MINIREVIEW

Corticotropin-Releasing Factor (CRF) and Endocrine Responses to Stress: CRF Receptors, Binding Protein, and Related Peptides (44108)

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Abstract. **Corticotropin-releasing factor (CRF) is a 41-amino acid neuropeptide, which is recognized as a critical mediator of complimentary, stress-related endocrine, autonomic, and behavioral responses in mammalian species. CRF belongs to a family of structurally related peptides including frogskin sauvagine and fish urotensin I. The effects of CRF and related peptides are mediated by two distinct receptors, which differ in their anatomical distribution, as well as in their pharmacological characteristics. In addition, CRF is bound with high affinity by a CRF binding protein (CRF-BP), which is a putative inhibitor of CRF action. CRF is probably not the sole endogenous ligand for CRF receptors or the CRF-BP, since a second mammalian member of the CRF family, urocortin, has recently been identified. This article describes recent findings with respect to CRF, its receptors, binding protein, and CRF-related peptides, which provide further insights into the role and mechanisms of CRF action in stress responses.** [P.S.E.B.M. 1997, Vol 215]

Corticotropin-releasing factor (CRF) is acknowledged as the primary hypothalamic factor driving stress-induced adrenocorticotropin (ACTH) secretion from the anterior pituitary (1, 2). However, the actions and distribution of CRF and its receptors extend beyond hypophysiotropic neurosecretory neurons (3–8). Indeed, CRF is recognized as a critical neuropeptide mediator of complimentary stress-related endocrine, autonomic, and behavioral responses (7). Furthermore, the extensive distribution of CRF-like protein and its receptors, coupled with the multifaceted effects of this peptide within the immune, gastrointestinal, and cardiorespiratory systems, as well as endocrine organs (e.g., testes, ovaries, adrenal, and human placenta), suggests a role for peripheral CRF in the regulation of several stress-responsive systems. Finally, CRF has been implicated in the pathophysiology of variety of diseases associated with dysregulation of stress responses and autoimmunity (7, 9).

Until recently, major questions pertaining to the molecular identity of CRF receptors, the impact of the CRF-binding protein (CRF-BP) on responses to CRF, and speculation over the existence of alternative endogenous CRF receptor/BP ligands prevented detailed analyses of the modes and mechanisms of CRF action. The purpose of this article is to highlight recent findings in these areas and to describe how these affect our

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understanding of the mechanisms of CRF action in response to stress.

The CRF Peptide Family

CRF is a 41–amino acid, single-chain polypeptide, which has now been described in many species (including rat, human, pig, sheep, horse, goat, cow, Xenopus, and white suckerfish). Rat and human CRF have identical amino acid sequences. CRF is synthesized as a precursor polypeptide (prepro CRF) which is 187–196 amino acids long, with the mature CRF peptide sequence present near the carboxy terminus. CRF has sequence homology and shares many biological properties with two other vertebrate peptides: sauvagine, a 40–amino acid peptide isolated from the frog skin (10); and urotensin I, a 41–amino acid peptide isolated from the caudal neurosecretory system of teleost fish (11). CRF also has sequence homology to two diuretic hormones, Mas-DPI and Mas-DPII, from the tobacco hornworm, *Manduca sexta* (12, 13).

CRF and Endocrine Responses to Stress

Numerous experimental studies provide overwhelming evidence that CRF is the principal physiological regulator of pituitary ACTH secretion in many species, including humans. This concept derives from observations that (i) the primary site of CRF synthesis within the CNS is in the parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus, and CRF protein is abundant in PVN nerve terminals within the median eminence (3, 4), where it is released into the hypophyseal portal circulation (2); (ii) high-affinity CRF receptors are present in the anterior pituitary (14), where CRF stimulates the pituitary secretion of ACTH and other pro-opiomelanocortin (POMC) products (β-endorphin) (1); and (iii) passive immunoneutralization of CRF or administration of CRF receptor antagonists reduces ACTH secretion (2, 15). In addition, during stress hypothalamic CRF secretion increases, a change that is commonly accompanied by enhanced CRF mRNA expression in the PVN, and inhibition of CRF action prevents or abrogates elevations in plasma ACTH concentration (2, 16, 17).

