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Corticotropin-releasing factor receptor type 1 and type 2 interaction in irritable bowel syndrome.

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Abstract

Irritable bowel syndrome (IBS) displays chronic abdominal pain or discomfort with altered defecation, and stress-induced altered gut motility and visceral sensation play an important role in the pathophysiology. Corticotropin-releasing factor (CRF) is a main mediator of stress responses and mediates these gastrointestinal functional changes. CRF in brain and periphery acts through two subtype receptors such as CRF receptor type 1 (CRF1) and type 2 (CRF2), and activating CRF1 exclusively stimulates colonic motor function and induces visceral hypersensitivity. Meanwhile, recent several studies demonstrated that CRF2 has a counter regulatory action against CRF1, which may imply that CRF2 inhibits stress response induced by CRF1 in order to prevent it from going into an overdrive state. Colonic contractility and sensation may be explained by the state of the intensity of CRF1 signaling. CRF2 signaling may play a role in CRF1-triggered enhanced colonic functions through modulation of CRF1 activity. Blocking CRF2 further enhances CRF-induced stimulation of colonic contractility and activating CRF2 inhibits stress-induced visceral sensitization. Therefore, we proposed the hypothesis, i.e. balance theory of CRF1 and CRF2 signaling as follows. Both CRF receptors may be activated simultaneously and the signaling balance of CRF1 and CRF2 may determine the functional changes of gastrointestinal tract induced by stress. CRF signaling balance might be abnormally shifted toward CRF1, leading to enhanced colonic motility and visceral sensitization in IBS. This theory may

lead to understand the pathophysiology and provide the novel therapeutic options targeting altered signaling balance of CRF1 and CRF2 in IBS.

Key words: Irritable bowel syndrome; Corticotropin-releasing factor; receptor; Colonic motility; Visceral sensation

Introduction

Irritable bowel syndrome (IBS) displays chronic abdominal pain or discomfort with altered defecation which is not explained by structural or biochemical abnormalities. The prevalence is quite higher in the general population (10 to 20%) [1-4], and it impairs patients' quality of life and has an enormous economic impact including direct costs of health care use and indirect costs of absenteeism from work [2]. The pathophysiology of IBS has not been determined definitely but it is generally accepted that dysfunction of the bidirectional communication system between brain and gut, i.e. brain-gut axis, contributes to the symptom generation [5, 6].

Stress induces behavioral, neuroendocrine and autonomic responses, and corticotropin-releasing factor (CRF) is a main mediator of these responses in the brain-gut axis [7-13]. Stress also alters colonic motor and sensory functions, which are thought to play an important role in IBS pathophysiology [14-16]. Several animal and few human studies proved that CRF mediates these gut responses [16-19]. Administration of CRF alters colonic motility and increases plasma adrenocorticotropic hormone (ACTH), and these responses are exaggerated in IBS patients [17]. These lines of evidence suggest that altered brain-gut axis resulting from exaggerated response to CRF, leading to changes in colonic functions is thought to relevant to the pathophysiology of IBS.

In this paper, we will review the actions and mechanisms of central and peripheral CRF signaling in colonic motor and visceral sensory functions, and discuss the possible role of CRF signaling in the pathophysiology of IBS.

And we will also present the balance theory of CRF receptors signaling, which may well explain the actions of CRF in gastrointestinal (GI) functions. Finally, the therapeutic role of CRF signaling according to this theory will be also discussed.

CRF receptors and ligands

CRF is a 41-amino acid residue peptide which was originally isolated from ovine brain [20] and named for its property to stimulate anterior pituitary secretion of ACTH. During the last twenty years, three new mammalian CRF-related peptides, urocortins (Ucns) such as urocortin 1 (Ucn1), urocortin 2 (Ucn2), and urocortin 3 (Ucn3) have been characterized [21-24].

CRF and Ucns exert its action through the activation of two receptors, CRF receptor type 1 (CRF1) and type 2 (CRF2) [25, 26]. CRF receptors are members of the G-protein coupled receptors family. The dominant mode of signaling for both CRF1 and CRF2 is the Gs-coupled adenylate cyclase-phosphokinase cascade [24]. However, CRF receptors coupled to other types of G proteins have also been demonstrated [25, 27], and phospholipase C-protein kinase C and extracellular signal-regulated kinase-mitogen activated protein kinase cascades are also reported [25].

