Stress Neurobiology and Corticotropin-Releasing Factor

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Educational Objectives:
After reading this article, you will be familiar with:

- The evidence linking hypothalamic-pituitary-adrenal axis abnormalities and psychiatric symptoms.
- The role of corticotropin-releasing factor (CRF) in depressed patients.
- Extrahypothalamic CRF circuits and their impact on depression.

Who will benefit from reading this article?
Psychiatrists, primary care physicians, neurologists, nurse practitioners, psychiatric nurses, and other mental health care professionals. Continuing medical education credit is available for most specialties. To determine whether this article meets the CE requirements for your specialty, please contact your state licensing board.

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The concept that stressful life events may render one vulnerable to psychiatric disease has been a mainstay of the psychiatric literature for more than a century. This idea was initiated, in part, by the pioneering work of Sigmund Freud, who used psychoanalytic methods to explore the relationship between stressful life events and psychopathology. These concepts slowly evolved into more biologically based theories with the early work of Hans Selye, who studied the relationship between stress, illness, and emotions. The occurrence of depression, anxiety, and other psychiatric symptoms in both Cushing and Addison diseases, which are associated with excessive or markedly reduced levels of circulating glucocorticoids, respectively, served as a further impetus for researchers to scrutinize hypothalamic-pituitary-adrenal (HPA) axis function in psychiatric disorders.
HPA axis abnormalities in depression

Evidence linking HPA axis abnormalities and psychiatric symptoms dates back over 100 years, and numerous studies have been conducted in this area. Some of the earliest controlled clinical studies in psychiatry, dating back to the 1950s, demonstrated a number of abnormalities in glucocorticoid (ie, cortisol) function in depressed patients, including elevated plasma cortisol concentrations, increased 24-hour urinary free cortisol concentrations, and increased levels of cortisol metabolites in urine. Elevated cortisol secretion in depression is among the most reproducible findings in all of biologic psychiatry. Structural changes in the components of the HPA axis have also been documented in depressed patients, including pituitary gland enlargement demonstrated by MRI and enlargement of the adrenal glands, presumably due to adrenocorticotropin hormone (ACTH) hypersecretion in depressed patients postmortem and in suicide victims. The adrenal gland enlargement seen in depression has been confirmed using MRI, and it appears to be state-dependent, waxing and waning in parallel with exacerbation and resolution of depressive symptoms, respectively.

Corticotropin-releasing factor

Although Saffran and colleagues identified a crude extract that promoted the release of ACTH from the pituitary in 1955, the ultimate regulator of ACTH and cortisol release—corticotrophin-releasing factor (CRF)—was not isolated and chemically characterized until 1981. Working with extracts derived from 500,000 sheep hypothalami, Vale and colleagues at the Salk Institute isolated, synthesized, and elucidated the structure of CRF. This discovery led to the availability of synthetic CRF, which allowed for a comprehensive assessment of the HPA axis. Based on findings from numerous studies, it is clear that CRF coordinates the endocrine, immune, autonomic, and behavioral responses of mammals to stress.

In the hypothalamus, CRF is synthesized primarily in the paraventricular nucleus (PVN). These PVN CRF neurons receive input from a variety of brain regions, including the amygdala, the bed nucleus of the stria terminalis, and the brain stem. Hypothalamic CRF-containing neurons project to the median eminence. In response to stress, this neural circuit becomes activated, thereby releasing CRF from median eminence nerve terminals into the hypothalamoshypophysial portal system, where it activates CRF receptors on corticotrophs in the anterior pituitary to promote the synthesis of pro-opiomelanocortin and the release of its major posttranslation products, ACTH and β-endorphin. ACTH, released from the anterior pituitary, stimulates the production and release of cortisol from the adrenal cortex. These same hypothalamic CRF neurons also project to the spinal cord and brainstem nuclei, including the locus caeruleus (LC), the major noradrenergic nucleus in the brain.

