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Brain and Gut CRF Signaling: Biological Actions and Role in the Gastrointestinal Tract

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Abstract

Background: Corticotropin-releasing factor (CRF) pathways coordinate behavioral, endocrine, autonomic and visceral responses to stress. Convergent anatomical, molecular, pharmacological and functional experimental evidence supports a key role of brain CRF receptor (CRF-R) signaling in stress-related alterations of gastrointestinal functions. These include the inhibition of gastric acid secretion and gastric-small intestinal transit, stimulation of colonic enteric nervous system and secretory-motor function, increase intestinal permeability, and visceral hypersensitivity. Brain sites of CRF actions to alter gut motility encompass the paraventricular nucleus of the hypothalamus, locus coeruleus complex and the dorsal motor nucleus while those modulating visceral pain are localized in the hippocampus and central amygdala. Brain CRF actions are mediated through the autonomic nervous system (decreased gastric vagal and increased sacral parasympathetic and sympathetic activities). The activation of brain CRF-R2 subtype inhibits gastric motor function while CRF-R1 stimulates colonic secreto-motor function and induces visceral hypersensitivity. CRF signaling is also located within the gut where CRF-R1 activates colonic myenteric neurons, mucosal cells secreting serotonin, mucus, prostaglandin E2, induces mast cell degranulation, enhances mucosal permeability and propulsive motor functions and induces visceral hyperalgesia in animals and humans. CRF-R1 antagonists prevent CRF- and stress-related gut alterations in rodents while not influencing basal state.

Discussion: These preclinical studies contrast with the limited clinical positive outcome of CRF-R1 antagonists to alleviate stress-sensitive functional bowel diseases such as irritable bowel syndrome.

Conclusion: The translational potential of CRF-R1 antagonists in gut diseases will require additional studies directed to novel anti-CRF therapies and the neurobiology of brain-gut interactions under chronic stress.

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CONFLICT OF INTEREST

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Keywords

CRF peptides; CRF receptor antagonists; irritable bowel syndrome; gut secreto-motor function; stress; visceral pain

1. INTRODUCTION

R. Guillemin and A. Schally demonstrated independently the existence of a corticotropin-releasing factor (CRF) stimulating adrenocorticotropic hormone (ACTH) release from the pituitary in the mid nineteen fifties [1]. After decades of intensive research, Wylie Vale (Guillemin's former PhD's student) [2] and his colleagues made a series of milestone discoveries. They identified the structure of CRF as a 41 amino acid peptide [3], the related peptides, urocortins (Ucns) including, Ucn 1 [4], Ucn 2 [5] or stresscopin-related peptide [6], and Ucn 3 [7] or stresscopin [6]. In addition, they cloned the first cognate G-protein coupled receptors, CRF receptor subtype 1 (CRF-R1) [8] and almost simultaneously with Lovenberg *et al.* [9], the CRF receptor subtype 2 (CRF-R2) [10]. Both CRF receptors are products derived from distinct genes [10] and subject to extensive alternative RNA splicing [11–13]. Radioligand binding studies revealed that CRF is a preferential CRF-R1 agonist with low affinity to CRF-R2, whereas Ucn 1 displays high affinity to both receptor subtypes and Ucn 2 and Ucn 3 are selective ligands for CRF-R2 [14].

The release of CRF from the paraventricular nucleus of the hypothalamus (PVN) into the median eminence plays a primary role in mediating the pituitary ACTH secretion induced by various stressors. This was established by the use of CRF antibody [15], various peptide CRF-R1 and/or CRF-R2 antagonists developed by J. Rivier [16], selective non peptide CRF-R1 antagonists [17] (Table 1), and genetically modified mice [18]. However, reports that CRF ligands and receptors are also expressed outside the hypothalamus [19–21], provided neuroanatomical support for their involvement beyond the endocrine component of the stress response. Early on, compelling reports demonstrated that CRF acutely injected into the lateral brain ventricles of rodents induces behavioral, autonomic and visceral changes mimicking the effects of stress [22–26]. Moreover, CRF ligands and receptors are also expressed in viscera, prominently in the heart and the gut in experimental animals and human subjects [27–31] where they exert biological actions [31–34]. A vast pre-clinical literature also indicates that the dysregulation of CRF/CRF-R1 signaling system contributes to the etiology or the exacerbation of several stress-sensitive diseases such as anxiety/depression, relapse to addiction, and functional bowel diseases [35, 36–39].

This review will focus on the biological actions of CRF/Ucns systems in the brain and the periphery, namely within the gut, to influence gastrointestinal secreto-motor functions and the role of CRF receptor subtypes in stress-related alterations of gut functions and visceral pain. We will also address whether inhibition of the CRF-R1 signaling system will hold promises to curtail stress-sensitive functional bowel disorders namely the irritable bowel syndrome (IBS).

2. BRAIN CRF AND UROCORTINS: ACTIONS TO INFLUENCE GASTRIC FUNCTION

2.1. Brain CRF Peptides Inhibit Gastric Acid Secretion

Two years after the CRF identification, the central action of the peptide to influence gastric acid secretion in rats was the first report indicating that CRF can alter visceral function by acting in the brain [40]. Thereafter, several reports established that CRF or the amphibian CRF-related peptide, sauvagine, injected into the cerebrospinal fluid (CSF) either into the lateral brain ventricle (intracerebroventricular, ICV) or the brainstem at the level of the cisterna magna (intracisternally, IC) induces a dose-related, rapid in onset, and sustained inhibition of gastric acid secretion [40–45]. The inhibitory effect occurred under conditions of stimulated gastric acid secretion induced by either vagal activation (central injection of thyrotropin releasing hormone, TRH, 2-deoxy-D-glucose, pylorus ligation or gastric distention) or peripheral hormone (pentagastrin) while not influencing the acid secretory response to histamine in rats [40–45]. Similarly in conscious dogs, the injection of CRF into the 3rd ventricle inhibits 2-deoxy-D-glucose-, a peptone meal- and pentagastrin- but not histamine-stimulated acid secretion [46, 47] suggesting a conserved mechanism among species. CRF's inhibitory action is mediated through interaction with brain CRF receptor and not related to leakage of the peptide into the periphery [40] where CRF or sauvagine can also inhibit acid secretion [48]. Further studies showed that Ucn 1 injected ICV reduces gastric acid secretion in rats, an effect that is blocked by ICV astressin$_2$-B, a CRF-R2 antagonist, but not NBI-27914, a CRF-R1 antagonist [49]. This indicates a primary role of brain CRF-R2 in mediating the gastric acid inhibitory effect of Ucn 1.

Responsive sites of CRF action are located in the hypothalamus, namely the PVN, ventromedial (VMH) and lateral (LH) hypothalamic nuclei where peptide microinjection suppresses gastric acid secretion in conscious pylorus-ligated rats [40, 44, 50–51]. By contrast, CRF microinjected into the caudate putamen or dorsomedial frontal cortex, under the same conditions, did not influence acid secretion, showing selectivity to specific hypothalamic nuclei [40, 50, 51]. In the hindbrain, other responsive sites include the locus coeruleus/subcoeruleus (LC/SC) where CRF suppresses the acid response to intravenous (IV) infusion of pentagastrin in rats while CRF microinjected into sites within the vicinity but outside the LC/SC had no effect [52].

The autonomic nervous system (ANS) mediates the inhibitory effect of CRF from the brain to the stomach. In rats, central CRF inhibits gastric vagal efferent discharges [53] and stimulates sympathetic outflow as shown by the rise in circulating levels of epinephrine and norepinephrine in rats and dogs [46, 54]. Ganglionic blockade with chlorisondamine, spinal cord transection at the fifth cervical level, noradrenergic blockade with bretylium, α_2-adrenergic blockade with yohimbine and acute adrenalectomy, abolish the inhibitory effect of IC, ICV or LC/SC injections of CRF on gastric acid secretion supporting a primary role of the sympathetic nervous system in rats [40, 41, 52]. It is to note that vagal-dependent mechanisms also contribute to the inhibitory effect of IC but not ICV or LC/SC injections of CRF [40, 41, 52]. This is consistent with the direct access of CRF injected IC to influence neurons in the dorsal motor nucleus of the vagus, which regulate gastric function through

efferent axonal projections from their cell bodies to the gastric enteric nervous system [55]. These data combined with the unaltered gastric acid inhibitory response to central injection of CRF and sauvagine by hypophysectomy and peripheral injection of naloxone [40–41] further ascertain that the central action of CRF is mediated by the ANS and unrelated to the activation of the hypothalamic-pituitary-adrenal (HPA) hormones in rats. In dogs, peripheral mediators involve arginine vasopressin as shown by the increased plasma levels of peptide by ICV CRF and the dampening of ICV CRF inhibitory effect by the peripheral injection of a vasopressin receptor antagonist [47, 56]. Although gastrin is a hormone well established to regulate gastric acid secretion, the inhibition of gastric acid secretion induced by central CRF does not involve gastrin in rats and dogs [40, 51].

