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# From Hans Selye's Discovery of Biological Stress to the Identification of Corticotropin Releasing Factor signaling pathways: Implication in Stress-Related Functional Bowel Diseases

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## Abstract

Selye's pioneer the concept of biological stress in 1936 culminating to the identification of the corticotropin releasing factor (CRF) signaling pathways by Vale's group in the last two decades. The characterization of the 41 amino-acid CRF and other peptide members of the mammalian CRF family, urocortin 1, urocortin 2 and urocortin 3, the cloning of $CRF_1$ and $CRF_2$ receptors, which display distinct affinity for CRF ligands, combined with the development of selective CRF receptor antagonists enable to unravel the importance of $CRF_1$ receptor in the stress-related endocrine (activation of pituitary-adrenal axis), behavioral (anxiety/depression, altered feeding), autonomic (activation of sympathetic nervous system) and immune responses. The activation of $CRF_1$ receptors is also part of key mechanisms through which various stressors impact the gut to stimulate colonic propulsive motor function and to induce hypersensitivity to colorectal distension as shown by the efficacy of the $CRF_1$ receptor antagonists in blunting these stress-related components. The importance of $CRF_1$ signaling pathways in the visceral response to stress in experimental animals provided new therapeutic approaches for treatment of functional bowel disorder such as irritable bowel syndrome, a multifactor functional disorder characterized by altered bowel habits and visceral pain for which stress has been implicated in the pathophysiology and is associated with anxiety-depression in subset of patients.

### Keywords

CRF; CRF receptor; CRF antagonists; colonic motor function; irritable bowel syndrome; stress

Hans Selye pioneered the concept of biological stress borrowing the word stress from the physic terminology that defines stress as the interaction between a deforming force and the resistance to it. His initial report in 1936 provided experimental evidence that the adrenal cortex, the immune system and the gut are commonly altered organs as shown by the hypertrophy of the adrenals, involution of the lymphatic nodes along with the occurrence gastric erosions in rats exposed to various nocuous chemical or physical stimuli.1 2 Subsequent contributions by Geoffrey Harris' in the 1950's established that stress-induced adrenocorticotropic hormone (ACTH) secretion involves "neural control via the hypothalamus and the hypophyseal portal vessels of the pituitary stalk".3 Biochemical support for this pathway came fifteen years later when Guillemin, a former Selye's Ph.D.

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student, and Schally's group independently demonstrated the existence of hypothalamic factor(s) that elicited ACTH release from the rat pituitary.[4,5] The name, corticotropin-releasing factor (CRF), was established in line with its ability to stimulate ACTH release and in keeping with the fact that its chemical structure was yet to be identified.[4,5] Interestingly, although CRF was one of the first hypothalamic releasing factor to be named, its biochemical identification lingered for three subsequent decades. However, intense research to isolate CRF in the 1970's led to the identification of other hypothalamic releasing factors composed of 3–10 amino acid (a.a.) including thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LH-RH) for which Guillemin and Schally obtained the Nobel Price in 1977.[6] However in 1981, Vale and his group contributed major milestones with the identification of the 41-a.a. peptide, CRF characterized from ovine hypothalami, and subsequently the cloning of CRF receptors and the development of specific CRF receptor antagonists (Fig. 1).[7–10]

A broad number of studies have documented the pathways through which CRF mediates the HPA axis limb of the stress response. The peptide is synthesized in a discrete population of hypophysiotrophic neurons in the parvocellular part of the paraventricular nucleus of the hypothalamus (pPVN) that also co-express arginine vasopressin (AVP). These peptides are released into the hypophyseal-portal blood vessels from axon terminals located in the *Zona externa* of the median eminence. Then CRF binds to specific CRF subtype 1 ($CRF_1$) receptor located on membranes of anterior pituitary corticotrope cells to induce ACTH secretion, while AVP interacts with V1b pituitary receptors to potentiate the ACTH release.[11,12]

