44 Corticotropin-Releasing Factor Receptors as a Potential Target in the Developments of Antidepressant Drugs

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Abbreviations

ACTH	Adrenocorticotropic hormone
ASVG-30	Antisauvagine-30
BNST	Bed nucleus of the stria terminalis
CRF	Corticotropin-releasing factor
FRL	Flinders resistant line
FSL	Flinders sensitive line
HAM-A	Hamilton anxiety scale
HAM-D	Hamilton depression scale
HPA axis	Hypothalamic-pituitary-adrenal axis
SSRI	Selective serotonin reuptake inhibitor
Ucn 1	Urocortin 1
Ucn 2	Urocortin 2
Ucn 3	Urocortin 3

44.1 Introduction

Depression is a highly prevalent form of mental illness that affects an estimated 350 million people [1]. In the coming decades, major depression is projected to become the second leading cause of disability worldwide, as well as the leading cause of disability in high-income nations [1]. Because stressful life events frequently trigger depressive symptoms, this illness is often characterized as a stress-related psychiatric disorder. A classic definition of stress is any response to demands, usually noxious, placed on the body [2]. An alternate definition describes stress as any alteration in the psychological homeostatic process [3]. Although body's response to acute stressors allows it to adapt to environmental demands, chronic exposure to stress has been hypothesized to lead to long-term alterations of the physiological systems that are thought to mediate the stress response. It is these long-term changes that may also underlie the symptoms of stress-related psychiatric disorders such as depression.

Corticotropin-releasing factor (CRF) is a 41-amino-acid neuropeptide that has long been considered to be one of the body's major regulators of the stress response. It is involved in mediating the neuroendocrine response to stress [4], as well as autonomic [5, 6] and behavioral responses to environmental demands [7, 8]. CRF is the main regulator of the hypothalamic-pituitary-adrenal (HPA) axis stress response, which has been traditionally used by biologists as a method of quantifying stress [4]. When the body is faced with a stressor, CRF neurons are activated in the paraventricular nucleus of the hypothalamus where they send axon terminals to the median eminence. From this area, CRF is released into the portal blood system and carried

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to the anterior lobe of the pituitary gland where it stimulates the synthesis and release of adrenocorticotropic hormone (ACTH). ACTH subsequently stimulates the release of glucocorticoids by the outer shell of the adrenals. In addition, CRF in the medulla can activate the sympathetic nervous system, stimulating the release of adrenaline from the adrenal medulla and other parts of the sympathetic nervous system [5, 6].

Chronic elevations in CRF and the hormones involved in the endocrine stress response can result in various detrimental physiological effects. Increased CRF levels can lead to the decreased toxicity of natural killer cells in the immune system [9] and induce stress-like changes in gastrointestinal, cardiovascular, and metabolic functions [10–12] via activation of the HPA axis and sympathetic nervous system. ACTH secretion directly influences immune function and acts within the brain to regulate sleep [13, 14]. Hypersecretion of glucocorticoids can result in infection via suppressed immune function, whereas low levels of these hormones can result in inflammatory conditions in laboratory animals [14]. Although the neuroendocrine stress response is important in the regulation of physiological responses to stress, the behavioral response to stress appears to occur independent of HPA axis activation. Hypophysectomy and blockade of the HPA axis response via dexamethasone suppression do not alter the behavioral response to stress produced by central administration of CRF [15, 16]. Thus, it appears that a central site of action is responsible for coordinating stress-related behavior.

Two genes encoding distinct G-protein-coupled CRF receptors have been identified (Fig. 44.1). The CRF_1 receptor is found mainly in the pituitary, amygdala, hippocampus, cerebellum, and cortex and is generally associated with increases in stress-related behaviors [8]. CRF, which preferentially binds to the CRF_1 receptor [17], leads to increases in the behavioral stress response in animal models [18–22]. Clinical research has also shown that depressed individuals show increased levels of CRF in cerebrospinal fluid [23]. A number of nonpeptide CRF_1 receptor antagonists have been developed with the hope that these drugs may be of therapeutic value in the treatment of depression and other stress-related psychiatric illnesses [24–26], although none have demonstrated clinical utility to date.