Early clinical and experimental observations demonstrated that emotional stimuli inhibit gastric acid secretion. Beaumont noticed when his fistulous subject experienced “fear, anger or whatever depresses or disturbs the nervous system”, that gastric secretion was inhibited [57]. Cannon showed that the flight-or-fight response resulted in decreased gastric acid secretion in cats [58]. The involvement of CRF receptor in the brain in acute stress-related inhibition of gastric acid secretion has been demonstrated in rats. The CRF-R1/CRF-R2 antagonist, α-helical $CRF_{9–41}$ injected IC or ICV prevents brain surgery-and partial restraint-induced reduction of gastric acid secretion while the antagonist injected peripherally has no effect [54, 59]. Moreover, the activation of central CRF signaling pathways is part of the brain mechanisms through which specific anorexic brain peptides inhibit gastric acid secretion. In particular, CSF injection of cocaine- and amphetamine-regulated transcript (CART) or neuromedin U-induced inhibition of gastric acid secretion in pylorus-ligated rats is completely blocked by the pretreatment into the CSF with α-helical $CRF_{9–41}$ or anti-CRF immunoglobulin G while the inhibitory effect of gastrin-releasing peptide, and interleukin-1ß are not altered [54, 60, 61]. Lastly, the activation of cell bodies into the hypothalamic arcuate nucleus (ARC) by microinjection of kainic acid inhibits pentagastrin-stimulated acid secretion, a response that the peptide CRF-R1/R2 antagonist, astressin microinjected into the PVN prevented [62]. These data indicate that the activation of CRF receptors by endogenous CRF in the PVN reduces gastric acid secretion consistent with the effect of exogenous injection of the peptide at this site [50]. Of importance is the lack of change in basal gastric secretion in rats injected with CRF receptor antagonists into the CSF. This indicates that brain CRF signaling is not involved in the basal regulation of gastric acid secretion [54, 59].

2.2. Brain CRF Stimulates Gastric Bicarbonate Secretion

The first evidence that the CRF in the brain can influence gastric bicarbonate secretion came from observations that the peptide microinjected into hypothalamic nuclei (PVN, LH and VMH) increases the volume of gastric secretion while inhibiting total acid output and acid concentration in conscious rats [50]. Such a response was established to be peptide- and site-specific. Bombesin, calcitonin gene-related peptide, or calcitonin microinjected into these hypothalamic sites have no effect, and CRF and sauvagine injected IC inhibits both gastric secretory volume and acid concentration in rats [40, 43]. Subsequent reports established that CRF microinjected into the VMH stimulated gastric bicarbonate concentration and output and potassium secretion in conscious pylorus-ligated rats [51].

2.3. Brain CRF Peptides Inhibit Gastric Transit and Motility

The central action of CRF to suppress gastric motor function is well established in various experimental conditions. All mammalian CRF ligands (CRF, Ucn 1, Ucn 2, and Ucn 3) and amphibian/fish CRF-related peptides (sauvagine and urotensin I) injected into the CSF at the level of the lateral or 4^{th} ventricle, cisterna magna or L5-L6 spinal cord segments, induce a rapid in onset and long-lasting inhibition of gastric transit of non-caloric or caloric liquid or solid meal in several non-primate mammal species [63–74].

CRF and Ucn 1 alter gastric contractility pattern and the propagation of motor events in various species including rats, dogs and sheep [70, 75–80]. In fed rats, CRF injected ICV induces a long-lasting inhibition of postprandial antral motility [81]. Other studies in fasted rats showed that CRF or Ucn 1 injected IC suppresses vagally stimulated high amplitude gastric contractions evoked by IC TRH agonist or 2-deoxy-D-glucose [82, 83]. In fasted dogs, ICV CRF induces an immediate and sustained disruption of the interdigestive pattern of gastric motility with abolition of the cyclic activity front in the antrum and the occurrence of irregular contractions of small amplitude [84, 85]. Several lines of evidence established that the activation of brain CRF-R2 signaling mediates CRF and Ucns action to impair gastric propagative motor function. The selective CRF-R2 ligand, Ucn 2 injected into the brain ventricle mimicked CRF inhibitory action. In addition, the ICV or IC injection of non-selective peptide, CRF-R1/CRF-R2 antagonists, α-helical $CRF_{9–41}$, D-$Phe^{12}CRF_{12–41}$, astressin, or astressin-B and the selective CRF-R2, $astressin_2$-B, blocked ICV or IC injection of CRF-, Ucn 1- and Ucn 2-induced delayed gastric emptying and motility while administration of selective CRF-R1 antagonists, NBI-35965 or NBI-27914 have no effect in rats, mice or dogs [49, 64, 66, 68–71, 73, 77, 81, 86–91].

The PVN and dorsal vagal complex (DVC) are brain responsive sites to microinjection of CRF to inhibit basal gastric emptying or vagally-stimulated gastric contractility while delivering the peptide into the LH, other medial hypothalamic sites, central amygdala (CeA) or LC/SC, had no effect [80, 83, 92–95]. These sites of action express CRF-R2 mRNA [20] and regulate gastric motor function through parasympathetic pathways [55]. In particular, the dorsal and ventral caps of the parvocellular division of the PVN contain neurons that send direct projections to the DVC and spinal cord intermedio-lateral column [96]. Consistent with these neuroanatomical connections, the majority of studies using combined pharmacologic, surgical or electrophysiological approaches to assess the pathways involved in CRF inhibitory action have established the primary involvement of the vagus. Subdiaphragmatic vagotomy prevents ICV or IC CRF and Ucn 1-induced delayed gastric emptying in mice, rats and dogs [64–66, 70, 73, 81, 85, 92, 95, 97] and the alterations of gastric motility in rats or dogs [70, 81, 85, 95]. Electrophysiological recording of efferent activity in the gastric branch of the vagus also shows that CRF, and more prominently sauvagine, injected IC decreases vagal efferent outflow in anesthetizes rats [53]. However, a few reports also indicate the role of the sympathetic nervous system in mediating ICV or IC CRF-induced delayed emptying and the inhibition of postprandial antral and pyloric contractions [65, 72, 78]. In our studies, only IC Ucn 2-induced delayed gastric emptying is mediated by sympathetic pathway and peripheral alpha-adrenergic receptors while the inhibitory action of IC CRF and Ucn 1 is vagal-dependent in rats [73]. CRF injected into the

PVN or CSF increases sympathetic outflow to the viscera [54, 98]. It may be speculated that the discrepancies in neuronal pathways are related to differences in peptides (CRF or urocortins) and doses used along with brains sites of injection that may differentially influence sympathetic activity and thereby the modulation of peripheral cholinergic transmission to the stomach.

The end effectors through which alterations of autonomic outflow suppress gastric motor function may involve gut peptides. A few data suggest the contribution of peripheral somatostatin in ICV CRF-induced delayed gastric emptying in rats [99] but not in dogs [76]. The suppression of the circulating prokinetic hormones, motilin in dogs and acyl ghrelin in rats in response to ICV CRF may contribute in the inhibition of gastric propulsive motor function [49, 76]. However, this needs to be ascertained using peripheral injection of motilin and ghrelin antagonists. Other studies clearly established that the effects of centrally injected CRF are not secondary to the stimulation of pituitary-adrenal hormones as shown by the unaltered delayed emptying induced by ICV or IC CRF in hypophysectomized or acute adrenalectomized rodents [64–66, 97].

Support for a role of brain CRF receptor activation in stress-induced inhibition of gastric propulsive motor function comes from consistent reports using pharmacological CRF receptor blockade. The IC, ICV or PVN injection of CRF-R1/CRF-R2 antagonists, α-helical $CRF_{9–41}$, D-Phe_{12} $CRF_{12–41}$, astressin, or astressin-B, abrogate the gastric stasis induced by various stressors [25]. These encompass psychological/physical (swim stress, wrap restraint), visceral (trephination, abdominal surgery, peritoneal irritation induced by intraperitoneal, IP injection of 0.6% acetic acid), immunological (IV or ICV injection of interleukin-1ß), or chemical (ether) [54, 63, 69, 87, 90, 100–102]. All these stressors, including abdominal surgery and immune challenge activate CRF neurons and upregulate CRF mRNA in the PVN [21, 90, 103–110]. However, the respective involvement of CRF and/or Ucns to activate CRF receptors leading to stress-related inhibition of gastric motor function is still largely unexplored and may vary with the type of stressors. A recent study points to a primary role of hypothalamic Ucn 3 in the inhibition of antral contractions in the stomach induced by chronic psychological stress of a communication box paradigm whereby rats receive visual, auditory and olfactory stimuli from rats subjected to foot shock for 1-h/day for 5 days. Under these conditions, there is an upregulation of Ucn 3 gene expression in the hypothalamus, unlike that of Ucn 1 or Ucn 2, and the ICV injection of Ucn 3 antiserum prevented the inhibition of antral motility (although the selectivity of the antiserum to Ucn 3 *vs.* Ucn 1 or 2 was not mentioned) [79].