Despite sharing 70% amino acid sequence similarity, CRF1 and CRF2 display distinct characteristic affinities for CRF and Ucns [21-23]. CRF has a higher affinity (10- to 40-fold higher) for CRF1 than for CRF2. Ucn1 binds CRF2 with 100-fold greater affinity than CRF, and

CRF1 with 6-fold greater affinity than CRF [21-23]. Ucn2 and Ucn3 exhibit high selectivity only for CRF2 [22, 23].

Role of CRF in stress-induced stimulation of colonic motor function

Although no specific or consistent abnormal changes in GI motility definitely related to abdominal pain or discomfort are determined, many studies reported altered colonic motility in IBS [14, 28, 29]. Several studies (but not all studies) showed accelerated colonic transit is observed in diarrhea predominant IBS [29]. In addition, IBS patients display exaggerated motility response to stress as compared to healthy controls [18], suggesting the importance of stress and altered colonic motility in symptom generation in IBS.

Central CRF receptors

Various stressors such as psychological (water avoidance), physical (restraint), or immunological (interleukin-1β) accelerate colonic transit and stimulate colonic contractions in rodents [30-36]. Central administration of CRF stimulates colonic motility such as reduced colonic transit time, stimulation of defecation and colonic contractions [33-37]. These responses are blocked by central administration of a non-selective CRF receptor antagonist such as α-helical $CRF_{(9-41)}$ or astressin, and central administration of CRF mimics the responses [30, 31, 35, 38].

Meanwhile, CRF1 and CRF2 are known to display the different response in colonic motor function. CRF, Ucn1 or Ucn2 administered

intracerebroventricularly (icv), increases fecal pellet output, and Ucn1 has similar potency as CRF. However, Ucn 2 is about 10 and 8 times less potent than CRF and Ucn1, respectively in mice [39]. In addition, restraint stress or icv CRF-induced stimulation of pellet output and acceleration of distal colonic transit were prevented by icv, a selective CRF1 antagonist, NBI-35965 but not by icv, a selective CRF2 antagonist, astressin$_2$-B [40]. These results indicate that activation of brain CRF1 is involved in the stress-induced stimulation of colonic motor function.

Central CRF-induced altered motor function is independent from the activation of hypothalamic–pituitary–adrenal axis, because this response is observed in hypophysectomized rats [37]. Chlorisondamine or atropine but not bretylium blocked central CRF-induced stimulation of colonic transit, but vagotomy only reduced this response by 19% in rats [33, 34]. Meanwhile other study demonstrated vagotomy completely abolished this response by CRF [37]. Thus stimulation of central CRF receptors may activate vagal and sacral parasympathetic neurons resulting in increased enteric nervous system activity, thereby stimulating colonic motor function.

In addition, central CRF or restraint stress-induced stimulation of defecation was blocked by peripheral administration of 5-hydroxytryptamine (5-HT)$_3$ antagonist or 5-HT$_4$ antagonist [38, 41]. Moreover, increase in 5-HT content in the feces of rat proximal colon by intracisternal (ic) CRF or restraint stress was observed and it was inhibited by ic, a selective CRF1 antagonist, NB1-27914. These results suggest that parasympathetic cholinergic activation of colonic 5-HT$_3$ and 5-HT$_4$ receptors also mediates the

151 action of CRF.

152 Microinjection of CRF into the specific brain nuclei reveals the
153 responsive site to CRF. Mönnikes et al. showed it is localized in the
154 hypothalamus (paraventricular nucleus; PVN, arcuate nucleus) and pontine
155 areas, such as locus coeruleus (LC) [33, 34, 42]. These brain nuclei are known
156 to be involved in CRF-induced anxiety and depression [43-45]. PVN contains
157 numerous CRF like immunoreactive neurons and receptors, and sends direct
158 projections to dorsal vagal complex and spinal preganglionic neurons
159 controlling autonomic nervous system activity [46, 47]. LC noradrenergic
160 neurons during stress can supply norepinephrine across the central nervous
161 system and modulate the stress response [48]. Activation of LC by CRF
162 induces increased vigilance and anxiogenic behavior [49, 50]. These results
163 may support the role of brain CRF receptors in the pathophysiology of IBS,
164 because IBS patients are frequently comorbid with psychiatric disorders such
165 as anxiety and depression [51], and display greater reactivity to stress [52].

