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CRF AND CRF RECEPTORS: Role in Stress Responsivity and Other Behaviors

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■ **Abstract** Since corticotropin-releasing factor (CRF) was first characterized, a growing family of ligands and receptors has evolved. The mammalian family members include CRF, urocortinI (UcnI), UcnII, and UcnIII, along with two receptors, CRFR1 and CRFR2, and a CRF binding protein. These family members differ in their tissue distribution and pharmacology. Studies have provided evidence supporting an important role of this family in regulation of the endocrine and behavioral responses to stress. Although CRF appears to play a stimulatory role in stress responsivity through activation of CRFR1, specific actions of UcnII and UcnIII on CRFR2 may be important for dampening stress sensitivity. As the only ligand with high affinity for both receptors, UcnI's role may be promiscuous. Regulation of the relative contribution of the two CRF receptors to brain CRF pathways may be essential in coordinating physiological responses to stress. The development of disorders related to heightened stress sensitivity and dysregulation of stress-coping mechanisms appears to involve regulatory mechanisms of CRF family members.

INTRODUCTION

CRF Receptors and Ligands

Corticotropin-releasing factor (CRF) was first characterized in 1981 (1). Since then, a growing family of ligands and receptors has been discovered. First shown to be important regulators of the endocrine stress response, this family of neuropeptides and receptors is now known to be involved in diverse roles of homeostatic balance important in rapid mobilization of resources and behaviors for responses to stress. In recent years, vast new endogenous functions have been attributed to this family, including regulation of food intake and satiety, GI motility, vascular tone and development, hearing, and cardiac function, demonstrating the ubiquitous importance of the CRF family (3–13, 199). The mammalian family members

include CRF, urocortinI (UcnI), UcnII, and UcnIII. These ligands differ in their tissue distribution and receptor pharmacology.

Pharmacology of CRF Receptors

CRF receptors belong to the class B subtype of G protein–coupled receptors (GPCR). CRFR1 and CRFR2 are produced from distinct genes and have several splice variants expressed in various central and peripheral tissues. CRFR1 has α and β isoforms in addition to subtypes designated c–h, which have been detected in human and rodent tissues. Several of these isoforms have been shown to be nonfunctional [see (140) for detailed review of structural comparisons of receptor subtypes] (141–144). CRFR2 is expressed in three functional subtypes, α, β, and γ (145). These isoforms differ in their N-terminal sequence as well as their distribution in both tissues and species. Both CRFR2α and CRFR2β have been detected in human and rodents (146, 147). However, to date, CRFR2γ has only been reported in humans (148). There is nearly 70% identity between CRFR1 and CRFR2 at the amino acid level. As is consistent with other GPCR family members, the transmembrane and intracellular domains of the CRF receptors have the highest homology (over 80% identity) (145). The third intracellular loop is the receptor region thought to interact with the G-proteins for most GPCRs. In the CRF receptor family, the third intracellular loops are identical between receptors (149, 150). Specific sites of ligand action on CRF receptors have been identified through mutagenesis and chimaeric-receptor studies in which the N terminus, second and third extracellular domains, and N terminus juxtamembrane region have been shown to be important in determining the ligand binding and receptor specificity (151–155).

As a key stimulator of the stress response, CRF is found in the paraventricular nucleus of the hypothalamus (PVN), central nucleus of the amygdala (cAmyg), and hindbrain regions in the CNS, and in the gut, skin, and adrenal gland in the periphery. UcnI is predominantly expressed in cell bodies of the Edinger Westphal nucleus (EW) in the brain (14). In the periphery, it has been found in the gastrointestinal tract (GI), testis, cardiac myocytes, thymus, skin, and spleen. Both CRF and UcnI have high affinities for the CRF binding protein (CRF-BP). The most recently cloned ligand family members, UcnII and UcnIII, are also found in the CNS and periphery. UcnII (also known as stresscopin related peptide) is expressed in the hypothalamus, brainstem, and spinal cord in the CNS, and in the heart, blood cells, and adrenal gland in the periphery (15, 16). UcnIII (also known as stresscopin) expression has been found in the hypothalamus and amygdala in the CNS, and in the GI and pancreas in the periphery (16, 17). Neither UcnII nor UcnIII binds the CRF-BP. These ligand family members act via their two known receptors, CRFR1 and CRFR2. These receptors are seven transmembrane, G protein–coupled, and predominantly linked to the activation of adenylate cyclase through Gs [although recent evidence has suggested possible tissue-specific promiscuous signaling of these receptors through other second messenger systems (18, 19)]. CRFR2 has

two splice variants, CRFR2α and CRFR2β, found in rodents and humans, as well as a third variant found only in humans, CRFR2γ (20, 21). Although CRF has tenfold higher affinity for CRFR1 than for CRFR2, UcnI has equal affinities for both receptors (22). UcnII and UcnIII appear to be selective for CRFR2, although UcnII may activate CRFR1 at higher concentrations. The tissue distribution of these receptors has aided in the delineation of their endogenous functions. Both receptors are found in the CNS, with CRFR1 being more pervasive (23). CRFR1 is distributed throughout the cerebral cortex, cerebellum, olfactory bulb, medial septum, hippocampus, amygdala, and pituitary (24). Central CRFR2 is predominantly limited to sites in the lateral septum and hypothalamus, but is widely expressed in peripheral tissues, including the heart, GI, lung, skeletal muscle, and vasculature (20, 22, 25). The choroid plexus is also a major site of CRFR2 expression. It appears to be the coordinate adaptive functions of these two receptors that act to maintain homeostatic balance in response to stress.

The CRF-BP is a 37-kDa N-linked glycoprotein expressed in rodent and primate brain and pituitary (24, 26–29). In humans, CRF-BP is found in the liver and in the circulation, where it inactivates CRF and has been proposed to prevent inappropriate pituitary-adrenal stimulation during pregnancy (27). The association of CRF with its binding protein forms a dimer complex (CRF2/BP2) and is thought to modulate the endocrine activity of CRF (30). Recombinant CRF-BP has been shown to block CRF-induced adrenal corticotrophic hormone (ACTH) secretion from rat anterior pituitary cells (27). CRF-BP has also been detected in brain regions not associated with CRF activity, suggesting that it may also have CRF-independent actions.

The CRF family of ligands and receptors has been implicated in the regulation of stress responsivity and behaviors associated with these responses. As this plethora of attributable behaviors has seen tremendous growth in recent years due to the discovery of new ligands and the generation of transgenic mouse models, for brevity we review data most closely related to hypothalamic-pituitary-adrenal (HPA) axis stress responses, including anxiety and depression. We compare results obtained from peptide administration to those from genetic mouse models. These studies may support the continued search for as yet undiscovered CRF receptors and ligands whose detection will no doubt fill in the gaps.

ENDOCRINE STRESS RESPONSE

Stress has been defined biologically as various physiologic alterations, including homeostatic imbalances and activation of the pituitary adrenal axis. During a stress response, CRF activates the HPA axis, acting at CRFR1 on anterior pituitary corticotropes to stimulate the release of ACTH. ACTH then enters the blood stream and acts at receptors in the adrenal gland cortex to stimulate the synthesis and releases of glucocorticoids. A prominent negative-feedback system acts to inhibit further CRF production and release from the hypothalamus. Vasopressin (AVP) has been

shown to act synergistically with CRF to augment the release of ACTH in rodents and humans, suggesting that AVP may also play a physiologic role in modulating the ACTH response mediated by CRF (31–34). These studies demonstrated, using specific AVP receptor antagonists, that the actions of AVP on corticotropes were primarily mediated through vasopressin V1b receptors (33). There are many levels at which the HPA axis is regulated and the stress response can be affected. Acute responses to stress are necessary in order to maintain homeostasis in the organism. However, chronic stress or dysregulation of this system can lead to cell death, mood and affective disorders, and other diseases. CRF has also been implicated in allostasis, the ability of an organism to maintain stability through change, as a critical component by which organisms actively deal with stress in their environment. Results from experimental modulation of components of the HPA axis have helped decipher specific roles for each of these receptors and ligands.

Agonist and antagonist studies as well as transgenic and knockout mouse models have confirmed the importance of CRF family members in the HPA axis stress response (see Table 1). Direct infusion into the brain or peripheral administration of CRF or UcnI generates a potent release of ACTH from the pituitary (14, 33,

TABLE 1 Summary of stress and anxiety measures in transgenic and knockout mice

	HPA Axis				
Condition	**Basal**	**Stress**	**Anxiety**	**Other behaviors analyzed**	**References**
ICV CRF	↑	↓ ↑*	↑*	Learning/memory, feeding, fear, reproduction	(60)
ICV UcnI	↑	NE	↑*	Feeding, learning/memory	(3, 169)
ICV UcnII	NE	NE	↓	Feeding	(13, 83)
ICV CRF Antag (R1)	↓	↓*	↓*	Depression, feeding	(2, 93, 199)
ICV CRF Antag (R2)	↑↓	↑↓–	↑↓	Feeding	(60, 181)
CRFR1 KO	↓	↓	↓	Learning/memory#, alcohol addiction	(70, 71, 116, 212)
CRFR2 KO	–	↑	↑	Depression, feeding#	(74–76)
CRFR1/R2 KO	↓	↓	↑↓	Grooming#	(73, 77)
CRFKO	↓	↓	–	Grooming, feeding#	(46)
CRF Tg	↑	↓–	↑	Reproduction#	(43, 45)
CRF-BP KO	–	–	↑	Weight gain	(69)
CRF-BP Tg	–	↓	↑	Feeding, locomotor activity	(67, 68)
UcnI KO	–	–	↑–	Hearing	(11)

*Reviewed in Reference 60.

#Reviewed in Reference 128.

34, 207). Peptide and small-molecule antagonists of CRFR1 have been well documented to decrease or blunt this hormone release following a stress response in humans and animal models while not diminishing basal hormone levels (36–41). In one study, chronic administration of a CRFR1 antagonist (CRA1000) at a low dose of 10 mg/kg in rats did not affect basal HPA axis hormone levels, but effectively decreased the maximal stress response for both ACTH and corticosterone (40).

The role CRFR2 plays in the endocrine stress response remains unclear, but has been examined by intracerebroventricular (icv) antagonist infusion and knockout mouse models. Central administration of the CRFR2 peptide antagonist antisauvagine-30 (ASV-30) in mice has been shown to produce little effect on the ACTH response to or recovery from a restraint stress (187). Interestingly, in this study there was an inhibitory effect of the lower dose of ASV-30 on ACTH release with no restraint stress, which is reversed with a tenfold higher dose of ASV-30. Further studies are necessary to better elucidate the effects of CRFR2 antagonism on HPA axis stress responses.

CRF-OE

Transgenic mice centrally overexpressing CRF (CRF-OE) were developed to study the effects of chronic HPA axis activation (43). These mice developed Cushing's syndrome–like symptoms due to constitutive heightened production of ACTH and corticosterone. Delayed and attenuated HPA axis hormone responses to stress that may result from desensitization of the HPA axis were also reported in these mice (44). More recently, a second line of CRF-OE mice has been generated (45). These mice were produced utilizing a Thy-1 promoter construct that drives constitutive transgene expression in postnatal and adult neurons. Similar to the first line of CRF-OE mice, these mice also showed elevated basal plasma corticosterone levels. Unlike the first line of overexpressing mice, the Thy-1 generated mice had normal basal ACTH levels and normal hormone levels in response to stress (45). As usage of the Thy-1 promoter causes elevated gene expression in many brain regions normally not expressing CRF, these results may not represent a true model of stress hyperresponsivity.

