Corticotropin Releasing Factor Receptors and Their Ligand Family

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ABSTRACT: The CRF receptors belong to the VIP/GRF/PTH family of G-protein coupled receptors whose actions are mediated through activation of adenylate cyclase. Two CRF receptors, encoded by distinct genes, CRF-R1 and CRF-R2, and that can exist in two alternatively spliced forms, have been cloned. The type-1 receptor is expressed in many areas of the rodent brain, as well as in the pituitary, gonads, and skin. In the rodent, one splice variant of the type-2 receptor, CRF-R2α, is expressed mainly in the brain, whereas the other variant, CRF-R2β, is found not only in the CNS, but also in cardiac and skeletal muscle, epididymis, and the gastrointestinal tract. The poor correlation between the sites of expression of CRF-R2 and CRF, as well as the relatively low affinity of CRF for CRF-R2, suggested the presence of another ligand, whose existence was confirmed in our cloning of urocortin. This CRF-like peptide is found not only in brain, but also in peripheral sites, such as lymphocytes. The broad tissue distribution of CRF receptors and their ligands underscores the important role of this system in maintenance of homeostasis. Functional studies of the two receptor types reveal differences in the specificity for CRF and related ligands. On the basis of its greater affinity for urocortin, in comparison with CRF, as well as its brain distribution, CRF-R2 may be the cognate receptor for urocortin. Mutagenesis studies of CRF receptors directed toward understanding the basis for their specificity, provide insight into the structural determinants for hormone-receptor recognition and signal transduction.

INTRODUCTION

It is a matter of life or death for an organism that is exposed to environmental perturbations (stressors) to be able to respond in a manner that maintains physiological homeostasis. A major regulator of homeostasis is the corticotropin-releasing-factor (CRF), which mediates the autonomic, behavioral, and neuroendocrine responses to stress. In addition, there are many data suggesting that CRF and its family members are involved in the function of the immune, reproductive, and cardiovascular systems,[1–3] as well as in pathophysiologic states, such as depression and Alzheimer's disease.[4–8] Recently, the observation that CRF affects the growth of mammary and lung cancer cells, and that it protects neuronal cells from hypoxia-induced cell death,[9–11] suggests that CRF may play a role in cell growth and survival.

The family of CRF agonists includes (fish) urotensin, (frog) sauvagine, and the new mammalian CRF agonist, urocortin. The actions of these CRF agonists are initiated by binding and activating receptors that belong to the family of seven-

transmembrane domain, G-protein–coupled receptors that activate adenylate cyclase. In order to understand the mechanism of CRF action, a detailed description of the ligand-receptor interaction at a molecular level is required. This paper describes the cloned CRF receptors and the structural determinants for ligand recognition. Data on the tissue distribution and receptor binding of urocortin are included.

CRF RECEPTOR TYPES AND VARIANTS

To date, two types of CRF receptors, each encoded by a different gene, have been cloned. Using an expression cloning approach, the cDNA for CRF-R1 was obtained from a human Cushing's tumor[12] and from the mouse AtT-20 cell line.[13] The homologous receptors in rat and human were also cloned and were found to have only minor amino acid differences.[13–15] Type-1 CRF receptors have subsequently been cloned in sheep (Meyers, Trinh, Myers, Gene Bank #AF054582), chicken,[16] and *Xenopus*.[17] A splice variant of CRF-R1, in which 29 amino acids are inserted into the first intracellular loop, was cloned from the Cushing's tumor.[12] To date, the latter variant has not been found in any other tissue. Another variant, from which the third exon—encoding amino acids 40–80—is deleted, was cloned from human hypothalamus.[18] This variant has been detected in human fetal and placental tissue,[19] as well as in rat testis.[20]

After CRF-R1 had been cloned, its expression sites in the rat brain were found to be widespread, but not completely overlapping with previously detected binding sites. For example, there are CRF binding sites in the hypothalamus,[21] but there is very little binding to CRF-R1,[22] or expression of its message.[23,24] The existence of another CRF receptor was confirmed when rodent CRF-R2 was cloned.[25–28] In the rodent, there are two splice variants, CRF-R2α and CRF-R2β, that differ in their N-terminal domains. Subsequently, the human homologs of CRF-R2α and CRF-R2β were cloned,[29,30] as well as a third N-terminal splice variant, CRF-R2γ.[31] The sequences of cloned CRF receptors are compared in FIGURE 1.

The CRF receptors belong to the family that includes the growth hormone releasing factor, parathyroid hormone, glucagon, vasoactive intestinal peptide, calcitonin, and secretin receptors. The rodent CRF-R1 and CRF-R2β are overall 68% similar, at the amino acid level, but they are 79% similar in the transmembrane domains, and 84% similar in the intracellular domains. The major sequence differences between the two types of receptors occur in the extracellular domains (FIG. 1).

All receptors have putative signal peptides, N-glycosylation sites in their N-termini, and eight conserved cysteine residues in their extracellular domains. Consistent with the signal transduction pathway of the native receptors, the cloned receptors transduce a CRF-stimulated accumulation of intracellular cAMP in transfected cells.[26] It is relevant, in this respect, that all receptors have the same sequence in the third intracellular loop, which is presumed to play an important role in coupling to the G-proteins (FIG.1).