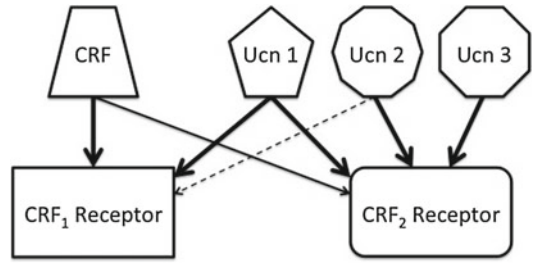

Fig. 44.1 Schematic representation of CRF-receptor subtypes and their associated ligands. Corticotropin-releasing factor (*CRF*) shows preferential binding affinity for the CRF_1 receptor. Urocortin 1 (*Ucn 1*) is equipotent in binding to the CRF_1 and CRF_2 receptor. Urocortin 2 (*Ucn 2*) and urocortin 3 (*Ucn 3*) are 1,000-fold more selective in binding to the CRF_2 receptor compared to Ucn 1. However, Ucn 2 induces low levels of activity at the CRF_1 receptor, whereas Ucn 3 does not induce CRF_1 receptor signaling

The CRF_2 receptor is found mainly in the lateral septum, ventromedial hypothalamus, and choroid plexus and exists in three splice variants: the CRF_{2a}, CRF_{2b}, and CRF_{2c} receptors [27, 28], and the discovery of additional neuropeptides belonging to the CRF family has aided in characterizing the role of the CRF_2 receptor in the stress response (Fig. 44.2). Urocortin 1 (Ucn 1) is a 40-amino-acid neuropeptide that is thought to be an endogenous ligand for the CRF_2 receptor, as it shows equally high binding affinity for both the CRF_1 and CRF_2 receptors [29] and produces a distinct behavioral profile compared to CRF. Central injections of this peptide more potently reduce feeding in food-deprived rats but only mildly increase locomotor activation compared to CRF [30]. Ucn 1 injections also lead to delayed increases in stress-related behaviors [31].

Urocortin 2 (Ucn 2) is another CRF-related neuropeptide composed of 38 amino acids [32]. Ucn 2 shares a similar sequence with stresscopin-related peptide, differing only in the cleavage site of the mature peptide [33]. A putative human sequence, originally identified as human urocortin-related peptide, shares 76% amino acid identity with the identified mouse sequence

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hCRF      SEEPPISLDLTFHLLREVLEMARAEQLAQQAHSNRKLMDII
oCRF      SQEPPISLDLTFHLLREVLEMTKADQLAQQAHSNRKLLDIA

hUcn 1     DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV
rUcn 1     DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV

hUcn 2       IVLSLDVPIGLLQILLEQARARAAREQATTNARILARV
mUcn 2       VILSLDVPIGLLRILLEQARYKAARNQAATNAQILAHV

hUcn 3       FTLSLDVPTNIMNLLFNIAKAKNLRAQAAANAHLMAQI
mUcn 3       FTLSLDVPTNIMNILFNIDKAKNLRAKAAANAQLMAQI
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Fig. 44.2 Amino acid sequence structure of mammalian neuropeptides within the corticotropin-releasing factor peptide family. Represented are the structures of human corticotropin-releasing factor (*hCRF*), ovine corticotropin-releasing factor (*oCRF*), human urocortin 1 (*hUcn 1*), rat urocortin 1 (*rUcn 1*), human urocortin 2 (*hUcn 2*), mouse urocortin 2 (*mUcn 2*), human urocortin 3 (*hUcn 3*), and mouse urocortin 3 (*mUcn 3*)