CRF-KO

To further investigate the involvement of CRF in the HPA axis stress response, mice deficient for CRF (CRF-KO) were developed (46). These mice confirmed the importance of CRF in the stress response pathway, as both basal and acute stress exposed plasma corticosterone levels were significantly blunted in the absence of CRF. This group went on to further characterize these mice in several studies, demonstrating that while UcnI distribution is normal, its mRNA expression level is elevated in the EW in the absence of CRF (47, 48). These studies also revealed an elevation of UcnI in the EW following acute stress exposure, which

was blocked with glucocorticoid pretreatment, while not affecting basal UcnI expression. These results may suggest dual regulatory mechanisms for UcnI expression, where a low basal expression is insensitive to HPA axis regulation, whereas a higher expression pattern is tightly regulated by glucocorticoids and stress. A blunted or absent HPA axis stress response to footshock and interleukin exposure has also been demonstrated in these mice (49). These data clearly support the critical role CRF plays in mediation of the HPA axis stress response and the deficiency for developmental compensatory mechanisms for this pathway. Perhaps actions of the autonomic stress response in these mice may have developed as a means for compensation in the absence of an endocrine response. To examine the role of CRF in stress-induced release of epinephrine and norepinephrine, CRF-deficient mice were compared to wild-type littermates (50). In keeping with numerous results obtained from passive immunoneutralization of CRF, this study found that in the absence of CRF, mice produced delayed and reduced epinephrine and elevated norepinephrine plasma levels as a result of reduced adrenal phenylethanolamine N-methyltransferase (PNMT) expression and enzymatic activity. Glucocorticoid insufficiency in the CRF-KO mice was shown to be responsible for this reduction. These results suggest that in the absence of a normal HPA axis stress response, as found in CRF-deficient mice, endocrine factors also influence the autonomic stress response. Previous findings support an important role for CRF in activation of the autonomic nervous system, including activation of norepinephrine-containing sites as well as other key neurotransmitters important for mood regulation (51–59, 61–65, 198, 203).

UcnI-KO

Unlike CRF-deficient mice, mice deficient in UcnI (UcnI-KO) display normal basal and stress-stimulated HPA axis plasma hormone levels (11, 66). Two separate groups have generated UcnI-deficient mice using homologous recombination technology. One study demonstrated normal plasma ACTH and corticosterone levels following a time course of restraint stress (11). The second group revealed additional normal autonomic responses to stress, including plasma corticosterone, epinephrine, and norepinephrine, as well as changes in heart rate (66). Analysis of gene expression for other CRF family members in these mice found no change in CRF mRNA levels in the PVN (66) and a slight decrease in CRFR2 expression in the lateral septum (11). Results from these studies suggest that UcnI may not be a critical component of the HPA axis stress response or in stress-induced autonomic control.

CRF-BP-OE

As further testimony to the resiliency of the CRF/HPA axis interaction, transgenic mice overexpressing the CRF-BP (CRF-BP-OE) showed little alteration in basal or stress-induced plasma hormone levels in either of the two lines generated

(67, 68). Ubiquitous overexpression using the metallothionein-I promoter resulted in increased CRF-BP expression in the brain, pituitary, and peripheral tissues, including liver, kidney, and spleen (67). A significant attenuation of ACTH secretion was detected in male transgenic mice 3 h following a systemic inflammation stress. No differences were detected at earlier time points or in female mice. Although high levels of circulating CRF-BP were detected in these mice, no differences were found in basal HPA axis hormone levels. Specific overexpression of CRF-BP in the anterior pituitary was accomplished utilizing the pituitary glycoprotein hormone alpha-subunit promoter (68). These transgenic mice also have normal basal ACTH and corticosterone levels and a normal response to an acute restraint stress. However, increased levels of CRF and AVP were detected in the PVN of these mice, suggesting a compensation of increased ligand expression to overcome the increased production of CRF-BP.

CRF-BP-KO

Further exploration into the role of CRF-BP in regulation of the HPA axis stress response has been assessed using a mouse model of CRF-BP deficiency (CRF-BP-KO) (69). Basal ACTH and corticosterone levels are normal in CRF-BP-KO mice. Further, these mice show increases in HPA axis hormone levels in response to acute restraint stress similar to those of their wild-type littermates. When taken together, basal and stress-induced HPA axis hormone level results from CRF-BP-KO mice and CRF-BP-overexpressing mice suggest that CRF-BP is not a critical component in regulation of the stress response and that only under chronic stress exposure does it influence hormone production.

CRFR1-KO

The importance of CRFR1 in regulation of the HPA axis stress response is also evident in the examination of CRFR1-deficient (CRFR1-KO) mice. Two independent lines of CRFR1-KO mice have been generated. Both lines have demonstrated that in the absence of CRFR1, mice show a blunted response to restraint stress as revealed by a minimal increase in plasma ACTH and corticosterone compared to the significant release seen in wild-type littermates (70, 71). However, despite the diminished HPA axis hormonal stress response, basal levels in these mice are not different from wild-type levels. One study found that PVN AVP mRNA and protein levels were elevated in the CRFR1-deficient mice, thus suggesting that in the absence of CRFR1, AVP V1a receptors maintain a compensatory activation of basal HPA axis hormone levels (72). Other studies examining these mice, however, have detected no increases in AVP protein or mRNA expression in the PVN in the absence of CRFR1 (70, 73).

CRFR2-KO

Three independent laboratories developed mice deficient for CRFR2 (CRFR2-KO) (74–76). Although all three lines showed that CRFR2-KO mice have normal basal levels of ACTH and corticosterone and a normal circadian rhythm of hormone levels, two lines revealed the heightened sensitivity to stress of these mice (74, 75). CRFR2-KO mice exposed to a restraint stress showed rapid and elevated ACTH levels compared to the control animals. Similarly, corticosterone levels in the CRFR2-KO mice were significantly elevated following 2 min of restraint, compared to wild-type levels where an increase was detected following 5 min of the stress. These results demonstrating an elevated sensitivity of the HPA axis to stress in CRFR2-KO mice suggest that this receptor may function endogenously to dampen or modulate the stress response associated with CRFR1 activation. Other studies have shown that the recovery following stress is different in CRFR2-KO mice (75). Corticosterone levels in CRFR2-KO mice were still significantly elevated 90 min following exposure to a 5-min restraint stress when compared to wild-type levels. These results support a counteracting role for CRFR2 in the HPA axis stress response. Interestingly, no differences were found in basal CRF mRNA or protein levels in the PVN in CRFR2-KO mice (74, 75). However, we have found increased expression levels of AVP in the PVN, which may be augmenting the CRF response in these mice and thus resulting in the increased sensitivity and hormone levels detected (73).

CRFR1/2-KO

The response to stress in mice deficient for both CRF receptors (CRFR1/2-KO) has also been examined. In the absence of either known receptor, mice display remarkably little HPA axis response to a restraint stress (73, 77). ACTH and corticosterone levels following restraint stress are significantly lower in the CRFR1/2-KO mice compared to CRFR1-KO mice, suggesting a possible role of CRFR2 in mediation of HPA-axis sensitivity (73). Although CRFR1 is abundantly expressed in anterior pituitary corticotropes, CRFR2 is not, thus suggesting a possible new involvement of CRFR2 in HPA axis activation that may occur upstream of corticotropic cell stimulation, possibly in CRF cell bodies within the hypothalamus. Basal plasma levels of ACTH do not differ between groups, but we detected significantly lower basal morning plasma corticosterone levels in the double receptor-deficient mice compared to CRFR1-KO mice. Additional analyses of the HPA axis in mice deficient for both CRF receptors have also shown a deficiency in their response to restraint stress or social defeat stress (77). Taken together, these data suggest that both CRF receptors may participate in the maintenance and regulation of homeostasis in response to stress activation.

CRF mRNA and protein levels are elevated in the absence of CRFR1 (70, 73, 77). This increased expression of CRF is partially due to decreased endogenous

corticosterone negative feedback in the absence of CRFR1, as glucocorticoid replacement diminishes the increased CRF expression in these mice (73). However, CRF levels still remain elevated compared to wild-type mice, suggesting an additional regulatory mechanism for CRF expression that may involve CRFR2. AVP mRNA and protein levels are also elevated in the CRFR1/2-KO mice (73, 77).

These studies support a hypothetical model in which CRFR1 and CRFR2 may play important and opposing roles in regulation of organismal responses to stress and perturbations of homeostasis. This model suggests that following a challenge, CRF activation of CRFR1 stimulates the HPA axis and sympathetic nervous system in order to maintain physiologic equilibrium under acute perturbations for energy mobilization and redistribution and may also function in allostasis under more chronic insults. CRFR2, however, may function as an inhibitory or modulatory receptor activated by UcnI, -II, or -III to dampen these actions of CRFR1.

ANXIETY

CRF has been proposed to have an involvement in the development of anxiety-related and mood disorders. Studies have found that dysregulation of CRF or its family members in stress responsivity can lead to the onset of anxiety-like behaviors and depression. Evidence from research and clinical investigations demonstrates that depression encompasses a profound neurocircuitry failure. The central nucleus of the amygdala, Barrington's nucleus, locus coeruleus, and the dorsal raphe are possible sites involved in CRF actions and important regulators in endocrine control of behaviors symptomatic of anxiety and depression. Strong evidence links stress and the sensitivity of the individual to stressful encounters to the development of depression. A large body of evidence now ties CRF to the development of depression (78–82). Studies researching the involvement of CRF and its family members in stress responsivity have utilized antisense oligonucleotides, antagonists, and transgenic mouse technologies to discern specific roles for these neuropeptides and receptors.

Agonist/Antagonist Studies

Evidence in support of this model has shown that central administration of the CRFR2-specific ligand UcnII decreases stress-stimulated anxiety-like behaviors in rats (83). The mechanism for this response is unknown, but the delay in effectiveness indicates that activation of CRFR2 likely requires downstream effectors to dampen the behavioral stress response. Alternatively, UcnII permeation from ventricle application to access CRFR2-expressing brain sites involved in stress-induced behavior may require more time. In support of an anxiolytic role for CRFR2, a second study has shown that antagonism of CRFR2 by icv infusion of ASV-30 in mice revealed increased anxiety-like behaviors as measured by

significantly decreased time and entries in the open arms of the elevated plus maze (76). However, other studies utilizing antagonists for CRFR2 have reported conflicting results, showing either no effect or an anxiolytic effect (42, 84–86). The behavioral effects of CRFR2 antagonists seem to be variable and dependent on injection site, dose, time, stress, and behavior measured. These conflicting reports using CRFR2-specific agonists and antagonists no doubt support the existence of a complex regulation of stress responsivity and may indicate heterogeneous and diverse roles for CRFR2 (see Table 1).