The genomic structures of the rat and human receptors show that they contain 13 exons and 12 introns, and a general structure similar to that of the genes for parathyroid hormone and glucagon receptors.[20,32] The splice variant encoding the insertion

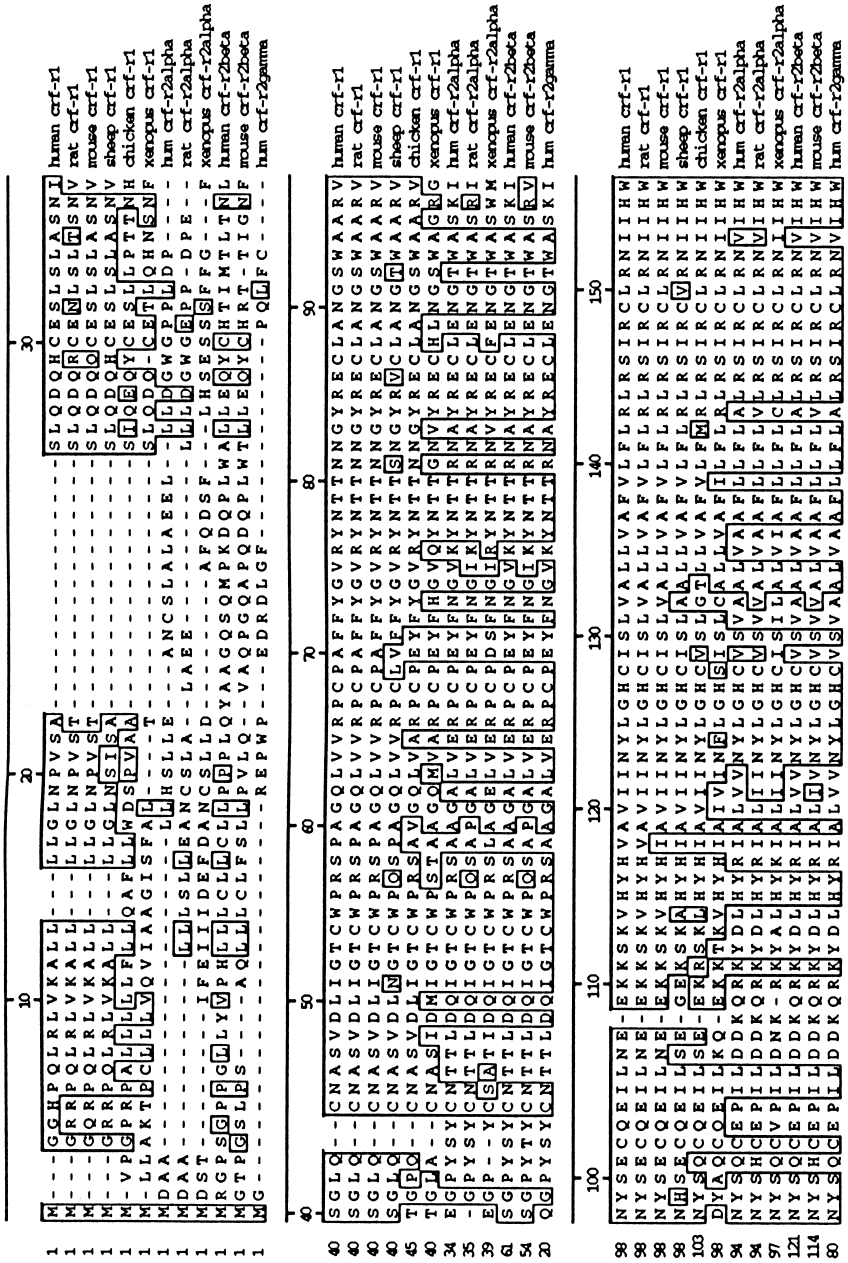


FIGURE 1. Sequence alignment of the cloned CRF receptors./Continued on next two pages.