and is proposed to be the human ortholog to mouse Ucn 2 [34]. Although Ucn 2 is equipotent in its binding affinity at the CRF_2 receptor when compared to Ucn 1, it is 1,000-fold more selective in binding to the CRF_2 receptor [32]. A third CRF-related neuropeptide known as urocortin 3 (Ucn 3) has also been identified [34]. Ucn 3 is a 38-amino-acid neuropeptide that shares a similar sequence with stresscopin, differing only in the cleavage site of the mature peptide [33]. Compared to Ucn 2, Ucn 3 displays a similar potency but higher selectivity in binding to the CRF_2 receptor [34]. Doses of up to 3 nM of Ucn 3 do not induce adenylate cyclase in CRF_1 transfectants in vitro, a marker of CRF_1 receptor activity [34]. Ucn 3 immunoreactive projections have been found in the hypothalamus, lateral septum, bed nucleus of the stria terminalis, and amygdala [35], areas believed to mediate the behavioral stress response [36–38]. These areas also are known to express high levels of the CRF_2 receptor [39], supporting the notion that Ucn 3 is an endogenous ligand for the CRF_2 receptor. The role of Ucn 2 and Ucn 3 in regulating stress-related behaviors is still unclear. Some studies have shown that administration of Ucn 2 and Ucn 3 attenuates the behavioral response to stress [40–42], whereas others have found that these selective CRF_2 receptor ligands enhance stress-related behavior [43].

Despite the seemingly contradictory findings regarding CRF_2 receptors and stress-related behavior, which will be discussed more in depth later in this chapter, characterization of the CRF receptors suggests that these CRF_1 and CRF_2 receptors may have distinct roles in the regulation of depressive behaviors. Preclinical studies strongly suggest that activation of the CRF_1 receptor increases depression-related behaviors [44–46]. Although there is no general consensus regarding the role of CRF_2 receptors in the behavioral stress response, preclinical evidence suggests that underactivation of this receptor may be involved in the regulation of increased depression-like behavior in animals [47, 48]. The present chapter will review the role of CRF-related ligands and CRF receptors in depression and proposes targeting the CRF system as a potential pharmacotherapy for depressive disorders.

44.2 Corticotropin-Releasing Factor and the Behavioral Stress Response

Much of the data regarding CRF itself and nonselective CRF-receptor antagonists focus more on animal models of anxiety rather than depression. These results should be strongly considered when examining the role of CRF in depression, however, given the multifaceted complexity and high incidence of comorbidity between these two disorders [49–51]. CRF injected into unstressed animals under familiar conditions leads to

increased locomotor activation [18, 19], whereas in unfamiliar settings, centrally administered CRF can lead to behavioral suppression [18]. In addition, CRF administration can lead to even greater reductions in operant responding during the conflict test [20], a model of anxiety in which a reward is accompanied by presentation of an aversive stimulus in order to suppress responding for the reward. Other examples of stress-related behavior induced by central administration of CRF include an enhanced acoustic startle response [21, 52], increases in the conditioned fear response [22], and decreased appetite [53, 54]. Furthermore, transgenic mice overproducing CRF show decreased exploration of a novel environment compared to wild types, an effect potentiated by exposure to social defeat stress [55]. Overexpression of CRF in the amygdala also leads to increases in stress-related behaviors in rats [56].

Further evidence that brain CRF systems play an important role in the regulation of the behavioral response to stress comes from studies using nonselective peptide CRF-receptor antagonists. These antagonists attenuate both CRF and stress-induced behavioral changes. α-Helical $CRF_{(9–41)}$, a CRF-receptor antagonist, decreases the CRF-enhanced acoustic startle response when centrally injected in rats [57]. This antagonist also increases exploratory behavior of the open arms of the elevated plus maze following administration in CRF-overproducing mice [55]. Open-arm preference in the elevated plus maze, as indicated by the ratio of open-arm to total-arm time and entries, has been proposed to relate inversely to anxiety [58]. A second peptide CRF-receptor antagonist, D-Phe-$CRF_{(12–41)}$, reduces both CRF- and stress-induced increases in locomotor activation [59]. Astressin, a third CRF-receptor antagonist, decreases CRF-induced locomotor activation and increases open-arm exploration in the elevated plus maze [60].

CRF-receptor antagonists have also been shown to reduce stress-induced behavioral changes. In the elevated plus maze, administration of α-helical $CRF_{(9–41)}$ leads to increased exploration of the open arms of the elevated plus maze in rats subjected to restraint stress, swim stress, or social conflict stress [61]. Astressin also increases open-arm exploration in the elevated plus maze in rats subjected to social conflict stress [60]. D-Phe-$CRF_{(12-41)}$ attenuates stress-induced increases in locomotor activation and decreases in exploration of the open arms of the elevated plus maze [59].