166 Water avoidance stress (WAS) induces numerous Fos-positive cells in
167 PVN, LC, nucleus tractus solitarius (NTS), and the parasympathetic nucleus
168 of the lumbosacral spinal cord (L6-S1) in rats [30, 53]. Bilateral microinfusion
169 of α-helical $CRF_{(9-41)}$ into the PVN before restraint or WAS abolished stress-
170 induced alterations of colonic transit [33, 34]. These results further support
171 the notion that stress or CRF activates PVN and LC, leading to stimulating
172 colonic motor function mediated through vagal and sacral parasympathetic
173 neurons.

174

175 *Peripheral CRF receptors*

176 Intravenous (iv) administration of CRF induces the stimulation of
177 pellet output and colonic transit with a potency similar to central injection
178 (icv) in rats [38, 54, 55]. Peripherally injected CRF antagonist, α-helical
179 $CRF_{(9-41)}$ or astressin which does not cross the blood-brain barrier, blunts the
180 stimulation of distal colonic transit and fecal pellet output induced by acute
181 wrap restraint or WAS in rats [38, 54-56]. Moreover, in in vitro studies, CRF
182 increases distal colonic myoelectric activity [56], and Ucn1 or CRF stimulates
183 contractions of colonic muscle strips [57, 58]. These results strongly suggest
184 that CRF also acts peripheral CRF receptors to stimulate colonic motility.

185 Enhanced colonic motility induced by peripheral CRF is mediated
186 through CRF1, which is supported by the following evidence. Peripheral
187 administration of CRF reduces colonic transit time but Ucn2 or Ucn3 does not
188 induce the response under the same conditions in rodents [59, 60].
189 Intraperitoneal (ip) cortagine, a selective CRF1 agonist decreases the distal
190 colonic transit time, increases distal and transverse colonic contractility,
191 increases defecation and induces watery diarrhea in rats [61]. In addition, ip
192 administration of NBI-27914 or CP-154,526, a selective CRF1 antagonist
193 abolishes the response by CRF [59, 60]. Since all now available CRF1
194 antagonists can cross the blood-brain barrier, these results do not indicate
195 directly the role of peripheral CRF1. However, as described above, stress-
196 induced stimulation of defecation is abolished by non-selective CRF receptor
197 antagonists with peripheral limited action, and moreover, subcutaneous (sc)
198 injection of $astressin_2$-B does not alter accelerated distal colonic transit

induced by restraint stress [60]. These results suggests that peripheral injection of CRF- or stress-induced stimulating colonic motor function is mediated through peripheral CRF1.

Recent studies demonstrated that the expression of CRF receptors and ligands in the colon in various cells such as neuronal (enteric nervous system), enterochromaffin (EC) and immune cells (mast cells, lymphocytes) in rodents and human [62-70]. Most of these studies also showed that CRF and Ucns are expressed in close proximity of the CRF receptors. Moreover, both EC cells and mast cells are not only a target of peripheral CRF to stimulate the release of chemical mediators such as serotonin, etc., but also secrete CRF itself [69, 71-73]. Luminally released serotonin from EC cells activates mucosal 5-HT_3 receptors located on the vagal afferents, which stimulates colonic motility via the vagovagal reflex [74]. These results suggest that peripheral CRF and Ucns may form autocrine/paracrine loop, thereby modulating the motility.

In addition, several studies suggested that colonic myenteric neurons are also possible action sites of peripheral CRF for the following reasons. Ip CRF induces colonic myenteric Fos expression through peripheral CRF1 and the nearly all Fos expressing cells are CRF1 immunoreactive [75]. Moreover, Fos activation by ip CRF is correlated with increased defecation [75]. Ucn1 evokes the contractions of rat colonic smooth muscle strips, which are blocked by a selective CRF1 antagonist, antalarmine or the neuronal blocker, tetrodotoxin [57]. Additionally, myenteric neurons in the guinea pig jejunum display an increased intracellular calcium concentration in response to CRF

application, and this neuronal activation is mediated through CRF1 [76].