In addition to direct actions on pituitary corticotropes, administration of CRF directly into the brain (intracerebroventricularly, i.c.v.) influences the secretion of a number of neuroendocrine hormones. It stimulates pituitary ACTH release (18), probably *via* activation of hypophysiotropic neurons (19), though it cannot be dismissed that CRF leaks into the hypophyseal portal vasculature (20). I.c.v. CRF inhibits the secretion of luteinizing hormone (LH) (21–23) and growth hormone (GH) (21, 24, 25) without acute effects on follicle-stimulating hormone, thyroid stimulating hormone, or prolactin. In contrast, peripheral administration of CRF has no significant impact on LH or GH secretion, indicating that the likely site of action of CRF in modulating these hormones is within the brain. Indeed, CRF inhibits hypothalamic gonadotropin-releasing hormone release into hypophyseal portal blood (23).

The involvement of CRF in stress-induced reductions in plasma LH and GH is demonstrated by the reversal of electrofootshock-induced hormone changes after i.c.v. pretreatment with the CRF receptor antagonist α-helical $CRF_{(9-41)}$ (26, 27). However, the role of CRF in stress-induced suppression of hypothalamo-pituitary-gonadal activity appears to be stress specific, since endogenous CRF participates in footshock-, fasting- and suckling-induced, but not cytokine-induced, inhibition of LH secretion (27–31). Despite CRF having no impact on prolactin secretion in unstressed rats, i.c.v. pretreatment with α-helical $CRF_{(9-41)}$ virtually abolishes the increases in plasma prolactin in ovariectomized, estrogen-primed female rats stressed by immobilization (32), suggesting that CRF plays a permissive role in stimulating prolactin release during stress.

The demonstration of peripheral sources of CRF raises the possibility of its involvement in stress-induced modulation of endocrine activity outside of its role within the brain and/or pituitary. CRF immunoreactivity and binding sites are present in the rat ovary (33), where it may play an autocrine role in the regulation of steroidogenesis and/or as a proinflammatory mediator during the inflammation-like phenomena of ovarian physiology, such as ovulation of luteolysis (34). CRF is also produced by the Leydig cells of the testes and acts through high-affinity receptors on Leydig cells to potently inhibit gonadotropin-induced cAMP generation and steroid production (35). Within the adrenal gland, CRF binding sites have been identified in medullary tissue (36, 37) and CRF is co-released with catecholamines following splanchnic nerve stimulation (38) or hemorrhage (39). A paracrine role of CRF in stimulating adrenocortical glucocorticoid secretion has been proposed (40, 41).

CRF Receptors

The effects of CRF are mediated by specific, cell surface receptors whose kinetics and pharmacology have been described extensively (14, 42). Radioligand binding studies utilizing [I^{125}]-labeled ovine or rat/human CRF have demonstrated CRF binding sites in the brain, the anterior and neurointermediate lobes of the pituitary, the AtT-20 mouse corticotropic cell line, and also the testes, ovaries, spleen, and small cell lung carcinoma (14, 33, 42, 43). CRF-induced secretion of POMC-derived peptides is paralleled, in a dose-related fashion, by the production of cAMP and the activation of a cAMP-dependent protein kinase, suggesting that CRF

exerts its effects *via* enzymatic pathways involving cAMP (44–47). Furthermore, CRF binding sites exhibit pharmacology consistent with G protein–coupled receptors—that is, binding of CRF is increased in the presence of divalent cations and decreased in the presence of GTP analogs (48, 49).

A number of *in vivo* studies have indicated at least two distinct pharmacological profiles of CRF-induced physiological effect. Intravenous (i.v.) CRF not only elicits pituitary ACTH secretion but also produces vascular effects, including vasodilatation, hypotension, a reduction plasma exudation from the vascular beds of injured tissues, and inhibition of the subsequent edema. However, the i.v. doses of CRF required to elicit ACTH are far lower (at least 10-fold) than those needed to produce such vascular effects (e.g., 50–53). Sauvagine, urotensin I, and CRF are approximately equipotent ACTH secretagogs in rats (54–56), but, in contrast, sauvagine and urotensin I are far more potent than CRF at inducing vasodilation and hypotension, and at inhibiting of edema (53, 57, 58). Finally, the ACTH and vascular responses to CRF differ markedly in their sensitivity to antagonism by the CRF receptor antagonist α-helical $CRF_{(9-41)}$. Indeed, complete reversal of CRF-induced hypotension or CRF inhibition of edema have been reported at antagonist (α-helical $CRF_{(9-41)}$) to agonist (CRF) ratios of around 4:1 to 6:1 (50, 51). By contrast, CRF-induced ACTH secretion is inhibited by only 50% at an α-helical $CRF_{(9-41)}$:CRF ratio as high as 200:1 *in vitro* (59) and 1000:1 *in vivo* (50).