In keeping with the insightful concept suggested by Selye in the 50's on the existence of a *"first mediator"* that integrates the adaptive bodily response to stress,[13] the biological actions of CRF expanded quickly far beyond the neuroendocrine component of stress response. Consistent experimental reports showed that CRF injected into the brain recapitulated the overall behavioral (anxiety/depression, alterations of feeding), autonomic (sympathetic and sacral parasympathetic activation), immune, metabolic and visceral responses induced by various systemic or cognitive stressors.[14–18] In particular, early observations in rats and dogs showed that exogenous administration of CRF into the brain or peripherally mimicked Walter Cannon's early experimental findings that stress inhibited gastric acid secretion and emptying.[19–22] In addition, blockade of CRF receptors by the injection of peptide CRF antagonist, α-helical $CRF_{9–41}$, prevented gastric inhibition of acid and emptying induced by exposing rats to restraint or abdominal surgery.[21,23] These data supported a physiological role of CRF signaling pathways in the alterations of gastric secretory and motor functions in rodents exposed to various stressors and paved the way to delineate the central and peripheral sites of CRF actions, the CRF receptor subtypes and autonomic effectors involved in mediating the alterations of gut function elicited by stress exposure.[16] In addition, these observations provided new venues for pharmacological interventions in stress-related functional bowel disorders.[24]

This review will address briefly the state-of-knowledge on CRF signaling system including the expanding members of mammalian CRF-related peptides, their pharmacological characterization on cloned $CRF_1$ and $CRF_2$ receptors and the development of selective CRF receptor subtype antagonists. We will integrate these advances to the understanding of stress-induced alterations of gut function particularly the stimulation of colonic propagative motility and the development of hyperalgesia in experimental animals. Lastly, emergent clinical evidence supporting therapeutic use of $CRF_1$ receptor antagonists to alleviate stress-responsive functional bowel diseases such as irritable bowel syndrome (IBS) often associated with anxiety and depression co-morbidity will be presented.

## CRF SIGNALING PATHWAYS

Seminal contributions to the identification of the CRF signaling pathways opened a new era of research which expended greatly the understanding of the biochemical coding of stress-related processes.25

### The Family of CRF Peptides

In mammals, CRF is a well-conserved 41-a.a. peptide with an identical primary structure in humans, primates, dogs, horses and rodents, while ovine CRF differs byseven a.a.26 In 1995, urocortin 1 (Ucn 1, also known as urocortin)27 was characterized from rat midbrain as a 40-a.a. peptide with 45% sequence identity with human/rat(r/h)CRF.28 Similarly to CRF, Ucn 1 structure is highly conserved across mammalian species since human shares 95% identity with rat, mice and sheep that are 100% homologous.28–30 Brain mapping studies revealed the existence of mismatches between the distribution CRF and Ucn 1 and that of CRF receptors in specific area.31 This triggered the search for additional endogenous CRF-related agonists resulting in the cloning of two novel putative CRF-related peptides named urocortin 2 (Ucn 2) and urocortin 3 (Ucn 3).27,32–34 The mouse Ucn 2 is a 38-a.a. peptide that displays 76% homology with the human Ucn 2 counterpart27,33,34 and more distant homology with r/hCRF (34%), and r/mUcn 1 (42%).33 Mouse urocortin 3 (mUcn 3) and human Ucn 3,32 also named human stresscopin, 34 are more distantly related to r/hCRF, and r/hUcn 1 with 18% and 21% homology respectively.32

### CRF Receptors

CRF ligands interact with $CRF_1$ and/or $CRF_2$ receptors. The receptors were cloned from two distinct genes that have 70% identity at the a.a. level.14 Both $CRF_1$ and $CRF_2$ belong to the B1 subfamily of seven-transmembrane domain receptors.35 Radioreceptor and functional assays have demonstrated that $CRF_1$ and $CRF_2$ receptors differ considerably in their binding characteristics.27,36 $CRF_1$ receptor displays high affinity to CRF and Ucn 1 but shows no appreciable binding affinity to Ucn 2 and Ucn 3. In contrast, $CRF_2$ binds to Ucn 1, Ucn 2 and Ucn 3 with greater affinity than CRF making this receptor subtype highly selective for Ucns signaling.27,28 Both $CRF_1$ and $CRF_2$ receptors exist in a number of splice variants.37,38 In rodents, $CRF_2$ receptor is expressed in two functional isoforms, α and β, that differ in their N-terminal domains.39 The $CRF_{2\alpha}$ and $CRF_{2\beta}$ variants have similar pharmacological profiles, but distinct central vs. peripheral distribution, respectively in rats.37,40,41 In mice brain, the presence of a soluble (s)$CRF_{2\alpha}$ splice variant that binds $CRF_1$ ligands and inhibits the cellular response to CRF or Ucn 1 indicates a possible functional relevance to modulate CRF/Ucn 1 actions.42 While ovine CRF and to a lesser extent r/hCRF are considered as preferential $CRF_1$ agonists, so far there is no selective endogenous $CRF_1$ agonist.43 To selectively activate $CRF_1$ receptor, $CRF_1$ peptide agonists, cortagine and $stressin_1$-A have been recently developed that display 100-fold greater affinity on $CRF_1$ versus $CRF_2$ receptors.44,45