Shortly after the isolation and characterization of CRF, a standardized intravenous CRF stimulation test was developed to assess HPA axis activity. In this paradigm, CRF is administered intravenously (usually at a dose of 1 µg/kg or a fixed dose of 100 µg) and the ACTH and cortisol responses are measured at 30-minute intervals over a 2- to 3-hour period. Numerous studies have now documented a blunted ACTH and β-endorphin response to exogenously administered ovine CRF or human CRF in depressed patients compared with nondepressed persons; the cortisol response in depressed patients and nondepressed control subjects did not consistently differ.

It has been hypothesized that the attenuated ACTH response to CRF is due to chronic hypersecretion of CRF from nerve terminals in the median eminence, which results in down-regulation of CRF receptors in the anterior pituitary, and/or to chronic hypercortisolism and its associated negative feedback. CRF receptor down-regulation results in a reduced responsivity of the anterior pituitary to CRF, as repeatedly demonstrated in laboratory animals.

Two CRF receptor subtypes, CRF1 and CRF2, with distinct anatomic localization and receptor pharmacology, have been identified in rats and humans. Both receptors are G-protein coupled receptors (GPCRs) and are positively coupled to adenyl cyclase via the protein Gs. The CRF1 receptor is predominantly expressed in the pituitary, cerebellum, and neocortex in the rat. Considerable evidence from laboratory animal studies has shown that CRF1 receptors may specifically mediate some of the anxiogenic-like behaviors observed after administration of CRF.

In agreement with these findings, mice with targeted knockouts of the CRF1 receptor were found to have an impaired stress response. The CRF1 receptor knockout mice were less anxious than their wild-type litter mates when tested in the elevated plus maze, a paradigm commonly used to assess anxiety-like behavior. In addition, data in these transgenic mice showed a significant reduction in stress-induced release of ACTH and corticosterone.
CRF2 receptor knockout mice have also been generated.36,37 Deletion of the CRF2 receptor gene during development provided an ambiguous profile, showing increased anxiety in some but not all anxiety tasks36,37: in males, but not females37; in males and females36, or not at all.38 Thus these studies suggest CRF2 receptor blockade may lead to states of increased anxiety, although it is likely that both the environment and the genetic background on which the knockouts were bred significantly contribute to the behavioral phenotype of these animals.

Research using selective CRF2 receptor agonists and antagonists has been even more inconsistent. Several studies have used the selective CRF2 receptor antagonist antisauvagine-30 (ASV-30),39 which has been reported to be between 100- and 1000-fold selective for the CRF2 receptor, depending on whether the radiolabeled ligand is sauvagine39 or ASV-30,40 respectively. Intraseptal administration of ASV-30 was shown to reduce anxious behavior induced by immobilization stress in the plus maze task or by previous association with foot shock in mice.41 These behavioral data were corroborated in rats, where intracerebroventricular ASV-30 reduced anxious behavior in the plus maze, defensive withdrawal, and a conditioned anxiety paradigm.32,42

Selective agonists at the CRF2 receptor have also been discovered. The peptides urocortin II and urocortin III are structurally and ancestrally related to CRF but show between 100- and 1000-fold selectivity at the CRF2 receptor versus the CRF1 receptor.43,44 Urocortin III has been shown to mildly suppress locomotion and has an anxiolytic-like profile in mice.45 However, another study from the same group demonstrated that urocortin II was inactive in the mice in the plus maze after acute administration but increased their exploratory behavior in the plus maze 4 hours later. Thus, compounds reported to be both selective agonists and antagonists at the CRF2 receptor have shown anxiolytic-like effects, making the exact role of this receptor in modulating stress-induced behaviors ambiguous.