Investigations of brain CRF receptor subtype(s) involved in various stressors-induced inhibition of gastric motor function point to the implication of either CRF-R1 or CRF-R2, depending upon their interoceptive or exteroceptive nature. For instance, the gastric ileus occurring immediately post-surgical stress (abdominal surgery and cecal palpation), is mediated by CRF-R1 as shown by the use of genetically modified CRF-R1 or CRF-R2 knockout mice or ICV injection of CRF-R1 antagonist in rats [111] (unpublished observations). In contrast, acute restraint stress-induced gastric ileus involves CRF-R2 in rats [72]. Additional studies are needed to identify the CRF receptor subtypes involved in other stress models. In particular, it will be important to dissect whether the activation of

differential brain circuitries by physical *vs.* psychological stressors [112] recruits different CRF ligands and receptor subtypes within these pathways and thereby their primary role.

Of interest, is also the evidence that CRF receptors in the brain serve as downstream mechanisms for specific brain peptides known to activate CRF neurons in the PVN. Those include CART, nociceptin/orphanin FQ (N/OFQ), glucagon-like peptide 1 (GLP-1) and des-acyl ghrelin that act in the brain to inhibit gastric motor function and appetite [113–116]. Studies have shown that the injection of α-helical $CRF_{9–41}$ into the 4th or lateral ventricle or astressin IC prevents respectively the delayed gastric emptying in rats induced by CART injected into the 4th ventricle, ICV N/OFQ and IC GLP-1 [113–115]. The ICV pretreatment with CRF-R1/CRF-R2 antagonist, astressin and the selective CRF-R2 antagonist, anti-sauvagine-30 abolishes ICV injection of des-acyl ghrelin-induced reduction of antral contractions in fasted rats while the CRF-R1 antagonist, NBI-27914 had no effect [116]. However, IC α-helical $CRF_{9–41}$ and ICV $astressin_2$-B do not alter IC adrenomedullin or ICV nesfatin-1-induced delayed gastric emptying in rats [117, 118] showing peptide specificity.

By contrast, CRF receptor antagonists injected into the CSF do alter neither basal gastric emptying of a liquid non-nutrient or solid nutrient meal nor motility in rats, mice or dogs in the large majority of studies [25, 64, 66, 70]. This indicates that brain CRF signaling is not involved in basal and postprandial regulation of gastric motor function.

3. CENTRAL ACTIONS OF CRF PEPTIDES TO INFLUENCE SMALL INTESTINAL SECRETORY MOTOR FUNCTION

3.1. Central CRF Stimulates Duodenal Bicarbonate Secretion

When injected ICV, CRF is one of the most potent peptides to stimulate duodenal bicarbonate secretion in rats compared with other known stimulants while a number of peptides (ICV bombesin, urotensin, calcitonin, vasoactive intestinal peptide, gastrin, growth hormone-releasing factor, gonadotropin-releasing hormone and β-endorphin) and norepinephrine tested under the same conditions in conscious or anesthetized rats are inactive [119–121]. The ICV injection of α-helical $CRF_{9–41}$ abolishes both ICV CRF and partial restraint stress-induced three-fold increase in duodenal bicarbonate secretion in rats [120, 122]. These data suggest that the activation of brain CRF receptors during stress promotes duodenal secretory function and may enhance the resistance of the duodenal mucosa similar to what reported in the gastric mucosa [51].

Peripheral mechanisms through which central CRF stimulates duodenal bicarbonate secretion are not mediated by the ANS as shown by the unaltered response by chlorisondamine, vagotomy, blockade of sympathetic nervous system with bretylium and adrenalectomy in rats [120]. Evidence indicates the involvement of the pituitary axis releasing β-endorphin, as hypophysectomy and naloxone prevent the duodenal bicarbonate response. In addition, β-endorphin, unlike ACTH, injected IV at doses reproducing the circulating levels induced by ICV CRF, stimulates duodenal bicarbonate secretion [120]. Lastly, ICV or IV injection of CRF shows similar potency to stimulate duodenal bicarbonate

secretion consistent with the activation of the HPA axis by CRF injected centrally or peripherally [120].

3.2. Central CRF Stimulates Ileal Water Absorption

CRF injected ICV stimulates water, chloride and sodium absorption in the ileum of conscious rats [122]. The enhanced ileal water transport is not altered by hypophysectomy, adrenalectomy, vagotomy and naloxone, is blocked by chlorisondamine, bretylium and phentolamine, and mimicked by peripheral injection of norepinephrine [122]. This is consistent with the known action of brain CRF to stimulate the sympathetic noradrenergic outflow to the gut [123]. So far, only one study indicates that brain CRF receptors are involved in restraint-induced increased ileal water absorption in rats as shown by the use of ICV α-helical $CRF_{9–41}$ before acute restraint stress [122].

3.3. Central CRF Inhibits Small Intestinal Motor Function

The influence of brain CRF on small intestine has been less investigated compared to the proximal (stomach) and distal (colon) segments of the gut. However, existing literature indicates that ICV injection of CRF also inhibits small intestinal transit in rats [54, 65–66]. Further studies to investigate the underlying alterations of small intestinal motility indicate that CRF injected into the brain alters the pattern of contractions that varies in function of the fed or fasted state and species. Under fed conditions, CRF injected ICV after a nutrient meal increases the frequency of proximal duodenal contractions in dogs, while not altering their amplitude and durations [70]. In fed rats, ICV Ucn 1 increases the amplitude of pressure waves in the duodenum [81], while in fed sheep, ICV CRF induces a long-lasting inhibition of duodenal motor activity [75]. In fasted dogs, ICV CRF inhibits the occurrence of migrating motor complex in the duodenum and proximal jejunum [84, 85]. Likewise, in rats the ICV injection of Ucn 1 acts through CRF-R2 to disrupt the fasted pattern of duodenal motor activity and replaces it with a fed pattern resulting in non-propagative irregular spike burst activity monitored in the duodenum and jejunum [81, 124].

In rats and dogs, peripheral mechanisms of actions of ICV CRF and Ucn 1 to inhibit small intestinal transit and disrupt fasted motility involve vagal cholinergic pathway and are independent from the activation of the HPA axis [65, 72, 81, 84, 124]. Collectively, these studies indicate that central CRF induces a vagally mediated suppression of small intestinal transit associated with alterations of coordinated propagative motor activity.

Studies on the implication of CRF receptors in stress-related inhibition of intestinal transit have been limited and yield discrepant results with ICV α-helical $CRF_{9–41}$ having no effect or reversing acute partial restraint stress-induced slowing of small intestinal transit [54, 63]. Collectively, existing evidence so far is scant to ascertain a role of brain CRF signaling in the alterations of small intestinal motor function by stress.

4. CENTRAL ACTIONS OF CRF PEPTIDES TO INFLUENCE COLONIC FUNCTION

4.1. Activation of Brain CRF-R1 Stimulates Colonic Secreto-Motor Function

A vast literature shows that, opposite to the upper gut, the ICV or IC injection of CRF or Ucn 1 evokes a rapid onset increased motility in the cecum, triggers spike burst activity and pressure wave in the proximal and distal colon, accelerates whole and distal colonic transit, decreases colonic fluid absorption, and induces defecation and diarrhea in freely moving rats, mice or gerbils [63, 65, 71, 87, 93, 125–131]. By contrast, ICV injection of the selective CRF-R2 agonist, Ucn 3 has no effect in mice [71]. The colonic changes induced by ICV CRF or Ucn 1 are blocked by the ICV injection of CRF-R1/CRF-R2 antagonists, α-helical $CRF_{9–41}$, D-$Phe^{12}CRF_{12–41}$, and astressin or by selective CRF-R1 antagonists, NBI-35965, NBI-27914, NGD 98–2, and NGD 9002. In contrast, selective CRF-R2 antagonists, $astressin_2$-B or anti-sauvagine, injected ICV are inefficient to block the central effects of CRF or Ucn 1 in rodents [65, 71, 87, 91, 130–133]. Other studies also showed increased release of mucin, prostaglandin E_2 (PGE_2) and mast cell protease II from the colonic mucosal explants of rats 30 min after ICV injection of CRF, mimicking the effects of immobilization stress [133]. These convergent findings establish that the activation of brain CRF-R1 signaling by CRF or Ucn 1 in the brain stimulates colonic secretory motor function in experimental animals.

