Corticotropin-releasing factor receptor signaling and modulation: implications for stress response and resilience

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Abstract

Introduction: In addition to their role in regulation of the hypothalamic-pituitary-adrenal-axis, corticotropin-releasing factor (CRF) and its related peptides, the urocortins, are important mediators of physiological and pathophysiological processes of the central nervous, cardiovascular, gastrointestinal, immune, endocrine, reproductive, and skin systems. Altered regulation of CRF-mediated adaptive responses to various stressful stimuli disrupts healthy function and might confer vulnerability to several disorders, including depression and anxiety.

Methodology: This narrative review was conducted through search and analysis of studies retrieved from online databases using a snowball method.

Results: This review covers aspects beginning with the discovery of CRF, CRF binding protein and their actions via interaction with CRF receptors type 1 and type 2. These are surface plasma membrane receptors, activation of which is associated with conformational changes and interaction with a variety of G-proteins and signaling pathways. We also reviewed the pharmacology and mechanisms of the receptor signaling modulatory activity of these receptors.

Conclusion: This review compiles and presents knowledge regarding the CRFergic system, including CRF related peptides, CRF binding protein, and CRF receptors, as well as some evidence that is potentially indicative of the biological roles of these entities in several physiological and pathophysiological processes.

Keywords: Corticotropin-releasing factor, corticotropin-releasing hormone, CRF, anxiety, depression, stress.

Corticotropin-releasing factor (CRF) and stress

The concept of stress is widely known in biomedical research. However, due to its popularization, the term "stress" has been used indiscriminately to define cause, processes, and responses.[1] There is a distinct temporal dynamic that characterizes the stress response: 1) a rapid, stimulatory, initial phase involving activation of the sympatho-adrenomedullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes, which is accompanied by behavioral changes resembling the 'fight or flight' response (this phase of the response increases energy expenditure and heart function, shuts down the immune system, and boosts the body to action[2-5]); and 2) a second phase, responsible for switching off this increased

activation, which is a lasting adaptation and restoration process.[2,6] The main regulatory mechanism known to take place at this time is the glucocorticoid negative feedback, which acts on the HPA-axis (Figure 1). Whenever this switch control is unbalanced, there might be a prolonged or absent activating step of the stress
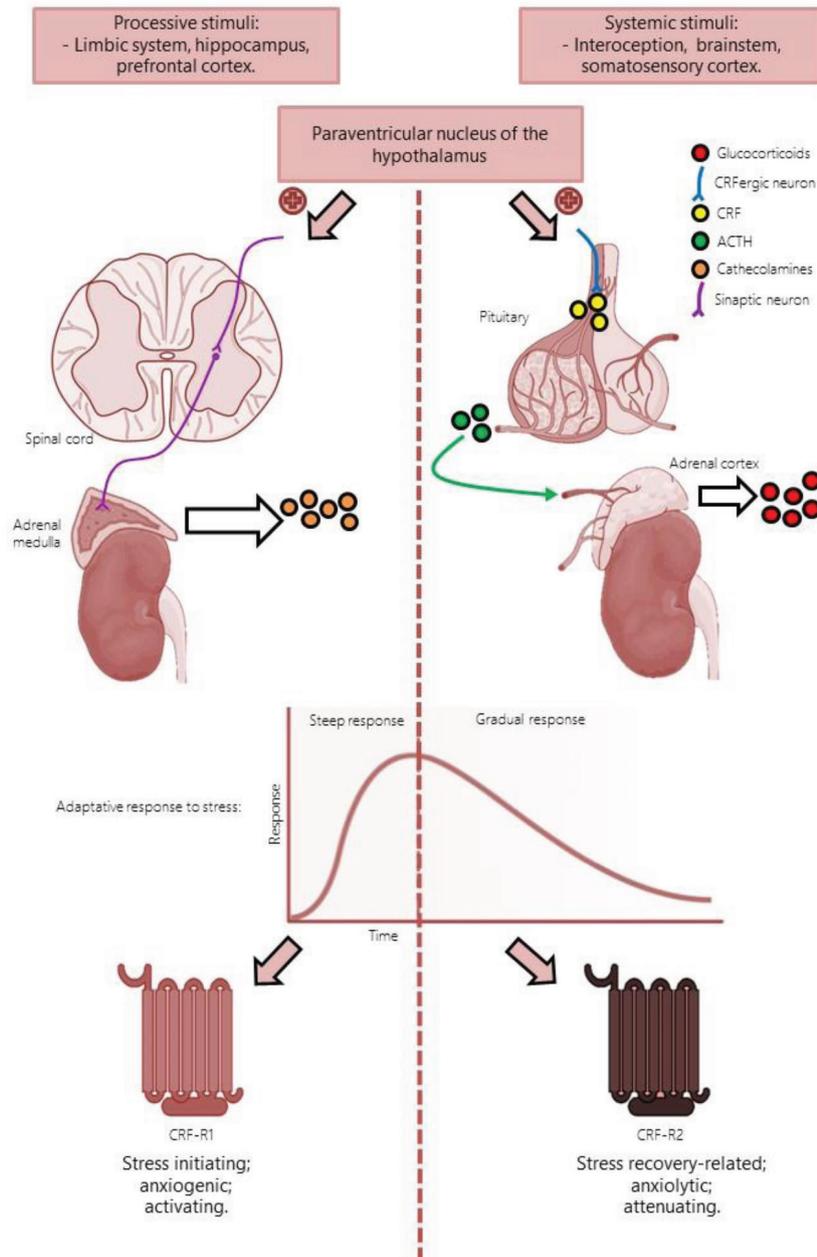