With regard to animal models of depression, D-Phe-$CRF_{(12–41)}$ reverses increases in intracranial self-stimulation reward thresholds induced by central CRF administration [62]. Injections of CRF [63, 64] and CRF promoter-induced overexpression of CRF [56] have also shown to increase immobility in rats observed in the forced swim test. The forced swim test is an animal model of depression in which immobility is thought to model a depressive-like state. Treatment with standard antidepressant drugs can reverse immobility, an effect correlated with antidepressant efficacy in humans [65]. These results suggest that increased CRF activity may be a key component in regulating depressive-like behaviors in animals.

44.3 CRF_1 Receptors, Stress-Related Behavior, and Animal Models of Depression

Preclinical evidence strongly suggests that activation of the CRF_1 receptor increases stress-related behavior [8]. As described above, CRF, which preferentially binds to the CRF_1 receptor, leads to increases in the behavioral stress response independent of HPA axis activation. For example, ovine CRF, which shows an 80-fold higher affinity in binding to the CRF_1 receptor vs. the CRF_2 receptor [17], produces increased motor activation and decreases open-arm exploration in the elevated plus maze [40]. CRF_1 receptor knockout mice lacking CRF_1 receptors also show a decreased responsiveness to stressful stimuli [66–68] and less spontaneous motor activity [69]. These data demonstrating that activation of the CRF_1 receptor increases the behavioral response to stress suggests the CRF_1 receptor may be a key biological component in regulating stress-related psychiatric disorders.

A number of CRF_1 receptor antagonists have been examined in animal models of depression, yielding mixed results. CP-154,526, one of the earliest nonpeptide CRF_1 receptor antagonists to be developed [70], reversed escape deficit in rats exposed to inescapable shock without affecting controls in the learned helplessness task [71]. During this task, animals were exposed to a series of inescapable shocks and then given the opportunity to escape shock following a period of time. Decreased escape behavior has been proposed to indicate a depressive-like state [72]. When administered 60 min before a test session, acute and chronic injections of CP-154,526 and CRA1000, another nonpeptide CRF_1 receptor antagonist, reduced escape failure in rats in a manner comparable to chronic treatment with the tricyclic antidepressant imipramine [73, 74]. Acute and chronic treatment with the CRF_1 receptor antagonist R278995/CRA0450 also reduced escape failures [75]. However, the CRF_1 receptor antagonists DMP696 and DMP904 were not effective in learned helplessness task [76].

Data obtained using the forced swim test have also been equivocal regarding the effectiveness of CRF_1 antagonists in animal models of depression. For example, CP-154,526, R121919, and antalarmin, CRF_1 antagonists that are structurally similar to CP-154,526 [77], have been found to be ineffective in reversing swim stress-induced immobility in the rat [78]. Similar results have also been found using R278995/CRA0450 [75], DMP696, and DMP904 [76]. In mice, antalarmin, DMP696, DMP904, and R121919 were also unable to reverse immobility in the forced swim test [45]. In contrast, the CRF_1 receptor antagonist LWH234 reduced immobility, but did not affect stress-induced increases in ACTH [78]. Another study has found that SSR125543A and antalarmin can also attenuate immobility due to swim stress [79].

In the tail suspension test, subchronic dosing of R121919 and DMP696 has been shown to decrease immobility in mice within a similar manner to that of the selective serotonin reuptake inhibitors (SSRI) fluoxetine and paroxetine or the selective norepinephrine reuptake inhibitor reboxetine [45]. In contrast, antalarmin, DMP 904 [45], CP154,526 [80], and R278995/CRA0450 [75] were found to be ineffective in the tail suspension test.

These conflicting findings may be explained, in part, by the baseline behavior of the animals. Similar to nonselective peptide CRF-receptor antagonists, CRF1 receptor antagonists may only be effective in reversing stress-induced changes in behavior. The development of the Flinders sensitive line (FSL) rats, showing higher a baseline level of immobility compared to other rat strains [44], has led to support for this hypothesis. FSL rats receiving chronic treatment with SSR125543A show decreases in immobility in the forced swim test comparable to that observed following chronic injections of fluoxetine and the tricyclic antidepressant desipramine. These drugs did not affect immobility in Flinders resistant line (FRL) rats [81]. Similar results have also been observed in FSL and FRL rats receiving chronic treatment with CP-154,526, imipramine, and the SSRI citalopram [82]. Given these results, it appears that CRF_1 receptor antagonists are most effective in animal models of depression when tested in animals that show behaviors indicative of a depressive-like state.