Despite the conflicting evidence as to the role CRFR2 plays in mediating stress-induced behaviors, there remains little question that CRFR1 activation is a key component in the development of anxiety (7, 60, 87–92, 169). These studies have well characterized the behavioral effects of infusions of CRF or UcnI as well as CRFR1 antagonists. An interesting dichotomy of CRF-stimulated anxiety-like behaviors is found in its activation of locomotor activity when measured in a familiar environment (94–97), but suppression of exploration in an unfamiliar one (94, 98–101). These effects on locomotion appear independent of the HPA axis, as hypophysectomy or pretreatment with glucocorticoids does not block the behavior (102). Administration of a CRF antagonist has been shown to block this locomotor response (88).

The recent development of CRFR1-specific small-molecule antagonists by several different groups for possible treatment of anxiety- and depression-related disorders has spurred an interest in defining the specific stressors and pathways involved in CRFR1-mediated anxiety by preferentially antagonizing CRFR1 (37–41, 103–108). Small molecules readily cross the blood-brain barrier, thus making their experimental administration of greater ease than peptides that must be infused directly into the ventricle or brain region of interest. Results have demonstrated an antidepressant action of the CRFR1-specific small-molecule antagonist antalarmin in the forced swim test for depression (106). Studies have demonstrated an involvement of unimpeded CRFR1 activity or increased production of CRF with the development of anxiety-like or depression-like behaviors in rodents and humans (80, 82, 106, 109, 110). These studies have also shown that treatment of these disorders can be accomplished without disruption of normal circadian HPA axis hormone production while still squelching peak hormone responses to stress (39, 40). These results provide promise for future drug development and treatment of CRF-related anxiety disorders.

Antisense Studies

Studies in which antisense oligonucleotides have been infused into the brain have produced variable results. This methodology allows the investigator to suppress the expression of a receptor or ligand in a specific brain region in order to examine the function of that protein by the behavior/physiology produced in its absence. Ideally, the decrease in expression should be large enough as to have a measurable effect. Controls for such experiments are normally sense, missense, or scrambled

oligonucleotides. Conclusions regarding CRFR1 antisense studies have generally shown results similar to those produced by pharmacological or genetic approaches. A study in which CRFR1 expression was diminished by 10%–12% compared to vehicle or missense controls produced effects in the defensive-withdrawal test such that rats with decreased CRFR1 in the frontal cortex and amygdala spent less time in the protective chamber and showed a shorter latency to emerge from the chamber (111). In this same study, animals infused with CRFR2 antisense oligonucleotides in which CRFR2 expression was reduced in the septum and amygdala by 20% were not different from vehicle- or missense-treated animals in the defensive-withdrawal test. However, locomotor activity did appear to be increased in the antisense groups. Despite the effects obtained in the defensive-withdrawal test, neither CRFR1 nor CRFR2 antisense infusions produced a behavioral effect on the elevated plus maze. As all of the oligonucleotide infusions in this study caused a reduction in body weight that was not seen in the vehicle-infused animals, concrete conclusions regarding activity and anxiety are difficult to make. In a second antisense study, oligonucleotides designed against CRFR1 or CRFR2 were infused icv into male rats and their responses measured on anxiety and depression tests (112). The CRFR1 antisense-infused rats displayed a substantial increase in anxiolytic-like behavior on the elevated plus maze, with a 20% increase in the number of entries and a 15% increase in the amount of time spent in the open arms. No difference was detected on the elevated plus maze for rats infused with the CRFR2 antisense oligos. In the Porsolt forced swim test for depression, rats infused with CRFR1 antisense oligos showed no differences in time spent immobile nor in their latency to the first float reaction. However, rats infused with antisense oligos to CRFR2 increased the percentage time spent immobile as well as decreased the latency to the first float. These results suggest that CRFR2 is directly involved in mediation of stress coping, in opposition to CRFR1 involvement in stress-inducing behaviors.

CRF-OE

CRF-OE mice have provided a valuable tool for examining the effects of elevated CRF on behavioral stress-responsivity. Studies in these mice have shown increased anxiety-like behaviors in the elevated plus maze and light-dark box, as well as decreased locomotor activity in a novel environment and decreased sensorimotor performance on a rotorod (109, 113, 114). Further, examination of specific spontaneous behaviors in the home cage were shown to be significantly decreased, including locomotor activity, time spent rearing, and time spent digging, and time spent grooming was increased compared to control mice (114). These changes in behavior are a reflection of increased anxiety in these mice. However, in a novel environment, CRF-OE mice display hypoactivity (109, 114). This phenotype is not detected in a familiar environment, suggesting a connection between stress activation, anxiety, and locomotor activity. CRF-OE females also display significantly diminished sexual receptivity compared to wild-type mice (113). This is further

evidence of elevated anxiety levels specific to CRF overexpression, as central CRF infusion produces similar effects in female rats (115).

CRF-KO

Despite strong evidence in support of CRF involvement in anxiogenic behaviors, CRF-KO mice display normal behavioral responses to stress (47, 49). CRF-KO mice exhibit normal decreases in activity in a multicompartment chamber (MCC) following restraint and CRF icv infusion (47). Further, no differences were found for basal or restraint behavioral responses for time or entries in the open arms of an elevated plus maze, or line crossings and rearings in an open-field test in these mice compared to controls (47, 49). CRF-KO mice also display normal startle- and fear-conditioned responses to stress stimuli. No difference was found between CRF-KO mice and controls for levels of shock-induced freezing. Interestingly, infusion of a specific CRFR1 antagonist (CP-154,526) blocked this shock-induced freezing equally well in both CRF-KO and wild-type mice, demonstrating that although activation of CRFR1 is necessary for the behavioral response, CRF itself is not (47). Although UcnI mRNA in the EW nucleus is elevated in CRF-KO mice, no alterations in central UcnI cellular expression patterns were detected (47, 48). As the CRFR1 antagonist blocks anxiety-like behaviors in the CRF-KO mice, and as the fiber distribution of the only other known mammalian CRFR1 ligand, UcnI, does not indicate a direct activation of known pathways involved in behavioral responses to stress, might other as yet undiscovered CRFR1 ligands exist? Certainly, it remains possible that UcnI could activate CRFR1-containing brainstem nuclei and thus indirectly stimulate anxiety-like behaviors via a direct action on autonomic stress systems. However, behavior data from UcnI-KO mice suggest this is not the case and, in fact, support the existence of additional CRFR1 ligands (11).

UcnI-KO

We have found a significant increase in anxiety-like behaviors in the UcnI-KO mice (11). Mice deficient for UcnI spent less time and made fewer entries into the open arms of the elevated plus maze compared to wild-type mice. Further, these mice also spent less time in the inner squares in the open-field test compared to wild-type mice. No differences in either test were found for locomotor activity as measured by total number of entries in the plus maze and total square crossings in the open-field test. However, these mice were shown to have a significant auditory deficit. A second line of UcnI-deficient mice reported no differences in anxiety-like behaviors in the elevated plus maze, open-field test, or light-dark emergence test (66). However, an impairment in acoustic startle was detected in these mice. Although the genetic background of both lines of UcnI-KO mice appears to be similar, differences in housing prior to testing and sexes tested can certainly cause conflicting results.

CRF-BP-OE

The ubiquitous CRF-BP-OE mice have not been tested for anxiety-like behaviors. Interestingly, mice overexpressing CRF-BP only in the pituitary demonstrate increased locomotor activity in a familiar environment (68). Against prediction, mice overexpressing the CRF binding protein in a limited capacity in the pituitary show a tendency toward anxiolytic-like behaviors with more entries and time in the open arms of an elevated plus maze. Total arm entries were also significantly greater for the CRF-BP-OE mice compared to controls. As CRF increases locomotor activity in a familiar environment, and suppresses it in an unfamiliar one, these data would suggest that brain CRF levels are elevated in regions important for stress-stimulated locomotor activation. Certainly, CRF mRNA levels are increased in the PVN in these mice. The expression levels of CRF in other brain regions as well as that of other CRF family member have not been analyzed to date.

CRF-BP-KO

The CRF-BP-KO mice display an expected increase in anxiety-like behaviors (69). These mice spent less time and made fewer entries into the open arms of an elevated plus maze, made fewer exits out of the dark chamber in a defensive withdrawal test, and had a significantly greater latency to first exit the dark chamber. Although statistical significance was not reached, a trend in the data shows that the CRF-BP-KO mice also spent less time and made fewer crosses into the center of an open field. These behavioral responses are likely the result of increased free CRF or UcnI in the absence of the binding protein, which is thus able to more easily activate CRF receptors and increase stress-induced anxiety-like behaviors. Although these results suggest an important regulatory role for CRF-BP in possible "dampening" functions in stress-induced behaviors, expression levels and comparisons of unbound CRF ligands have not yet been well characterized in these mice.

CRFR1-KO

Mice deficient for CRFR1 display the predicted phenotype of anxiolytic-like behavior and impaired stress-responsivity (70, 71). CRFR1-KO mice spend significantly more time on and make more entries into the open arms of an elevated plus maze as well as spend significantly more time out of the chamber in the dark-light emergence task (70). These mice also show a decreased latency to enter the lit compartment in the light-dark box test as well as increased time spent in the light compared to wild-type mice (71). The CRFR1-KO group had more entries into the lit compartment and fewer animals avoiding the lit compartment. During alcohol withdrawal, the KO mice demonstrate increased anxiety-like behavior when compared to their basal responses. However, this response is minimal and remains

below that of the wild-type basal level. These results obtained from global deletion of CRFR1 confirm previous studies for an anxiogenic role for this receptor. Interestingly, one study did find an increase in locomotor activity for the CRFR1-KO mice (71) that a second study did not detect (70). An explanation for this discrepancy has not been examined to date. A recent study examined alcohol consumption in the CRFR1-KO mice following exposure to stress. This study found that these mice display enhanced and delayed alcohol consumption compared to wild-type mice (116). Although these results suggest that alterations in CRFR1 may constitute a genetic risk factor for stress-induced alcohol consumption and alcoholism, the direct effects of decreased glucocorticoid production during stress (owing to deficient adrenal glands in the CRFR1-KO mice) on alcohol addiction cannot be ruled out (117). In opposition to the previous findings in CRFR1-KO mice, others have found that CRFR1 antagonist pretreatment of alcohol-withdrawn rats prior to a stress exposure inhibited alcohol-seeking reinstatement (118). A recent study has also found elevated levels of grooming in CRFR1-KO mice during examination of behavior in their home cage (119). As increased grooming is usually categorized as an anxiety behavior, this finding goes against the absence of CRFR1 producing an anxiolytic-like phenotype. An increase in grooming behavior may provide clues as to the specific endogenous functions attributable to CRF receptors in stress-related behaviors. Increased grooming behavior has also been reported for CRF-OE and CRF-KO mice (47, 49, 114) and in CRFR1/2-KO mice (73). Perhaps these data show that grooming behavior is more of a sign of central CRF dysregulation than a true indicator of anxiety.