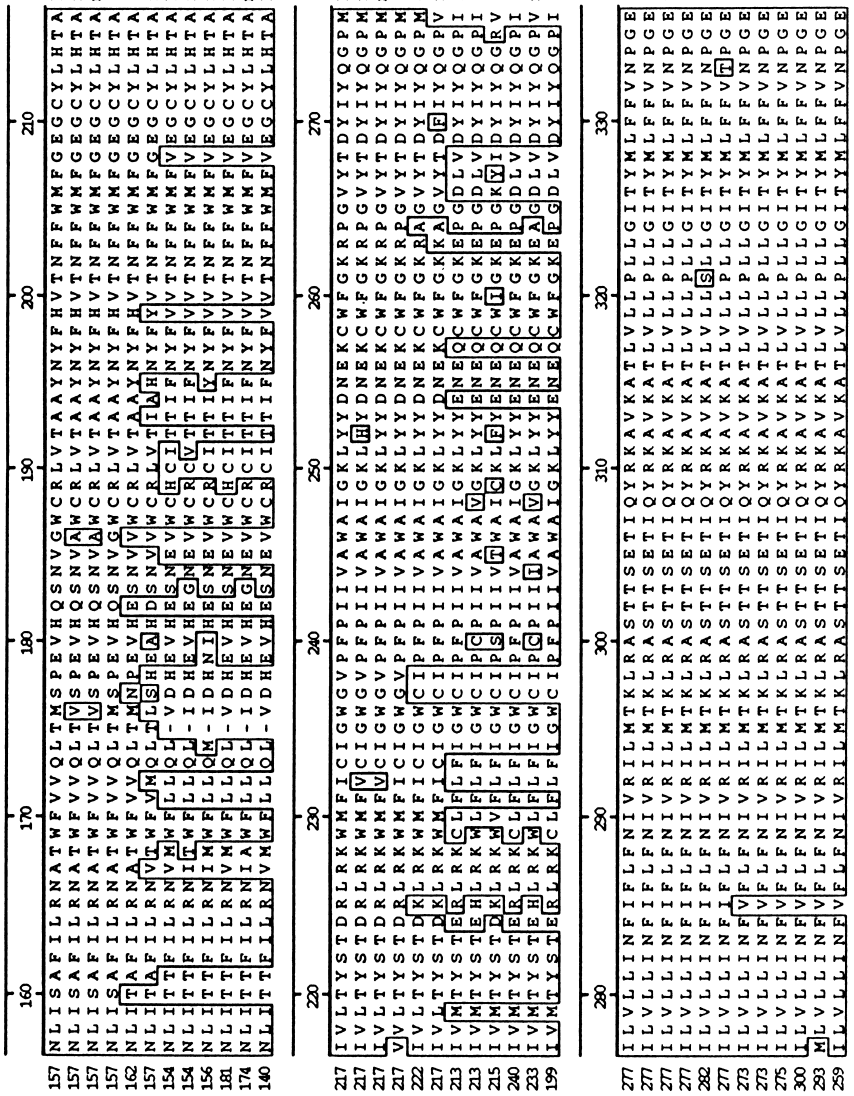


FIGURE 1/continued.

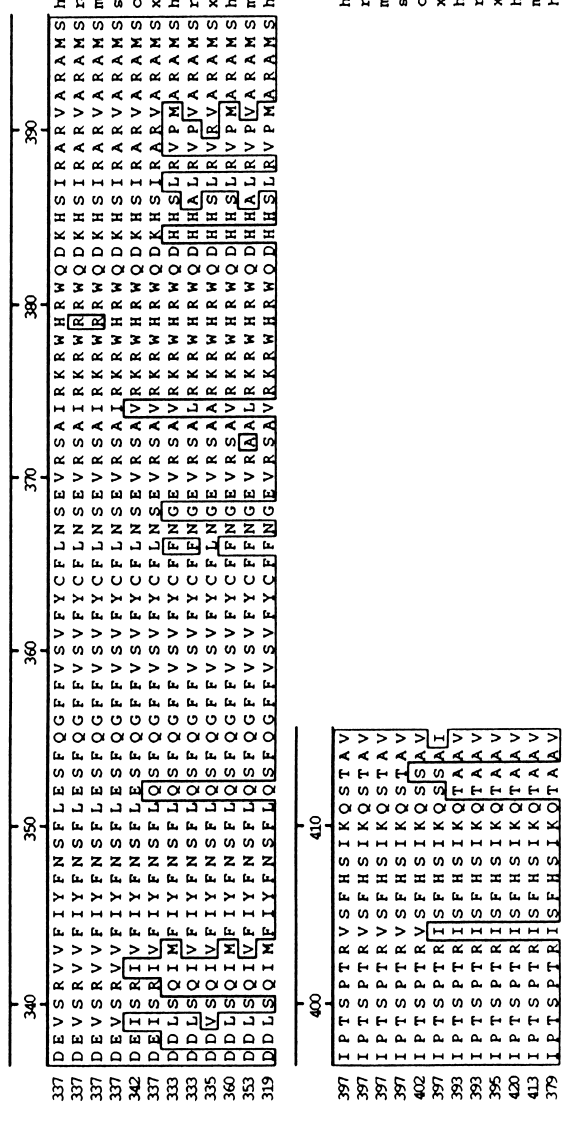


FIGURE 1/continued. Sequence alignment of the cloned CRF receptors.

in the first intracellular loop, isolated from the human Cushing's tumor, was not detected in the rat gene, but was found in the human gene.[32]

CRF RECEPTOR EXPRESSION

After the receptors had been cloned, their expression sites were determined by means of *in situ* hybridization, RNAse protection, and RT-PCR. These results show that there is a wide distribution of CRF-R1 in the rat brain, with high levels in the forebrain, subcortical limbic structures in the septal region, amygdala, cerebellar cortex, and deep nuclei; and low or no expression in the hypothalamus. In keeping with the effects of CRF on the pituitary, CRF-R1 is also expressed in the anterior and intermediate lobes of the rat pituitary.[23] Additionally, CRF-R1 expression is found in mouse testis,[33] human ovary,[34] skin,[35,36] human endometrial stromal cells,[37] human leukemic mast cells,[38] rat mammary carcinoma,[10] and melanoma cells.[39]

In the rodent, CRF-R2α is found mainly in the brain, where it is confined to subcortical structures; with high expression in the lateral septal nucleus, the ventromedial hypothalamus, and in the choroid plexus; with very low expression in the pituitary.[24] A distinct site of expression for CRF-R2β is in cerebral arterioles throughout the rat brain.[24] Interestingly, in the rodent, CRF-R2β is expressed both in the brain and in the periphery—namely, the epididymis, skeletal muscle, gastrointestinal tract, and heart.[26–28,40] Additionally, CRF-R2β is expressed in mouse AT-1 myocyte tumor cells.[41] In contrast to the rodent, the human CRF-R2α is found in the brain and periphery, whereas CRF-R2β and CRF-R2γ are found mainly in the brain.[30,31]