More consistent results have also been seen in animals examined in the chronic mild stress model. BALB/c mice exposed to a series of mild stressors, including restraint, food restriction, and changes in housing, show a deteriorated physical state and decreased body weight. Chronic treatment with antalarmin or fluoxetine reverses the decrease in physical state [83]. Chronic treatment with SSR125543A or fluoxetine can also reverse deterioration of physical state in mice. In addition, both of these drugs attenuate the stress-induced reduction of cell proliferation in the dentate gyrus [84]. Interestingly, fluoxetine treatment led to an increase in cell proliferation in the dentate gyrus in nonstressed mice, whereas SSR125543A had no effects on cell proliferation in these animals [84], further supporting the hypothesis that CRF_1 antagonists may only be effective in altering depressive-like behaviors related to stressful conditions.

Recent data also suggest that CRF_1 antagonists may be a useful alternative to those resistant

to the therapeutic properties of traditional antidepressants [46]. In a series of experiments, mice exposed to chronic mild stress were examined in a number of stress-related behavioral tests following treatment with fluoxetine. Animals were then further divided into responder and nonresponder groups to fluoxetine treatment. Mice that did not respond to fluoxetine showed increases in nest-building behavior, an indication of increased motivation, and decreases in aggression when administered SSR125543A [46]. The findings of this study suggest that while traditional antidepressants may be beneficial to some, CRF_1 receptors may provide an alternative target for those who do not benefit from these medications.

44.4 CRF_1 Antagonists in Clinical Trials

The earliest clinical trials examining the effectiveness of nonpeptide CRF_1 antagonists in the treatment of depression were performed at the Max Planck Institute of Psychiatry in Munich, Germany, examining the CRF_1 antagonist NBI-30775. The initial open-label trial designed to assess the safety of NBI-30775 did not show significant alterations in liver enzymes or heart rate in patients with major depression. Moreover, consistent with the hypothesis that behavioral measures of stress occur independently of HPA axis activation, NBI-30775 did not alter the normal neuroendocrine response in response to intravenous administration of CRF. These patients also showed reduced scores in the Hamilton depression (HAM-D) and Hamilton anxiety (HAM-A) scales [85].

A subsequent trial showed that a 30-day treatment of low- or high-dosing regimens of NBI-30775 did not affect various endocrine measures, including HPA axis, hypothalamic-pituitary-gonadal axis and plasma renin activity, and aldosterone, human growth hormone, and insulin-like growth factor vasopressin and thyroid hormone levels. In addition, reports of adverse side effects such as headache, nausea and dizziness that were observed during the trial did not appear to be the direct result of the experimental compound [86]. NBI-30775 has also been shown to normalize electroencephalogram sleep patterns in patients diagnosed with major depression [87]. Finally, subsequent analysis of plasma leptin levels and body weight of the patients examined in the Zobel et al. study [85] showed that neither of these measures was affected by NBI-30775 treatment [88]. Although these data suggested that NBI-30775 is relatively safe and may be clinically useful, an unpublished study in the UK found elevated liver enzyme levels in two patients. This finding led to the termination of the development of NBI-30775, according to a media release from Janssen Pharmaceuticals.

Phase I clinical trials have been conducted to assess the effects of another CRF_1 receptor antagonist, NBI-34041, on neuroendocrine function [89]. For 14 days, 24 healthy male subjects received either NBI-34041 or placebo, and the HPA axis response to CRF and psychosocial stress was examined. NBI-34041 reversed the increases in ACTH and cortisol due to intravenous CRF or psychosocial stress but did not impair normal HPA axis function. Although these initial results demonstrate that NBI-34041 is effective in reversing physiological responses to stress without affecting basal hormone levels, further work is clearly needed to assess the safety and efficacy of this drug in the treatment of depression. Currently, there is no available literature concerning clinical trials for NBI-34041 in depression.