4.2. Brain Sites of Action and Peripheral Mechanisms

The PVN and pontine areas, namely the LC/Barrington nucleus complex in rats have been identified as brain nuclei responsive to CRF to induce increased tonic and phasic colonic motility, stimulation of colonic transit and induction of watery fecal output [92, 93, 134–136]. By contrast, microinjection of CRF into the LH and other medial hypothalamic sites, the CeA, and pontine sites adjacent but outside of the boundaries of the LC/SC, have no effect on colonic transit or fecal pellet output [92, 93, 134–136]. Of significance is the demonstration that these hypothalamic and pontine sites are also brain nuclei involved in central CRF-induced CRF-R1 mediated anxiety and depression [137, 138] providing anatomical and biochemical substrata for the early use of defecation in rodents as an index of emotionality [139, 140].

CRF-R1 is the main receptor subtype mediating the pituitary release of ACTH and downstream glucocorticoids [15]. However, peripheral mechanisms involved in the stimulation of colonic secreto-motor function in response to central injection of CRF are not secondary to the activation of the HPA axis and implicate the ANS [65]. Tracing studies identified a proportion of CRF immunoreactive neurons in the Barrington’s nucleus and PVN transynaptically linked to the colon [141, 142]. This provides neuroanatomical support for CRF signaling in the PVN and pontine sites to influence colonic motor functions through the activation of sacral parasympathetic cholinergic outflow. In addition, peripheral ganglionic blockade using chlorisondamine and atropine prevents, and subdiaphragmatic vagotomy reduces, the stimulation of colonic transit, phasic and tonic contractions, and defecation induced by CRF injected IC-, ICV- or into the PVN, while the noradrenergic blockade by bretylium has no effect [65, 92, 135, 143].

Within the colon, effector mechanisms recruited by central CRF relate to the release of serotonin (5-HT) interacting with serotonin receptor type 3 (5-HT_3) and type 4 (5-HT_4). There is evidence that IC injection of CRF, like restraint stress, increases 5-HT content in the feces harvested in the proximal colon [144]. In addition, convergent pharmacological studies show that several 5-HT_3 antagonists such as granisetron, ramosetron, ondansetron and azasetron, or the 5-HT_4 antagonist, SB-204070, given orally or into the lumen of the proximal colon, block the stimulation of colonic transit and defecation induced by ICV or IC injection of CRF [127, 131, 144, 145]. Additional mechanisms of ICV CRF-induced defecation may involve the activation of colonic myenteric cholinergic and substance Pergic neurons interacting with neurokinin 1 receptor located on colonic smooth muscles [146].

4.3. Role of Brain CRF-R1 Signaling

CRF-R1 signaling in the brain plays a role in stress-related stimulation of colonic propulsive motor function while not being involved in basal or postprandial regulation of colonic secreto-motor function under non-stress conditions [39, 147]. First, the ICV injection CRF and the high affinity CRF-R1 ligand, Ucn 1 [14] recapitulate the colonic secreto-motor responses to stressors while that of selective CRF-R2 agonists, Ucn 2 and Ucn 3 either have no effect on basal colonic transit or inhibit stimulated colonic contractions in rats [63, 65, 87, 127, 129, 130, 143, 148–150]. However the ICV, IC or PVN injection of α-helical $CRF_{9–41}$, D-Phe_{12} $CRF_{12–41}$, or astressin prevents the reduction of colonic transit time or defecation evoked by acute restraint, water avoidance stress and the increased frequency of colonic spike-bursts induced by conditioned fear in rats [54, 63, 87, 91, 126, 127, 129, 135, 142, 143, 151]. Lastly, several brain penetrant non-peptide CRF-R1 antagonists such as antalarmin, CP-154,526, CRA1000, JTC-017, NBI-27914, NBI-35965, NGD 98–2 and NGD 9002 [17, 130, 152] injected either centrally or peripherally (subcutaneous, IP and for some compounds, orally) dampen various acute stressors-(restraint, water avoidance stress, elevated plus maze, social intruder, morphine withdrawal) or 5 days exposure to heterotypic stressors-induced stimulation of defecation, colonic transit, motility and luminal 5-HT release in rodents [63, 71, 87, 91, 130–132, 153–157]. Likewise, in primates, antalarmin attenuates the defecation occurring in animals exposed to a social intruder [23]. By contrast, the selective CRF-R2 antagonist, $astressin_2$-B injected centrally at doses that block CRF-R2-mediated inhibition of gastric emptying, does not alter restraint stress-related defecation in mice [71]. In addition, CRF-R1 deficient mice display significantly lower defecation in an open field test than their wild-type littermates [158]. Conversely, CRF-overexpressing mice show enhanced defecation to a novel environment [159]. Microinjection of CRF receptor antagonist or CRF oligonucleotide antisense before water avoidance stress into specific brain nuclei point to the PVN and LC/SC nuclei as main sites of CRF/CRF-R1 pathway to stimulate colonic motor function induced by stress while CRF receptors in the CeA are not involved [92, 126, 160–162]. Further supporting the key role of these brain nuclei, ICV injection of CRF-R1/CRF-R2 antagonist, α-helical $CRF_{9–41}$ before exposure to water avoidance stress curtails the activation of neurons selectively in the PVN and LC neurons in correlation with the decrease in fecal output [126]. In addition, studies used strains of rats with different susceptibility to stress, namely the stress resilient Lewis which has a defective CRF synthesis/release compared to Fischer rats [163]. The Lewis and Fischer rats showed respectively low and high activation of neurons in the PVN and intermediolateral columns of

the lumbosacral segment of the spinal cord correlated with the magnitude of defecation [142].

A number of brain peptides influencing food intake and activating CRF neurons in the PVN, such as neuropeptide Y, GLP-1, CART, and ghrelin, also stimulate colonic motor function through activation of brain CRF receptor [164–168]. Other studies showed that CRF receptor located in the PVN mediated the activation of neurons in the ARC-induced stimulation of colonic motor function [144]. Therefore, CRF/CRF-R1 signaling in the PVN induced by various stressors and specific brain peptides is an important convergent signaling pathway that leads to the stimulation of colonic secretory motor function.

5. CENTRAL ACTIONS OF CRF PEPTIDES TO INFLUENCE VISCERAL SENSITIVITY

Recurrent abdominal pain in the absence of detectable organic diseases is the hallmark of irritable bowel syndrome (IBS) [169]. Clinical evidence indicates that IBS is a stress-sensitive disease as various stressors of psychosocial, physical or immune origin and early adverse life events can contribute to the development and/or exacerbation of IBS symptoms [170–172]. Several stress-related animal models developed to reproduce some common features of IBS [173, 174] provided valuable tools to examine the implication of CRF signaling in stress-related visceral hypersensitivity.

5.1. Role of Brain CRF-R1 in Stress-Related Visceral Hypersensitivity

Earlier studies demonstrated that the ICV injection of CRF in rats enhances the pain response to colorectal distension (CRD) mimicking the effects of restraint stress [175]. CRF action is blocked by the ICV injection of α-helical $CRF_{9–41}$, or the CRF-R1 antagonist, antalarmin given intraperitonerally [175, 176] pointing to brain CRF-R1 activation in the induction of visceral hypersensitivity. The role of this signaling pathway was further established to mediate the hypersensitivity to CRD induced by various stressors in rodents using pharmacological or genetic approach [39]. The selective CRF-R1 antagonists (Table 1) including NBI-35965 [130], CP-154,526 [177–180], NBI-27914 [181], antalarmin [176, 182–184], JTC-017 [155], NBI-30775 [185], DMP696 [186], NGD 98–2 and NGD 9002 [132], inhibit the visceral hyperalgesia-induced by a variety of conditions such as early life adverse events, repeated water avoidance stress [130, 178, 186], chronic high anxiety in the Wistar Kyoto rats [176, 183], colonic inflammation [180], repeated nociceptive activation of colonic mechanoreceptors [132, 155, 176, 178, 182, 183] or post-natal intracolonic injection of acetic acid [181]. Likewise, CRF-R1 knockout mice show a reduction of visceral response to phasic CRD compared to wild type littermate [185]. Collectively these pre-clinical studies support a pivotal role of brain CRF-R1 signaling in the visceral hyperalgesia resulting from different stress context: early life adverse events, repeated psychological stress in adulthood, chronic high anxiety as well as peripherally initiated mechanisms associated with colonic inflammatory event or repeated CRD at nociceptive range.