In contrast to these above results, Tsukamoto et al. [77] demonstrated that the stimulatory effect of peripherally administered CRF on colonic motility was abolished by truncal vagotomy, hexamethonium, atropine and ic astressin, and suggested the possibility that peripheral injection of CRF reaches the area postrema (AP) and activates the dorsal nucleus of vagus via central CRF receptors, resulting in activation of the vagal efferent, leading to stimulating colonic motility. CRF does not penetrate to the brain but circumventricular organs including AP are relatively unprotected by the blood-brain barrier [78].

There is also the evidence that peripheral injection of CRF activates several brain nuclei such as PVN, central amygdala (CeA), NTS and AP [79, 80]. Additionally, CRF injection also induces Fos expression in lumbosacral spinal intermediolateral column and dorsal horn [80], which are known to contain cells that engage in ascending supraspinal projections to the NTS [81]. Moreover, it is also known that NTS receives a large proportion of efferents from AP [82]. CRF receptors are present on AP, and the cervical and subdiaphragmatic vagus [83, 84]. These results suggest that peripheral CRF may stimulate NTS possibly through humoral i.e. by directly activating AP, and/or neural mechanisms, i.e. through vagus afferents and/or ascending projections from lumbosacral spinal cord, then NTS may transfer convergent information to the dorsal nucleus of vagus [85], leading to modulating colonic motility. As described before, PVN is a responsive site to central CRF inducing the stimulation of colonic motor function. In addition, as will be described

247 later, CeA is thought to be one of the responsive area to brain CRF inducing
248 visceral sensitization. In this context, we would emphasize that the possibility
249 of contribution of central pathways to modulating colonic functions by
250 peripheral administration of CRF has not been denied.
251
252 **Role of CRF in stress-induced altered visceral sensation**
253 It is now widely accepted that an altered visceral sensitivity plays an
254 important role in the pathogenesis of IBS [14, 86, 87]. Previous studies
255 indicate that 33-90% of IBS patients display increased visceral sensitivity to
256 rectal balloon distention [88-93]. Several factors such as various methods
257 determining the sensitivity etc. may contribute to the observed wide range of
258 hypersensitivity, but in any event, these results also suggest that significant
259 portion of the patients does not develop visceral hypersensitivity in the basal
260 state. Meanwhile, we and other researcher demonstrated that conditioning
261 such as repetitive colon or rectal distention induces visceral hypersensitivity
262 in IBS patients regardless of the baseline sensitivity, and this response is not
263 observed in healthy controls [94, 95], which may be a reliable marker for IBS.
264 It was reported that visceral stimulation can be interpreted as stress to IBS
265 patients, because it evokes daily symptoms and negative emotion [86]. These
266 lines of evidence further support the importance of stress and altered visceral
267 sensation in pathophysiology of IBS.
268
269 *Central CRF receptors*
270 Several stress models evoke visceral hypersensitivity and this

response is blocked by central injection of CRF antagonist [96, 97]. Meanwhile, central administration (icv) of CRF induces visceral hypersensitivity to colorectal distention (CRD) in rats [96], which is mediated through CRF1 [98]. However, the studies evaluating the brain sites responsible for modulating visceral sensation has been limited so far. Kosoyan et al. [99] showed that LC neurons were activated by CRD or ic CRF, which was abolished by iv NBI-35965, which can cross the blood-brain barrier in rats, indicating that CRF1 signaling plays a role in visceral hypersensitivity through activating LC.

Su et al. [100] very recently demonstrated that CRF microinjected into CeA increased visceromotor response (VMR) to CRD and the response was blocked by injection of CP-15426, a selective CRF1 antagonist into this site. CRF-like immunoreactivity and gene expression in CeA are increased in response to CRD [101]. It is also known that amygdala is an important site contributing to the persistent pain inducing negative affective states such as fear, anxiety, and depression [102].