Figure 1 - Both emotional and systemic stimuli have the capacity to induce a stress response in the paraventricular nucleus of the hypothalamus. A rapid response occurs through the SAM axis, culminating in release of catecholamines from the adrenal medulla. The second response is usually gradual, since the final product is released minutes after the presence of a stressor. From the paraventricular nucleus, CRFergic neurons projecting to the pituitary gland release CRF, stimulating production and release of ACTH in the bloodstream. Once it reaches the adrenal cortex, ACTH stimulates production and release of glucocorticoids. CRF receptors have different expression patterns and roles in response to stress. CRFR1 participates in the initial phases of the stress response, at a faster and more intense rate when compared to CRFR2, triggering anxiety behaviors. In contrast, CRFR2 activity is mainly involved in a later phase, opposing the activating role of CRFR1, thereby generating a stress-attenuating response. ACTH = adrenocorticotropic hormone; CRF = corticotropin-releasing factor; CRFR1 = corticotropin-releasing factor receptor type 1; CRFR2 = corticotropin-releasing factor receptor type 2; HPA = hypothalamus-pituitary-adrenal; SAM = sympatho-adrenomedullary.

response leading to maladaptive consequences and potential harm.[7] In the case of persistent release of glucocorticoids, the resources to maintain the internal physiology (i.e. "allostasis") are overused and a diverse range of different kinds of damage affect several body systems, especially in vulnerable individuals.[8]

Beyond the sympathetic and HPA axis components (i.e. neurotransmitters and hormones), several other molecules are involved in the adaptive stress response.[9] CRF has emerged as a central molecule capable of integrating and orchestrating such responses, adapted to mastering challenging situations. In this sense, CRF was rapidly identified as the initiator of both neuroendocrine and sympathetic stress responses.[10-12] Moreover, CRF is also recognized as the link between stress and psychiatric disorders, since exposure to major stressful events or recurrent exposure to mild stressors are risk factors for development of adverse conditions such as depression, anxiety, and substance abuse disorders.[7,13,14] CRF was termed "corticotropin-releasing factor" (also referred as "corticotropin-releasing hormone") because of its ability to stimulate release of the adrenocorticotropic hormone (ACTH) from the pituitary.[10] However, this neuropeptide also has additional functions beyond the single one described in its name: CRF acts as a hormone and a neuromodulator in multiple brain areas, interacting with distinct neurotransmitter systems.[15,16] More than thirty years on from its discovery, extensive efforts have been made to uncover the physiological and pathophysiological significance of CRF. Dysregulation of the HPA-axis and CRF signaling has been associated with the etiology of many human diseases that seem to be related to exposure to stress and maladaptive stress responses (e.g. depression, anxiety, anorexia, obesity, inflammatory diseases, and Alzheimer's disease).[17-21]

The family of CRF receptors and their ligands

The CRFergic system comprises a family of molecules whose members include CRF, urocortin (UCN), corticotropin-releasing factor receptor type 1 (CRFR1), corticotropin-releasing factor receptor type 2 (CRFR2), and the corticotropin-releasing factor binding protein (CRFBP).[22-25] Both CRF and CRF-like molecules bind to these CRF receptors (CRFRs). The first CRF receptor discovered, CRFR1, was initially identified in rats, mice, and humans in 1993.[26-28] The second CRF receptor, CRFR2, was cloned and identified as an alternative spliced form of CRFR1.[29,30] However, the numbers of functional

CRFRs and differential splicing subtypes vary depending on the body tissues and taxa involved. The importance of the CRFergic system ranges from its involvement in the accelerated metamorphosis in response to pond-drying in some amphibians to intrauterine fetal stress syndromes in humans.[31,32] CRF and UCNs may share a common ancestral origin in a precursor peptide related to helping organisms deal with environmental changes during their developing stages.[33] This significance can, in part, explain how this system presents such a wide range of actions over multiple physiological functions.[34]