CP-316,311 is an additional CRF_1 receptor antagonist developed by Pfizer that has undergone clinical testing. Patients with recurring major depression were examined in a 6-week fixed dose double-blind placebo- and sertraline-controlled trial [90]. Although bioavailability data demonstrated adequate serum concentrations of CP-316,311 and urinary cortisol levels showed evidence of CRF_1 receptor blockade, patients administered CP-316,311 did not show any significant differences in HAM-D scores compared to those given placebo. Development of CP-316,311 was discontinued due to this negative result.

A multicenter clinical trial has also been conducted to determine the effectiveness of the CRF_1

antagonist pexacerfont in the treatment of generalized anxiety disorder [91]. Similar to CP-316,311, patients given pexacerfont had similar HAM-A scores compared to those given placebo, despite achieving efficacious serum concentrations of the drug. Clinical trials are also underway for CRF_1 antagonists in the treatment of post-traumatic stress disorder, substance use disorders, and stress-induced food cravings [92–95]. Although there remains interest in the field of developing CRF_1 antagonists with therapeutic value for depression and other stress-related psychiatric disorders, none have demonstrated clinical utility to date.

44.5 CRF_2 Receptors, Stress-Related Behavior, and Animal Models of Depression

Although the main focus in the development of antidepressants that target the CRF system has been on the CRF_1 receptor, CRF_2 receptors may present a compelling alternative given the lack of clinical success of CRF_1 receptor antagonists. The CRF_2 receptor is found mainly in the lateral septum, ventromedial hypothalamus, and choroid plexus [27, 28]. Activation of the CRF_2 receptor is most strongly associated with alterations in feeding behavior [30, 96]. Due to conflicting experimental findings, however, there is currently no general consensus regarding the role of CRF_2 receptors in the behavioral stress response.

CRF_2 receptor knockout mice show an anxiogenic-like phenotype when examined in the elevated plus maze, open-field, and light-dark emergence tests [97, 98]. Central infusion of CRF_2 receptor antisense decreases stress-coping behaviors and induces an anxiogenic-like response in rats [99]. Furthermore, rats examined in a model of post-traumatic stress disorder in which they were exposed to predator order over a 10-week period showed chronic upregulation of CRF_1 receptors and downregulation of CRF_2 receptors in the bed nucleus of the stria terminalis (BNST) [100]. This effect was attenuated by injections of *Lentivirus* overexpressing CRF_2 receptors [100]. As described previously, amidated 38-amino-acid synthetic peptides encoded by the Ucn 2 [32] and Ucn 3 [34] genes have been identified as selective CRF_2 receptor agonists. Central injections of Ucn 2 and Ucn 3 lead to suppressed motor activity in the locomotor activity test and an increase in open-arm exploration in the elevated plus maze [40, 41]. Ucn 3 also increases exploratory behavior in mice examined in the open-field test [42].

In contrast, there have also been findings that suggest an anxiogenic role for the CRF_2 receptor. Contrary to the findings discussed above, studies have found that central injections of antisauvagine 30 (ASVG-30) produced anxiolytic-like effects in the rat [101] and the mouse [43]. In addition, antisense inhibition of CRF_2 receptors in the lateral septum attenuates fear conditioning [102], and Ucn 2 has also been shown to produce a decrease in open-arm exploration in the elevated plus maze in mice [43]. However, further research has yielded additional insight to these conflicting results.

One possible explanation for these seemingly contradictory findings is that the doses used in these experiments may not be selective for the CRF_2 receptor. For example, ASVG-30 blocks footshock-induced freezing equally in CRF_2 receptor knockout mice and controls [103]. In contrast, the CRF_2 receptor antagonist astressin$_2$-B did not affect freezing in these mice [103]. ASVG-30 does have a moderate binding affinity for the CRF_1 receptor, whereas astressin$_2$-B is much more selective for the CRF_2 receptor [104, 105], and review of the previous literature shows that many of the studies that found anxiolytic-like effects following ASVG-30 administration used doses approximately threefold greater than the IC_{50} of this compound. Although Ucn 2 has been shown to induce an anxiogenic-like response when tested in the elevated plus maze [43], this ligand does have a low affinity for binding to the CRF_1 receptor [32]. In contrast, Ucn 3, which does not induce receptor signaling at the CRF_1 receptor, [34], appears to reduce behavioral measures of stress in the elevated plus maze on a more consistent basis [41, 106]. It is possible that the anxiolytic- and anxiogenic-like properties of

ASVG-30 and Ucn 3, respectively, may be due to low levels of binding activity at the CRF_1 receptor.