5.2. Brain Sites of Action

The CeA, bed nucleus stria terminalis, LC and hippocampus are brain sites through which the activation of CRF/CRF-R1 signaling results in visceral hypersensitivity [39, 155, 162, 187, 188]. A few reports showed that α-helical $CRF_{9–41}$ microinjected directly into the hippocampus decreases the frequency of abdominal contractions evoked by tonic nociceptive CRD [155]. Other study showed that the CRF-R1 antagonist, CP-376,395 microinjected into the anterolateral bed nucleus stria terminalis reduces the hypersensitivity to CRD induced by repeated water avoidance stress [188]. Additionally, several convergent studies support a role CRF/CRF-R1 in the CeA [162,187]. CRF microinjected into the CeA increased sensitivity to CRD in Wistar rats that was blocked by the intra-CeA microinjection of CP-154,526 [189]. Moreover, the CRF-R1 antagonist, CP-376,395 microinjected into the CeA reduced visceral hyperalgesia to CRD in a high anxiety strain of rats [187]. Neuroanatomical studies further support the role of CRF-R1 signaling in the CeA to modulate visceral pain. CRF is densely expressed in cell bodies of CeA that send axonal projections to basal forebrain and pontine/brainstem including the LC [190, 191]. There is also evidence of upregulation of CRF and CRF-R1 gene expression at these brain sites in rat models of anxiety associated with altered visceral pain [192]. Likewise, CRF gene and peptide expression are upregulated in the CeA by CRD, repeated exposure to water avoidance and other conditions inducing visceral pain or hyperalgesia in rats [193–199].

With regard to the influence of brain CRF-R2 activation on visceral pain, still little is known and further studies are required to establish its role. One report indicates that the CRF-R2 antagonist, $astressin_2$-B microinjected into the anterolateral bed nucleus stria terminalis reduced visceral pain induced at low CRD pressure in rats but had no effect at higher CRD pressure or even enhanced visceral pain in rats submitted to water avoidance stress [188].

5.3. Central and Peripheral Mechanisms of Action

Several possible mechanisms are involved in the prevention of stress- or mechanosensitization-related visceral hyperalgesia induced by CRF-R1 antagonist pretreatment. Previous studies on somatic pain provided molecular, electrophysiological and biochemical evidence that endogenous CRF/CRF-R1 signaling within the CeA participates to the mechanisms of pain sensitization [196, 200, 201]. The laterocapsular division of the CeA known as the “nociceptive amygdala” is the recipient of nociceptive-specific inputs originating from the parabrachial area as part of the spinal-parabrachial-amyloidal pathway [202]. In the CeA, CRF/CRF-R1 facilitates synaptic transmission of pain by switching on silent N-methyl-D-aspartate receptors through protein kinase A-dependent signaling [201, 203–205]. Further studies in genetically modified mice with deletion of CRF-R1 selectively on glutamatergic CeA neurons clearly ascertained that CRF facilitates the excitatory transmission by a CRF-R1-mediated direct action on glutamatergic neurons [205]. Whether similar cellular mechanisms in the CeA are involved in CRF/CRF-R1 mediated visceral hyperalgesia need to be explored. Additional components may involve the inhibition of noradrenergic output from the LC [189, 206–208], the largest of all noradrenergic nuclei [138]. Electrophysiological studies showed that CRF-R1/CRF-R2 antagonist, D-$Phe^{12}CRF_{12–41}$ or astressin injected ICV or directly into the LC and the selective CRF-R1 antagonist, NBI-35965, injected peripherally, blocked the activation of LC neurons

responsive to central injection of CRF and CRD in rats [206–208]. Additionally, the CRF-R1 antagonist, JTC-017 or CP-154,526 reduced CRD-induced noradrenaline release in the hippocampus and CeA, which are sites of rostral efferent projections from the LC [155, 189, 209]. The cortical and limbic rostral noradrenergic efferent projections from the LC are known to induce arousal and anxiogenic responses along with hypervigilance to visceral input that is a common feature in IBS patients [172, 210, 211]. It is to note that the brain sites (CeA, hippocampus, LC, hypothalamus) involved in CRF-R1-induced visceral hypersensitivity are also those implicated in anxiety disorders [212] and may have relevance in the co-morbidity of IBS with anxiety disorder [172, 213].

Among peripheral mechanisms, there is evidence of an important role of intestinal mucosal mast cells. Rats exhibiting visceral hypersensitivity after exposure to crowding stress display an increased number of mast cells in the colon mucosa [214, 215]. The ICV injection of CRF or restraint stress activates colonic mast cells monitored by the increase in colonic release of mast cell protease II, PGE_2 and histamine [133, 216]. Additionally, the mast cell stabilizer doxantrazole blocked the visceral hyperalgesia to CRD induced by ICV CRF or restraint [175] and ICV injection of α-helical $CRF_{9–41}$, abolished acute restraint stress–induced increase of colonic histamine content [216].

6. PERIPHERAL CRF SIGNALING: INFLUENCE ON COLONIC SECRETORY MOTOR FUNCTION

Early on, there was an enormous stride made in the biology of the CRF system to assess its actions and role in the brain due to the widely held consensus that the stress response is coordinated exclusively within the central nervous system [102]. The past few years, the CRF signaling systems within the gut are now emerging as important components of the stress response [27, 33, 217]. *In vitro* as well as whole animal and human studies indicate that the peripheral CRF signaling systems can modulate gut motility, secretion and barrier functions as well as visceral pain [33, 217]. Moreover, CRF-R1 and CRF-R2 and their ligands are localized throughout the gastrointestinal tract in various cell types including neuronal (the enteric nervous system), endocrine (enterochromaffin cells) and immune (mast cells, eosinophils, T-helper lymphocytes in the lamina propria) in experimental animals and human subjects [28, 218–225]. This provides support for a local role of the CRF systems within the gut as established in other viscera such as the cardiovascular system [226]. In addition, stressors modulate the expression of CRF peptides and CRF receptors within the gastrointestinal tract [28, 220, 227, 228].

6.1. Activation of Peripheral CRF-R1 Stimulates Secreto-Motor Function

Peripheral injections (IP, IV or subcutaneous) of CRF and Ucn 1 are equipotent to central injections to stimulate whole and distal colonic transit, defecation and to induce prominent diarrhea in rats, mice or dogs [63, 65, 127, 130, 133, 154, 222, 229–237]. These functional alterations are associated with an increase of clustered spike-burst propagative activity in the cecum, proximal and distal colon in rodents [154, 233, 235]. In addition, CRF injected IV induces the release of mucin, PGE_2 and mast cell protease II in colonic mucosal explants in rats indicative of the stimulation of goblet and mast cells [133].

Peripheral administration of CRF- or Ucn 1-induced the activation of CRF-R1 signaling mediates the acceleration of colonic transit and induction of watery diarrhea as shown by the use of selective CRF agonists and receptor antagonists. The selective CRF-R1 peptide agonists, cortagine, or stressin$_1$ [238, 239] injected IP stimulate colonic contractions distal colonic transit, defecation with a peak response within 30 min post injection, and a rapid onset diarrhea in rodents [154, 219, 222, 229, 231, 235, 238, 240]. Contrasting, the IP or IV injection of Ucn 2 or Ucn 3 has no effect on basal distal colonic transit, colonic contractions and defecation [222, 229, 235, 241]. In addition, astressin or selective CRF-R1 antagonists, NBI-35965, NBI-27914, CP-154,526 given peripherally (IP, SC or orally) block IP CRF, Ucn 1-or cortagine-induced accelerated colonic contractions and distal transit, defecation and diarrhea while the CRF-R2 antagonist given IP has not effect in rats and mice [130, 154, 229, 231, 232, 235, 240].

Peripheral *vs*. central injections of CRF-induced CRF-R1-mediated stimulation of colonic motor function involve distinct target sites of action. First, the CRF receptor antagonists given centrally (IC astressin or ICV α-helical CRF$_{9–41}$) do not alter IP or IV CRF-induced stimulation of fecal pellet output and colonic transit in rats [54,154]. Second, IP CRF-induced acceleration of colonic transit is not abolished by ganglion blockade or truncal vagotomy that contrasts with the role of the ANS in centrally injected CRF-induced stimulation of colonic propulsive motor function [65,134]. Further support for direct action within the colon comes from *in vitro* studies in isolated colonic rat preparations or isolated rat colonic muscle strips where CRF and Ucn 1 increase basal myoelectrical peristaltic activity, phasic contractions and electric field stimulation off-contractions while Ucn 2 has no effect on basal activity [154, 221, 235, 242].