These observations suggest the possibility of pathogenetic role of LC and CeA in IBS. CeA contains a high density of CRF neurons [103, 104], and these neurons project to the LC and increase their firing rate resulting in the stimulation of the ascending noradrenergic system [105]. The release of noradrenaline in the cortical and limbic rostral efferent projections from the LC or CeA [106] is known to induce arousal and anxiogenic responses along with hypervigilance to visceral input which is a commonly observed in IBS [107].

Early maternal separation, which is one of the models of IBS

displaying visceral sensitization [108], induces heightened basal tone of CRF gene expression, increased levels of CRF and upregulation of CRF1 signal transduction in the specific brain area such as LC and CeA, leading to enhanced reactivity to stress in adult rats [109-112]. Therefore, LC and CeA may be responsive sites of brain CRF-CRF1 signaling and mediate stress-induced visceral sensitization.

Peripheral CRF receptors

Peripheral CRF1 signaling also contributes to the visceral hypersensitivity. It was shown that WAS-induced visceral hyperalgesia was prevented by sc astressin [113]. We also demonstrated that CRD-induced visceral hyperalgesia was prevented by ip astressin but not by ip astressin$_2$-B [58]. In addition, peripheral CRF1 activation by ip cortagine increased VMR to CRD, which was blocked by ip astressin but not by icv [61]. These results suggest that stress-induced visceral hypersensitivity is also mediated through peripheral CRF1.

The definite action sites of peripheral CRF in modulating visceral sensation has not been determined. Since CRF receptors are proved to be expressed in dorsal root ganglia (DRG) [114], CRF may modulate visceral sensation through CRF receptors on spinal afferents directly.

As mentioned earlier, EC cells have CRF receptors and release serotonin through activating the receptors [65, 71]. Serotonin from EC cells is thought to contribute to visceral hypersensitivity through activating spinal afferents [115]. In addition, it became certain that mast cells of GI tract also

play an important role in stress-induced visceral sensitization [116]. Partial restraint stress-induced colonic hypersensitivity is prevented by doxantrazole, mast cell stabilizer in rats [96]. Mast cells have CRF receptors at their surface [66, 67] and their degranulation is triggered by peripheral CRF in GI tract [72]. They contain and release a large variety of mediators such as serotonin, prostaglandins and cytokines in response to various stimuli, and these mediators were demonstrated to activate visceral afferents or DRG neurons [117, 118], leading to induction of visceral sensitization. Therefore, peripheral CRF not only acts directly on visceral afferents but also indirectly through stimulating the release of chemicals from EC and mast cells leading to activating the afferents.

Meanwhile, acute stress-induced hypersensitivity to CRD was found to be linked to increase in colonic paracellular permeability [119]. Ait-Belgnaoui et al. [119] demonstrated that restraint stress-induced increased colonic permeability was blocked by ip α-helical $CRF_{(9-41)}$, and ip CRF mimicked this response. Moreover, CRF-induced increased permeability was blocked by ip doxantrazole. Therefore visceral sensitization induced by peripheral CRF signaling may result from altered colonic permeability possibly through mast cell-dependent mechanisms.

A balance theory of CRF1 and CRF2 signaling to modulate colonic motor and visceral sensation

As described above, central and peripheral CRF-CRF1 signaling are involved in the stimulatory action on colonic motility and sensation induced

343 by stress. However, stress activates both CRF1 and CRF2 signaling. For
344 example, restraint stress induces delayed gastric transit through CRF2 [60,
345 120], and simultaneously, it also results in the stimulation of colonic motility
346 through CRF1 [40]. Stress may stimulate to release CRF and Ucns in brain
347 and periphery, which could activate both CRF receptors according to the
348 distinct affinity for each CRF receptor. Thus it is thought that CRF2 may also
349 contribute to stress-induced altered colonic functions.

350 In fact, we and other researchers showed that activation of peripheral
351 CRF2 by peripheral administration of selective CRF2 agonist such as
352 sauvagine or Ucn2 blocked repetitive CRD-induced visceral hyperalgesia in
353 rats [58, 114, 121], suggesting that CRF2 signaling may have a counter action
354 to CRF1 in modulating visceral sensation. Moreover, recently this counter
355 action was also observed in modulation of colonic motility. Gourcerol et al.
356 [62] showed that ip Ucn2 inhibited ip CRF-induced stimulation of defecation
357 and ip astressin$_2$-B further enhanced the response in rats. Moreover,
358 restraint stress-induced stimulation of colonic contractions and WAS-induced
359 stimulation of pellet output were prevented by ip Ucn2.