**Extrahypothalamic CRF circuits and depression**

Although initially investigated for its role as one of the key modulators of the HPA axis, further research has revealed that CRF controls not only the neuroendocrine but also the autonomic, immune, and behavioral responses to stress in mammals. Results from both clinical studies and a rich body of literature conducted primarily in rodents and lower primates has highlighted the importance of extrahypothalamic CRF neurons.12,46,47 In rodents, primates, and humans, CRF and its receptors have been heterogeneously localized in a variety of regions, including the amygdala, thalamus, hippocampus, and prefrontal cortex, among others.48-51 These brain regions are important in regulating many aspects of the mammalian stress response and affect.

The presence of CRF receptors in both the dorsal raphe and LC, the origin of the major serotonergic and noradrenergic-containing perikarya, respectively, also deserves comment because most available antidepressants, including the tricyclics and SSRIs, are believed to act via modulation of the serotonergic and/or noradrenergic systems. The neuroanatomic proximity of CRF and monoaminergic systems provides evidence for an interaction between CRF systems and antidepressants, thereby suggesting a mechanism by which antidepressants may effect the CRF system.

The involvement of extrahypothalamic CRF systems in the pathophysiology of depression is suggested by numerous studies that have demonstrated elevated CRF concentrations in the cerebrospinal fluid (CSF) of depressed patients,52-56 although discrepant results have been reported.57 Elevated CSF CRF concentrations have also been detected in depressed suicide victims.55 A reduction in concentrations of CRF in CSF has been reported in healthy volunteers treated with the tricyclic antidepressant desipramine58 and in depressed patients following treatment with fluoxetine59 or amitriptyline,60 providing further evidence of an interconnection between antidepressants, monoamine neurons, and CRF systems. Similar effects have been reported after electroconvulsive therapy in depressed patients.59,61

Elevated CSF CRF concentrations appear to represent a state, rather than a trait, marker of depression (ie, a marker of current depression rather than a marker of vulnerability to depression).61 Furthermore, high and/or increasing CSF CRF concentrations despite symptomatic improvement of major depression during antidepressant treatment may be a harbinger of early relapse.62 Elevated CSF concentrations of CRF are believed to be due to CNS neuronal CRF hypersecretion,63 which may be acting at sites throughout the brain and contribute to many of the behaviors characteristic of depression.

Consistent with altered concentrations of CRF found in clinical studies of depression, CRF binding site density and messenger RNA (mRNA) expression have shown alterations in both preclinical and clinical studies, presumably in response to changes in CRF availability. Our group has previously reported a marked (23%) reduction in the number of CRF binding sites in the frontal cortex of suicide victims compared with controls64; we have now replicated this finding in a second study. Two later studies demonstrated an increase in CRFmRNA expression in
the PVN of depressed patients compared with controls.65,66

Increased CRF mRNA and decreased CRF₁ mRNA have also been detected in the brains of suicide victims in subregions of the frontal cortex.67 Although conducted in different laboratories and on different tissue, and keeping in mind the relative difficulty in obtaining and analyzing human tissue, the general pattern of increased CRF concentrations and/or CRF mRNA and the relative decrease in CRF binding sites is consistent with the well-documented phenomenon of receptor up- and down-regulation.

While the exact mechanism contributing to CRF hyperactivity remains obscure, studies from our group and others have documented long-term persistent increases in HPA axis activity and extrahypothalamic CRF neuronal activity after exposure to early untoward life events, for example, neglect and child abuse, respectively, in both laboratory animals (rats and nonhuman primates) and patients.68,69 Early life stress apparently permanently sensitizes the HPA axis and extrahypothalamic CRF neurons and leads to a greater risk of depression developing later in life. In several paradigms, early sensitization of CRF systems results in heightened responses to stress later in life.

To measure HPA axis responsivity to stress in humans, the Trier Social Stress Test (TSST) was developed. This laboratory paradigm involves a simulated 10-minute public speech and a difficult mental arithmetic task. The TSST has been validated as a potent activator of the HPA axis in humans.70 Recently, our group reported increased HPA axis responsivity (ie, elevated plasma ACTH and cortisol concentrations), presumably due to hypersecretion of CRF, after exposure to the TSST in both depressed and nondepressed women who were exposed to severe physical and emotional trauma as children.71 These data provide evidence that CRF systems are particularly sensitive to the effects of early adverse life events.