The role of CRFR1, along with CRF, in modulation of HPA axis activity, as well as in stress-induced behavior and cognitive alterations, has been extensively studied. Some thought was given to the therapeutic potential of CRFR antagonists to treat stress-related psychiatric disorders. In the first decade of the current century, several preclinical experiments and clinical trials were conducted for a wide range of stress-related conditions. Unfortunately, many candidate-drugs, designed as CRFR1 antagonists, have failed in double-blind, placebo-controlled trials.[35] Table 1 shows a list of registered clinical trials designed to evaluate the effects of CRFergic drugs under various clinical conditions. It is worth noting that few of these studies were registered for treatment of psychiatric conditions. Some studies resulted in drug withdrawal, while others remain to ascertain drug safety as aids in diagnosis rather than treatment of conditions.

Due to the high prevalence of studies focusing on CRFR1, the specific roles of CRFR2, CRFBP, and UCNs remain to be established. Although there is no consensus yet, it is presumed that CRF interacts with CRFR1 to mediate the initial reaction to stress, whereas UCNs and CRFR2 interactions are thought to be related to modulation of a post-stressor presentation phase[36] (Figure 1). This review aims to clarify CRF signaling, specifically with relation to CRFRs and their binding protein, and how the mechanisms that underlie the cellular responses to CRF and UCNs might be implicated in responses to stress. Briefly, we review data from pharmacology and behavioral neuroscience that are most closely associated with this topic.

It has been described that the CRF peptide is expressed in the paraventricular nucleus of the hypothalamus, in the central nucleus of the amygdala, and in hindbrain regions of the central nervous system (CNS). In the periphery, CRF has been found expressed in the gut, skin, and adrenal glands.[15] Meanwhile, the UCNs exhibit differing degrees of structural homology to the CRF protein and homology between regions where they are expressed. There are three of these CRF-like peptides, often named urocortins: stresscopin-related

peptide-I and -II (or UCN-I and UCN-II, respectively), and stresscopin (or UCN-III).[34] Each UCN presents a specific pattern of distribution in different brain structures. Several terminal fields of UCN-III fibers are expressed in regions with high levels of CRFR2, suggesting that UCN-III is an endogenous ligand for CRFR2.[34,37] Apparently, the affinity between ligands and receptors obeys a certain pattern that resembles their brain region distribution, which is a possible explanation for part of their biological significance. CRF has a tenfold greater affinity for CRFR1 than for CRFR2. Thus, CRF and UCN-I would be unselective agonists for CRFR1, whereas UCN-II and UCN-III appear to be selective for CRFR2.[15,34] CRF cognate receptors exhibit distinct distribution patterns in the CNS and peripheral tissues. In general, CRFR1 represents the major CRF receptor in the brain, while CRFR2 is predominantly expressed outside the CNS.[38] It seems that CRFR1 has only one known functional splice variant, the CRFR1α, which is expressed in the brain.[34] The CRFR1β isoform and its subtypes are originated by differential splicing of the CRFR1 gene.[15] They have been detected in human and rodent tissues, although several of these isoforms have been shown to be nonfunctional.[15] In mammals, three known CRFR2 splicing variants are found: CRFR2α and CRFR2β are expressed in both humans and rodents, while CRFR2γ has only been isolated in human limbic regions.[34,39]

CRFR1 is expressed in the cerebral cortex, cerebellum, olfactory bulb, medial septum, hippocampus, amygdala, pituitary, and peripheral tissues.[40] Studies using genetic knockout mice shed some light on the importance of this CRF receptor subtype. CRFR1-deficient mice display decreased levels of behaviors related to anxiety and dysfunctional HPA-axis response.[41] In regard to CRFR2 distribution and function, it is highly expressed in the lateral septum, medial and posterior bed nucleus of the stria terminalis, ventral medial hypothalamic nuclei, olfactory bulb, dorsal raphe nuclei, and peripheral tissues.[40] Alternatively, compared to CRFR1-deficient mice, CRFR2-deficient animals display increased levels of behaviors related to anxiety, with accelerated HPA-axis response and impaired coping behaviors.[42,43] Pharmacological manipulation of CRFR2 yielded conflicting evidence about its role in anxiety related behaviors.[15] In this sense, the role of CRFR2 in the endocrine response to stress is still under debate, with attempts to determine its biological function pointing to a prominent behavioral significance (i.e. anxiolytic or reducing arousal), probably mediated by its activation in other brain structures apart from the HPA-axis components.[44]

Table 1 - Drugs and respective CRF receptor targets registered for clinical trials*