Consistent with this anecdotal evidence, recent studies have demonstrated the existence of two related molecular species of CRF receptors, which differ from each other in anatomical distribution as well as pharmacological profile (60–71). They belong to a family of neuropeptide receptors (including receptors for vasoactive intestinal peptide, calcitonin, parathyroid hormone, glucagon, secretin, growth hormone-releasing hormone, and pituitary adenylate cyclase activating peptide), which contain seven transmembrane domains and are coupled to G proteins. CRF receptor type 1 (CRF-R1) has been cloned and characterized from a number of species, and displays 87%–98% homology among rat, human, mouse, and chicken forms (60–62, 64, 72). Identified mammalian versions are 415–amino acid proteins and possess putative glycosylation sites in the extracellular domain and potential phosphorylation sites in the intracellular loops and C-terminal tail. Variants of CRF-R1 have been reported in human and rat brain (61, 73), and also in human pituitary (60). A second, molecularly distinct CRF receptor has been identified in the rat (65), mouse (66–68), and human (74). In the rat at least, there are two known forms of the second CRF receptor (65). CRF-R2α is a 411–amino acid protein, which is expressed almost exclusively in brain (69). The alternatively spliced form, CRF-R2β, has the first 34–amino acids of R2α replaced with a 54–amino acid sequence, giving a full-length receptor of 431 amino acids (65). CRF-R2β is expressed both in the brain and in peripheral tissues (69). CRF-R2 contains similar putative extracellular N-glycosylation and transmembrane phosphorylation sites to CRF-R1, with R1 and R2 displaying around 70% sequence homology, overall.

In situ hybridization studies have described the relative distributions of CRF-R1 and R2. CRF-R1 mRNA is expressed in both the anterior and intermediate lobes of the pituitary. In the anterior pituitary, hybridization signal is clustered around a subset of cells identified as corticotropes by staining for ACTH immunoreactivity (63, 70, 71). In contrast, hybridization signal for CRF-R2 mRNA in the anterior pituitary is either undetectable (71) or detected only in scattered cells (70). Within the rat brain, CRF-R1 mRNA is present in discrete regions, including neo-, olfactory, and hippocampal cortices, cerebellum, septum, amygdala, and brainstem sensory relay structures, with only low levels being observed in thalamic and hypothalamic nuclei (63, 70, 71). Additional strong CRF-R1 expression in the reticular nucleus of the thalamus and a broader distribution in the amygdala are observed in the mouse (71). In contrast, the expression of CRF-R2 mRNA within rat or mouse brain is more confined to subcortical structures: in the lateral septal nucleus, ventromedial hypothalamic nucleus, olfactory bulb, amygdala, and the choroid plexus (70, 71). Additional hybridization signal for CRF-R2 mRNA has been observed in the bed nucleus of the stria terminalis and the nucleus of the solitary tract in one study (71), and in the PVN and supra-optic nucleus (SON) in another (70). CRF-R2 mRNA is also found within the cerebral arterioles (66, 70). CRF-R2α appears to be the predominant R2 isoform which is expressed on neuronal tissue, where R2β is localized to non-neuronal elements such as the choroid plexus and cerebral arterioles (70). CRF-R2β is also present within the periphery, in the heart, skeletal muscle, lung, epididymis, kidney, and intestine (65–69), while CRF-R1 has been detected in the skin (75) and ovary (76).