Using pharmacological differences in affinities for ovine CRF, the binding sites that correspond to CRF-R1 and CRF-R2 in the rat brain were shown to be in distinct, nonoverlapping regions each associated with a specific message expression.[22] Pharmacological characterization of CRF-like effects on relaxation of mesenteric small arteries of the rat suggests that the receptors that mediate these effects are CRF-R2;[42] as are the mediators for rat neonatal cardiomyocytes and a murine cardiomyocyte tumor cell line.[41]

A SECOND MAMMALIAN CRF AGONIST, UROCORTIN

The impetus behind the search for another mammalian, CRF-like ligand was based on four different observations. First, the peptides, urotensin and sauvagine, in fish and frogs, respectively, were thought to be the corresponding orthologs of CRF until peptides more closely related to CRF were cloned in these species.[43,44] Second, urotensin and sauvagine are nearly equipotent with CRF on CRF-R1, but are more potent than CRF on CRF-R2.[25–27] Third, there was specific urotensin-like immunoreactivity in rat brain and other tissues. Finally, the sites of expression of CRF-R1 and CRF-R2 in the rat brain are largely nonoverlapping,[24] showing a lack of correspondence, in some areas, with the expression of CRF. Using an antibody to urotensin, a peptide named urocortin was cloned from rat brain.[45] Subsequently, the human homolog was cloned.[46] Urocortin is 63% homologous to urotensin and 43%

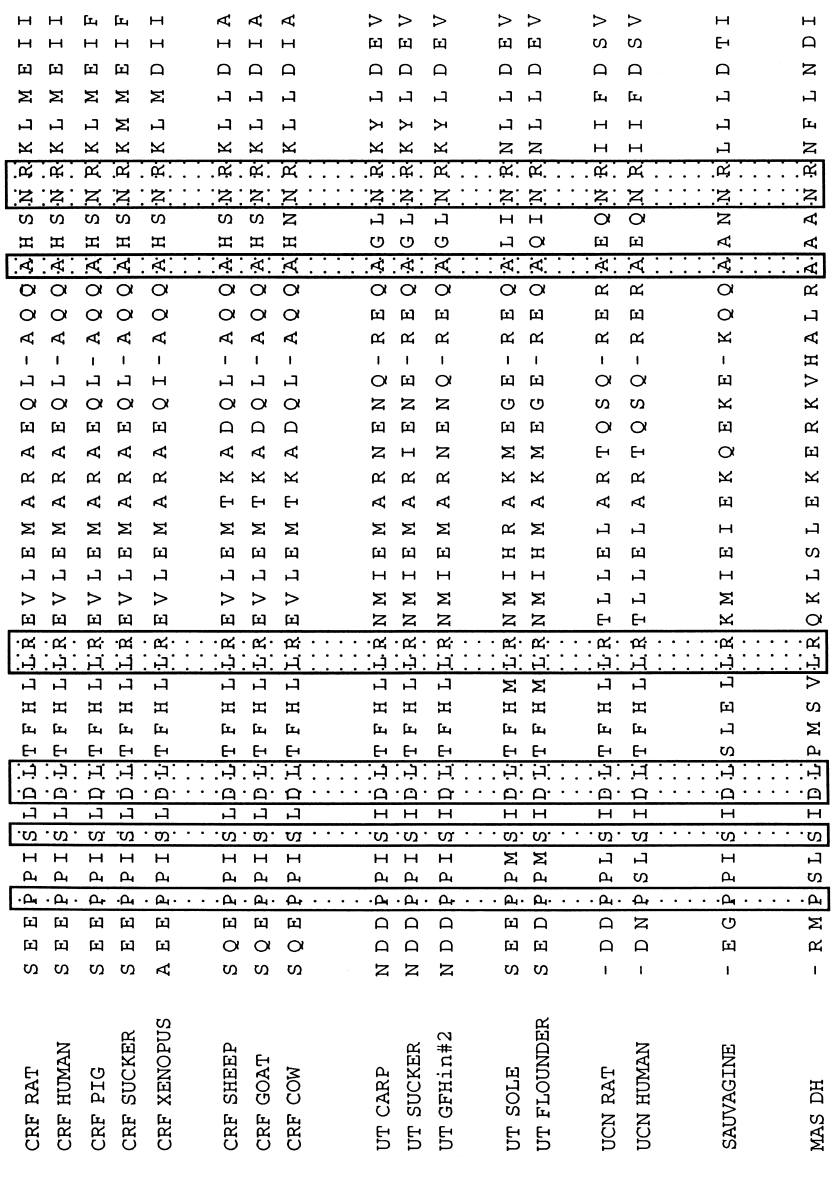


FIGURE 2. Sequences of CRF family members.