Drug (target)	Trial for	Status	Study results
CT38 (CRFR2 antagonist)	Myalgic encephalomyelitis/chronic fatigue syndrome	Completed	No results available
Emicerfont - GW876008 (CRFR1 antagonist)	Irritable bowel syndrome	Two completed trials	Results not yet publicly available
Pexacerfont - BMS-562086 (CRFR1 antagonist)	Alcoholism and anxiety	Completed	Results available
Verucerfont - NBI-77860 - GSK561679 (CRFR1 antagonist)	Congenital adrenal hyperplasia	Withdrawn	Study was withdrawn before participants were enrolled
	Alcoholism	Completed	Results available
	Posttraumatic stress disorders	Completed	Results available
Xerecept - Corticorelin - Achtrel (hCRF - non selective agonist)	Child functional dyspepsia	Withdrawn	Withdrawn (funding not obtained)
	Pituitary neoplasm/Cushing's disease	Completed	No results available
	Cocaine dependence	Completed	No results available
	Child brain edema/brain tumor	Terminated	No results available
	Brain edema/brain tumor	One terminated trial, one withdrawn, and two completed trials	Some results, not all results available

* Registered on ClinicalTrials.gov.
CRF = corticotropin-releasing factor; CRFR1 = corticotropin-releasing factor type receptor 1; CRFR2 = corticotropin-releasing factor receptor type 2; hCRF = human corticotropin releasing factor.

Corticotropin-releasing factor binding protein is a 37 kDa protein expressed predominantly in the brainstem, amygdala, bed nucleus of the stria terminalis, ventral premammillary and dorsomedial nuclei of the hypothalamus, and cerebral cortex.[45,46] It binds to CRF with an affinity equal to or greater than the CRFRs, which led researchers to consider it as an important modulator of CRF and its receptors.[47] Clinical and preclinical studies have demonstrated the importance of CRFBP in stress-related psychiatric illness, including anxiety, depression, and substance abuse disorders.[48-51] We recently demonstrated that stress-impaired social behavior is recovered by a CRFBP antagonist, CRF_{6-33}, microinjected into the bed nucleus of the stria terminalis.[52]

Unfortunately, CRFBP has received less attention than CRF and its receptors. Multiple hypotheses have been proposed to explain the biological significance of CRFBP.[53] Along with its initial characterization, it has been proposed that CRFBP is a CRF-sequestering molecule, promoting antagonism of CRFR1. This hypothesis was based on experiments that demonstrated recombinant CRFBP interfering with the binding of CRF to a CRF antibody.[54,55] Other studies proposed a potential facilitating role for CRFBP in CRFR2 function: this second hypothesis was largely based on studies conducted in the ventral tegmental area.[48,56,57] Additionally, some studies suggest that CRFBP may have signaling properties independent of CRFRs[58] or may act as an escort protein to traffic CRFR2α from organelles to the cell surface.[59] It seems that the role and mechanism of action of CRFBP vary depending on several factors, including brain region, cell type, and CRF receptor subtype.

Still, it remains to be determined whether abnormalities in expression of CRFRs or CRFBP contribute to the pathogenesis of diseases, or whether it is a trait secondary to disease onset and initiation of stress. To date, no abnormalities in the expression or function of these receptors and binding protein have been directly observed in human diseases.[34]

CRF as a neuromodulator, hormone, and neuropeptide

CRF is considered a neuromodulator rather than a neurotransmitter, since concentrations higher than 1 mM are required to induce direct depolarization of neurons. Thus, in the physiological nanomolar range (up to 250 nM), low CRF concentrations are not sufficient to disturb neuronal resting states.[11] The neuromodulatory effects of CRF seem to be related to excitatory transmission, neuronal plasticity, and consequent synaptic efficacy. In cortical layers, such as in the olfactory bulb, CRFergic

projections innervate new neurons to stabilize and promote new synapses, thus consolidating the shaping of new circuits.[60] In the hippocampus, CRF induces spontaneous action potentials while it also shortens after-hyperpolarization; these effects are linked to the facilitation of signal propagation.[61,62] Microinjection of CRF into the hippocampus improves context-dependent fear conditioning, and this effect can be prevented by CRFR1 antagonism or protein kinase inhibition (using Astressin and KN-62, respectively).[63] Knowledge about CRF-induced plasticity effects extends beyond the hippocampus, including brain regions such as the amygdala,[64,65] lateral septum,[66] bed nucleus of the stria terminalis,[67] and brainstem.[56,68] In the locus coeruleus and ventral tegmental area, CRF administration leads to dose-dependent increases in excitatory noradrenergic and dopaminergic transmission.[56,68] CRFergic excitatory activity induced by stress seems to promote pruning and rearrangement of the dendritic tree.[69] Again, these effects are dose-dependent, since high levels of CRF induce retraction of thin spines, preventing their remodeling, with consequent harm to learning and memory formation.[70]