Convergent evidence substantiates that the stimulatory action of peripherally injected CRF, Ucn 1 or cortagine involves the activation of cholinergic and nitrergic neurons in colonic myenteric ganglia promoting peristaltic through interaction with CRF-R1 in rats or guinea pigs [143, 219, 221, 222, 240, 243, 244]. The use of laser-capture microdissection combined with reverse transcriptase polymerase chain reaction or immunohistochemical detection in myenteric whole-mount preparation of the colon reveals that CRF-R1 are primarily expressed at the gene and protein levels on myenteric neurons compared with other layers of the rat or guinea colon [219, 221–222, 243]. In addition, the use of a neuronal blocker, tetrodotoxin, abolishes Ucn 1-evoked phasic contractions in rat colonic smooth muscle strips further supporting the role of enteric nervous system [221]. Other evidence shows that IP CRF or IP cortagine in rats or partial restraint in mice induces Fos expression in CRF-R1 expressing cholinergic and nitrergic myenteric neurons in the colon while Ucn 2 under the same conditions has no effect [219, 222, 240, 245, 246]. Of note, atropine, a muscarinic blocker, does not influence IP CRF-induced neuronal activation in colonic myenteric ganglia, indicating that the Fos response is not secondary to the activation of muscarinic receptors either on the myenteric ganglia (which possess both nicotinic and muscarinic receptors) or on colonic muscles [245]. Electrophysiological recording demonstrated that the administration of CRF or Ucn 1 onto colonic myenteric and submucosal plexus preparations of guinea pig directly excites myenteric and submucosal neurons through CRF-R1 [243, 244, 247].

The colonic CRF/CRF-R1 signaling may have relevance as local effector of the acute stress response. There is evidence that stressors that stimulate colonic secreto-motor function upregulate CRF and CRF-R1 in the colonic mucosa and submucosa plus muscle layers [28, 227]. Pharmacological studies show that peripherally restricted peptide CRF antagonists, α-helical $CRF_{9–41}$, astressin or astressin-B injected IP or IV abolish or dampen the stimulation of propulsive colonic motor function (distal colonic transit, high amplitude high frequency contractions and defecation) and the colonic release of mucin, PGE_2 and mast cell protease II evoked by acute exposure to restraint, water avoidance stress and novel environment in rats or mice [63, 127, 130, 133, 154, 234, 248].

6.2. Peripheral CRF-R2 Inhibits CRF-R1 Stimulatory Action

The inhibitory effect of CRF-R2 was suspected initially by the demonstration that IP CRF (CRF-R1>CRF-R2 agonist) was more potent to stimulate defecation in rats than IP Ucn 1 which has not only high affinity to CRF-R1 but also higher affinity than CRF on CRF-R2 [154]. Subsequent studies showed that the IP injection of the selective CRF-R2 agonist, Ucn 2 induces a CRF-R2-mediated inhibition of colonic contractility and defecation stimulated by IP injection of CRF or Ucn 1 (Ucn 1), restraint stress in rats or CRF overexpression in genetically-modified mice [154, 159, 222, 235]. Likewise, *in vitro* studies on colonic muscle strips showed that Ucn 2 blocks the amplitude of contractions elevated by CRF [235]. Conversely, rats injected IP with $astressin_2$-B or CRF-R2 knockout mice showed enhanced colonic motility, defecation and diarrhea responses to IP CRF- and Ucn 1- or restraint [159, 222, 235]. These results are consistent with CRF and Ucn 1 activating both CRF receptor subtypes [14], and under conditions of CRF-R2 blockade, only the CRF-R1-mediated stimulatory effect is maintained without the dampening action of CRF-R2. These findings open new insight into the dual mechanisms through which acute stress influences colonic function, engaging not only the colonic CRF-R1-mediated enteric stimulatory pathway, but also CRF-R2 to dampen the response suggestive of an adaptive counter-regulatory role of CRF-R2 in the colonic response to stress [222, 235]. However, in the absence of stress, peripheral CRF-R2 agonists or antagonists do not influence basal colonic motor function [222, 235]. The opposite effects of CRF-R1 and CRF-R2 are also observed at the level of intestinal mucosal barrier function altered by stress. Pigs exposed to early-weaning stress display intestinal barrier dysfunction and hypersecretion that involve CRF-R1 activation whereas activation of peripheral CRF-R2 prevents the intestinal barrier dysfunction induced by early life stress [249].

One target of Ucn 2 inhibitory action involved the colonic myenteric neurons where both CRF-R1 and CRF-R2 are localized [222, 228]. The IP injection of Ucn 2 reduces the neuronal activation evoked by IP CRF and the phosphorylation of extracellular signal regulated kinase (ERK) ½ [222]. The localization of CRF-R2 on nitrergic inhibitory myenteric neurons may also play a role [222].

7. PERIPHERAL ACTIONS OF CRF PEPTIDES TO INFLUENCE VISCERAL SENSITIVITY IN EXPERIMENTAL ANIMALS

7.1. Peripheral CRF-R1 Induces Visceral Hyperalgesia

Recent reports suggest that the activation of CRF/CRF-R1 in the colon is involved in the development of visceral hyperalgesia. Peripheral administration of CRF or CRF-R1 peptide selective agonist, cortagine, induces visceral hypersensitivity to CRD in naïve rats and mice [235, 240, 250] that is abolished by IP, but not ICV, injection of the CRF-R/CRF-R2 antagonist, astressin [235, 240]. The role of endogenous peripheral CRF-R1 signaling is further supported by the demonstration that IP pretreatment with peripherally restricted CRF antagonists, astressin, or α-helical $CRF_{9–41}$ prevents visceral hyperalgesia induced by repeated water avoidance stress, tonic CRD, 4% acetic acid or maternal separation in rats [179, 235, 250, 251]. In addition, chronic psychosocial stress-induced visceral hyperalgesia in rats up-regulates CRF-R1 gene expression in the colon while not influencing CRF-R2 [215]. Collectively these data support the participation of a peripheral CRF-R1 component in the development of visceral hyperalgesia in rats.

Several mechanisms contribute the visceral hypersensitivity induced by the activation of CRF-R1 in the colon [215, 248]. Alteration of intestinal permeability appears to be a prerequisite for the development of visceral hypersensitivity and nociceptor sensitization in rodents [252, 253]. Consistent evidence showed that peripheral CRF-R1 activation is involved in various stressors-induced mast cell dependent disruption of the intestinal epithelial barrier function [217]. Namely acute or chronic exposure to water avoidance stress, early life adverse event, or sustained overcrowding increased the paracellular and transcellular permeability in the intestine which is mimicked by peripheral injection of CRF *in vivo* and *in vitro* [26, 217, 248, 254–257]. The alterations of intestinal permeability evoked by various stressors or IP CRF do not occur under conditions of pretreatment with peripheral injection of peptide CRF receptor antagonists [26, 240, 248, 254, 256–259] and selective CRF-R1 antagonists [26]. The beneficial effect of peptide CRF receptor antagonist is associated with increases in the protein expression of the tight junction protein, occludin, in the distal colon [251]. Additional insights into mechanisms come from the strong link between peripheral CRF receptor activation and colonic mucosal mast cell degranulation. This is supported by the localization of CRF receptors on mucosal mast cells [248, 260], the degranulation of mast cells by peripheral injection of CRF and the blockade of acute stressors-evoked mucosal mast cell degranulation by peripheral injection of peptide CRF receptor antagonists in rats and pigs [26, 133, 216, 217, 248, 251, 253, 254, 261]. The content of mast cells can in turn lead to the release of several preformed (histamine, 5-HT, protease) or newly synthesized compounds (PGE_2, cytokines, nerve growth factor, tumor necrosis factor-α) [261] known to influence permeability and also to activate or sensitize sensory afferents [262, 263]. Lastly CRF-R1 are localized on enterochromaffin cells and CRF or Ucn 1 through CRF-R1 activation stimulates 5-HT release [11] which is known to active spinal afferents [264].

7.2. Peripheral CRF-R2 Dampens Visceral Hyperalgesia

Opposite to the visceral hyperalgesia to CRD induced by peripheral injection of selective or preferential CRF-R1 agonist, sauvagine and of the selective CRF-R2 agonist, Ucn 2, inhibit visceral sensitization induced by repeated CRD [182, 235, 241]. Conversely, IP injection of CRF-R2 antagonist, astressin$_2$-B, enhances the visceral response to CRD induced by IP CRF [235]. Ucn 2 inhibitory effect is associated with the reduction of ERK 1/2 activation in the L6/S1 segment of the spinal cord in rats [241]. In addition, *in vitro* studies showed that Ucn 2 reduces CRD-induced increase in the activity of inferior splanchnic afferent fibers [241] indicating the modulation of the nociceptive visceral input by Ucn 2. However, additional mechanisms may be involved. CRF-R2 is expressed on colonic enterochromaffin and mucosal mast cells, and myenteric cell bodies and fibers [27, 222, 265–267]. Ucns-CRF-R2 activation can modulate transmitter release from entechromaffin cells, mast cells and myenteric neurons stimulated by CRF-R1.

