticotropin-releasing factor-immunoreactive neurons in the guinea pig enteric nervous system. J Comp Neurol 494(1): 63-74.

75. Sand E, Thernner-Persson A, Ekblad E (2011) Corticotropin releasing factor distribution in rat intestine and role in neuroprotection. Regul Pept 166(1-3): 65-70.

76. Munamatsu Y, Fukushima K, Iino K, Totsune K, Takahashi K, et al. (2000) Urocortin and corticotropin-releasing factor receptor expression in the human colonic mucosa. Peptides 21(12): 1799-1809.

77. Saruta M, Takahashi K, Suzuki T, Torii A, Kawakami M, et al. (2004) Urocortin 1 in colonic mucosa in patients with ulcerative colitis. Clin Endocrinol Metab 89(1): 5332-5361.

78. Mahajan S, M Liao, P Barkan, K Takahashi, A Bhargavaa (2014) Urocortin 3 expression at baseline and during inflammation in the colon: corticotropin releasing factor receptors cross-talk. Peptides 54: 58-66.

79. Chatzaki E, Crowe PD, Wang L, Million M, Tache Y, et al. (2004) CRF receptor type 1 and 2 expression and anatomical distribution in the rat colon. J Neurochem 90(2): 309-316.
Central and Peripheral Modulation of Visceral Pain and Visceral Hypersensitivity by the CRF-CRFR System

80. Yuan PQ, S Vincent Wu, J Elliott, PA Anton, E Chatzakic (2012) Expression of corticotropin releasing factor receptor type 1 (CRF1) in the human gastrointestinal tract and up regulation in the colonic mucosa in patients with ulcerative colitis. Peptides 38(1): 62-69.

81. Chatzakic E, Anton PA, Million M, Lambropoulos M, Constantindis Tet al. (2013) Corticotropin-releasing factor receptor subtype 2 in human colonic mucosa: down-regulation in ulcerative colitis. World J Gastroenterol 19(9): 1416-1423.

82. Gue, M, Del Rio-Lo, Eutamene H, Theodorou V, Fioramonti J, et al. (1997) Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. Neurogastroenterol Motil 9(4): 271-279.

83. Million M, Grigoridias DE, Sullivan S, Crowe PD, McRoberts JA, et al. (2003) A novel water-soluble selective CRF1 receptor antagonist, NB135965, blunts stress-induced visceral hyperalgesia and colonic motor function in rats. Brain Res 985(1): 32-42.

84. Larache M, Bradesi S, Million M, McLean P, Tache Y, et al. (2008) Corticotropin-releasing factor type 1 receptors mediate the visceral hyperalgesia induced by repeated psychological stress in rats. Am J Physiol Gastrointest Liver Physiol 294(4): G1033-G1040.

85. Saito-NK, Hasegawa R, Nagura Y, Ito H, Fukudo S (2008) Corticotropin-releasing hormone receptor 1 antagonist blocks colonic hypersensitivity induced by a combination of inflammation and repetitive colorectal distension. Neurogastroenterol Motil 20(10): 1147-1156.

86. Million M, Zhao JF, Luckey A, Cizmert J, Maynard GD (2013) The newly developed CRF1-receptor antagonists, NG8-92 and NG8-9002, suppress acute stress-induced stimulation of colonic function and visceral hypersensitivity in rats. Pain 155(9): e17349.

87. BG-V Meerveld, Johnson AC, Cochrane S, Schulkin J, Myers DA (2005) Corticotropin-releasing factor 1 receptor-mediated mechanisms inhibit colonic hypersensitivity in rats. Neurogastroenterol Motil 17(3): 415-422.

88. Taguchi R, Shikata K, Furuya Y, Hirakawa T, Ino M, et al. (2017) Selective corticotropin-releasing factor 1 receptor antagonist E2508 reduces restraint stress-induced defecation and visceral pain in rat models. Psychoneuroendocrinology 75: 110-115.

89. Rasnick KS, Heinrichs SC, Britton KT Koch GF (1993) Microinjection of a corticotropin-releasing factor receptor antagonist into the central nucleus of the amygdala reverses anxiety-like effects of ethanol withdrawal. Brain Res 605(1): 25-32.

90. Johnson AC, Tran L, Schulkin J, BG-V Meerveld (2012) Importance of stress receptor-mediated mechanisms in the amygdala on visceral pain perception in an intrinsically anxious rat. Neurogastroenterol Motil 24(5): 479-486.