The excitatory properties of CRF play a pivotal role in neurodevelopment. Activation of CRFR1 promotes neuronal differentiation and projection elongation in cultured hippocampal cells.[71] Accordingly, CRF provides protection for neural stem cells against excessive glucocorticoid of maternal origin, while deletion of the CRF gene results in compromised proliferation and enhanced apoptosis during neurogenesis.[72] In later developmental stages, the CRF function appears to be quite the opposite in response to stress. For instance, CRFR1 activation mediates stress-induced loss of apical dendritic spines in the hippocampus with associated impairments of spatial memory.[73] Whereas, CRFR2 UCN-II-activation induces nerve growth factor production in astrocytes, promoting synapse formation in cultured hippocampal cells.[74] In terms of a broader CRFergic circuit effect, there are few studies extending these findings to other brain structures. Nevertheless, it was demonstrated that exposure to stressors or stress-induced c-Fos activation in the nucleus incertus impaired long-term potentiation in a hippocampal-medial prefrontal cortex circuit.[75] Opposite effects can be observed in studies focusing on regions such as the amygdala and bed nucleus of the stria terminalis.[65,76,77] Chronic exposure to stressors or administration of UCNs seem to reinforce long-term potentiation and excitability in the basolateral amygdala.[78,79] Although contrasting, these plasticity effects could be abolished by administration of CRFR1 antagonists (e.g. Antalarmin and NBI27914).[75,78]

In the HPA-axis, CRF activates CRFR1 on pituitary corticotropes to stimulate release of ACTH, which activates receptors in the adrenal gland cortex to stimulate synthesis and release of glucocorticoids.[36] This response is shut down by a negative-feedback system: CRF production and release from the hypothalamus is inhibited, ceasing the stress response.[25,80] Aside from induction of expression of proopiomelanocortin (POMC) genes and release of β-endorphins, activation of CRFR1 under stressful conditions induces upregulation of expression of the CRFR1 gene in the hypothalamus, hippocampus, and prefrontal cortex[81] (Figure 2). More detailed information of the role of CRF in the HPA-axis is given in subsequent subsections in this review.

Since hyperactivation of the neuroimmune system was recognized as a powerful element in development of neuropsychiatric disorders,[82,83] CRF has also been implicated as one of the modulators of the intricate association between the immune system, stress, and mood disorders.[84] Beyond neurotransmission, CRF signals to neighboring glial cells affecting inflammatory responses. Activated microglial cells express high levels of CRFR1[85] and therefore CRF can stimulate microglia to release signal molecules (e.g. cytokines, chemokines) that influence brain function and disease states.[85,86] In turn, cytokines exert rapid effects over the paraventricular nucleus, stimulating CRF release and promoting the stress response.[87-89] Other studies have found evidence of the role of CRF in the immune system through regulation of the nuclear factor kappa B (NF-κB) pathway. For instance, CRF-induced upregulation of this transcription factor also promotes pituitary POMC gene expression,[90] while its inhibition results in CRF-mediated neuroprotection.[91] In sum, CRF appears to have several modes of action: 1) as a hormone, orchestrating the neuroendocrine response to stress; 2) as a neuromodulator, potentiating signal transduction and inducing arousal-like responses during the stress experience; and 3) performing other functions like immunosuppression (via glucocorticoid secretion) or boosting inflammation (via direct actions on glial cells).

Mechanisms of CRF receptor signaling

The CRFRs belong to the family of G protein-coupled receptors (GPCRs), also known as seven-transmembrane domain receptors.[92] The CRFRs are part of the class B or secretin receptor family of GPCRs, a small family of receptors that are activated by peptide ligands.[93] Therefore, CRFR1 and CRFR2 are responsible for detecting molecules outside the cell (in this case,

CRF and CRF-like molecules), then activating internal signal transduction pathways and, ultimately, cellular responses. Coupling of CRFRs and G proteins helps to stabilize the receptor and confers an active state of high ligand affinity.[94] The initial studies of the effects of CRF inducing pituitary ACTH release demonstrated that this response takes place through activation of Gαs/cyclic adenosine monophosphate (cAMP).[95] Both CRFRs act to activate primarily adenylyl/cyclase and cAMP signaling pathways,[27,29] through activation of Gαs.[34] However, other studies also demonstrated that CRFRs are highly promiscuous (in fact several GPCRs seem to be) and can activate other types of Gα besides Gαs.[11] It seems that the patterns of activation of signaling pathways are unique to each tissue where the CRFRs are expressed. The mechanisms underlying this diversity are not well understood. It is possible that even the type of agonist bound to the CRFR might be involved in receptor/G-protein coupling selectivity.