The CRF ligand-CRF receptor interactions is primarily coupled to Gαs and adenyl cyclase activation, which leads to cAMP-dependent cascades including protein kinase A.36,37,46 Another sensitive and consistent intracellular signaling resulting from the activation of CRF receptors is the phosphorylation of extracellular–signal regulated kinases-1 and -2 (ERK1/2) that is cell type and ligand specific.38,47,48 Activation of ERK1/2 pathway is involved in memory, learning process, and stress related behaviors, especially in hippocampus, amygdala and cortex.49

### CRF Receptor Antagonists

Key to the assessment of the role of endogenous CRF ligands and CRF receptors in the stress response was the development of specific CRF antagonists. Earlier studies relied on the use of non-selective $CRF_1$/$CRF_2$ peptide antagonists, mainly α-helical $CRF_{9–41}$,9 D-Phe$^{12}CRF_{12–41}$,50 followed by the potent and long acting, astressin and astressin-B.51 Recently, selective peptide $CRF_2$ receptor antagonists, namely antisauvagine-30 and the more potent, long acting analog, astressin$_2$-B, were developed.10,52 As a whole, peptide antagonists generally have a poor penetrance into the brain. For instance, astressin injected intravenously (iv) at a dose blocking iv CRF-induced delayed gastric emptying, did not influence the inhibition of gastric transit induced by CRF-injected into the cerebrospinal fluid (CSF) at the level of the cisterna magna in rats.53 Due to the intense interest to target the $CRF_1$ system in the context of various human pathologies including anxiety disorders, 54,55 extensive pharmaceutical industry efforts resulted in the development of a variety of $CRF_1$ antagonists.56–58 These compounds are small hydrophobic orally active molecules that cross the blood-brain barrier. 57,58 Their availability has been instrumental in enabling a wide range of preclinical studies that established the role of brain CRF-$CRF_1$ signaling pathways in stress-related endocrine, anxiogenic behavior, autonomic and visceral responses.14,59,60 For instance the role of CRF-$CRF_1$ receptor activation in the HPA induced by stress was demonstrated using CRF antibody along with various $CRF_1$ receptor antagonists which inhibited the rise in circulating levels of ACTH and corticosterone in response to various psychological, physical and immune stressors while Ucn 1 antibody did not.11,61 With regard to the visceral response, growing evidence indicates that $CRF_1$ signaling pathways contribute also to altered colonic function and hyperalgesia induced by stress independently from the HPA activation.60

## CENTRAL CRF SIGNALING PATHWAYS: FUNCTIONAL ROLE IN STRESS-RELATED COLONIC STIMULATION AND VISCERAL HYPERALGESIA: PRECLINICAL EVIDENCE

### Central Injection of CRF induces a $CRF_1$ Receptor Mediated Stimulation of Colonic Motor Function

In rodents, several stressors as diverse as restraint, open field test, conditioned fear, loud sound, restraint, cold exposure, fear conditioning, water avoidance, inescapable foot or tail shocks, and central injection of interleukin-1 stimulate colonic motor function monitored by the shortening of colonic transit time, increased motility index and/or defecation.21,62–67 Primates exposed to a social stressor, manifested stress responses including urination and defecation.15 Likewise, in humans, various stressors, such as dichotomous listening, painful stimuli of intermittent hand immersion in cold water, fear, anxiety and stressful interviews, increased colonic motility in healthy subjects.68–72

CRF injected into the lateral brain ventricle (icv) mimicked stress-related colonic functional alterations as shown by the stimulation of colonic transit, defecation and at highest doses, the induction of diarrhea in experimental animals as reviewed recently. 24,73 Ucn 1 injected icv also stimulates colonic transit in mice.66 Consistent with an acceleration of propulsive colonic transit, icv CRF stimulates motor activity in the proximal and distal colon and induces the occurrence of colonic spike burst activity in rats.62,67,74–76 Pharmacological studies showed that the $CRF_1$ receptors is the subtype involved in CRF action. This was supported by the rank order of potency of icv ovine CRF >r/hCRF and Ucn 1>Ucn 2>Ucn 3 to induce defecation in mice consistent with a $CRF_1$ mediated effect.66 In addition, the icv injection of selective $CRF_1$ antagonists, NBI-35965 and NBI-27914 blocked icv CRF- and Ucn 1-induced acceleration of colonic transit, and increased in the colonic motility index

while icv injection of the selective $CRF_2$ agonist, $astressin_2$-B or anti-sauvagine had no effect in rats and mice.66,76,77