CRFR2-KO

Similar to results showing increased stress sensitivity in these mice, our studies also found an increase in anxiety-like behaviors (74). Results from examination on an elevated plus maze showed that both male and female CRFR2-KO mice spent less time on and entered less frequently the open arms of the elevated plus maze compared to wild-type mice. The increase in anxiety-like behavior was not due to altered locomotor activity, as overall activity in the closed-arm entries and total-arm entries was not different between the two genotypes. Results from the open-field test also showed increased anxiety-like behaviors. The CRFR2-KO mice spent less time in and displayed a lower percentage of inner square crosses than did wild-type mice. Unlike the robust differences in anxiety-like behaviors detected in the elevated plus maze and open-field tests in our line of CRFR2-KO mice, discrepancies in other lines have reported differences in anxiety-like behaviors only in male CRFR2-KO mice (76) or have demonstrated similar trends in behaviors that did not reach statistical significance (75). These differences are likely due to differences in genetic background between lines of mice.

As a possible animal model of depression, we have examined CRFR2-KO mice for depression-like behaviors in the forced swim test. Male and female CRFR2-KO

mice showed increased immobility as an indicator of depression compared to wild-type mice of the same sex. CRFR2-KO mice also displayed lower levels of swimming and climbing behaviors compared to wild-type mice. Treatment of CRFR2-KO mice with the CRFR1 antagonist, antalarmin, decreased immobile time and increased swimming and climbing time in both sexes. As differences in these behaviors in the forced swim test are good indicators of serotonergic and catecholaminergic involvement, our results may reveal an interaction of CRF pathways with other known antidepressant systems, and may further support an involvement of CRF receptors in the development of depression such that elevated CRFR1 activity, in the absence of CRFR2, increases depression-like behaviors.

We have reported increased CRF mRNA levels in the central nucleus of the amygdala in CRFR2-KO mice that may explain the increased anxiety-like and depression-like behaviors detected in these mice (74), as this nucleus is an important part of the limbic system and plays a major role in transduction of stress signals (120). In addition, the septum that contains an abundance of CRFR2 has been shown to modulate the activity of the amygdala (121–123) and lesions of this nucleus result in decreased ACTH secretion following restraint stress (124–127). We have also detected increased UcnI mRNA in the EW and UcnIII mRNA in the lateral perifornical region in CRFR2-KO mice (74).

CRFR1/2-KO

Results from testing double receptor–deficient mice for anxiety-like behaviors have revealed that their responses are sexually dichotomous (73). Female CRFR1/2-KO mice display decreased levels of anxiety-like behaviors compared to wild-type mice. Anxiolytic-like profiles were obtained in the behavioral parameters derived from the two different anxiety models tested. These mice entered the open arms of the elevated plus maze more frequently and spent more time in the open arms than control mice. Female CRFR1/2-KO mice produced significantly fewer fecal boli during the experiment, an indicator of decreased anxiety-like behaviors. Further anxiolytic-like data from these mice was recently published showing a decreased latency to first enter the lit compartment in a light-dark box test (128). These results suggest an anxiolytic-like phenotype for the female CRFR1/2-KO mice. However, male CRFR1/2-KO mice spent significantly more time grooming than either controls or CRFR1-deficient mice, indicative of anxiogenic-like behavior. Females also spent significantly more time in the open arms of the elevated plus maze and in the center of the open field and produced significantly fewer fecal boli during testing than the males.

Surprisingly, during behavioral testing we discovered that male CRFR1-KO mice did not display the characteristic robust anxiolytic-like behavior previously reported for these mice (70, 71, 129). In examination of differences in breeding schemes, we have found a significant correlation between the genotype of the dam and the anxiety-like behavior detected in their male offspring. If the dam

was heterozygous or homozygous for the CRFR2 deletion, the pup, regardless of its genotype, was significantly more likely to display anxiogenic-like behaviors (73). This correlation was significant for male but not female pups. Previous studies have well defined the nurturing role of the dam and how this behavior may affect the adult stress responsivity of the pup, suggesting that a low nurturing mother leads to a high-anxiety pup (130, 131). Further, studies have also revealed that elevated HPA axis stress-hormone levels can affect the anxiety-like behaviors of the pups (132–137). We are currently examining both of these hypotheses.

CRF Receptor Antagonists

The development of possible therapeutically relevant CRF receptor antagonists has become a current focus in drug discovery for treatment of stress-related disorders, such as depression and anxiety. Several small-molecule CRFR1 antagonists have been produced and studied in recent years. Antalarmin, a pyrrolopyrimidine compound, has been well examined in both rodents and nonhuman primates (see Table 2) (156). In rats, antalarmin inhibited conditioned fear responses (157). Prevention of both stress-induced behaviors and increased cerebrospinal fluid CRF levels was also found in rhesus monkeys treated with antalarmin (158). In this study, the antagonism of CRFR1 had only minimal effects on basal HPA axis hormone levels while still decreasing generalized anxiety behaviors. CP-154,526 is an analog of antalarmin and a CRFR1-specific nonpeptide antagonist also tested for effects on anxiety and stress-related behaviors in rodents. These studies have found a reduction of conditioned fear (159), increased open-arm time in an elevated plus maze (160), as well as decreased ultrasonic vocalizations by pups separated from their dams (161) following treatment with CP-154,526. These antianxiety effects have been shown to occur without sedation or ataxia; effects common

TABLE 2 Peptide and small-molecule CRF receptor antagonists

Compound	Receptor	Model examined	References
Antalarmin	CRFR1	Rat, mouse, primate	(157, 158, 167–172)
CP-154,526	CRFR1	Rat, mouse	(159, 173–178)
R121919	CRFR1	Rat, human	(163, 165, 179)
NBI 27914	CRFR1	Rat	(172, 180–182)
NBI 30775	CRFR1	Mouse	(166)
SSR125543A	CRFR1	Rat	(167)
Astressin	CRFR1, CRFR2	Rat, mouse	(183–188)
Astressin2B	CRFR2	Rat, mouse	(189–191)
Anti-sauvagine30	CRFR2	Rat, mouse	(181, 183, 187, 192–195)
Alpha-helical CRF (9-41)	CRFR2 $\geq$ CRFR1	Rat, mouse	(193, 196–203)

following treatment with other anxiolytics, such as benzodiazepines (162). A third small-molecule CRFR1 antagonist, R121919, has been examined both in rodents and in human clinical trials. This compound reduced anxiety scores and symptoms of depression in a small open-labeled trial in humans (163) in addition to producing anxiolytic-like effects in rats where acute administration of R121919 reduced measures of anxiety in a rodent defensive withdrawal paradigm (164). CRFR1 antagonists NBI 27914 (165) and NBI 30775 (formerly R121919) (166) have shown promise in treatment of stress-related behaviors in rodents. Chronic treatment of mice with NBI 30775 caused complex changes in hippocampal serotonergic neurotransmission that may reveal molecular mechanisms of CRFR1 involvement in the development of depression. A recently developed CRFR1 antagonist, SSR125543A, was shown to antagonize stress-induced hyperthermia, distress vocalization, and cortical norepinephrine release in rats (167). In the forced swim test, SSR125543A produced clear antidepressant-like effects, indicating that SSR125543A shows consistent antagonistic activity in acute and chronic tests of unavoidable stress exposure.

Additional Peripheral Roles for CRF Family Members

In addition to central roles, studies have demonstrated profound peripheral functions for CRF family members. The peripheral administration of CRF receptor agonists and antagonists have revealed potent effects on GI motility. Both CRFR1 and CRFR2 are found in the GI tract and stimulation of either receptor produces significant changes in gastric emptying. Whereas administration of CRF increases gastric motility, UcnI treatment appears to delay gastric emptying (204–206). UcnII also inhibited gastric emptying while not influencing distal colonic transit (189). The CRFR2 peptide antagonist astressin2B blocked this effect of Ucn II. Interestingly, gastric responses to CRF and restraint stress are blocked by astressin2B but not by CP-154,526, whereas the colonic response is blocked only by CP-154,526 (189, 190). These studies demonstrate that stress-induced delayed gastric emptying likely involves CRFR2, whereas stimulation of distal colonic transit appears to involve CRFR1, revealing pharmacologically distinct functions of these receptors.

A second major peripheral action of CRF family members involves cardiovascular hemodynamics, vascularization, and cardioprotection. Almost as soon as CRF had been isolated, studies demonstrated its profound ability to decrease mean arterial pressure and increase superior mesenteric artery flow when peripherally administered (207). Upon the identification of a second CRF receptor expressed in the vasculature and cardiac myocytes, further investigations revealed dramatic inotropic actions and further characterized the vasodilatory effects of CRF ligands (22, 209–210). Results from mice deficient in CRFR2 confirmed these actions were specific to this receptor, as peripheral administration of UcnI in these mice produced no change in mean arterial pressure or inotropic actions in the heart (75, 211). CRFR2 is also found in both endothelial and smooth muscle cells (SMCs) in the vasculature. We have reported that CRFR2-KO mice are hypervascularized

(213, 214). We have also examined in vitro the effects of CRFR2 activation on cell proliferation, cell cycle protein phosphorylation, and capillary tube formation. Our results demonstrate that activation of CRFR2 in vitro results in reduced vascular endothelial growth factor (VEGF) release from SMCs, an inhibition of SMC proliferation, and inhibition of capillary tube formation in collagen gels. Treatment of a subcutaneously injected gel matrix with UcnI also inhibited growth factor–induced vascularization. Western blots found that the cell cycle retinoblastoma protein (Rb), essential for cell cycle progression, is decreased following UcnI treatment of SMCs. These results suggest that CRFR2 is a critical component of a pathway necessary for tonic inhibition of adult neovascularization. These results open new doors in research for CRF family members, as CRFR2 may be a potential target for therapeutic modulation of angiogenesis in cancer and ischemic cardiovascular disease.

CRF family ligands have also been shown to be important in the protection of cardiac myocytes from lethal ischaemic injury (215, 216). Mechanistically, UcnI treatment causes rapid phosphorylation of ERK1/2-p42/44, and PD98059, which blocks the MEK1-ERK1/2-p42/44 cascade, inhibits this survival-promoting effect of UcnI (217). These studies suggest a novel function of UcnI as a cardioprotective agent that might act when given during reperfusion following an ischemic injury.

CONCLUSIONS

A vast and growing body of evidence has demonstrated the importance of the CRF family in regulation of organismal homeostasis. This review has focused on the specific roles that the CRF family of ligands, binding protein, and receptors play in stress and the development of anxiety-like behaviors. The main controversy surrounding the role the CRF receptors play in regulation of stress responsivity is that of CRFR2 functioning to either enhance behavioral responses to stress or to impede them. Although genetic mouse models have shown that in the absence of CRFR2, anxiety-like behaviors and stress sensitivity are increased, icv infusions of antagonists to CRFR2 do not agree. One question that hales around the infusion methodology is whether or not the infused drug accesses the receptors being examined, and further, at what concentration and which sites? A thorough study examining this issue has demonstrated that ventricularly infused CRF-related peptides do gain access to cognate receptors (138). This study showed that both centrally infused CRF and UcnI activated cell groups expressing CRF receptors as measured by Fos-immunoreactivity at these sites. However, the question of dose and pharmacology at each specific cell region still remains and is a key factor in discerning roles via attributing behaviors to receptors at these nuclei. Certainly, transgenic mouse models also have problems, as “developmental compensation” can be an issue in the ability to directly attribute phenotypic results with the absence of the protein of interest. We have demonstrated in mice that

TABLE 3 Ligand and receptor expression levels in transgenic and knockout mouse models

Mouse	CRF	UcnI	UcnII	UcnIII	CRFR1	CRFR2	AVP	References
CRF-OE Ubiq	↑PVN ↑cAmyg	NE	NE	NE	NE	NE	NE	(43)
CRF-OE Thy-1	↑	NE	NE	NE	NE	NE	NE	(45)
CRF-BP-OE Pituitary	↑PVN	NE	NE	NE	NE	NE	↑PVN	(68)
CRF-BP-OE Ubiq	NE	NE	NE	NE	NE	NE	NE	(67)
CRFKO	X	↑EW	NE	NE	NE	NE	NC PVN	(47, 48)
CRF-BPKO	NE	NE	NE	NE	NE	NE	NE	(69)
CRFR1 KO	↑PVN	NE	NE	NE	X	NC	↑PVN NC PVN	(70–72)
CRFR2 KO	NC PVN ↑CA	↑EW	NC	↑LPF	NC	X	↑PVN	(74)
CRFR1/R2 KO	↑PVN NC CA	NC EW	NE	↑LPF	X	X	↑PVN	(73)
UcnI KO	NC PVN	X	NE	NE	NC	↓LS	NE	(11)

NE: not examined.