TABLE 1. Relative potencies of CRF ligands[a]

Compound	Inhibitory binding constant, K_i (nM)[b]						cAMP, EC_{50} (nM)[c]	
	hCRF-R1[d]	hCRF-R1[e]	rCRF-R2α[d]	rCRF-R2α[e]	mCRF-R2β[d]	mCRF-R2β[e]	rCRF-R1	mCRF-R2β
Astressin	2.0 (1.8–2.3)	1.6 (0.95–2.7)	1.5 (0.81–2.8)	2.8 (1.7–4.7)	1.0 (0.39–2.8)	0.87 (0.56–1.4)	n/a	n/a
α-helical CRF(9–41)	17 (13–21)	49 (29–82)	5.0 (2.6–10)	4.1 (2.5–6.7)	0.97 (0.43–2.2)	0.81 (0.52–1.3)	n/a	n/a
[DPhe^{12},Nle^{21,38}]r/hCRF(12–41)	56 (41–75)	75 (64–88)	5.2 (2.6–10)	31 (19–51)	8.4 (4.4–16)	6.9 (5.0–9.5)	n/a	n/a
r/hCRF	11 (8.4–15)	5.2 (2.9–9.3)	44 (26–75)	13 (7.2–22)	38 (21–67)	17 (10–29)	1.9 ± 0.8	1.7 ± 0.4
rUcn	13 (0.71–2.6)	0.79 (0.43–1.4)	1.5 (0.74–3.2)	0.58 (0.42–0.82)	0.97 (0.46–2.0)	0.41 (0.26–0.66)	0.8 ± 0.1	0.18 ± 0.04
sfUrotensin I	3.1 (2.4–4.0)	2.8 (2.4–3.3)	9.8 (5.5–17)	3.4 (2.6–4.4)	6.4 (3.8–11)	3.0 (1.8–4.8)	3.1 ± 1.7	0.74 ± 0.1
Sauvagine	9.4 (7–13)	11 (8.8–13)	9.9 (4.4–22)	1.4 (1.1–1.8)	3.8 (2.3–6.2)	2.0 (1.1–3.6)	2.5 ± 1.4	0.5 ± 0.2

[a] The values for this table are taken from Refs. 45 and 60.
[b] 95% confidence limits in parentheses.
[c] ± SEM.
[d] ^{125}I-[DTyr^{1}]Astressin as radioligand.
[e] ^{125}I-[Tyr^{0}]rUcn as radioligand.

homologous to CRF. The sequences for urocortin and the other CRF family members are shown in FIGURE 2, from which it can be seen that urocortin is more closely related to urotensin than to CRF.

Urocortin is detected in many regions of the rat brain, including the Edinger-Westphal nucleus, lateral superior olive,[45] substantia nigra, ventral tegmental area, linear and dorsal raphe nuclei,[47,48] and the hypothalamus.[49] In the human brain, urocortin-like immunoreactivity and message are observed in every region, with the highest concentrations found in the frontal cortex, temporal cortex, and hypothalamus.[50]

Urocortin is also found in peripheral sites. For example, immunoreactive urocortin is detected in the rat digestive system and the pituitary.[49,51] Human anterior pituitary was also found to express both the message and immunoreactivity for urocortin.[52] Other sites of expression of urocortin mRNA and of the peptide are mucosal inflammatory cells in the human gastrointestinal tract,[53] human placenta, and fetal membranes.[54] Using RT-PCR, urocortin mRNA is detected in primary cultures of rat cardiomyocytes as well as in cardiac myocyte cell lines.[55]

By combining RT-PCR and radioimmunoassay, it was found that normal human lymphocytes produce urocortin but not CRF.[56] The involvement of CRF-like ligands in immune function is further highlighted by the observation that both CRF and urocortin suppress experimental autoimmune encephalomyelitis in rats.[57] These neuropeptides may act directly on the components of the immune system, rather than indirectly through the activation of the HPA.

The regions of urocortin immunoreactivity overlap with the regions of expression of CRF-R2 in many areas of the brain[47] and some effects of urocortin appear to be mediated by CRF-R2. For example, urocortin was found to be approximately six times more potent than CRF as an inhibitor of heat-induced edema.[58] Furthermore, blockade of CRF-R1 does not affect the CRF- and urocortin-induced decrease in food intake in rats.[59] Also, urocortin protects myocytes, cells that express CRF-R2, from hypoxia-induced cell death.[55]

Pharmacologic studies have shown that urocortin is more potent than CRF on both types of CRF receptors, but in general, the difference in potencies is greater for CRF-R2.[45,60] The affinities of selected CRF ligands and the potencies in stimulating cAMP in cells expressing the two types of CRF receptors are given in TABLE 1. Astressin is a CRF peptide antagonist that has high affinity for both types of CRF receptors.[61] From TABLE 1 it can be seen that the antagonist α-helical CRF(9–41) has a greater affinity for CRF-R2 than for CRF-R1. This observation may explain the fact that α-helical CRF(9–41) blocks heat-induced edema at doses that do not block CRF-stimulated ACTH release.[58]

STRUCTURAL REQUIREMENTS FOR CRF RECEPTOR BINDING—MUTAGENESIS STUDIES

One approach to assessing the structural determinants for receptor function uses mutagenesis to determine regions of the receptor that may be involved in binding and signaling. In one study, chimeric receptors were created in which domains of the GRF receptor were replaced by the corresponding domains of the CRF-R1. A chi-

meric receptor in which the ECD-1 of the CRF-R1 was replaced by the ECD-1 of the GRF-R, does not bind either labeled astressin or labeled urocortin. A chimera in which the ECD-1 of the GRF-R was replaced by the corresponding domain of the CRF-R, binds astressin and urocortin with a dissocuation constant K_d ~10 nM. Additional chimeras were created to explore the role of the extracellular loops, and it was found that a chimera expressing all three extracellular loops, together with the N-terminus of the CRF-R, displays nearly the same affinity as the wild-type receptor.[62]

As a further test of the role of the ECD-1 in binding CRF analogs, a chimera was created, in which the ECD of the activin type-2 receptor was replaced by the ECD-1 of CRF-R1 (CRF-R/Act-R). The activin receptor is a single transmembrane serine/threonine kinase.[63] This chimera binds astressin with nanomolar affinity (see TABLE 2). In another study, the ECD-1 was expressed as a soluble protein and it was found to display only low binding affinity for a CRF agonist.[64] These data suggest that the N-terminal domain of the CRF-R1 contains major binding determinants for CRF agonists and antagonists, but that it must be anchored to the cell membrane.