Activation of the cAMP/protein kinase A (PKA) signaling pathway engenders both cytoplasmic events (acute post translational changes in target proteins) and nuclear events (regulation of genetic transcription through cAMP response element-binding [CREB] proteins).[96,97] In the HPA-axis, activation of the cAMP pathway via CRF induces expression of Nur77 and Nurr1 messenger ribonucleic acids (mRNAs), as well as transcription of the NurRE site, leading to transcriptional activation of the POMC gene.[98] Moreover, specific activation of cAMP via CRFR1 induces transcription of several responsive genes related to attenuation of CRF signaling properties that constitute several mechanisms that promote the HPA-axis negative feedback.[99] Other effects that have been identified as activated by CRFR1 via CRF and UCN-I binding include expression of several enzymes belonging to the cortisol synthesis pathway.[100] As previously mentioned, CRF signaling also promotes self-upregulation of CRFR1 gene transcription[99,100] (Figure 2).

There are also cAMP-independent signaling pathways activated by CRFRs. MAPK/ERK cascades can be activated by CRFR1 and CRFR2 when activated by specific agonists.[34] These enzymes constitute the pathways that mediate physiological functions of CRF and CRFRs, such as cardioprotection against injury-induced hypoperfusion,[101] behavioral and memory adaptation to stress,[102] neuroprotection,[103] vasodilatation,[104] and smooth muscle contractility.[105] ERK and p38 MAPK can also participate in transcription of Nur factors and consequent induction of POMC secretion[106] (Figure 2). Additionally, it has been demonstrated that CRFRs can also signal through induction of protein kinase C (PKC), protein kinase B (PKB/ATK), important second

messengers such as Ca^{2+}, nitric oxide synthase, guanylyl cyclase, prostaglandins, Fas, and Fas-ligand.[106-111] It is reasonable to think that the vast range of signaling modes built-in to the CRFergic system demonstrate its importance, specially mediating the CRF biological effects throughout several biological tissues.

Regulatory mechanisms of CRF receptor signaling

Knowledge of the cellular mechanisms that regulate the effects of CRF and CRF-like molecules on their cell-targets is still unsatisfactory. However, GPCR-mediated signaling can rapidly be attenuated by protein kinase phosphorylation and consequent interaction with arrestins. This interaction leads to receptor desensitization, G-protein uncoupling and subsequent receptor internalization.[112,113] Moreover, studies have demonstrated that exposure to high levels of CRF desensitizes the CRFR1 signaling response.[55,114-116] The coupling of a GPCR with an arrestin initiates two processes: 1) receptor membrane uncoupling and 2) internalization. Both of these processes are involved in cell desensitization and normalization of

cell responses. Possibly, in the case of CRF, once an agonist binds to a receptor, a β-arrestin is translocated from the cytosol to the plasma membrane close to CRFR1 to conduct receptor internalization.[117] There is some evidence suggesting that the process occurs with the involvement of clathrin-coated vesicles.[117,118] This receptor internalization mechanism is not completely understood for CRFR1 and a β-arrestin-independent mechanism has also been proposed.[117] It is highly probable that after internalization, CRFRs are trapped into lysosomes for degradation or stored in intracellular compartments, being available for future redirection to the membrane.[119]

The activity of CRFRs is susceptible to intracellular mechanisms that rapidly attenuate signaling output and prevent cell overstimulation. Efforts to unravel the plethora of CRFR biological actions are recent, and what can be inferred is related to what is known about the GPCR family. Usually, such processes of signaling cessation begin with receptor phosphorylation by second messenger-activated protein kinases (PKs), followed by recruitment of arrestin family proteins, leading to receptor desensitization.[94] The main PKs identified as regulators of CRFR1 phosphorylation and desensitization is the family of G protein-coupled receptor kinases
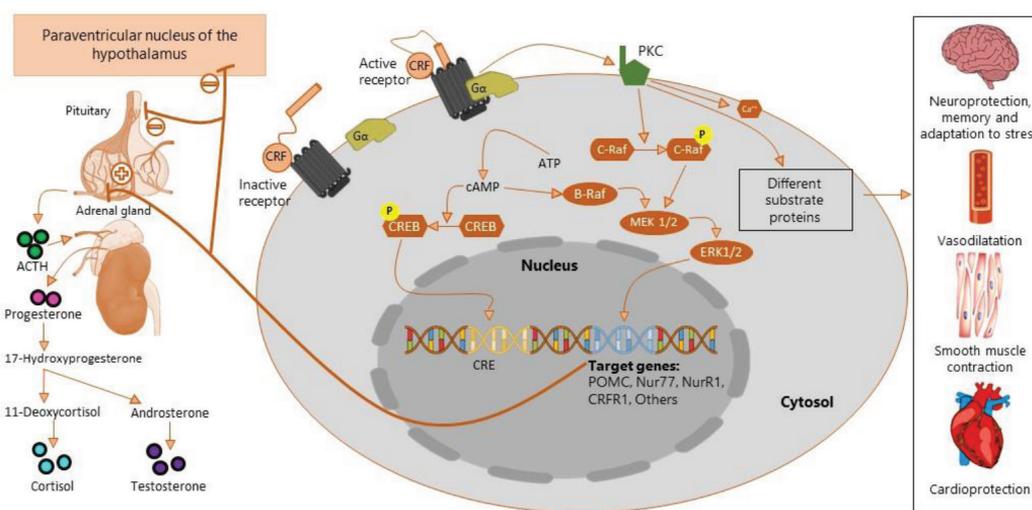