NC: no change.

in the absence of CRFR2, mRNA levels of CRF and UcnI in important stress-responsive brain regions are increased (74). CRFR1 and CRFR2 are, for the most part, not found in similar brain regions. Therefore, a hypothesis might suggest that the removal of an inhibitory influence stemming from one brain site could result in increased ligand expression levels then acting on receptors in a second brain site. Studies of CRF transgenic mouse models have reported alterations in ligand expression patterns and mRNA levels, but there remains a great deal more to be examined (see Table 2). By mapping the changes in expression patterns and levels in these mice, we can compile greater details regarding the brain regions involved in stress regulation in association with changes in behavioral and endocrine responses. As exposure to chronic stress or the propensity to a hypersensitive stress response contributes to the development of anxiety-related disorders and depression, CRF transgenic and knockout mouse models are valuable tools with which we can delineate mechanisms and design drug targets for therapeutic benefit. The development of conditional and site-specific gene deletion mouse models will also aid in the deciphering of the roles the CRF receptors and ligands play.

CRF receptors display distinct specificities for CRF family ligands. Studies have demonstrated the importance of various receptor domains in the determination of ligand specificity. The first extracellular domain (ECD1) of CRFR1 was shown to be important in determining ligand affinities (139). Recently, similar work has demonstrated, using a soluble form of the first ECD of CRFR2, that this domain can also regulate ligand-receptor interactions (139). This CRFR2 ECD1 binds UcnI and UcnII with high affinity, but surprisingly has a very low affinity for UcnIII, suggesting that alternate receptor domains or structure must be important for high-affinity binding of UcnIII. These studies confirm the importance of receptor domain structure for ligand specificity and may support a molecular mechanism by which receptors with multiple ligands discriminate in vivo.

In the two decades since CRF was first characterized, studies have demonstrated the importance of the CRF family of ligands and receptors in regulation of organismal allostasis. From the initial agonist and antagonist infusion studies to the more recent transgenic mouse models and clinical trials, results have illustrated an involvement of this family in the endocrine and behavioral responses to stress (see Figure 1, color insert). CRF itself appears to play a stimulatory role in stress responsivity through activation of CRFR1. Specific actions of UcnII and UcnIII on CRFR2 may be important for dampening stress sensitivity. Regulation of the relative contribution of the two CRF receptors to brain CRF pathways may be essential in coordinating physiological responses to stress. The development of disorders related to heightened stress sensitivity and dysregulation of stress-coping mechanisms appear to involve regulatory mechanisms of CRF family members. Further studies utilizing newly synthesized receptor-specific antagonists and gene deletion mouse models will aid in the elucidation of the roles these family members play as well as in the designation of therapeutic drug targets for the treatment of anxiety-related disorders and depression.

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LITERATURE CITED

1. Vale W, Spiess J, Rivier C, Rivier J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213:1394–97
2. Kalin NH, Sherman JE, Takahashi LK, 1998. Antagonism of endogenous CRH systems attenuates stress-induced freezing behavior in rats. *Brain Res.* 457:130–35
3. Spina M, Merlo-Pich E, Chan RK, Basso AM, Rivier J, et al. 1996. Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. *Science* 273:1561–64
4. Parkes DG, Vaughan J, Rivier J, Vale W, May CN. 1997. Cardiac inotropic actions of urocortin in conscious sheep. *Am. J. Physiol. Heart Circ. Physiol.* 272:H2115–22
5. Okosi A, Brar BK, Chan M, De Souza L, Smith E, et al. 1998. Expression and protective effects of urocortin in cardiac myocytes. *Neuropeptides* 32:167–71
6. Deleted in Proof
7. Koob GF, Heinrichs SC. 1999. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res.* 848:141–52
8. Maillot C, Million M, Wei JY, Gauthier A, Tache Y. 2000. Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. *Gastroenterology* 119:1569–79
9. Contarino A, Dellu F, Koob GF, Smith GW, Lee K-F, et al. 2000. Dissociation of locomotor activation and suppression of food intake induced by CRF in CRFR1-deficient mice. *Endocrinology* 141:2698–702
10. Terui K, Higashiyama A, Horiba N, Furukawa KI, Motomura S, Suda T. 2001. Coronary vasodilation and positive inotropism by urocortin in the isolated rat heart. *J. Endocrinol.* 169:177–83
11. Vetter DE, Li C, Zhao L, Contarino A, Liberman MC, et al. 2002. Urocortin-deficient mice show hearing impairment and increased anxiety-like behavior. *Nat. Genet.* 31:363–69
12. Deleted in proof
13. Inoue K, Valdez GR, Reyes TM, Reinhardt LE, Tabarin A, et al. 2003. Human urocortin II, a selective agonist for the type 2 corticotropin-releasing factor receptor, decreases feeding and drinking in the rat. *J. Pharmacol. Exp. Ther.* 305:385–93
14. Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, et al. 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 378:287–92
15. Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, et al. 2001. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. *Proc. Natl. Acad. Sci. USA* 98:2843–48
16. Hsu SY, Hsueh AJ. 2001. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. *Nat. Med.* 7:605–11
17. Lewis K, Li C, Perrin MH, Blount A, Kunitake J, et al. 2001. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc. Natl. Acad. Sci. USA* 98:7570–75
18. Grammatopoulos DK, Randeva HS,

Levine MA, Kanellopoulou KA, Hillhouse EW. 2001. Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins. *J. Neurochem.* 76: 509–19

19. Blank T, Nijholt I, Grammatopoulos DK, Randeva HS, Hillhouse EW, Spiess J. 2003. Corticotropin-releasing factor receptors couple to multiple G-proteins to activate diverse intracellular signaling pathways in mouse hippocampus: role in neuronal excitability and associative learning. *J. Neurosci.* 23:700–7
20. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, et al. 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc. Natl. Acad. Sci. USA* 92:836–40
21. Kostich WA, Chen A, Sperle K, Largent BL. 1998. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. *Mol. Endocrinol.* 12:1077–85
22. Perrin M, Donaldson C, Chen R, Blount A, Berggren T, et al. 1995. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Proc. Natl. Acad. Sci. USA* 92:2969–73
23. Lovenberg TW, Chalmers DT, Liu C, De Souza EB. 1995. CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* 136:4139–42
24. Potter E, Sutton S, Donaldson C, Chen R, Perrin M, et al. 1994. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc. Natl. Acad. Sci. USA* 91:8777–81
25. Stenzel P, Kesterson R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP. 1995. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. *Mol. Endocrinol.* 9:637–45
26. Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW. 1992. The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc. Natl. Acad. Sci. USA* 89:4192–96
27. Potter E, Behan DP, Fischer WH, Linton EA, Lowry PJ, Vale WW. 1991. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 349:423–26
28. Chen R, Lewis KA, Perrin MH, Vale WW. 1993. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc. Natl. Acad. Sci. USA* 90:8967–71
29. Cortright DN, Nicoletti A, Seasholtz AF. 1995. Molecular and biochemical characterization of the mouse brain corticotropin-releasing hormone-binding protein. *Mol. Cell. Endocrinol.* 111:147–57
30. Lowry PJ, Koerber SC, Woods RJ, Baigent S, Sutton S, et al. 1996. Nature of ligand affinity and dimerization of corticotrophin-releasing factor-binding protein may be detected by circular dichroism. *J. Mol. Endocrinol.* 16:39–44
31. DeBold CR, Sheldon WR, DeCherney GS, Jackson RV, Alexander AN, et al. 1984. Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. *J. Clin. Invest.* 73:533–38
32. Liu JH, Muse K, Contreras P, Gibbs D, Vale W, et al. 1983. Augmentation of ACTH-releasing activity of synthetic corticotropin releasing factor (CRF) by vasopressin in women. *J. Clin. Endocrinol. Metab.* 57:1087–89
33. Rivier C, Rivier J, Mormede P, Vale W. 1984. Studies of the nature of the interaction between vasopressin and

corticotropin-releasing factor on adrenocorticotropin release in the rat. *Endocrinology* 115:882–86
34. Bilezikjian LM, Vale WW. 1987. Regulation of ACTH secretion from corticotrophs: the interaction of vasopressin and CRF. *Ann. NY Acad. Sci.* 512:85–96
35. Deleted in proof
36. Baram TZ, Mitchell WG, Haden E. 1996. Inhibition of pituitary-adrenal secretion by a corticotropin releasing hormone antagonist in humans. *Mol. Psychiatry* 1:320–24
37. Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. 1996. In vivo and in vitro characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 137:5747–50
38. Bornstein SR, Webster EL, Torpy DJ, Richman SJ, Mitsiades N, et al. 1998. Chronic effects of a nonpeptide corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. *Endocrinology* 139:1546–55
39. Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, et al. 1999. The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *Endocrinology* 140:79–86
40. Ohata H, Arai K, Shibasaki T. 2002. Effect of chronic administration of a CRF(1) receptor antagonist, CRA1000, on locomotor activity and endocrine responses to stress. *Eur. J. Pharmacol.* 457:201–6
41. Gutman DA, Owens MJ, Skelton KH, Thrivikraman KV, Nemeroff CB. 2003. The corticotropin-releasing factor1 receptor antagonist R121919 attenuates the behavioral and endocrine responses to stress. *J. Pharmacol. Exp. Ther.* 304:874–80
42. Deleted in proof
43. Stenzel-Poore MP, Cameron VA, Vaughan J, Sawchenko PE, Vale W. 1992. Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 130:3378–86
44. Coste SC, Murray SE, Stenzel-Poore MP. 2001. Animal models of CRH excess and CRH receptor deficiency display altered adaptations to stress. *Peptides* 22:733–41
45. Groenink L, Dirks A, Verdouw PM, Schipholt M, Veening JG, et al. 2002. HPA axis dysregulation in mice overexpressing corticotropin releasing hormone. *Biol. Psychiatry* 51:875–81
46. Muglia L, Jacobson L, Dikkes P, Majzoub JA. 1995. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* 373:427–32
47. Weninger SC, Dunn AJ, Muglia LJ, Dikkes P, Miczek KA, et al. 1999. Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. *Proc. Natl. Acad. Sci. USA* 96:8283–88
48. Weninger SC, Peters LL, Majzoub JA. 2000. Urocortin expression in the Edinger-Westphal nucleus is up-regulated by stress and corticotropin-releasing hormone deficiency. *Endocrinology* 141: 256–63
49. Dunn AJ, Swiergiel AH. 1999. Behavioral responses to stress are intact in CRF-deficient mice. *Brain Res.* 845:14–20
50. Jeong KH, Jacobson L, Pacak K, Widmaier EP, Goldstein DS, Majzoub JA. 2000. Impaired basal and restraint-induced epinephrine secretion in corticotropin-releasing hormone-deficient mice. *Endocrinology* 141:1142–50
51. Brown MR, Fisher LA, Rivier J, Spiess J, Rivier C, Vale W. 1982. Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. *Life Sci.* 30:207–10
52. Valentino RJ. 1989. Corticotropin-releasing factor: putative neurotransmitter in the noradrenergic nucleus locus