In CRF-R1, there eight conserved cysteine residues in ECD-1, -2, and -3. The role of these residues was studied by mutating them, either singly or in pairs. It was found that there is potential pairing of the Cys residues, #44 with #102, #68 with #87; and the two residues in ECD-2 and -3, #188 with #258. Mutations of C30 and C54 appear to have little effect on receptor binding. Additionally, mutations of the cysteine residues within the transmembrane and intracellular domains have no effect on either binding or signaling.[65]

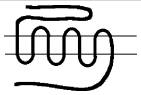
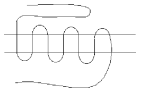
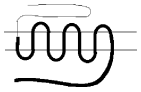
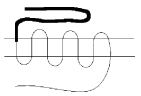
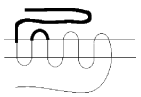
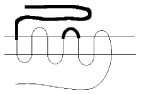
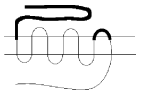
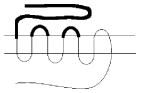
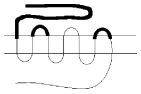
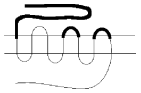
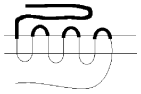
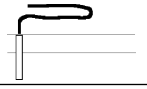
Another question relates to the origin of CRF receptor selectivity as manifested by greater potency of CRF in signaling by CRF-R1 compared to CRF-R2.[25] The amino acids that differ between CRF-R1 and CRF-R2 were exchanged and it was found that substitution of Val266, Tyr267, and Thr 268 in CRF-R1 by the corresponding residues in CRF-R2 increases the EC-50 for CRF-stimulated cAMP accumulation by a factor of about ten. Other residues in ECD-2 (#175–#178, and #189) also appear to be involved in the CRF response of CRF-R1.[66,67]

At the amino acid level, the *Xenopus* CRF-R1 is about 80% homologous to the human CRF-R1, but the two receptors differ in their affinities for sauvagine, r/h CRF, and ovine CRF.[17] Whereas hCRF-R1 is not selective for these ligands, the xCRF-R1 has about one tenth the affinity for ovine CRF and sauvagine, as compared to CRF. Using mutagenesis, it was found that the five amino acids #76, #81 #83, #88, and #89 in the N-terminal domain are responsible for the observed differences.[68] These data are consistent with some published results,[62] but they contrast with others.[66,67]

The avian CRF-R1 is about 88% identical to that of the human, rat, and mouse CRF-R1, with many amino acid differences in the ECD-1, and the remainder of the differences scattered throughout the rest of the sequence.[16] In the avian receptor the relative potencies are urotensin ≅ sauvagine > CRF, for both binding and signaling. This pattern is more like that seen for the CRF-R2 than for CRF-R1. No mutagenesis study has yet been reported for the avian receptor, but the ECD-1 is again implicated in the ligand specificity because most of the amino acid differences appear in this domain.

Recently a series of nonpeptide CRF antagonists have been developed that bind, specifically and with high affinity, to type-1 receptors; and inhibit, both *in vitro* and

TABLE 2. Inhibitory dissociation constants, K_i (nM), for astressin and urocortin bound to COSM6 cells transfected with various chimeric and mutant receptors[a]

	Receptor	Ast/Ast* K_i (nM)[b]	Ucn/Ucn* K_i (nM)[b]
	rCRF-R (wt)	1.8 (1.1–2.9)	3.0 (1.6–5.7)
	rGRF-R (wt)	—[c]	—[c]
	$E1_g$/CRF-R	—[c]	—[c]
	$E1_c$/GRF-R	13 (7.7–22)	12 (1.9–74)
	$E1_c$/$E2_c$/GRF-R	10 (7.1–14)	10 (4.5–22)
	$E1_c$/$E3_c$/GRF-R	7.1 (3.8–13)	—[c]
	$E1_c$/$E4_c$/GRF-R	4.2 (3.0–5.8)	1.6 (0.58–4.4)
	$E1_c$/$E2_c$/$E3_c$/GRF-R	5.4 (3.5–8.4)	—[c]
	$E1_c$/$E2_c$/$E4_c$/GRF-R	7.9 (5.3–12)	7.0 (3.5–14)
	$E1_c$/$E3_c$/$E4_c$/GRF-R	11 (5.2–23)	7.6 (2.1–27)
	$E1_c$/$E2_c$/$E3_c$/$E4_c$/GRF-R	4.3 (1.9–9.6)	1.3 (0.71–2.4)
	$E1_c$/ActIIB-R	3.5 (1.8–7.0)	—[c]

[a]The K_i values were determined from homologous displacement assays, assuming that the K_d values for Ast* and Ucn* were not significantly different from those for astressin and urocortin, respectively.