Brain nuclei responsive to CRF resulting in the stimulation of colonic motor function have been localized in specific hypothalamic (PVN) and pontine areas such as the noradrenergic, locus coeruleus (LC)/subLC and Barrington nucleus.78–81 These responsive sites are also those involved in CRF-induced anxiety and depression.82,83 Pharmacologic and surgical approaches established the pathway through which central CRF stimulates colonic motor function. It is not related to the concomitant stimulation of HPA but involves the activation of celiac vagal and sacral parasympathetic outflow to the pelvic organs.62,67,78,81,84 Effector mechanisms within the colon that activate colonic transit involve parasympathetic mediated activation of colonic serotonin (5-HT) acting on 5-$HT_3$ and 5-$HT_4$ receptors as shown by the blockade of colonic motor stimulation to icv CRF by atropine and by subcutaneous or intracolonic administration of 5-$HT_3$ antagonists, granistron, ramosteron, ondansetron and azasetron, and 5-$HT_4$ antagonist, SB-204070 while icv injection of these antagonists had no effects.67,76,85 This is also supported by the demonstration that icv injection of CRF increases the 5-HT content in the feces of the rat proximal colon.67

## $CRF_1$ Receptor Antagonists Alleviate Stress-Induced Stimulation of Colonic Motor Function

Substantial preclinical evidence has accumulated to support that stress-related stimulation of colonic motor function is primarily mediated by the activation of $CRF_1$ signaling pathway. First, there is mimicry between the colonic response to stress and that induced by centrally administered $CRF_1$ receptor agonists.60,73 Moreover, the CRF receptor antagonist, α-helical $CRF_{9–41}$ injected icv abolishes wrap restraint- and partial body restraint-induced stimulation of colonic transit in female and male rats.21,86 α-Helical $CRF_{9–41}$, D-$Phe^{12}CRF_{12–41}$, and astressin injected icv also antagonized the increased frequency of colonic spike-bursts induced by conditioned fear stress and reduces the defecatory response to wrap restraint in rats.21,62,87–89 Furthermore an array of selective $CRF_1$ antagonists, namely CP-154,526, CRA 1000, NBI 27914, NBI 35965, antalarmin and JTC-017 injected either icv or peripherally alleviate various stressors (restraint, water avoidance stress, elevated plus maze, social intruder)-induced stimulation of colonic motor function.24,66,73 By contrast $astressin_2$-B injected icv at doses that blocked $CRF_2$ mediated inhibition of gastric emptying did not alter the stress-related defecation in mice.66 In an open field test, $CRF_1$ knockout mice had significantly less defecation than the wild type.90 Taken together these data are consistent with the involvement of $CRF_1$ signaling pathways in the colonic motor response to acute stress. Of particular interest for future clinical use of these compounds are convergent reports showing that CRF receptor antagonists did not impact on the basal and postprandial functioning of the colon in non-stress conditions in rodents.60

## $CRF_1$ Receptors Mediate Stress-Induced Colonic Hyperalgesia

Gué et al. provided the first evidence in rats that icv injection of CRF mimicked stress-induced colonic hyperalgesia to colorectal distention (CRD) and that icv injection of α-helical $CRF_{9–41}$ blocked icv CRF and restraint-induced colonic sensitization to CRD.91 Thereafter the stress-related visceral hyperalgesia was characterized to involve central $CRF_1$ receptors and expanded to a number of experimental models of CRD-induced hypersensitivity using several selective $CRF_1$ antagonists as recently reviewed.24 For instance, icv CRF-induced colonic hypersensitivity to a tonic CRD is no longer observed in rats pretreated with antalarmin.92 In the anxiety prone Wistar Kyoto rats, intracolonic instillation of acetic acid-induced colonic hyperalgesia to a 2nd set of tonic CRD is inhibited by antalarmin.92 In a model of neonatal maternal separation, the colonic hypersensitivity in adult rats exposed acutely to water avoidance stress and phasic CRD is blocked by oral

pretreatment with NBI 35965.93 Likewise, acute water avoidance-induced a delayed colonic hypersensitivity to phasic CRD is abolished by CP-154,526.94 and colonic hypersensitivity induced by two sets of tonic CRD in female rats is alleviated by antalarmin.95 Lastly, microinjection of α-helical $CRF_{9–41}$ into the hippocampus or peripheral injection of the $CRF_1$ antagonist, JTC-017 results in the reduction of visceral pain induced by noxious tonic CRD along with the anxiety response to CRD in rats.96