ceruleus. *Psychopharmacol Bull.* 25:306–11
53. Butler PD, Weiss JM, Stout JC, Nemeroff CB. 1990. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J. Neurosci.* 10:176–83
54. Deleted in proof
55. Curtis AL, Valentino RJ. 1994. Corticotropin-releasing factor neurotransmission in locus coeruleus: a possible site of antidepressant action. *Brain Res. Bull.* 35:581–87
56. Schulz C, Lehnert H. 1996. Activation of noradrenergic neurons in the locus coeruleus by corticotropin-releasing factor. A microdialysis study. *Neuroendocrinology* 63:454–58
57. Lechner SM, Curtis AL, Brons R, Valentino RJ. 1997. Locus coeruleus activation by colon distention: role of corticotropin-releasing factor and excitatory amino acids. *Brain Res.* 756:114–24
58. Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ. 1997. Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J. Pharmacol. Exp. Ther.* 281:163–72
59. Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I. 1998. Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* 18: 492–502
60. Cullen MJ, Ling N, Foster AC, Pelleymounter MA. 2001. Urocortin, corticotropin releasing factor-2 receptors and energy balance. *Endocrinology* 142:992–99
61. Page ME, Abercrombie ED. 1999. Discrete local application of corticotropin-releasing factor increases locus coeruleus discharge and extracellular norepinephrine in rat hippocampus. *Synapse* 33:304–13
62. Kawahara H, Kawahara Y, Westerink BH. 2000. The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. *Eur. J. Pharmacol.* 387:279–86
63. Kirby LG, Rice KC, Valentino RJ. 2000. Effects of corticotropin-releasing factor on neuronal activity in the serotonergic dorsal raphe nucleus. *Neuropsychopharmacology* 22:148–62
64. Price ML, Lucki I. 2001. Regulation of serotonin release in the lateral septum and striatum by corticotropin-releasing factor. *J. Neurosci.* 21:2833–41
65. Valentino RJ, Liouterman L, Van Bockstaele EJ. 2001. Evidence for regional heterogeneity in corticotropin-releasing factor interactions in the dorsal raphe nucleus. *J. Comp. Neurol.* 435:450–63
66. Wang X, Su H, Copenhagen LD, Vaishnav S, Pieri F, et al. 2002. Urocortin-deficient mice display normal stress-induced anxiety behavior and autonomic control but an impaired acoustic startle response. *Mol. Cell. Biol.* 22:6605–10
67. Lovejoy DA, Aubry JM, Turnbull A, Sutton S, Potter E, et al. 1998. Ectopic expression of the CRF-binding protein: minor impact on HPA axis regulation but induction of sexually dimorphic weight gain. *J. Neuroendocrinol.* 10:483–91
68. Burrows HL, Nakajima M, Lesh JS, Goosens KA, Samuelson LC, et al. 1998. Excess corticotropin releasing hormone-binding protein in the hypothalamic-pituitary-adrenal axis in transgenic mice. *J. Clin. Invest.* 101:1439–47
69. Karolyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, et al. 1999. Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. *Proc. Natl. Acad. Sci. USA* 96:11595–600
70. Smith GW, Aubry JM, Dellu F,

Contarino A, Bilezikjian LM, et al. 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 20:1093–102
71. Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, et al. 1998. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor. *Nat. Genet.* 19:162–66
72. Muller MB, Landgraf R, Preil J, Sillaber I, Kresse AE, et al. 2000. Selective activation of the hypothalamic vasopressinergic system in mice deficient for the corticotropin-releasing hormone receptor 1 is dependent on glucocorticoids. *Endocrinology* 141:4262–69
73. Bale TL, Picetti R, Contarino A, Koob GF, Vale WW, Lee KF. 2002. Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. *J. Neurosci.* 22:193–99
74. Bale TL, Contarino A, Smith GW, Chan R, Gold LH, et al. 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat. Genet.* 24:410–14
75. Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, et al. 2000. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. *Nat. Genet.* 24:403–9
76. Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, et al. 2000. Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat. Genet.* 24:415–19
77. Preil J, Muller MB, Gesing A, Reul JM, Sillaber I, et al. 2001. Regulation of the hypothalamic-pituitary-adrenocortical system in mice deficient for CRH receptors 1 and 2. *Endocrinology* 142:4946–55
78. Nemeroff CB. 1988. The role of corticotropin-releasing factor in the pathogenesis of major depression. *Pharmacopsychiatry* 21:76–82
79. Nemeroff CB. 1992. New vistas in neuropeptide research in neuropsychiatry: focus on corticotropin-releasing factor. *Neuropsychopharmacology* 6:69–75
80. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. *J. Endocrinol.* 160:1–12
81. Reul JM, Holsboer F. 2002. Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr. Opin. Pharmacol.* 2:23–33
82. Holsboer F. 1999. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J. Psychiatr. Res.* 33:181–214
83. Valdez GR, Inoue K, Koob GF, Rivier J, Vale W, Zorrilla EP. 2002. Human urocortin II: mild locomotor suppressive and delayed anxiolytic-like effects of a novel corticotropin-releasing factor related peptide. *Brain Res.* 943:142–50
84. Radulovic J, Ruhmann A, Liepold T, Spiess J. 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. *J. Neurosci.* 19:5016–25
85. Takahashi LK, Ho SP, Livanov V, Graciani N, Arneric SP. 2001. Antagonism of CRF(2) receptors produces anxiolytic behavior in animal models of anxiety. *Brain Res.* 902:135–42
86. Bakshi VP, Smith-Roe S, Newman SM, Grigoriadis DE, Kalin NH. 2002. Reduction of stress-induced behavior by antagonism of corticotropin-releasing hormone 2 (CRH2) receptors in lateral septum or CRH1 receptors in amygdala. *J. Neurosci.* 22:2926–35
87. Koob GF, Thatcher -Britton K. 1985. Stimulant and anxiogenic effects of

corticotropin releasing factor. *Prog. Clin. Biol. Res.* 192:499–506
88. Britton KT, Lee G, Vale W, Rivier J, Koob GF. 1986. Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. *Brain Res.* 369:303–6
89. Rassnick S, Heinrichs SC, Britton KT, Koob GF. 1993. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res.* 605:25–32
90. Menzaghi F, Howard RL, Heinrichs SC, Vale W, Rivier J, Koob GF. 1994. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. *J. Pharmacol. Exp. Ther.* 269:564–72
91. Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, et al. 1996. Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J. Pharmacol. Exp. Ther.* 276:56–64
92. Spina G, Merlo-Pich E, Akwa Y, Balducci C, Basso M, et al. 2002. Time-dependent induction of anxiogenic-like effects after central infusion of urocortin or corticotropin-releasing factor in the rat. *Psychopharmacology* (*Berlin*) 160:113–21
93. Swerdlow NR, Britton KT, Koob GF. 1989. Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by alpha-helical CRF (9–41). *Neuropsychopharmacology* 2:285–92
94. Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W. 1982. Corticotropin releasing factor produces behavioural activation in rats. *Nature* 297:331–33
95. Koob GF, Swerdlow N, Seeligson M, Eaves M, Sutton R, et al. 1984. Effects of alpha-flupenthixol and naloxone on CRF-induced locomotor activation. *Neuroendocrinology* 39:459–64
96. Sherman JE, Kalin NH. 1987. The effects of ICV-CRH on novelty-induced behavior. *Pharmacol. Biochem. Behav.* 26:699–703
97. Dunn AJ, Berridge CW. 1990. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res. Brain Res. Rev.* 15:71–100
98. Britton DR, Koob GF, Rivier J, Vale W. 1982. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci.* 31:363–67
99. Berridge CW, Dunn AJ. 1986. Corticotropin-releasing factor elicits naloxone sensitive stress-like alterations in exploratory behavior in mice. *Regul. Peptides* 16:83–93
100. Takahashi LK, Kalin NH, Vanden Burgt JA, Sherman JE. 1989. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behav. Neurosci.* 103:648–54
101. Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT. 1991. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharmacology* (*Berlin*) 103:227–32
102. Eaves M, Thatcher-Britton K, Rivier J, Vale W, Koob GF. 1985. Effects of corticotropin releasing factor on locomotor activity in hypophysectomized rats. *Peptides* 6:923–26
103. Griebel G, Perrault G, Sanger DJ. 1998. Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* (*Berlin*) 138:55–66
104. Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. 1998. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor 1 receptor attenuates stress-induced relapse to drug

seeking in cocaine- and heroin-trained rats. *Psychopharmacology* (*Berlin*) 137: 184–90
105. Millan MJ, Brocco M, Gobert A, Dorey G, Casara P, Dekeyne A. 2001. Anxiolytic properties of the selective, non-peptidergic CRF(1) antagonists, CP154,526 and DMP695: a comparison to other classes of anxiolytic agent. *Neuropsychopharmacology* 25:585–600
106. Griebel G, Simiand J, Steinberg R, Jung M, Gully D, et al. 2002. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl) ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR12-5543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J. Pharmacol. Exp. Ther.* 301:333–45
107. McElroy JF, Ward KA, Zeller KL, Jones KW, Gilligan PJ, et al. 2002. The CRF(1) receptor antagonist DMP696 produces anxiolytic effects and inhibits the stress-induced hypothalamic-pituitary-adrenal axis activation without sedation or ataxia in rats. *Psychopharmacology* 165:86–92
108. Blank T, Nijholt I, Vollstaedt S, Spiess J. 2003. The corticotropin-releasing factor receptor 1 antagonist CP-154,526 reverses stress-induced learning deficits in mice. *Behav. Brain Res.* 138:207–13
109. Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. 1994. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J. Neurosci.* 14:2579–84
110. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, et al. 2000. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatr. Res.* 34:171–81
111. Heinrichs SC, Lapsansky J, Lovenberg TW, De Souza EB, Chalmers DT. 1997. Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. *Regul. Peptides* 71:15–21
112. Liebsch G, Landgraf R, Engelmann M, Lorscher P, Holsboer F. 1999. Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. *J. Psychiatr. Res.* 33:153–63
113. Heinrichs SC, Min H, Tamraz S, Carmouche M, Boehme SA, Vale WW. 1997. Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. *Psychoneuroendocrinology* 22:215–24
114. van Gaalen MM, Stenzel-Poore MP, Holsboer F, Steckler T. 2002 Effects of transgenic overproduction of CRH on anxiety-like behaviour. *Eur. J. Neurosci.* 15:2007–15
115. Sirinathsinghji DJ, Rees LH, Rivier J, Vale W. 1983. Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. *Nature* 305:232–35
116. Sillaber I, Rammes G, Zimmermann S, Mahal B, Zieglgansberger W, et al. 2002. Enhanced and delayed stress-induced alcohol drinking in mice lacking functional CRH1 receptors. *Science* 296:931–33
117. Fahlke C, Engel JA, Eriksson CJ, Hard E, Soderpalm B. 1994. Involvement of corticosterone in the modulation of ethanol consumption in the rat. *Alcohol* 11:195–202
118. Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y. 2000. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology* (*Berlin*) 150:317–24
119. Penalva RG, Flachskamm C, Zimmermann S, Wurst W, Holsboer F, et al. 2002. Corticotropin-releasing hormone receptor type 1-deficiency enhances hippocampal serotonergic neurotransmission: an in vivo microdialysis study in mutant mice. *Neuroscience* 109:253–66
120. Liang KC, Melia KR, Campeau S, Falls