The discrete distributions of CRF-R1 and R2 imply that they subserve distinct physiological functions, a suggestion that is supported by the dissimilar pharmacologies of the two cloned receptor subtypes (Table I). A number of studies have described the binding constants and potencies of various CRF receptor ligands in cells transfected with the cDNAs of the different CRF receptors. First, CRF appears to be a less potent ligand at R2 compared with R1 (65, 68, 77). The majority of studies have described sauvagine, urotensin, and CRF as having similar binding constants or potencies at CRF-R1. In contrast, at CRF-R2, a rank order of sauvagine > urotensin > CRF is apparent (55, 65, 67, 68, 77). CRF-R2α and R2β display virtually identical pharmacologies with respect to these ligands (55, 77). Finally, the CRF

Table I. Pharmacological Characterizations of CRF Effects on Physiological Parameters and CRF Receptor Subtypes

	Effect of CRF		CRF receptor subtype	
	ACTH secretion	Vascular effects	CRF-R1	CRF-R2
Relative threshold dose of CRF to elicit response	Lower	Higher	Lower	Higher
Relative potency of CRF, sauvagine, and urotensin I	CRF = sauvagine = urotensin I	sauvagine ≥ urotensin I > CRF	CRF = sauvagine = urotensin I	sauvagine > urotensin I > CRF
α-Helical $CRF_{(9-41)}$: CRF ratio for 50% antagonism	1000:1 (*in vivo*) 200:1 (*in vitro*)	1:1 (hypotension) <4:1 (edema)	>300:1	ca. 1:1
Localization to anterior pituitary/vascular tissues	—	—	Hybridization signal clustered over corticotropes	Found in heart and vascular tissues

receptor antagonist α-helical $CRF_{(9-41)}$ is a far more effective antagonist at R2 than at R1 (68). Thus the pharmacologies and distributions of the molecular subtypes of CRF receptors appear to bear out observations made *in vivo* regarding the pharmacology of CRF responses. In particular, the data discussed above strongly suggest that CRF-R1 is the major receptor isoform regulating pituitary ACTH secretion. Indeed, treatment of rat anterior pituitary cells within a CRF-R1 antisense oligonucleotide blunts CRF binding and CRF-induced ACTH secretion (78). In contrast, the receptor pharmacologies and anatomical distributions imply that CRF's effects on vasculature (e.g., vasodilatation, hypotension, and inhibition of injury-induced edema) are mediated by CRF-R2. Furthermore, the relative potencies of sauvagine, urotensin I, and CRF are suggestive of CRF-R2 mediated events in several other instances. For example, a rank order of sauvagine ≥ urotensin I > CRF is apparent in the induction of hypertension and elevation in plasma catecholamines after injection of peptides i.c.v. (53), and in producing increases in plasma glucose concentration (53, 79), inhibition of gastric emptying (80), decreased gastric acid secretion (80, 81), and reduced feeding (80, 82) after either i.c.v. or peripheral peptide administration.

Studies investigating the regulation of the expression of CRF receptor subtype have thus far been restricted to CRF-R1. CRF-R1 mRNA levels are decreased in anterior pituitary cells in culture following treatment with CRF or glucocorticoids (83, 84) or *in vivo* after glucocorticoid treatment (85), acute or repeated immobilization stress (86), or endotoxin treatment (87). While barely detectable in the hypothalamic PVN of control, unstressed rats, CRF-R1 mRNA is markedly upregulated in this nucleus by systemic endotoxin treatment (88, 89), immobilization stress (86, 88, 90, 91), or the central injection of CRF (92). Similar upregulation of CRF-R1 mRNA by stressful stimuli has been noted in the SON, but CRF-R1 mRNA in extrahypothalamic regions of the brain appears to be unaffected by these manipulations (86, 88, 90, 91). The observation that CRF-R1 mRNA is dramatically upregulated by stress/CRF in the PVN and SON suggests, though does not prove, that receptor protein levels in this nucleus are increased during stress. In this regard, the demonstration of increased binding of radiolabeled CRF in this region (90) following stress lends support to this suggestion. To what extent increased expression of CRF-R1 in the hypothalamus contributes to responses to stress is presently unknown.

CRF-Binding Protein

In addition to interacting with its cognate receptors, CRF is bound with high affinity by a CRF-binding protein, CRF-BP, which inhibits the biological activity of CRF in a number of *in vitro* assay systems. CRF-BP was originally identified, isolated, and purified from human plasma, and is thought to inhibit the remote actions (particularly pituitary ACTH secretion) of CRF produced from the placenta of women in the third trimester of pregnancy (93–98).