The central and peripheral mechanisms through which activation of $CRF_1$ receptor and their blockade influence the development of visceral hyperalgesia are still to be defined. $CRF_1$ receptor antagonists may act by dampening CRD-induced activation of brain noradrenergic pathways. Recent electrophysiological studies in anesthetized rats showed that $[DPhe^{12}]CRF_{12–41}$, administered icv or microinfused into the LC, astressin injected into the cisterna magna and selective $CRF_1$ antagonist, NBI 35965 given iv prevented LC neuronal activation in response to central injection of CRF and CRD at submaximal distention (40 mmHg).97،98 In addition, peripheral injection of $CRF_1$ antagonists JTC-017 reduced the rise in noradrenaline levels in the hippocampus induced by CRD. Bursting activity in the LC is associated with the release of noradrenaline in the cortical and limbic rostral efferent projections of the LC leading to arousal and anxiogenic response.99 Still to be delineated is how blockade of $CRF_1$ receptors influence the neural pain pathways in the brain and spinal cord involved in the development of hyperalgesia. In the periphery, central CRF may contribute to colonic hypersensitivity by activating colonic mast cells. The icv injection of CRF induced a rapid increase in the release of rat mast cell protease II, prostaglandin $E_2$ and histamine levels in the colon.100،101 α-Helical $CRF_{9–41}$ injected icv prevents both icv CRF and stress-induced enhancement of colonic mast cell content of histamine.101 In addition, mast cell stabilizer doxantrazole prevents icv CRF and stress-induced colonic hypersensitivity to a 2nd set of CRD.91

## $CRF_1$ SIGNALING PATHWAYS AS A NEW THERAPEUTIC TARGET FOR IRRITABLE BOWEL SYNDROME

### IBS, Stress, Co-Morbidity with Anxiety/Depression

According to ROME-III, IBS is classified as a functional bowel disorder (category C-1), associated with recurrent changes in bowel habits, increased sensitivity to CRD, and abdominal discomfort/pain/bloating that are occurring in the absence of detectable organic disorders in routine examination.102 IBS subgroups have been based on the predominance of symptoms: diarrhea, constipation, alternating constipation and diarrhea, and abdominal pain.103 In the US, 10–15% of the population suffers from this condition with women seeking healthcare services 2 or 3 times more frequently than men.104 IBS patients also complain of additional symptoms not being included into the general diagnostic criteria of IBS leading to the concept of co-morbidity which is to be found in 29% to 92% of IBS patients.105 In particular, IBS patients have a high prevalence of co-existent psychiatric disorders, predominantly anxiety/depression and it has also been associated with fibromyalgia.105–108 Moreover, stressful life events, including history of major traumatic events in childhood are important risk factors for IBS and influence the onset and severity of symptoms.109،110 Prospective studies established that there is a 4%–31% incidence of post infectious IBS following bacterial gastroenteritis and stressful life events increase the risk to develop such post-infection IBS.111،112

### CRF Signaling System and IBS

The convergent preclinical data pointing to the role of central and peripheral $CRF_1$ signaling pathways in stress-related processes including those related to altered colonic function and visceral hypersensitivity increased interest to target CRF receptors as a new promising

therapeutic intervention for IBS diarrhea predominant symptoms (Table 1).24,73,113 The role of CRF signaling system at central and/or peripheral levels or a combination of both is gaining clinical recognition as part of the neurobiological common denominator of IBS symptoms susceptible to stress and anxiety/depression.114,115 For instance, there is evidence for elevated levels of CRF in the LC in patients with major depression.116 Elevated concentrations of CRF in the CSF is also present in patients with anxiety and vulnerability to stress as well as those suffering from obsessive compulsive disorders, posttraumatic stress disorders or childhood trauma.117–119 Carpenter et al. showed in a controlled study with depressed and healthy subjects that CRF in the CSF is a predictor of perceived aversive early life experiences.120 In patients suffering from fibromyalgia, CSF levels of CRF are associated with both pain symptoms and autonomic dysfunction but not with fatigue.121 Investigations in IBS patients indicate also that there is an overactivity of the HPA and enhanced plasma CRF response to mental stress.122,123