WA, Miserendino MJ, Davis M. 1992. Lesions of the central nucleus of the amygdala, but not the paraventricular nucleus of the hypothalamus, block the excitatory effects of corticotropin-releasing factor on the acoustic startle reflex. *J. Neurosci.* 12:2313–20
121. King FA, Meyer PM. 1958. Effects of amygdaloid lesions upon septal hyperemotionality in the rat. *Science* 128:655–56
122. Melia KR, Davis M. 1991. Effects of septal lesions on fear-potentiated startle, and on the anxiolytic effects of buspirone and diazepam. *Physiol. Behav.* 49:603–11
123. Lee Y, Davis M. 1997. Role of the septum in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J. Neurosci.* 17:6424–33
124. Allen JP, Allen CF. 1975. Amygdalar participation in tonic ACTH secretion in the rat. *Neuroendocrinology* 19:115–25
125. Beaulieu S, Di Paolo T, Barden N. 1986. Control of ACTH secretion by the central nucleus of the amygdala: implication of the serotoninergic system and its relevance to the glucocorticoid delayed negative feedback mechanism. *Neuroendocrinology* 44:247–54
126. Beaulieu S, Di Paolo T, Cote J, Barden N. 1987. Participation of the central amygdaloid nucleus in the response of adrenocorticotropin secretion to immobilization stress: opposing roles of the noradrenergic and dopaminergic systems. *Neuroendocrinology* 45:37–46
127. Marcilhac A, Siaud P. 1996. Regulation of the adrenocorticotrophin response to stress by the central nucleus of the amygdala in rats depends upon the nature of the stressor. *Exp. Physiol.* 81:1035–38
128. Contarino A, Gold LH. 2002. Targeted mutations of the corticotropin-releasing factor system: effects on physiology and behavior. *Neuropeptides* 36:103–16
129. Contarino A, Dellu F, Koob GF, Smith GW, Lee KF, et al. 1999. Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. *Brain Res.* 835:1–9
130. Zaharia MD, Kulczycki J, Shanks N, Meaney MJ, Anisman H. 1996. The effects of early postnatal stimulation on Morris water-maze acquisition in adult mice: genetic and maternal factors. *Psychopharmacology* (*Berlin*) 128:227–39
131. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. 1998. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl. Acad. Sci. USA* 95:5335–40
132. Takahashi LK, Kalin NH, Barksdale CM, Vanden Burgt JA, Brownfield MS. 1988. Stressor controllability during pregnancy influences pituitary-adrenal hormone concentrations and analgesic responsiveness in offspring. *Physiol. Behav.* 42:323–29
133. Takahashi LK, Baker EW, Kalin NH. 1990. Ontogeny of behavioral and hormonal responses to stress in prenatally stressed male rat pups. *Physiol. Behav.* 47:357–64
134. Takahashi LK, Kalin NH. 1991. Early developmental and temporal characteristics of stress-induced secretion of pituitary-adrenal hormones in prenatally stressed rat pups. *Brain Res.* 558:75–78
135. Takahashi LK, Haglin C, Kalin NH. 1992. Prenatal stress potentiates stress-induced behavior and reduces the propensity to play in juvenile rats. *Physiol. Behav.* 51:319–23
136. Cratty MS, Ward HE, Johnson EA, Azzaro AJ, Birkle DL. 1995. Prenatal stress increases corticotropin-releasing factor (CRF) content and release in rat amygdala minces. *Brain Res.* 675:297–302
137. Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. 1997. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J. Neurosci.* 17:2626–36

138. Bittencourt JC, Sawchenko PE. 2000. Do centrally administered neuropeptides access cognate receptors? An analysis in the central corticotropin-releasing factor system. *J. Neurosci.* 20:1142–56
139. Perrin M, DiGruccio M, Koerber S, Rivier J, Kunitake K, et al. 2003. A soluble form of the first extracellular domain of mouse type 2beta corticotropin releasing factor receptor reveals differential ligand specificity. *J. Biol. Chem.* 278:15595–600
140. Grammatopoulos DK, Chrousos GP. 2002. Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. *Trends Endocrinol. Metab.* 13:436–44
141. Chen R, Lewis KA, Perrin MH, Vale WW. 1993. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc. Natl. Acad. Sci. USA* 90:8967–71
142. Ross PC, Kostas CM, Ramabhadran TV. 1994. A variant of the human corticotropin-releasing factor (CRF) receptor: cloning, expression and pharmacology. *Biochem. Biophys. Res. Commun.* 205:1836–42
143. Grammatopoulos DK, Dai Y, Randeva HS, Levine MA, Karteris E, et al. 1999. A novel spliced variant of the type 1 corticotropin-releasing hormone receptor with a deletion in the seventh transmembrane domain present in the human pregnant term myometrium and fetal membranes. *Mol. Endocrinol.* 13:2189–202
144. Pisarchik A, Slominski AT. 2001. Alternative splicing of CRH-R1 receptors in human and mouse skin: identification of new variants and their differential expression. *Faseb J.* 15:2754–56
145. Dautzenberg FM, Hauger RL. 2002. The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol. Sci.* 23:71–77
146. Lovenberg TW, Chalmers DT, Liu C, De Souza EB. 1995. CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* 136:4139–42
147. Liaw CW, Lovenberg TW, Barry G, Oltersdorf T, Grigoriadis DE, de Souza EB. 1996. Cloning and characterization of the human corticotropin-releasing factor-2 receptor complementary deoxyribonucleic acid. *Endocrinology* 137:72–77
148. Kostich WA, Chen A, Sperle K, Largent BL. 1998. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. *Mol. Endocrinol.* 12:1077–85
149. Perrin MH, Vale WW. 1999. Corticotropin releasing factor receptors and their ligand family. *Ann. NY Acad. Sci.* 885:312–28
150. Arai M, Assil IQ, Abou-Samra AB. 2001. Characterization of three corticotropin-releasing factor receptors in catfish: a novel third receptor is predominantly expressed in pituitary and urophysis. *Endocrinology* 142:446–54
151. Hofmann BA, Sydow S, Jahn O, van Werven L, Liepold T, et al. 2001. Functional and protein chemical characterization of the N-terminal domain of the rat corticotropin-releasing factor receptor 1. *Protein Sci.* 10:2050–62
152. Perrin MH, DiGruccio MR, Koerber SC, Rivier JE, Kunitake KS, et al. 2003. A soluble form of the first extracellular domain of mouse type 2beta corticotropin-releasing factor receptor reveals differential ligand specificity. *J. Biol. Chem.* 278:15595–600
153. Dautzenberg FM, Higelin J, Brauns O, Butscha B, Hauger RL. 2002. Five amino acids of the Xenopus laevis CRF (corticotropin-releasing factor) type 2 receptor mediate differential binding of CRF ligands in comparison with its human counterpart. *Mol. Pharmacol.* 61:1132–39
154. Perrin MH, Fischer WH, Kunitake KS, Craig AG, Koerber SC, et al. 2001. Expression, purification, and characterization of a soluble form of the first ex-

tracellular domain of the human type 1 corticotropin releasing factor receptor. *J. Biol. Chem.* 276:31528–34
155. Assil IQ, Qi LJ, Arai M, Shomali M, Abou-Samra AB. 2001. Juxtamembrane region of the amino terminus of the corticotropin releasing factor receptor type 1 is important for ligand interaction. *Biochemistry* 40:1187–95
156. Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. 1996. In vivo and in vitro characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 137:5747–50
157. Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, et al. 1999. The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *Endocrinology* 140:79–86
158. Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, et al. 2000. Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. *Proc. Natl. Acad. Sci. USA* 97:6079–84
159. Hikichi T, Akiyoshi J, Yamamoto Y, Tsutsumi T, Isogawa K, Nagayama H. 2000. Suppression of conditioned fear by administration of CRF receptor antagonist CP-154,526. *Pharmacopsychiatry* 33:189–93
160. Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, et al. 1996. A non peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur. J. Pharmacol.* 309:195–200
161. Kehne JH, Coverdale S, McCloskey TC, Hoffman DC, Cassella JV. 2000. Effects of the CRF(1) receptor antagonist, CP 154,526, in the separation-induced vocalization anxiolytic test in rat pups. *Neuropharmacology* 39:1357–67
162. McElroy JF, Ward KA, Zeller KL, Jones KW, Gilligan PJ, et al. 2002. The CRF(1) receptor antagonist DMP696 produces anxiolytic effects and inhibits the stress-induced hypothalamic-pituitary-adrenal axis activation without sedation or ataxia in rats. *Psychopharmacology* 165:86–92
163. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, et al. 2000. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatr. Res.* 34:171–81
164. Keck ME, Welt T, Wigger A, Renner U, Engelmann M, et al. 2001. The anxiolytic effect of the CRH(1) receptor antagonist R121919 depends on innate emotionality in rats. *Eur. J. Neurosci.* 13:373–80
165. Chen C, Dagnino R Jr, De Souza EB, Grigoriadis DE, Huang CQ, et al. 1996. Design and synthesis of a series of non-peptide high-affinity human corticotropin-releasing factor1 receptor antagonists. *J. Med. Chem.* 39:4358–60
166. Oshima A, Flachskamm C, Reul JM, Holsboer F, Linthorst AC. 2003. Altered serotonergic neurotransmission but normal hypothalamic-pituitary-adrenocortical axis activity in mice chronically treated with the corticotropin-releasing hormone receptor type 1 antagonist NBI 30775. *Neuropsychopharmacology* doi:10.1038/sj.npp.1300267
167. Griebel G, Simiand J, Steinberg R, Jung M, Gully D, et al. 2002. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclo-propyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR-125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J. Pharmacol. Exp. Ther.* 301:333–45
168. Bornstein SR, Webster EL, Torpy DJ, Richman SJ, Mitsiades N, et al. 1998. Chronic effects of a nonpeptide

corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. *Endocrinology* 139:1546–55