360 Acute stress induces integrated responses to maintain homeostasis
361 and warrant survival of organisms. In the absence of proper counter
362 regulation, the stress response runs in an overdrive state that can become
363 maladaptive and fatal [122]. In this context, existence of counter action by
364 CRF2 signaling may be suitable for the survival of organisms under stressful
365 condition.

366 In this context, we hypothesized as follows. Colonic contractility and

sensation may be explained by the state of the intensity of CRF1 signaling. CRF2 signaling may play a role in CRF1-triggered enhanced colonic functions through modulation of CRF1 activity. The signaling balance of CRF1 and CRF2 might determine the functional colonic changes induced by stress. We designated this hypothesis as balance theory of CRF1 and CRF2 signaling.

We [58] have very recently demonstrated several results supporting the hypothesis. Ip CRF increased the colonic contractions and selective CRF1 stimulation by cortagine also increased the contractility in rats. Blocking or activating peripheral CRF2 by itself did not alter the basal contractility, while blocking CRF2 enhanced the response by CRF. These results may be explained by the following (schematic illustrations are shown in Fig. 1).

In the basal condition, both types of CRF signaling are not activated (Fig. 1a). CRF activates both CRF1 and CRF2, and CRF has a much higher affinity for CRF1 [21-23]. CRF induces strong activation of CRF1 signaling prevailing over the inhibition by CRF2 signaling, leading to stimulation of colonic contractility (Fig. 1b). CRF1 agonist stimulates colonic contractility without modulation of CRF2 signaling (Fig. 1c). The CRF2 agonist or antagonist by itself does not change colonic contractility because of a lack of activation of CRF1 signaling (Fig. 1d and e). Meanwhile, CRF2 antagonist induces disinhibition of CRF1 signaling, and enhances the stimulatory action of colonic contractility by CRF (Fig. 1f). The signaling balance of CRF1 and CRF2 may determine the state of colonic contractions (Fig. 1g). Moreover, this hypothesis was also tested in in vitro study using colonic muscle strips. CRF evoked the contractions of strips and Ucn2 abolished this response [58].

We also showed the results regarding visceral sensation which was consistent with the hypothesis in that paper. Namely, CRD induced visceral sensitization which was blocked by ip astressin. Ip cortagine enhanced but Ucn2 abolished the response. Meanwhile, ip CRF did not alter CRD-induced sensitization, but ip CRF together with CRF2 blocking further enhanced the response by CRD. These results may be explained by the balance theory as follows.

CRD may activate peripheral CRF1 and induce CRF1-dependent visceral sensitization. Then CRF1 agonist further enhances and CRF2 agonist reduces the response induced by CRD. When exogenous CRF is administered in this condition, both signaling are activated simultaneously and increases the signal intensity in addition to the one induced by CRD. Although CRF has higher affinity for CRF1, activating CRF2 by ip CRF may be enough to suppress the intensity of CRF1 signaling in modulation of visceral sensation, resulting that an overall response by exogenous CRF is not remarkable. Therefore CRF2 blocking with ip CRF further enhances the sensitization by disinhibition of CRF1 signaling.

The balance theory could explain well CRF and stress-induced altered colonic functions as described above, and moreover, we also suggested that peripheral CRF-induced altered gastric contractility may follow the same rule [123]. In this context, CRF-induced altered upper and lower GI functions might be explained by the theory.

The balance may be determined by the injected or released peptides during stress such as CRF and Ucns, and expression profile of CRF1 and

CRF2 may also contribute to the signaling balance. CRF1 and CRF2 receptors are expressed in colon, and stress such as open field or CRD alters these receptor expression [124], suggesting the dominant signaling may depend on the mode of stress.

The mechanisms of interaction between CRF1 and CRF2 signaling

How does the CRF2 signaling modulate the CRF1 signaling? Several studies showed the following evidence.