Human and rat CRF-BP cDNAs display a high degree of sequence homology and encode a precursor protein 322 amino acids in length, with one putative glycosylation site and 11 conserved cysteine residues (99). Recently, highly homologous cDNAs encoding mouse (100) and sheep (101) CRF-BP have also been cloned. CRF-BP has been localized to human liver (the likely source in blood), brain, and placenta (99, 102, 103), and to the brain and pituitary of rats and mice (104). The absence of CRF-BP in nonprimate blood presumably reflects the lack of its synthesis in the liver. CRF-BP in brain appears to be membrane associated, as indicated by its solubilization profiles and its partitioning upon sucrose gradient purification (96). However, there is no evidence of a receptor function for CRF-BP, since unlike the identified CRF receptors, it

lacks obvious transmembrane domains or intracellular signaling motifs in its sequence.

Within the rat brain, CRF-BP mRNA and protein is expressed predominantly in the cerebral cortex, including all major archi-, paleo-, and neocortical fields (104). Similarly, CRF-BP immunoreactivity is present in several regions of the human cerebral cortex (103). Other prominent CRF-BP expression sites in the rat are subcortical limbic structures such as the amygdala, the bed nucleus of the stria terminalis, raphe nuclei, brainstem reticular formation, and olfactory, auditory, trigeminal, and vestibular sensory relay systems (104). Hypothalamic CRF-BP expression is limited to the ventral premammilary and dorso-medial nuclei. CRF-BP is synthesized in, and secreted by, both neurons and astrocytes (105). While there is substantial, but incomplete, regional overlap in CNS distributions, there are only a few instances of cellular co-localization of CRF-BP with either CRF or CRF-R1 (104). Within the anterior pituitary CRF-BP is expressed almost exclusively within corticotropes, with the majority of ACTH immunopositive cells showing CRF-BP mRNA hybridization signal (104). CRF-BP mRNA expression within the anterior pituitary is inhibited by stress and adrenalectomy (106).

The exact function of CRF-BP under normal or pathological conditions remains uncertain. Present evidence indicates that CRF-BP is an endogenous inhibitor of CRF action by acting as a CRF "sink" and/or that it plays a role in CRF clearance or degradation. Human CRF-BP binds r/h CRF with high affinity. Indeed the affinity of CRF-BP for r/h CRF is at least as high as that of CRF-R1 and greater than that for CRF-R2 (55, 77). CRF-BP also binds urotensin I with extremely high affinity and sauvagine with much lower affinity (97). Consistent with the high affinity of CRF-BP for CRF, a number of studies have demonstrated that CRF-BP can inhibit the biological effects of CRF when the two are incubated together (99, 107–109). However, the "on-rate" of this interaction appears to be relatively slow. When CRF is preincubated with CRF (as would be the case when the pituitary is exposed to plasma CRF of placental origin) CRF-BP inhibits ACTH release due to CRF in a perfused pituitary cell column. In contrast, when the pituitary cells are exposed to short pulses of CRF while being constantly perfused with CRF-BP (as would be the case when the pituitary is exposed to CRF of hypothalamic origin), no such inhibition is observed (107). These data apparently explain how, despite having high circulating levels of CRF, late pregnancy women maintain relatively normal levels of plasma ACTH and retain hypothalamic control of pituitary ACTH secretion. However, a slow "on-rate" questions whether CRF-BP can play any role in modulating acute transynaptic CRF–CRF receptor interactions within brain. Recent studies have shown that free "CRF" levels are elevated when human brain tissue is treated with a CRF analog that competes for CRF at the CRF-BP (103). Furthermore, when treated with a mid-sequence analog of CRF ($CRF_{(6-33)}$), which displaces CRF from its BP, but does not interact with known CRF receptors, it induces some CRF-like effects (i.e., increased learning and memory) (103). These data clearly demonstrate the ability of CRF-BP in brain to modulate the availability of "free" CRF to interact with its receptors.

Alternate CRF Ligands

A number of lines of evidence indicate the likely presence of one or more mammalian peptides that are ligands for CRF receptors and/or CRF-BP but are molecularly distinct from hypothalamic $CRF_{(1-41)}$. First, the recent demonstration that amphibian sauvagine and fish urotensin I are not CRF orthologs, but that peptides more closely related to CRF are present in these same vertebrate classes (110, 111), suggests the existence of sauvagine– and urotensin I–like peptides in mammalian species. Secondly, while CRF-BP levels are relatively high in the blood of both pregnant/nonpregnant and male humans alike, CRF concentrations in blood are elevated only in the third trimester of pregnancy, questioning the function and possible ligands of CRF-BP in plasma at other times. Furthermore, only partial coincidence of CRF-BP and CRF immunostaining within the rat brain is apparent. Similarly, discordance between the localization of CRF receptor mRNA and CRF immunoreactivity have been noted. The higher affinity of CRF-R2 for sauvagine and urotensin I than CRF also suggests the possibility of equivalent mammalian peptides. Indeed, CRF-BP ligand activity that cannot be accounted for by authentic CRF has been described in sheep brain and in the synovial fluid of arthritic patients (98, 112).