169. Zorrilla EP, Valdez GR, Nozulak J, Koob GF, Markou A. 2002. Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat. *Brain Res.* 952:188–99
170. Bale TL, Vale WW. 2003. Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. *J. Neurosci.* 23:5295–301
171. Charmandari E, Kino T, Souvatzoglou E, Chrousos GP. 2003. Pediatric stress: hormonal mediators and human development. *Horm. Res.* 59:161–79
172. Hoare SR, Sullivan SK, Ling N, Crowe PD, Grigoriadis DE. 2003. Mechanism of corticotropin-releasing factor type I receptor regulation by nonpeptide antagonists. *Mol. Pharmacol.* 63:751–65
173. Griebel G, Perrault G, Sanger DJ. 1998. Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* 138:55–66
174. Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. 1998. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* 137:184–90
175. Isogawa K, Akiyoshi J, Hikichi T, Yamamoto Y, Tsutsumi T, Nagayama H. 2000. Effect of corticotropin releasing factor receptor 1 antagonist on extracellular norepinephrine, dopamine and serotonin in hippocampus and prefrontal cortex of rats in vivo. *Neuropeptides* 34:234–39
176. Kawahara H, Kawahara Y, Westerink BH. 2000. The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. *Eur. J. Pharmacol.* 387:279–86
177. Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y. 2000. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology* 150:317–24
178. Blank T, Nijholt I, Vollstaedt S, Spiess J. 2003. The corticotropin-releasing factor receptor 1 antagonist CP-154,526 reverses stress-induced learning deficits in mice. *Behav. Brain Res.* 138:207–13
179. Gutman DA, Owens MJ, Skelton KH, Thrivikraman KV, Nemeroff CB. 2003. The corticotropin-releasing factor1 receptor antagonist R121919 attenuates the behavioral and endocrine responses to stress. *J. Pharmacol. Exp. Ther.* 304:874–80
180. Baram TZ, Chalmers DT, Chen C, Koutsoukos Y, De Souza EB. 1997. The CRF1 receptor mediates the excitatory actions of corticotropin releasing factor (CRF) in the developing rat brain: in vivo evidence using a novel, selective, non-peptide CRF receptor antagonist. *Brain Res.* 770:89–95
181. Pelleymounter MA, Joppa M, Carmouche M, Cullen MJ, Brown B, et al. 2000. Role of corticotropin-releasing factor (CRF) receptors in the anorexic syndrome induced by CRF. *J. Pharmacol. Exp. Ther.* 293:799–806
182. Bakshi VP, Smith-Roe S, Newman SM, Grigoriadis DE, Kalin NH. 2002. Reduction of stress-induced behavior by antagonism of corticotropin-releasing hormone 2 (CRH2) receptors in lateral septum or CRH1 receptors in amygdala. *J. Neurosci.* 22:2926–35
183. Radulovic J, Ruhmann A, Liepold T, Spiess J. 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles

of CRF receptors 1 and 2. *J. Neurosci.* 19:5016–25
184. Turnbull AV, Vaughan J, Rivier JE, Vale WW, Rivier C. 1999. Urocortin is not a significant regulator of intermittent electrofootshock-induced adrenocorticotropin secretion in the intact male rat. *Endocrinology* 140:71–78
185. Spina MG, Basso AM, Zorrilla EP, Heyser CJ, Rivier J, et al. 2000. Behavioral effects of central administration of the novel CRF antagonist astressin in rats. *Neuropsychopharmacology* 22:230–39
186. Conti LH, Shannon MH, Murry JD, Printz MP. 2001. Repeated restraint stress-induced increase in baroreceptor reflex sensitivity: role of corticotropin-releasing factor. *Neuropeptides* 35:71–81
187. Pelleymounter MA, Joppa M, Ling N, Foster AC. 2002. Pharmacological evidence supporting a role for central corticotropin-releasing factor(2) receptors in behavioral, but not endocrine, response to environmental stress. *J. Pharmacol. Exp. Ther.* 302:145–52
188. Chang FC, Opp MR. 2002. Role of corticotropin-releasing hormone in stressor-induced alterations of sleep in rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283:R400–7
189. Million M, Maillot C, Saunders P, Rivier J, Vale W, Tache Y. 2002. Human urocortin II, a new CRF-related peptide, displays selective CRF(2)-mediated action on gastric transit in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 282:G34–40
190. Rivier J, Gulyas J, Kirby D, Low W, Perrin MH, et al. 2002. Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. *J. Med. Chem.* 45:4737–47
191. Rivier CL, Grigoriadis DE, Rivier JE. 2003. Role of corticotropin-releasing factor receptors type 1 and 2 in modulating the rat adrenocorticotropin response to stressors. *Endocrinology* 144:2396–403
192. Ruhmann A, Bonk I, Lin CR, Rosenfeld MG, Spiess J. 1998. Structural requirements for peptidic antagonists of the corticotropin-releasing factor receptor (CRFR): development of CRFR2beta-selective antisauvagine-30. *Proc. Natl. Acad. Sci. USA* 95:15264–69
193. Brauns O, Liepold T, Radulovic J, Spiess J. 2001. Pharmacological and chemical properties of astressin, antisauvagine-30 and alpha-helCRF: significance for behavioral experiments. *Neuropharmacology* 41:507–16
194. Takahashi LK, Ho SP, Livanov V, Graciani N, Arneric SP. 2001. Antagonism of CRF(2) receptors produces anxiolytic behavior in animal models of anxiety. *Brain Res.* 902:135–42
195. Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, et al. 2003. Corticotropin releasing hormone type 2 receptors in the dorsal raphe nucleus mediate the behavioral consequences of uncontrollable stress. *J. Neurosci.* 23:1019–25
196. Britton KT, Lee G, Vale W, Rivier J, Koob GF. 1986. Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. *Brain Res.* 369:303–6
197. Berridge CW, Dunn AJ. 1987. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. *Horm. Behav.* 21:393–401
198. Melia KR, Duman RS. 1991. Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. *Proc. Natl. Acad. Sci. USA* 88:8382–86
199. Heinrichs SC, Cole BJ, Pich EM, Menzaghi F, Koob GF, Hauger RL. 1992. Endogenous corticotropin-releasing factor modulates feeding induced by neuropeptide Y or a tail-pinch stressor. *Peptides* 13:879–84
200. Adamec RE, McKay D. 1993. Amygdala kindling, anxiety, and corticotrophin

releasing factor (CRF). *Physiol. Behav.* 54:423–31
201. Koob GF, Heinrichs SC, Pich EM, Menzaghi F, Baldwin H, et al. 1993. The role of corticotropin-releasing factor in behavioural responses to stress. *Ciba Found. Symp.* 172:277–89
202. Rassnick S, Heinrichs SC, Britton KT, Koob GF. 1993. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res.* 605:25–32
203. Koob GF. 1999. Corticotropin-releasing factor, norepinephrine, and stress. *Biol. Psychiatry* 46:1167–80
204. Nozu T, Martinez V, Rivier J, Tache Y. 1999. Peripheral urocortin delays gastric emptying: role of CRF receptor 2. *Am. J. Physiol. Gastrointest. Liver Physiol.* 276:G867–74
205. Wang L, Martinez V, Rivier JE, Tache Y. 2001. Peripheral urocortin inhibits gastric emptying and food intake in mice: differential role of CRF receptor 2. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281:R1401–10
206. Maillot C, Million M, Wei JY, Gauthier A, Tache Y. 2000. Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. *Gastroenterology* 119:1569–79
207. Brown MR, Fisher LA, Spiess J, Rivier J, Rivier C, Vale W. 1982. Comparison of the biologic actions of corticotropin-releasing factor and sauvagine. *Regul. Pept.* 4:107–14
208. Deleted in proof
209. Stenzel P, Kesterson R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP. 1995. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. *Mol. Endocrinol.* 9:637–45
210. Parkes DG, Vaughan J, Rivier J, Vale W, May CN. 1997. Cardiac inotropic actions of urocortin in conscious sheep. *Am. J. Physiol. Heart Circ. Physiol.* 272:H2115–22
211. Bale TL, Contarino A, Smith GW, Chan R, Gold LH, et al. 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat. Genet.* 24:410–14
212. Bradbury MJ, McBurnie MI, Denton DA, Lee KF, Vale WW. 2000. Modulation of urocortin-induced hypophagia and weight loss by corticotropin-releasing factor receptor 1 deficiency in mice. *Endocrinology* 141:2715–24
213. Bale TL, Giordano FJ, Hickey RP, Huang Y, Nath AK, et al. 2002. Corticotropin-releasing factor receptor 2 is a tonic suppressor of vascularization. *Proc. Natl. Acad. Sci. USA* 99:7734–39
214. Bale TL, Giordano FJ, Vale WW. 2003. A new role for corticotropin-releasing factor receptor-2: suppression of vascularization. *Trends Cardiovasc. Med.* 13:68–71
215. Okosi A, Brar BK, Chan M, D'Souza L, Smith E, et al. 1998. Expression and protective effects of urocortin in cardiac myocytes. *Neuropeptides* 32:167–71
216. Brar BK, Stephanou A, Okosi A, Lawrence KM, Knight RA, et al. 1999. CRH-like peptides protect cardiac myocytes from lethal ischaemic injury. *Mol. Cell Endocrinol.* 158:55–63
217. Brar BK, Jonassen AK, Stephanou A, Santilli G, Railson J, et al. 2000. Urocortin protects against ischemic and reperfusion injury via a MAPK-dependent pathway. *J. Biol. Chem.* 275:8508–14

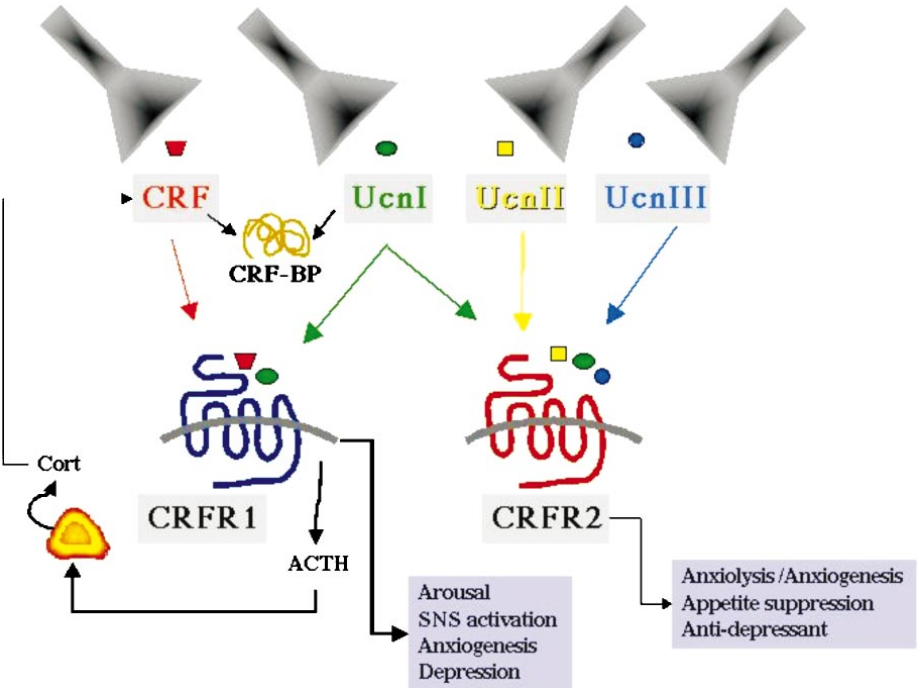

Figure 1 Summary of CRF ligand and receptor specificity and physiological and behavioral effects of receptor activation.