Liu et al. [125] demonstrated in myenteric plexus of guinea pig colon that CRF1 was mainly expressed in ganglion cell somas and CRF2 was expressed in varicose nerve fibers. CRF1 and CRF2 evoked depolarization of different types of myenteric neurons. In addition, they also suggested immunohistochmically that CRF2 might be expressed at pre-synaptic transmitter release sites. Therefore it is possible to think that CRF2 might regulate a neurotransmitter release, thereby modulating the neuronal activity induced by CRF1.

Gourcerol et al. showed that CRF1 and CRF2 were colocalized in the colonic myenteric plexus and CRF2 was expressed with neuronal nitric oxide in rats. On the basis of these results, they speculated the possibility that CRF2 inhibits CRF1 signaling through the release of inhibitory neurotransmitter such as nitric oxide [62].

These above findings may be possible mechanisms of the CRF1 and CRF2 interaction in modulating colonic motility. Meanwhile, there are also the results suggesting the mechanisms in modulating visceral sensation.

CRF2 is proved to be expressed in DRG, and CRD induces activation of splanchnic afferents in in vitro experiment using colorectal preparation with the attached mesenteric artery and splanchnic afferent nerve, which is blunted by intra-arterial injection of Ucn2 [114]. In this context, CRF may modulate visceral sensation through CRF receptors on spinal afferents, and the interaction of CRF1 and CRF2 might occur in this level.

As described before, EC cells and mast cells are targets of CRF. Both cells have CRF1 and CRF2 [65-67] and the mediators released from these targets can modulate the visceral sensation. Therefore, CRF1 and CRF2 interaction may also occur at these cells, possibly in cellular level. Gourcerol et al. speculated that CRF2 activation may share intracellular signaling targets of CRF1, leading to inhibition of CRF1 signaling [62].

The rationale of our proposed theory was only suggested by the studies regarding peripheral CRF receptors-induced altered GI functions. It would be possible that the actions induced by central CRF or ones other than GI response, such as endocrine, immune, autonomic, behavioral response, etc. are also explained by the balance theory. Further studies are needed.

CRF signaling as a therapeutic target for IBS

IBS patients have exaggerated responsivity of the gut, neuroendocrine and the brain to stress [6, 18, 126, 127]. Stress induces onset and/or exaggeration of GI symptoms in the majority of IBS patients [128, 129]. In addition, as described above so far, altered colonic motility and visceral sensation induced by CRF-CRF1 signaling are thought to play a key role in

463 the pathophysiology of IBS.

464 Exaggerated stress response in IBS patients may be explained by the
465 abnormal expression of CRF receptors and their function. In animal studies,
466 differential alterations of the receptors expression in colon are observed
467 between Sprague Dawley and Wister Kyoto (WKY) rats, which may explain
468 the high stress susceptibility of WKY rats [124]. WKY rats are stress-
469 sensitive strain, which spontaneously exhibit a high anxiety phenotype and
470 altered stress responses [130], and display visceral hypersensitivity [131] and
471 increased stress-related defecation [132]. Recently, it was also demonstrated
472 that genetic polymorphisms and haplotypes of CRF1 are associate with IBS
473 and related bowel patterns [133]. Single-nucleotide polymorphisms in the
474 regulatory region of the CRF1 gene might influence the expression of CRF1
475 [134] and generation of CRF1 variants with distinct structural and signaling
476 properties [25, 135].

477 In any event, altered stress response in IBS may be due to increased
478 CRF-CRF1 signaling. In other words, CRF signaling balance might be
479 abnormally shifted toward CRF1 in IBS, particularly diarrhea-predominant
480 type, according to our balance theory (Fig 2a). In this context, blocking CRF1
481 signaling is thought to be effective in treating IBS (Fig. 2b).

482 Contrary to expectation, clinical trials in IBS-diarrhea predominant
483 female patients did not show any significant beneficial effect of CRF1
484 antagonist, pexacerfont (BMS-562086) in IBS symptoms [136]. However, this
485 result does not deny the usefulness of CRF1 antagonist itself. Tested dose of
486 the compound might not be optimal for the treatment. Additionally, IBS

patients may be heterogeneous population. Even in diarrhea-predominant IBS, colonic accelerated transit is not consistent feature [29]. CRF1 antagonist might be effective only in the subpopulation of IBS patients, having exaggerated response to CRF-CRF1 signaling. Further studies with different protocol are needed to examine the effectiveness.