[b]Values in parentheses are 95% confidence limits.

[c]The specific binding was too low to measure the K_i accurately.

in vivo, CRF actions mediated by them.[69–72] One study found that the mutations H199V and M276I, two of the residues in transmembrane domains 3 and 5 that differ between CRF-R1 and CRF-R2, reduce the affinity of CRF-R1 for a nonpeptide antagonist but have no effect on the affinity for CRF.[67] In another study, a different nonpeptide antagonist, antalarmin,[72] displaced the labeled antagonist, astressin, to a much smaller degree than it displaced labeled oCRF from both the cloned CRF-R1 and the native cerebellar receptor—a tissue that predominantly expresses CRF-R1 (Perrin, unpublished). These data suggest that the binding domains of peptide and nonpeptide antagonists are different.

CONCLUSIONS

To date, four CRF receptors and one novel CRF ligand have been cloned. The type-1 receptor, widely expressed in the central nervous system and in the pituitary, is the major mediator for activation of the HPA axis. The fact that CRF-R1 is found in the reproductive and immune systems, as well as in spleen, adrenal, and skin, suggests that this receptor mediates many diverse actions. The type-2 receptor exists in different splice-variant forms that differ in their N-terminal sequences and in their tissue expression. In both the rodent and human, one variant is restricted to the central nervous system, whereas the other variants are found not only in the brain but also in peripheral sites.

A novel CRF agonist, urocortin, has been cloned and found to have characteristics that are similar, but not identical, to those of CRF. Urocortin is widely expressed in the brain, as well as in many of the peripheral tissues that express the type-2 receptor. Urocortin binds with high affinity to both types of receptors but is significantly more potent than CRF on the type-2 receptor.

Studies of mutant CRF receptors have shown that recognition of CRF peptides is governed, to a large extent, by residues in the extracellular domains, especially the N-terminus. Furthermore, the binding of nonpeptide CRF-R1–selective antagonists involves receptor domains that differ from those involved in binding of the peptide antagonists.

The future holds exciting prospects for cloning other CRF receptors and discovering novel sites of expression and action. The cloning of urocortin raises the possibility that other CRF-like molecules exist in mammals. Future studies of CRF receptor and/or urocortin knock-out mice will undoubtedly disclose expanded roles for the CRF family and increase our understanding of the roles of CRF in normal and pathological states.

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REFERENCES

1. TORPY, D.J. & G.P. CHROUSOS. 1996. The three-way interactions between the hypothalamic-pituitary-adrenal and gonadal axes and the immune system. Baillieres Clin. Rheumatol. **10:** 181–198.
2. RICHTER, R.M. & M.J. MULVANY. 1995. Comparison of hCRF and oCRF effects on cardiovascular responses after central, peripheral, and in vitro application. Peptides **16:** 843–849.
3. FISHER, L.A. 1993. Central actions of corticotropin-releasing factor on autonomic nervous activity and cardiovascular functioning. Ciba Found. Symp. **172:** 243–253.
4. NEMEROFF, C.B., E. WIDERLOV, G. BISSETTE *et al.* 1984. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science **226:** 1342–1344.
5. PLOTSKY, P.M., M.J. OWENS & C.B. NEMEROFF. 1998. Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. Psychiatr. Clin. North Am. **21:** 293–307.
6. NASMAN, B., T. OLSSON, M. FAGERLUND *et al.* 1996. Blunted adrenocorticotropin and increased adrenal steroid response to human corticotropin-releasing hormone in Alzheimer's disease. Biol. Psychiatry. **39:** 311–318.
7. BEHAN, D.P., S.C. HEINRICHS, J.C. TRONCOSO *et al.* 1995. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. Nature **378:** 284–287.
8. RAADSHEER, F.C., J.J. VAN HEERIKHUIZE, P.J. LUCASSEN *et al.* 1995. Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. Am. J. Psychiatry **152:** 1372–1376.
9. EAGLES, E., G. SANTILLI, A. OKOSI *et al.* 1998. Endogenous corticotropin releasing hormone protects neuroblastoma cells from hypoxia-induced necrosis. (Abst. # P1-420). *In* Endocrine Soc. Meeting, June 1988, New Orleans, Louisiana.
10. TJUVAJEV, J., Y. KOLESNIKOV, R. JOSHI *et al.* 1998. Anti-neoplastic properties of human corticotropin releasing factor: involvement of the nitric oxide pathway. In Vivo **12:** 1–10.
11. MOODY, T.W., F. ZIA, R. VENUGOPAL *et al.* 1994. Corticotropin-releasing factor stimulates cyclic AMP, arachidonic acid release, and growth of lung cancer cells. Peptides **15:** 281–285.
12. CHEN, R., K.A. LEWIS, M.H. PERRIN *et al.* 1993. Expression cloning of a human corticotropin-releasing factor receptor. Proc. Natl. Acad. Sci. USA **90:** 8967–8971.
13. VITA, N., P. LAURENT, S. LEFORT *et al.* 1993. Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. FEBS Lett. **335:** 1–5.
14. CHANG, C.-P., R.V. PEARSE, II, S. O'CONNELL *et al.* 1993. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron **11:** 1187–1195.
15. PERRIN, M.H., C.J. DONALDSON, R. CHEN *et al.* 1993. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology **133:** 3058–3061.
16. YU, J., L.Y. XIE & A.-B. ABOU-SAMRA. 1996. Molecular cloning of a type A chicken corticotropin-releasing factor receptor with high affinity for urotensin I. Endocrinology **137:** 192–197.
17. DAUTZENBERG, F.M., K. DIETRICH, M.R. PALCHAUDHURI *et al.* 1997. Identification of two corticotropin-releasing factor receptors from Xenopus laevis with high ligand selectivity: unusual pharmacology of the type-1 receptor. J. Neurochem. **69:** 1640–1649.
18. ROSS, P.C., C.M. KOSTAS & T.V. RAMABHADRAN. 1994. A variant of the human corticotropin-releasing factor (CRF) receptor: cloning, expression and pharmacology. Biochem. Biophys. Res. Comm. **205:** 1836–1842.