91. Johnson AC L, Tran, BG-V Meerveld (2015) Knockdown of corticotropin-releasing factor in the central amygdala reverses persistent visceralomotor hyperalgesia. Transl Psychiatry 5: e157.

92. Su J, Tanaka Y, Muratsubaki T, Kano M, Kanazawa M, et al. (2015) Injection of corticotropin-releasing hormone into the amygdala aggravates visceral nociception and induces noradrenaline release in rats. Neurogastroenterol Motil 27(1): 30-39.

93. Ciprian AC, KS Gomes, RL Nunes-de-Souza (2016) CRF receptor type 1 (but not type 2) located within the amygdala plays a role in the modulation of anxiety in mice exposed to the elevated plus maze. Horm Behav 81: 59-67.

94. Nishii H, Nomura M, Aono H, Fujimoto N, Matsumoto T (2007) Up-regulation of galanin and corticotropin-releasing hormone mRNAs in the key hypothalamic and amygdaloid nuclei in a mouse model of visceral pain. Regul Pept 141(1-3): 105-112.

95. Rouwette T, Klemann K, Gaszner B, Scheffer GJ, Rouboos EW, et al. (2011) Differential responses of corticotropin-releasing factor and urocortin 1 to acute pain stress in the rat brain. Neuroscience 183: 15-24.

96. Saito K, Toshiyuki K, Y Nagura, Hitomi I, M Kanazawa, et al. (2005) Corticotropin-releasing hormone receptor 1 antagonist blocks brain-gut activation induced by colonic distention in rats. Gastroenterology 129(5): 1533-1543.

97. Tran L, J Schulkin, BG-V Meerveld (2014) Importance of CRF receptor-mediated mechanisms of the bed nucleus of the stria terminalis in the processing of anxiety and pain. Neuropsychopharmacology 39(11): 2633-2645.

98. Zhang G, Yu L, Chen ZY, Zhu JS, Hua R (2016) Activation of corticotropin-releasing factor receptor neurons and microglia in paraventricular nucleus precipitates visceral hypersensitivity induced by colorectal distension in rats. Brain Behav Immun 55: 93-104.

99. Lechner SM, Curtis AL, Brons R, Valentino RJ (1997) Locus coeruleus activation by colon distention: role of corticotropin-releasing factor receptor and excitatory amino acids. Brain Res 756(1-2): 114-124.

100. Kosoyan HP, DE Grigoridias, Y Tache (2005) The CRF (1) receptor antagonist, NB1-35965, abolished the activation of locus coeruleus neurons induced by colorectal distension and intracisternal CRF in rats. Brain Res 1056(1): 85-96.

101. Kravets JL, BAS Reyes, EM Unterwald, EJ Van Bockstaele (2015) Direct targeting of peptidergic amygdalar neurons by noradrenergic afferents: linking stress-integrative circuitry. Brain Struct Funct 220(1): 51-55.

102. Ji G, V Neugebauer (2007) Differential effects of CRF1 and CRF2 receptor antagonists on pain-related sensitization of neurons in the central nucleus of the amygdala. J Neurophysiol 97(6): 3893-3904.

103. Ji G, Yu Fu, H Advanikar, Volker Neugebauer (2013) Non-pain-related CRF1 activation in the amygdala facilitates synaptic transmission and pain responses. Mol Pain 9: 2.

104. Shepard JD, KW Barron, DA Myers (2000) Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res 861(2): 288-295.

105. Greenwood-VM, Gibson M, Gunter W, Shepard J, Foreman R, et al. (2001) Stereotactic delivery of corticosterone to the amygdala modulates colonic sensitivity in rats. Brain Res 893(1-2): 135-142.

106. Myers B, Greenwood-VM (2007) Corticotropin receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. Am J Physiol Gastrointest Liver Physiol 292(6): G1622-G1629.

107. Tran L, B Greenwood-VM (2013) Altered expression of glucocorticoid receptor and corticotropin-releasing factor in the central amygdala in response to elevated corticosterone. Behav Brain Res 234(2): 380-385.

108. Johnson AC, BG-V Meerveld (2015) Knockdown of steroid receptors
in the central nucleus of the amygdala induces heightened pain behaviors in the rat. Neuropharmacology 93: 116-123.

109. Moloney R, BG-V Meerveld (2015) Knockdown of the glucocorticoid receptor in the central nucleus of the amygdala induces visceral-somatic hypersensitivity. Psychoneuroendocrinology 61: 15.

110. Myers B, BG-V Meerveld (2010) Elevated corticosterone in the amygdala leads to persistent increases in anxiety-like behavior and pain sensitivity. Behav Brain Res 214(2): 465-469.