8. CRF PEPTIDES INFLUENCE ON GUT FUNCTION AND VISCERAL SENSITIVITY IN HUMAN SUBJECTS

8.1. Biological Actions of CRF in Healthy Subjects

Similar to rodent reports, CRF/Ucns and CRF receptors are localized in human colonic enteric nervous system, mast cells, macrophages and enterochromaffin cells [29, 30, 267–272]. Functional studies also demonstrated that peripherally injected CRF in healthy human subjects largely reproduces the biological actions observed in experimental animals, namely the increased colonic motility, intestinal permeability, mast cell activation and visceral hypersensitivity [273, 274].

In healthy human volunteers, CRF applied intranasally, a route established to maximize the delivery of peptides or drugs into the brain [275, 276] results in a rise in gastric pH associated with a change in mood while circulating cortisol levels are not altered indicating the recruitment of CRF central pathways to inhibit acid secretion [277]. Other functional studies showed that systemic injection of CRF increases the motility index in the descending colon in both healthy volunteers and more so in IBS patients [274]. Recent investigations also indicate that systemic injection of CRF constricts the small intestine, increases volume in the ascending colon, and induces a sense of distention and bloating in healthy volunteers [278]. There are also convergent reports that systemic bolus injection of CRF lowers pain threshold to repetitive rectal distensions and enhances the intensity of discomfort sensation in healthy human subjects [274, 279, 280]. Also consistent with preclinical data, systemic injection of CRF increases intestinal permeability in a mast cell-dependent fashion as shown by the blockade of CRF action by the mast cell stabilizer disodium cromoglycate [273]. *In vitro* studies in sigmoid colon biopsies of healthy subjects further established that CRF increases transcellular permeability and uptake of protein antigens through direct activation of subepithelial mast cells where CRF receptors are located [267]. The human enterochromaffin-like cells, the BON subclone 1 cells express CRF-R1, and CRF, unlike Ucn 2, stimulates 5-HT release and up-regulates the expression of tryptophan hydroxylase, the 5-HT synthesizing enzyme, through interaction with CRF-R1 [11]. In view of the prominent role of 5-HT in the pathophysiology of IBS [281], this may represent an

important site of action of CRF to increase colonic motility and visceral pain in human subjects in addition of mast cell activation and permeability changes.

8.2. Therapeutic future of CRF Receptor Antagonists in Stress-sensitive Functional Diseases

Irritable bowel syndrome is a functional bowel disease that involves alterations in the bidirectional communication between the brain and the gut and is commonly associated with psychosocial and psychiatric comorbidity [170, 282]. Both experimental and clinical studies in healthy subjects highlight that the activation of central/peripheral CRF-R1 recapitulates important features occurring in IBS patients (anxiogenic/hypervigilance behavior, colonic mast cell activation and serotonin release, stimulation of colonic propulsive motor function, increased intestinal permeability and mucus secretion, watery diarrhea and visceral hypersensitivity) [36]. Compelling preclinical studies also established the pivotal actions of CRF-R1 antagonists in the brain and/or the gut to abolish or reduce the functional gut alterations induced by exogenous administration of CRF /Ucn 1 and endogenously released by stress [39]. These lines of evidence supported the conceptual framework that sustained activation of the CRF-R1 system at central and/or peripheral sites may be part of underlying mechanisms triggering IBS symptoms [36, 39]. Therefore, it raised high expectation regarding the potential translational applications of CRF-R1 antagonists for treating IBS [283–285].

A large number of small molecules CRF-R1 antagonists have been developed and tested in a preclinical setting [17], however, only a few compounds so far have progressed to clinical trials [286]. This setback may be related to the formation of reactive metabolites, suboptimal pharmacokinetics with prominent tissue accumulation due to lipophilicity, long elimination half-life, or high affinity to protein binding [17, 286, 287]. Efforts are still ongoing to overcome these issues as shown by recent disclosure of new compounds [132, 288, 289]. Among those, NBI-30545, NBI-35965, NGD 98–2 and NGD 9002 have the distinct advantage to be orally active and water-soluble [17, 132, 290, 291].

Several double-blind, placebo-controlled clinical studies provided preliminary proof-of-concept that oral administration of CRF-R1 antagonists dampened stress-related biological responses in healthy human subjects. For instance, in a clinical model of generalized anxiety evoked by inhaling 7.5% CO_2 for 20 min, the CRF-R1 antagonist R317573 (40 mg once daily) given for 7 days significantly dampened panic symptom score and generalized anxiety compared to placebo [292]. Positron emission tomography studies indicate that the acute administration of R317573 at 30 and 200 mg results in dose-related changes in brain activity in regions (putamen, amygdala, orbital frontal cortex) behaviorally relevant to anxiety and mood regulation [293]. Of relevance, a recent functional magnetic resonance imaging study showed that oral administration of the CRF-R1 antagonist, GW876008 (single dose of 20 or 200 mg) reduced the amygdala activation in response to the anticipation of visceral pain in IBS female patients compared to placebo [294]. Consistent reports established that CRF/CRF-R1 signaling in the CeA is a key component of the neuronal circuitry contributing to anxiety-like behavior providing neuroanatomical and biochemical substrata on mechanisms that drive the existing reciprocal relationship between visceral pain and affective mood

[196]. It may be speculated that the overactivity of the CRF/CRF-R1 in the CeA underlies the comorbidity of subset of IBS patients who display hypersensitivity to CRD [295] and mood disorders [296].

A few studies using systemic injection of peripherally restricted, -helical $CRF_{9–41}$ showed also beneficial effects to reduce electrical rectal stimulation-induced higher colonic motility index, abdominal pain and anxiety in IBS patients compared with healthy subjects [297]. The peptide CRF-R1/CRF-R2 antagonist also normalizes the altered EEG activities in IBS patients under basal and in response to CRD [298].

However, so far these clinical data have not translated yet in therapeutic use of CRF-R1 antagonists. Whether the existing or newly developed CRF-R1 antagonists [17, 132] will progress to show therapeutic benefits in subsets of IBS patients is still to be established. A placebo-controlled, double-blind clinical trial in IBS-diarrhea predominant patients was performed using the CRF-R1 antagonist pexacerfont (BMS-562086). Results showed only a dose-related trend to reduce visceral pain [299]. The suboptimal treatment (changes in ACTH plasma levels under pexacerfont treatment were not assessed), lack of information on the central and peripheral CRF-R1 occupancy by CRF antagonist treatment in the absence of positron emission tomography ligands and potential role of CRF-R1 splice variants (some of which are functional) [11, 300] may be contributing factors. Importantly CRF-R1 antagonists may improve symptoms mainly under conditions of altered brain/gut CRF/CRF-R1 signaling that cannot be identified in the heterogeneous populations of IBS patients enrolled in clinical studies performed so far. Therefore, whether the existing or newly developed CRF-R1 antagonists will provide therapeutic benefits for stress-sensitive diseases including IBS [147] or cyclic vomiting syndrome [301] for a subset of patients is still a work in progress.

CONCLUDING REMARKS

CRF, urocortins and CRF receptors are expressed in both the brain and gut where they exert profound alterations of gastrointestinal functions (Figs. 1, 2). Increasing knowledge has been gained during the past decades to localize CRF sites of action in the brain and the gut and to characterize the CRF receptor subtypes involved in mediating CRF/urocortins actions (Figs. 1, 2 & Table 2). Additionally, changes in the ANS activity induced by brain CRF receptor activation have been delineated and shown to be largely responsible for the alterations of gut function in rodents (Figs. 1, 2). By contrast, the peripheral effects are conveyed by direct actions on enteric nervous system, immune and/or endocrine cells within the gut wall in experimental animals (Figs. 1, 2) and healthy human subjects. Consistent reports established that the activation of central or peripheral CRF signaling during stress plays a key role in stress-related alterations with CRF-R1 driving the stimulation of colonic secretory-motor function and the development of visceral hyperalgesia (Fig. 2 & Table 2). However, CRF-R1 antagonists have not yet hold the promise of therapeutic benefits in clinical setting of stress-sensitive functional bowel disease such as irritable bowel syndrome, in part because of lack of adequate knowledge on the biology of the more than 10 variants of CRF-R1 and brain CRF receptor occupancy of the antagonists used.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
ANS	Autonomic Nervous System
ARC	Arcuate
CART	Cocaine- and Amphetamine-Regulated Transcript
CeA	Central Amygdala
CRD	Colorectal Distension
CRF	Corticotropin Releasing Factor
CRF-R1	Corticotropin Releasing Factor Receptor Subtype 1
CRF-R2	Corticotropin Releasing Factor Receptor Subtype 2
CSF	Cerebrospinal Fluid
DVC	Dorsal Vagal Complex
ERK	Extracellular Signal Regulated Kinase
GLP-1	Glucagon-like Peptide
HPA	Hypothalamic-Pituitary Axis
5-HT	Serotonin
5-HT$_3$	Serotonin Receptor Type 3
5-HT$_4$	Serotonin Receptor Type 4
IBS	Irritable Bowel Disease
IC	Intracisternal(ly)
ICV	Intracerebroventricular (ly)
IgG	Immunoglobulin G
IP	Intraperitoneal
IV	Intravenous
LH	Lateral Hypothalamus