Figure 2 - This figure depicts alternate signaling pathways activated after agonist binding to CRFR1 and CRFR2. When CRF or UCN binds to the receptor, the N-terminal segment integrates the receptor transmembrane domains. This process enables activation of the receptor and binding with G-proteins. This complex catalyzes conversion from ATP to cAMP, which is involved in both activation of the mitogen-activated protein kinase/extracellular-regulated kinase (MAPK/ERK) pathways and in phosphorylation of CREB transcription factor. Both pathways will ultimately lead to activation of transcription of target genes (POMC, Nur77, NurR1, CRFR1, and others) and transcription of the CRE gene respectively. POMC leads to release of ACTH by the pituitary gland. This process stimulates conversion of progesterone into cortisol. Activation of PKC (Inositol triphosphate-PKC) leads to phosphorylation of C-Raf protein, which is also involved in activation of transcription of target genes. Intracellular Ca^{2+} release and other different substrate proteins are also activated by PKC, triggering another set of CRF physiological responses in the CNS, cardiovascular system, and muscular system. ACTH = adrenocorticotropic hormone; ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; CNS = central nervous system; CRE = cAMP response element; CREB = cAMP response-element binding protein; CRF = corticotropin-releasing factor; CRFR1 = corticotropin-releasing factor receptor type 1; CRFR2 = corticotropin-releasing factor receptor type 2; PKC = protein kinase C; POMC = proopiomelanocortin; UCN: urocortin.

(GRK).[116] Once a GPCR has been phosphorylated by a GRK, it binds with high affinity to β-arrestins. This might result in uncoupling of the receptor to the G-protein with subsequent receptor inactivation. Alternatively, the β-arrestins serve as an adaptor protein to couple the receptor to clathrin-coated pits with subsequent receptor endocytosis. This process of endocytosis culminates in either sequestration or degradation of the receptor and the final outcome appears to be dependent on the cell type, the concentration of the agonist, and the duration of the presence of these ligands at the binding site.[120]

Recently, other proteins that can regulate CRFRs have been discovered: these are called GPCR-interaction proteins (GIP).[11] One example of a GIP seems to be CRFBP itself, a protein capable of acting in different ways with respect to CRF and UCNs and their interactions with CRFRs.[121,122] The second class of molecules that interact with the CRFergic system components are the receptor activity modifying proteins (RAMP).[123] For instance, the interaction between CRFR1 and receptor activity modifying proteins 2 (RAMP-2) promotes their expression on the cell surface, modulates G protein coupling to CRFR1, and ultimately regulates the receptor signaling and desensitization processes.[124] It has been demonstrated that CRFRs, as well as other GPCRs, form dimers.[125] Homodimerization is mainly reported for CRFR1,[126,127] while heterodimerization between CRFR1 and CRFR2 is a mechanism that increases CRFR2 cell surface expression.[128] As will be discussed in the next subsection, this is an important process during the entire stress response. Finally, CRFR1 can form heterodimers with other classes of GPCRs (e.g. orexin[129] and vasopressin[130]). These abilities of CRFRs to form dimers along with interaction with GIPs extend the diversity of their functioning and regulatory mechanisms.[11] Malfunctions in these modulatory processes are often linked to neurological disturbances and psychiatric disorders. Dysfunctional CRF activity may underlie anxiety-like responses by G-protein-coupled CRF receptor signaling.[131] Exploring the activation of metabotropic receptors and the modulatory role of their second messenger system could be ideal for understanding pathological states and may also shed light on the biological basis of vulnerability and resilience to stress.