Recently, Vale and colleagues have described a putative alternate CRF receptor/CRF-BP ligand in rats (55). Immunostaining of the rat brain with antisera specific to urotensin I identified urotensin-positive regions (Edinger Westphal [EW] nucleus of the midbrain and the lateral superior olive) which lack CRF mRNA. Screening of a cDNA library constructed with mRNA from the rat EW nucleus with a urotensin probe yielded a clone encoding a deduced 122–amino acid protein, with a carboxy terminus including a putative 40–amino acid peptide flanked by proteolytic processing sites. This peptide was called urocortin, reflecting its sequence identity, and similarity of biological effects, to *uro*tensin I (63% sequence identity to carp urotensin I) and *corti*cotrop*in*-releasing factor (45% sequence identity to r/h CRF) (Fig. 1). Urocortin also shares 35% sequence identity with sauvagine. Human urocortin has subsequently been cloned and characterized (77).

	1 5 10 15 20 25 30 35 40	
r Ucn	D D P P L S I D L T F H L L R T L L E L A R T Q S Q R E R A E Q N R I I F D S V	100%
hUcn	- N - S -	95%
cUro	N - - - - I - - - - - - - - - - - - N M I - M - - N E N - - - Q - G L - - K Y L - E -	63%
r/hCRF	S E E - - I - L - - - - - - - - - E V - - M - - A E Q L A Q Q - H S - - K L M E I I	45%
Svg	E G - - I - - - - - S L E - - - K M I - I E K Q E K E K Q Q - A N - - L L L - T I	35%

Figure 1. Amino acid sequences of the CRF-like peptides rat (r) and human (h) urocortin (Ucn), carp urotensin I (cUro), rat/human CRF, and sauvagine (Svg). -, identical amino acid to rat urocortin; %, percentage sequence homology with rat urocortin.

In situ hybridization studies of rat brain tissues demonstrated that urocortin mRNA has a distribution that differs from CRF (55). Hybridization signal for urocortin mRNA is strong, and co-localizes with urotensin immunoreactivity in the EW nucleus and the lateral superior olive. Weaker signal is observed over large neurons of the facial, hypoglossal, and ambiggual motor nuclei, neurosecretory neurons in the supra-optic nucleus, and diffusely in the septal region of the lateral hypothalamus. The localization of urocortin in peripheral tissues has not been well described; however, urotensin-like immunoreactivity is present in duodenal extracts, indicating a possible peripheral distribution of urocortin (55).

Urocortin is a potent activator of CRF-R2 (55, 77). CRF-R2 binds urocortin with 10- to 40-fold greater affinity than CRF and urocortin has a 10-fold lower EC_{50} than CRF when inducing cAMP in cells overexpressing the CRF-R2β (55, 77). Indeed, urocortin is more potent at this form of the receptor than urotensin I or sauvagine. In agreement with these data, urocortin is a far more potent hypotensive agent than CRF when either peptide is administered intravenously to rats (55). Similarly, urocortin inhibits injury-induced edema in the rat with an ED_{50} about 6- to 7-fold lower than CRF (52). Furthermore, i.c.v. administration of urocortin induces c-*fos* expression (a marker of cellular activation) in cells expressing CRF-R2 (55). Indeed, it has been proposed that urocortin is an/the endogenous ligand for the second CRF receptor (55). However, not only does urocortin activate CRF-R2 and physiological events thought to be mediated by this receptor, it is also a potent agonist at the CRF-R1 receptor. CRF-1 has a 2- to 6-fold greater affinity for urocortin than CRF, and the EC_{50} for urocortin and CRF are similar when inducing cAMP in CRF-R1 expressing cells (55, 77). Urocortin is a potent ACTH secretagog, both *in vivo* (52, 55, 113) and *in vitro* (55, 77). It has a 7-fold lower EC_{50} than CRF in a rat anterior pituitary cell culture assay and produces a similar magnitude and pattern of ACTH responses to CRF *in vivo* at doses 5–10 times lower (52, 55, 113). In addition to interacting with CRF receptors, urocortin is bound by CRF-BP. The binding coefficient of this interaction is about 2-fold lower than for CRF (55), and urocortin displaces CRF bound to CRF-BP thus elevating "free" CRF levels in brain (114).