Alternatively, these findings may be the result of site-specific actions. Astressin, a nonselective CRF-receptor antagonist, but not antisauvagine-30, impairs CRF-enhanced fear conditioning when injected into the hippocampus. Both of these antagonists, however, attenuate fear conditioning when injected into the lateral septum [107]. Lesions of the lateral septum also produce anxiolytic-like effects in rat models of anxiety [108, 109]. Furthermore, mice exposed to high levels of stress show enhanced anxiety-like behavior when injected with Ucn 2 in the lateral septum [110].

Another possibility is that CRF_2 receptors may regulate specific aspects of the stress-coping response, such as sensory information. In C57BL/6J and 129S6/SvEvTac mice, both the CRF_1 receptor antagonist NBI-03775 and antisauvagine-30 attenuated enhancement of the acoustic startle response by CRF. In addition, Ucn 2 also increased the acoustic startle response, but with less efficacy than CRF [111]. Further investigation, however, showed that although NBI-30775 and ASVG-30 reduced CRF-induced increases in startle and CRF-induced deficits in prepulse inhibition, CRF_2 receptor activation via Ucn 2 and Ucn 3 injections enhanced prepulse inhibition of the acoustic startle response [112]. Also, rats injected with ASVG-30 in the anterolateral BNST show decreases in open-arm exploration similar to those seen in vehicle-injected controls following exposure to water-avoidance stress, whereas injections of the CRF_1 receptor antagonist CP376395 into the anterolateral BNST appears to reverse this effect [113]. However, both ASVG-30 and CP376395 decreased the acoustic startle reflex in this same study [113]. These data suggest that although it is possible that CRF_2 receptors may have some stress-inducing properties, they appear to be a critical component tempering the stress response.

With regard to depressive-like behaviors specifically, the role of CRF_2 receptors remains unclear. When observed in the forced swim test, CRF_2 receptor knockout mice display increased depression-like behavior as indicated by increased immobility [47]. In contrast, female, but not male, Ucn 2 knockout mice show less immobility time in the tail suspension and forced swim tests [114]. The difference in behavioral profiles between these strains of mice may be due to the body-wide absence of CRF_2 receptors and a different compensation in CRF_1 signaling in the CRF_2 receptor knockout mice. Ucn 2 knockout mice still have CRF_2 receptors available for Ucn 1 and Ucn 3 to bind and in fact show upregulated CRF_2 receptor expression in the dorsal raphe [114]. Conflicting results have also been found following administration of CRF_2 receptor-specific ligands. Ucn 2 injected into the dorsal raphe has been shown to potentiate learned helplessness behavior in response to inescapable shock, possibly due to interactions with the serotonergic system [115]. However, another study showed that Ucn 2 and Ucn 3 decreased immobility and increased climbing and swimming in mice examined in the forced swim test [48]. Clearly, further work is needed to fully understand the role of CRF_2 receptors in depression.

Conclusions

The data presented are clear evidence that the CRF receptors and CRF-related ligands are important in mediating heightened stress, which in turn may lead to stress-related psychiatric disorders. Although further study is needed, characterization of the CRF_1 and CRF_2 receptors suggests that these behavioral changes may be the result of an imbalance of CRF_1 and CRF_2 receptor activity, rather than simply CRF activation. The increases in CRF typically observed during the stress response appear to lead to an overactivation of the CRF_1 receptor given the binding profile of this neuropeptide [17]. This hypothesis is further supported by the data indicating an anti-stress function for CRF_2 receptor activation [40–42, 100, 112], leading to the hypothesis that the CRF_1 receptor is responsible for coordinating the activational components of stress, whereas the CRF_2 receptor acts as compensatory coping mechanism to oppose this action.