111. Tran L (2015) Epigenetic modulation of chronic anxiety and pain by histone deacetylation. Mol Psychiatry 20(10): 1219-1231.

112. Johnson AC, BG-V Meerveld (2015) Central amygdala mechanisms regulating visceral pain. Psychoneuroendocrinology 61: 8.

113. O’Malley D, Julio-Piepera M, Dinan TG, Cryan JF (2014) Strain differences in stress-induced changes in central CRF1 receptor expression. Neurosci Lett 561: 192-197.

114. Larache M, G Gourcerol, LWang, K Pambukchian, S Brunnhuber, et al (2009) Cortagine, a CRF1 agonist, induces stresslike alterations of colonic function and visceral hypersensitivity in rodents primarily through peripheral pathways. Am J Physiol Gastrointest Liver Physiol 297(1): G215-G227.

115. Nozu T, Miyagishi S, Nozu R, Takakusaki K, Okamura T (2016) Water avoidance stress induces visceral hypersensitivity through peripheral corticotropin releasing factor receptor type 2 and central dopamine D2 receptor in rats. Neurogastroenterol Motil 28(4): 522-531.

116. Akbar A, JR Walters, S Ghosh (2009) Review article: visceral hypersensitivity in irritable bowel syndrome: molecular mechanisms and therapeutics. Aliment Pharmacol Ther 30(5): 423-435.

117. Ait-Belgnaoui A, Bradesi S, Fioramonti J, Theodorou V, Bueno L (2005) Acute stress-induced hypersensitivity to colonic distension depends upon increase in paracellular permeability: role of myosin light chain kinase. Pain 113(1-2): 141-147.

118. Wallon C, Yang PC, Keita AV, Ericson AC, McKay DM, et al. (2008) Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. Gut 57(1): 50-58.

119. Overman EL, JE Rivier, AJ Moeser (2012) CRF induces intestinal epithelial barrier injury via the release of mast cell proteases and TNF-alpha. PLoS One 7(6): e39935.

120. Von Mentzer B, Murata Y, Ahlstedt I, Lindstrom E, Martinez V (2007) Functional CRF receptors in BON cells stimulate serotonin release. Biochem Pharmacol 73(6): 805-813.

121. Wu SV, Yuan PQ, Lai J, Wong K, Chen MC, et al. (2011) Activation of Type 1 CRH receptor isoforms induces serotonin release from human carcinoid BON-1N cells: an enterochromaffin cell model. Endocrinology 152(1): 126-137.

122. Sikander A, SV Rana, KK Prasad (2009) Role of serotonin in gastrointestinal motility and irritable bowel syndrome. Clin Chim Acta 403(1-2): 47-55.

123. Wheatcroft J, Wakelin D, Smith A, Mahoney CR, Maue G, et al. (2005) Enterochromaffin cell hyperplasia and decreased serotonin transporter in a mouse model of postinfectious bowel dysfunction. Neurogastroenterol Motil117 (6): 863-870.

124. Barbara G, Stanghellini V, De Giorgio R, Cremoson C, Gottrell BS (2004) Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterolgy 126(3): 693-702.

125. Agelaki S, Christos T, A Gravanis, AN Margioris (2002) Corticotropin-releasing hormone augments proinflammatory cytokine production from macrophages in vitro and in lipopolysaccharide-induced endotoxin shock in mice. Infect Immun 70(11): 6068-6074.

126. Nozu T, Miyagishi S, Nozu R, Takakusaki K, Okamura T (2016) Lipopolysaccharide induces visceral hypersensitivity: role of interleukin-1, interleukin-6, and peripheral corticotropin-releasing factor in rats. J Gastroenterol 52(1): 72-80.

127. Nozu T, Shima K, S Miyagishi, K Takakusaki T Okumura (2015) Colorectal distention induces acute and delayed visceral hypersensitivity: role of peripheral corticotropin-releasing factor and interleukin-1 in rats. J Gastroenterol 50(12): 1153-1161.

128. Million M, I Wang, Y Wang, DW Adelson, P-Q Yuan (2006) CRF2 receptor activation prevents colorectal distension induced visceral pain and spinal ERK1/2 phosphorylation in rats. Gut 55(2): 172-181.

129. Akiba Y, JD Kaunitz, M Million (2015) Peripheral corticotropin-releasing factor receptor type 2 activation increases colonic blood flow through nitric oxide pathway in rats. Dig Dis Sci 60(4): 858-867.