PhaseI clinical studies indicate that CRF administration reproduced features of IBS symptoms in healthy volunteers consistent with experimental animals and also enhanced those in IBS patients.73,113,124 Reports indicate that systemic administration of CRF increases colonic motility and the response is exaggerated in IBS compared with healthy subjects.124 In particular, CRF induces the occurrence of clustered contractions in the descending and sigmoid colon along with abdominal pain and discomfort in IBS patients that are not observed in healthy controls.124 Other studies showed that in healthy human subjects, the administration of CRF decreased the visceral pain threshold to repetitive rectal distensions, and enhanced the intensity of discomfort sensation to CRD. 125,126 Recently, CRF was found to activate subepithelial mast cells and stimulate transcellular uptake of protein antigens in the mucosa in colonic biopsies of healthy subjects127, as previously reported in experimental animals.113,128 Since increased uptake of antigen-sized macromolecules is associated with inflammation, a role of CRF in this process will be consistent with increasing evidence that IBS patients display a low graded colonic inflammation, including plasmatic cytokines (interleukin-6), intraepithelial lymphocytes, mast cell degranulation and increased permeability. 122,129

In support of CRF signaling pathways in the pathophysiology of IBS, Fukudo's group reported that the peripheral injection of α-helical $CRF_{9–41}$ prevents rectal electrical stimulation-induced enhanced sigmoid colonic motility, visceral perception and anxiety in IBS patients compared to healthy controls without altering the HPA axis.130 In addition the CRF antagonists was recently reported to almost normalize the altered EEG activities in IBS patients under basal and in response to CRD. 131

## CONCLUSIONS

As we are entering Selye's centennial anniversary (1907–1982), and as former Ph.D. Selye student (YT), it is gratifying to see how much has been accomplished since his pioneer description of the stress concept emphasing the activation of the HPA as key component. Through the relentless efforts of several groups, the biochemical coding of the CRF/Ucns-$CRF_1$/$CRF_2$ receptor signaling pathways has been identified and characterized to coordinate the various facets of the response to stress. In particular, the activation of $CRF_1$ receptors plays a pivotal role in the HPA stimulation and anxiogenic response to various stressors. In addition, consistent preclinical reports established that the activation of $CRF_1$ receptors by CRF also recapitulates key symptomes of IBS-diarrhea predominent patients as it relates to stimulation colonic motility, watery diarrhea, mucus secretion, mast cell activation, visceral hyperalgesia, and anxiogenic/hypervigilance that are alleviated by various selective $CRF_1$ receptors antagonists (Table 1). Preliminary clinical studies also support a role of the CRF signaling system in the induction of IBS-like symptoms in healthy subjects and highten

sensivity in IBS patients that are alleviated by a peptide CRF antagonist.115 Targetting $CRF_1$ receptor may provide a new therapeutic venue in the treatment of IBS.24

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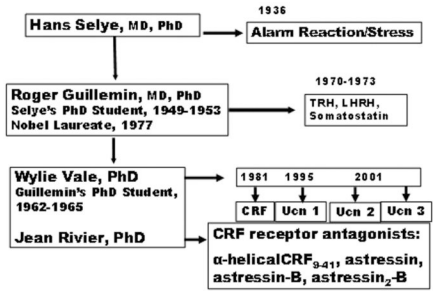


**Figure 1.**
From Selye to unraveling the biochemical coding of stress response: the mentoring linkage.

**Table 1**

Preclinical evidence to target $CRF_1$ receptors in IBS-diarrhea predominant symptoms

| **IBS-diarrhea: predominant features: Exacerbated by stress** | **$CRF_1$ antagonists: block stress-related:** |
|---|---|
| Anxiety/depression comorbidity | Anxiety/depression |
| Increased colonic motility | Stimulation of colonic motility |
| Ion transport dysfunction | Diarrhea |
| Change in mast cells | Activation of mast cells |
| Increase barrier permeability | Increase barrier permeability |
| Lower pain threshold to colorectal distention | Hypersensibility to colorectal distention |