When administered directly into the brain, urocortin mimics endocrine responses to stress, elevating plasma ACTH and decreasing plasma LH concentrations as has been observed for CRF (Fig. 2). However, what role, if any, endogenous urocortin plays in stress responses is presently unclear. While preliminary data indicate congruence of urotensin I-like immunoreactivity and CRF-R2 in the septal region of the rat brain, the relationship between urocortin terminal fields and CRF receptors has yet to be fully described. Furthermore, we do not know what effect stress has on urocortin synthesis/secretion. Investigation of the effects of CRF receptor antagonists on stress responses cannot distinguish between a CRF or urocortin action. Furthermore, CRF antisera/antibodies which recognize N-terminal epitopes of CRF may well cross-react with urocortin (Fig. 1). We recently reported (113) that antiserum specific to CRF abolishes the elevation in plasma ACTH concentrations due to electrofootshock in rats, while urocortin-specific antisera have no effect. These data indicate that, at least in response to acute stress, CRF (and not urocortin) is the primary mediator of primary ACTH secretion. Urocortin's involvement in other stress responses has not yet been tested.

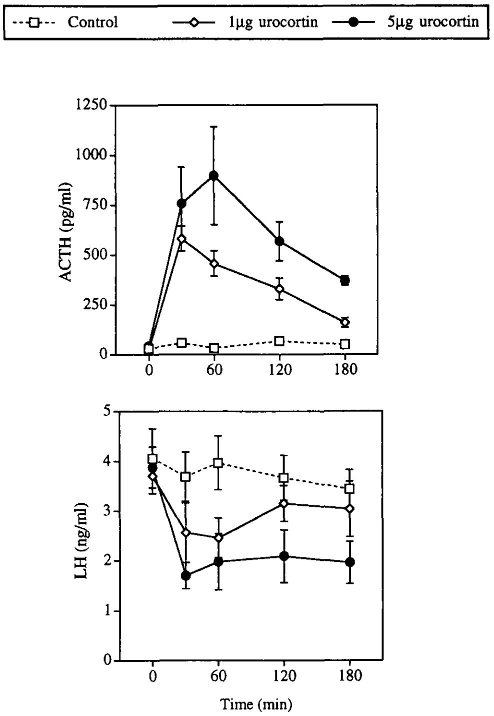

Figure 2. Effect of rat urocortin injected intracerebroventricularly (i.c.v.) on plasma ACTH and LH concentrations in conscious, castrated male rats.

Concluding Remarks and Future Directions

The last 5 years have seen important advances in our understanding of CRF and its mechanisms of action in eliciting responses to stress. Receptors mediating the effects of CRF have been identified, characterized, and localized, and a putative modulator of CRF bioavailability (CRF-BP) has been described. Furthermore, the existence of at least one further CRF-like mammalian peptide has been demonstrated.

These findings raise a number of new and important questions. The observations that the different CRF receptor subtypes display differing pharmacologies and discrete anatomical distributions suggest that they probably mediate distinct actions of CRF. Indeed, the greater affinity of this receptor for CRF homologs (urocortin, sauvagine, and urotensin I) than for CRF itself, raises the question of whether CRF really is an endogenous ligand for this receptor. Furthermore, determining the relative contribution of the different receptor subtypes to individual responses to stress will dictate the design of possible therapeutic agents. Whether CRF-BP plays a role in the regulation of CRF actions outside of human pregnancy is presently unknown. The demonstration that displacement of CRF from CRF-BP produces physiological, CRF-like effects indicates that the BP may be capable of modulating CRF availability for interaction with its receptor(s). However, whether CRF-BP moderates responses to CRF during stress remains to be determined. Finally, determining how CRF, its receptors, CRF-BP, and alternate CRF receptor ligands are regulated/dysregulated in various circumstances will aid our understanding of the mechanisms by which CRF elicits stress responses/contributes to the development of aberrant responses and disease.

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