Our proposed theory also suggests that in addition to CRF1 antagonist, CRF2 agonist may be a promising tool in treating IBS by resetting the abnormally shifted signaling balance to normal state (Fig. 2c). CRF2 in brain induces anxiolysis, while anxiety-related behavior is mediated through CRF1 [137]. Thus CRF2 agonist might be also beneficial for the comorbid psychological abnormality of IBS patients. Since stimulation of CRF2 reduces gastric emptying in rats [138], it might induce dyspeptic symptoms. Therefore CRF2 agonist with high organ selectivity, i.e. only targeted for colon and brain might be needed for clinical application.

The pathogenesis of IBS is thought to be multifactorial. We only mentioned colonic motility and visceral sensation, but also altered intestinal barrier [139], microbiota [140], low grade inflammation [141], abnormal pain processing in brain [142], etc. are known to contribute to the pathophysiology. Recent studies show that these factors are also able to be modulated by CRF signaling [72, 143-145]. These observations may further support the rationale of application of CRF receptors-related drugs for the treatment.

Conclusions

Altered colonic motility and visceral sensation are thought to

contribute to generation of IBS symptoms and CRF-CRF1 signaling plays a pivotal role in the pathophysiology of IBS through modulating these functions. In addition, CRF2 signaling is also demonstrated to modulate CRF and stress-induced altered colonic functions, and it has a counter regulatory action against CRF1. We proposed a balance theory of CRF1 and CRF2 signaling, i.e. both CRF receptors would be activated during stress simultaneously, and the signaling balance may determine the functional changes in GI tract. This theory is useful for understanding the pathophysiology of IBS and may also provide the novel therapeutic options targeting altered signaling balance of CRF1 and CRF2 in IBS.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1.

Schematic illustration of our theory on the mechanism of peripheral CRF-induced stimulation of colonic contractions. In the basal condition, both CRF1 and CRF2 signaling are not activated (a). CRF activates both CRF1 and CRF2 with higher affinity for CRF1. Thus CRF induces strong activation of CRF1 signaling prevailing over the inhibition by CRF2 signaling, resulting in enhanced colonic contractility (b). CRF1 agonist stimulates colonic contractility without interference of CRF2 signaling (c). The CRF2 agonist or antagonist does not change colonic contractility because of a lack of the activated CRF1 signaling (d and e). Meanwhile, CRF2 antagonist induces disinhibition of CRF1 signaling, and enhances the stimulatory action of colonic contractility by CRF (f). The signaling balance of CRF1 and CRF2 may determine the state of colonic contractions (g).

Figure 2.

The signaling balance of CRF1 and CRF2 might be abnormally shifted toward CRF1 by endogenously released CRF receptor ligands, i.e. CRF and Ucns, leading to enhanced colonic motility and visceral sensitization in IBS (a). According to the balance theory, both CRF1 antagonist and CRF2 agonist may be useful in treating IBS. CRF1 antagonist inhibits CRF1 signaling resulting in normalizing the signaling balance (b). CRF2 agonist increases the signal intensity of CRF2 in addition to the one induced by endogenous CRF2 ligands,

thereby resetting signaling balance to normal state (c).

a

Colonic contractions

CRF_1 CRF_2

Basal state

b

CRF_1 CRF_2

CRF

c

CRF_1 CRF_2

CRF_1 agonist

d

CRF_1 CRF_2

CRF_2 agonist

e

CRF_1 CRF_2

CRF_2 antagonist

f

CRF_1 CRF_2

CRF_2 antagonist
+
CRF

g

Basal state

Enhanced
Colonic contractions

CRF_1 CRF_2

a

Normal state

Enhanced
Colonic functions

CRF_1

CRF_2

b

CRF_1

CRF_2

c

CRF_1

CRF_2

Endogenous ligands

CRF1 antagonist

CRF2 agonist