LC/SC	Locus Coeruleus/Subcoeruleus
NE	Norepinephrine
N/OFQ	Nociceptin/Orphanin FQ
PGE_2	Prostaglandin E_2
PVN	Paraventricular Nucleus of the Hypothalamus
TRH	Thyrotropin Releasing Hormone
Ucns	Urocortin 1, 2 and 3
VMH	Ventromedial Hypothalamus

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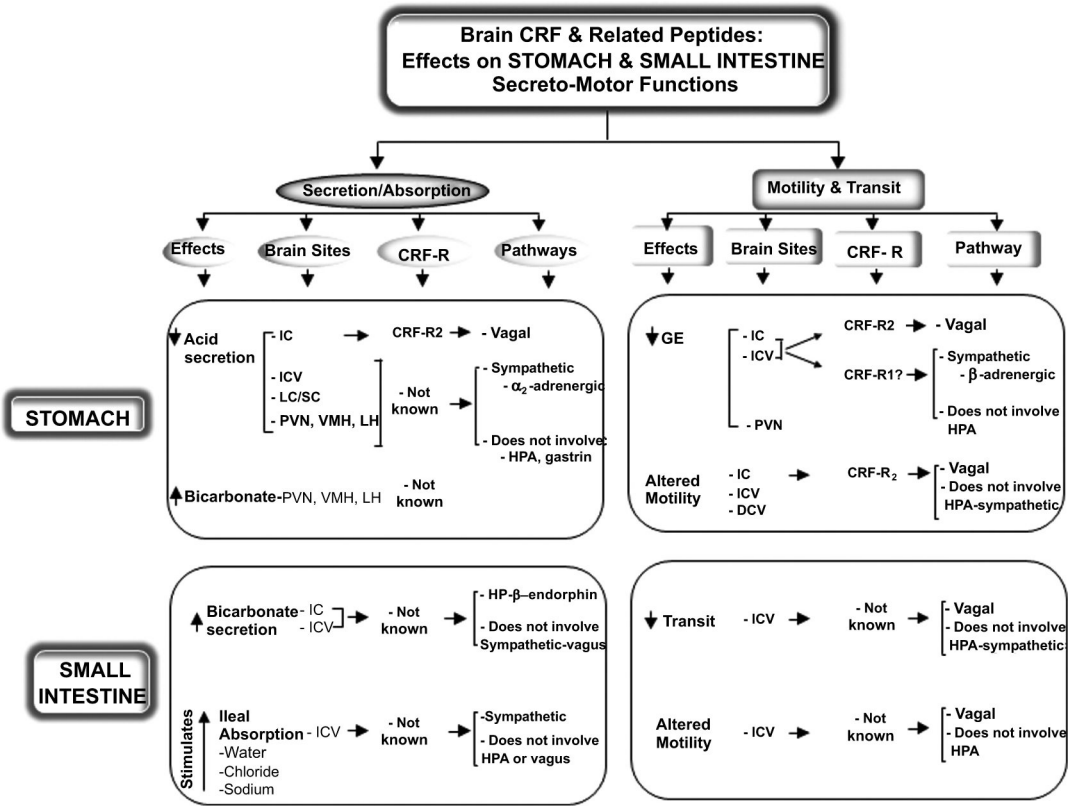

Fig. (1).

Summary of known influence of CRF and related peptides injected into the brain on gastric and small intestinal functions: CRF receptor mediating the effects, brain sites of action, and neural/hormonal pathways involved.

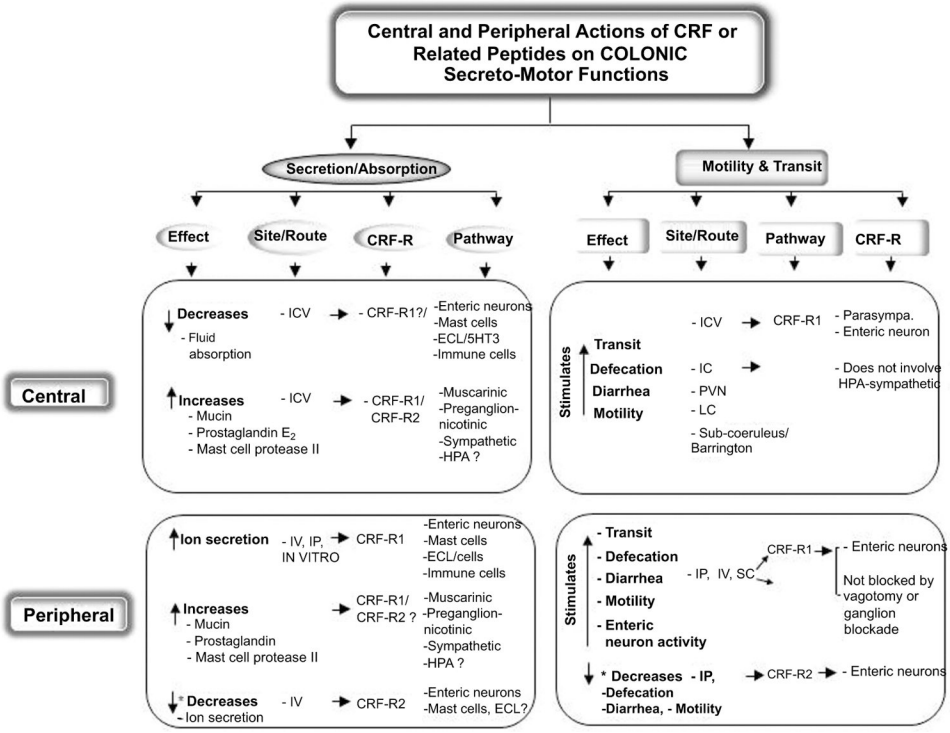

Fig. (2).

Summary of known influence of CRF and related peptides injected into the brain (central) or peripherally on colonic functions: CRF receptors mediating the effects, sites of action in the brain and the colon and the pathways involved.

Table 1.

List of non-selective CRF receptor antagonists (blocking both CRF-R1 and CRF-R2) and selective CRF-R1 or CRF-R2 antagonists.

CRF-R1 & CRF-R2 (peptide)	CRF-R1 (Non-Peptide)*	CRF-R2 (Peptide)
α-Helical $CRF_{9–41}$	CP-154, 526	Anti-sauvagine-30
D-$Phe^{12}CRF_{12–41}$	CP-376, 395	$Astressin_2$-B
Astressin	Antalarmin	
Astressin-B	NBI-30775	
	NBI-27914	
	NBI-35965	
	NBI-30545	
	R317573	
	GW876008	
	NGD 98–2	
	NGD 9002	
	CRA1000	
	DMP696	
	JTC-017	
	Pexacerfont (BMS-562086)	

*Cross the blood brain barrier.

Table 2.

Summary of known influence of CRF and related peptides injected into the brain (central) or peripherally on visceral pain of the gut: CRF receptors mediating the effects, sites of action in the brain and the colon and the pathways involved.

Species	Effect	Route/Site of Action	CRF Receptor	Pathways
Rodents	-	-	-	-
Brain CRF Urocortin 1	Visceral hyperalgesia	ICV LC CeA, BNST, Hippocampus	CRF-R1	↑ NE release in hippocampus and CeA following LC activation -Peripheral recruitment of mast cells?
Urocortin 2	↓ or ↑ visceral pain	BNST	CRF-R2	-
Peripheral CRF Urocortin 1	Visceral hyperalgesia	IP	CRF-R1	↑ Intestinal permeability Mast cells (histamine, proteases) Enterochromaffin cells (5-HT)
Urocortin 2	↓Visceral hyperalgesia	IP	CRF-R2	↓ ERK1/2 activation in L6-S1 ↓ Inferior splanchnic afferent fibers activity - Mast cell activity? - Enterochromaffin cells activation?
Human	-	-	-	-
Peripheral CRF	Visceral hyperalgesia	IV	-	↑ Intestinal permeability ↑ Mast cell activation