**Small-molecule CRF antagonists**

Although space constraints do not permit an extensive review of the preclinical literature, several additional points are worth noting. Findings from numerous studies have shown that when CRF is directly injected into the CNS of laboratory animals it produces effects reminiscent of the cardinal symptoms of depression, including decreased libido, reduced appetite, weight loss, sleep disturbances, and neophobia.13 Certainly by the late 1980s, a number of research groups, including our own, had hypothesized that a lipophilic, small-molecule CRF receptor antagonist that readily penetrates the blood-brain barrier after oral administration would represent a novel class of antidepressant and/or anxiolytic agents.

CRF₁ receptor antagonists have elicited activity in animal models of anxiety and depression. CRF receptor antagonists have been tested in many different paradigms, including the elevated plus maze, foot shock, restraint stress, and defensive withdrawal. Pretreatment with CRF receptor antagonists decreases measures of anxiety induced by stressors. There is also some evidence that CRF receptor antagonists may reduce the effects of drug withdrawal and stress-induced relapse to drug seeking in rats.47,72-74 Based on this premise, newly developed CRF₁ receptor antagonists represent a novel putative class of antidepressants. Such compounds show activity in nearly every preclinical screening test for antidepressants and anxiolytics.

Despite the rich preclinical and clinical literature supporting a potential role for CRF₁ receptor antagonists, there has only been 1 published study investigating the effects of a CRF₁ receptor antagonist in humans. A small open-label study examining the effectiveness of R121919, a CRF₁ receptor antagonist, in major depression was completed more than 5 years ago.75 This study of 20 patients showed that R121919 (5 to 40 mg/d or 40 to 80 mg/d for 30 days) was well tolerated by patients and did not significantly affect plasma ACTH or cortisol concentrations at baseline or following CRF challenge. It is important that the use of any potential CRF antagonist not lead to complete HPA axis blockade and adrenal insufficiency, which can, of course, result in a severe medical emergency. Hamilton Depression Rating Scale and Hamilton Anxiety Scale severity scores were both significantly reduced following 30-day treatment with this drug. Although this small open-label study does not provide unequivocal proof; it does provide further evidence that a selective CRF-receptor antagonist may provide antidepressant and anxiolytic properties in humans.75 Although this drug is no longer in clinical development because of hepatotoxicity, several novel CRF₁ antagonists are currently under investigation.

**Conclusions and future directions**

Since the discovery of CRF more than 25 years ago, evidence has accumulated indicating a preeminent role for this peptide in the pathophysiology of depression and anxiety. The recent introduction of small-molecule CRF receptor antagonists as a novel class of antidepressant and anxiolytic drugs remains very promising. These compounds block the actions of exogenous and endogenous CRF in a variety of in vivo models, supporting a putative role for these agents in the treatment of stress and/or anxiety and affective disorders. The promising clinical results in patients with depression in the completed open trial of R121919 is of great interest and the results of further studies are eagerly awaited.
As we await the results of additional clinical trials examining the efficacy of CRF₁ receptor antagonists in anxiety and mood disorders, it should be pointed out that these compounds may be beneficial in a broad array of neuropsychiatric disorders (including eating disorders, child abuse, and drug abuse), as well as irritable bowel syndrome and inflammatory diseases. Whether these drugs will be effective as monotherapy or whether they represent an important class of augmenting agents remains to be determined. Furthermore, the development of single photon emission CT and positron emission tomography ligands from these lead compounds for use in neuroimaging studies are likely to be useful in furthering our understanding of the pathophysiology of these mood and anxiety disorders.⁷⁶,⁷⁷

**Drugs mentioned in this article:**

- Amitriptyline (Limbitrol)
- Desipramine (Norpramin, Pertofrane)
- Fluoxetine (Prozac, Serafem)

**References**


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Evidence-based References

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