Currently, the effort with regard to medication development targeting the CRF system has focused on small molecule CRF_1 antagonists. Observations of both clinical and preclinical studies suggest that CRF_1 receptor antagonists are potentially effective candidate medications in the treatment of stress-related psychiatric illnesses. Patients diagnosed with affective disorders have shown increased CRF levels in cerebrospinal fluid and the hypothalamus, decreased CRF binding in the frontal cortex, and impaired HPA axis function [23, 116–118]. Preclinical evidence demonstrates that reducing brain CRF activity via antagonism of CRF_1 receptors can attenuate the behavioral changes observed in animal models of depression.

Although the evidence is compelling, there are still some issues that still need to be considered in the development of CRF_1 receptor antagonists. One highly encouraging characteristic of these drugs is their specificity in altering behavior only under stressed conditions, a feature that bodes well for side effect liability. Major side effects were not observed in clinical studies examining NBI-30775 [85]. However, animal studies have suggested that some CRF_1 antagonists may have slight sedative effects [75], endocrine disruptive actions [119], and transient abuse-related potential [120]. Another concern is that most structures have poor aqueous solubility and pharmacokinetic properties due to the fact that they are excessively lipophilic. Despite the efforts of the pharmaceutical industry, no CRF_1 receptor antagonists have demonstrated clinical utility to date. It is unclear if this lack of success is due to the lack of effectiveness of the compounds themselves, or the multifaceted complexity of the disorders they are designed to target, such as major depression. Nonetheless, the hope exists that further development of CRF_1 receptor antagonists and clinical trials that address these shortcomings may one day lead to new pharmacotherapies for the treatment of depression.

An alternative approach to targeting the CRF system in the development of new antidepressant medications may be to increase CRF_2 receptor activity. Although there is still no general consensus regarding the role of the CRF_2 receptor in the behavioral stress response, much of the data regarding the behavioral profiles of CRF-related neuropeptides and CRF-receptor knockout mice have led to a hypothesis that the CRF-receptor subtypes have an opposing role in the regulation of the stress-related behaviors. For example, CRF and Ucn 1, CRF-related neuropeptides with equal affinity for the CRF_1 and CRF_2 receptors [29], have been shown to increase overall behavioral activity in rats in a differential manner compared to Ucn 2 and Ucn 3. For example, de Groote et al. [121] found that CRF and Ucn 1 increase measures of exploratory behavior and grooming, whereas Ucn 2 and Ucn 3 only increase exploratory behavior. In addition, chronic exposure to stress leads to opposing profiles of CRF_1 and CRF_2 receptor expression [100]. In addition, it appears that some of the conflicting evidence may be due to the low-level binding activity at the CRF_1 receptor [103] and site-specific actions [107].

CRF_2 receptor-specific agonists have also been shown to reverse the effects of CRF. Ucn 2 and Ucn 3 decrease the stimulatory effect of CRF on locomotor activity in a familiar environment, and Ucn 2 injections attenuate CRF-induced decreases in exploratory behavior in the open field [122]. Although the precise mechanism of this action remains unclear, one hypothesis is that activation of the CRF_2 receptor may simply lead to a functional antagonism of the behaviors produced by CRF_1 receptor activation. Activation of the CRF_2 receptor may oppose the stress-inducing actions that result from CRF_1 receptor activation. Although this hypothesis must be further tested, the development of small molecule CRF_2 receptor agonists may also provide a novel approach for the treatment of depression.

The experiments discussed in this chapter suggest that the underlying mechanisms for increases in depressive behaviors may be the overactivation of CRF_1 receptors and possible underactivation of CRF_2 receptors. Studies describing the effects of CRF_1 receptor

antagonists imply that antagonizing the effects associated with activation of this receptor can attenuate the behavioral stress response. Although these compounds have also shown promising results when tested in preclinical models of depression, clinical trials have been unsuccessful thus far. Although data regarding CRF_2 receptor regulation of depressive behaviors is less clear, there is evidence that CRF_2 receptor ligands act opposing the effects of CRF_1 receptor activation. Thus, the development of small molecule CRF_2 receptor agonists may also be worth exploring as potential novel antidepressants. In conclusion, the data presented regarding CRF and its receptors and related ligands strongly suggest that targeting the CRF receptors has the potential to generate novel pharmacotherapies for depression.

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