CRF signaling and individual reactivity to stress

Although activation of the stress response is critical for survival, rapid counterregulation of this response is equally important for reestablishing normal functioning and emotional state after a threat subsides. Not all individuals experiencing stress develop depressive or anxiety states, which suggests that the response to stress is, to a significant extent, determined by individual vulnerability or resilience. "Resilience" can be described as a function determined by thresholds that characterize the boundary at which internal or external disturbances activate the stress response and a second threshold related to how quickly and effectively these stress responses can be shut down in response to disappearance of the stress event.[131]

It is possible that the *modus operandi* of the CRFergic system in a particular individual influences that individual's ability to cope with a stressor. This might be true, since CRF signaling is directly linked to initiation and to cessation of the stress response (Figure 1). Clinical and preclinical studies suggest that persistent dysfunctions of central CRF neurotransmission, possibly engendered by CRF hypersecretion or CRF receptor dynamics, contribute to the etiology of anxiety, depression-related illnesses and stress adjustment disorders. Genetic abnormalities, exposure to stress during developmental stages, exposure to traumatic events, or unpredictable stress at any stage can modulate an individual's resilience to stress.[131] In this sense, adversity in life is expected to modulate coping responses as well as how the CRFergic system is activated in response to a stressor. Several studies highlight the relationship between adopting a passive strategy to cope with stress and a consequent association with vulnerability to development of affective disorders in humans.[132,133] In experimental social stress paradigms, submissive individuals tend to develop a depressive-like endocrine and behavioral profile. In contrast, a proactive strategy is associated with resilience in both basic and clinical research settings.[134,135] Chronic administration of a CRFR1 antagonist (NBI-30775) promoted changes in the behavioral response to stress in the rat resident-intruder social defeat model, inducing shifts in coping strategies from a passive to an active style.[136] In animal models, NBI-30775 administration also prevents depressive-like symptoms induced by exposure to repeated social stress, represented by decreased immobility in the forced swim test and adrenal hypertrophy.[136] Later, these effects were reproduced with additional characterization of CRFRs ultrastructural distribution.[134] Understanding the cellular adaptations that enable active coping responses to stress may provide critical insight into mechanisms that promote resilience.

CRF signaling is involved in the modulating process of monoaminergic activity implicated in affective states. Within the dorsal raphe nuclei (DRN), CRF has opposing

effects on serotonergic neuronal activity, a dual CRF action that is influenced by prior exposure to stress. For example, a single exposure to forced swim stress qualitatively shifts the rat serotonergic DRN response to CRF from inhibition to excitation.[137] Repeated exposure to social stress also promotes dynamic changes in this neuronal subpopulation in rats that adopt an active coping strategy.[134] Furthermore, a cellular redistribution of CRFRs underlies this effect such that CRFR1 became internalized, while CRFR2 was recruited to the plasma membrane, leading to a shift in the types of response of serotonergic neurons to CRF, from inhibition to a CRFR2-mediated excitation.[134] Thus, CRFR1 internalization in response to stressful threatening stimuli seems to be related to development of resilience, inducing cessation of the stress response at the appropriate time, while recruitment of CRFR2 resumes the stress experience, at least in the affective state.

Unfortunately, preclinical studies that address the role of CRFR2 in stress and anxiety did not attain a reliable level of certainty. As mentioned earlier, pharmacological manipulation of CRFR2 produces conflicting results regarding anxiety-like behaviors and stress responses in animal models. Some authors suggest that the behavioral results produced by manipulation of CRFR2 can be largely influenced by cell type, neuroanatomic substrate, and peculiarities of the experimental procedures applied to obtain the evidence.[131] It is possible that CRFR2 activation counteracts the anxiety effects caused by CRFR1 activation, but in order to observe CRFR2 effects it would be necessary to achieve better control of the response time and even an initial CRFR1 activation.

Conclusion

The CRF family's involvement with the ability to cope with a stressor might be founded on a simplistic dual process of CRFR functioning. CRFR1 is thought to be the subtype through which CRF primarily initiates its HPA axis response to stress. The consequences of CRFR1 signaling amplify the stress response throughout the brain and trigger their own negative feedback. CRFR1 activation in the forebrain, hippocampus, and/or amygdala is linked to expression of anxiety-like behaviors, indicating that CRFR1 signaling might be required to initiate associated defensive responses interpreted as protective anxiety behaviors. Complementarily, a restorative process of the stress response has been attributed to UCNs, mainly UCN-II and III, that act as agonists of CRFR2.[22,24] CRFR2 signaling might be involved in reduction of CRFR1-induced activation, reduction of anxiety responses, and

restoration of cellular processes. The physiological state in which an individual can manage these two responses seems to be linked to that individual's ability to maintain health in adversity.

The evidence compiled by reviewing the literature in the present study demonstrates the ability of CRF to activate the stress response, constituting an initial defense of homeostasis.[10] Cumulative evidence supports the role of a CRFergic dysfunction in the pathogenesis of mood and stress adjustment disorders, driving the development of better strategies to discover and develop more suitable therapies for stress-related disorders.

Acknowledgements

Disclosure

No conflicts of interest declared concerning the publication of this article.

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