19. KARTERIS, E., D. GRAMMATOPOULOS, Y. DAI *et al.* 1998. The human placenta and fetal membranes express the corticotropin-releasing hormone receptor 1alpha (CRH-1alpha) and the CRH-C variant receptor. J. Clin. Endocrinol. Metab. **83:** 1376–1379.
20. TSAI-MORRIS, C.H., E. BUCZKO, Y. GENG *et al.* 1996. The genomic structure of the rat corticotropin releasing factor receptor. J. Biol. Chem. **271:** 14519–14525.
21. DE SOUZA, E.B., M.H. PERRIN, T.R. INSEL *et al.* 1984. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. Science **224:** 1449–1451.
22. PRIMUS, R.J., W. YEVICH, C. BALTAZAR *et al.* 1997. Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain. Neuropsychopharmacology **17:** 308–316.
23. POTTER, E., S. SUTTON, C. DONALDSON *et al.* 1994. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc. Natl. Acad. Sci. USA **91:** 8777–8781.
24. CHALMERS, D.T., T.W. LOVENBERG & E.B. DESOUZA. 1995. Localization of novel corticotropin-releasing factor receptor (CRF2)mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J. Neurosci. **15:** 6340–6350.
25. LOVENBERG, T.W., W.L. CHEN, D.E. GRIGORIADIS *et al.* 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. USA **92:** 836–840.
26. PERRIN, M., C. DONALDSON, R. CHEN *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:** 2969–2973.
27. KISHIMOTO, T., R.V. PEARSE II, C.R. LIN *et al.* 1995. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc. Natl. Acad. Sci. USA **92:** 1108–1112.
28. STENZEL, P., R. KESTERSON, W. YEUNG *et al.* 1995. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Mol. Endocrinol. **9:** 637–645.
29. LIAW, C.W., T.W. LOVENBERG, T. OLTERSDORF *et al.* 1996. Cloning and characterization of the human corticotropin-releasing factor-2 receptor complementary deoxyribonucleic acid. Endocrinology **137:** 72–77.
30. VALDENAIRE, O., T. GILLER, V. BREU *et al.* 1997. A new functional isoform of the human CRF2 receptor for corticotropin-releasing factor. Biochim. Biophys. Acta **1352:** 129–132.
31. KOSTICH, W.A., A. CHEN, K. SPERLE *et al.* 1998. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. Mol. Endocrinol. **12:** 1077–1085.
32. SAKAI, K., S. NAGAFUCHI, N. HORIBA *et al.* 1998. The genomic organization of the human corticotropin-releasing factor type-1 receptor. (Abst. # P3-571). *In* Endocrine Soc. Meeting, June 1988, New Orleans, Louisiana.
33. HEINRICH, N., M.R. MEYER, J. FURKERT *et al.* 1998. Corticotropin-releasing factor (CRF) agonists stimulate testosterone production in mouse leydig cells through CRF receptor-1. Endocrinology **139:** 651–658.
34. ASAKURA, H., I.H. ZWAIN & S.S. YEN. 1997. Expression of genes encoding corticotropin-releasing factor (CRF), type-1 CRF receptor, and CRF-binding protein and localization of the gene products in the human ovary. J. Clin. Endocrinol. Metab. **82:** 2720–2725.
35. SLOMINSKI, A., G. ERMAK, J. HWANG *et al.* 1995. Proopiomelanocortin, corticotropin releasing hormone and corticotropin releasing hormone receptor genes are expressed in human skin. FEBS Lett. **374:** 113–116.

36. ERMAK, G. & A. SLOMINSKI. 1997. Production of POMC, CRH-R1, MC1, and MC2 receptor mRNA and expression of tyrosinase gene in relation to hair cycle and dexamethasone treatment in the C57BL/6 mouse skin. J. Invest. Dermatol. **108:** 160–165.
37. DIBLASIO, A.M., F.P. GIRALDI, P. VIGANO *et al.* 1997. Expression of corticotropin-releasing hormone and its R1 receptor in human endometrial stromal cells. J. Clin. Endocrinol. Metab. **82:** 1594–1597.
38. THEOHARIDES, T.C., L.K. SINGH, W. BOUCHER *et al.* 1998. Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. Endocrinololgy **139:** 403–413.
39. SLOMINSKI, A., G. ERMAK, J.E. MAZURKIEWICZ *et al.* 1998. Characterization of corticotropin-releasing hormone (CRH) in human skin. J. Clin. Endocrinol. Metab. **83:** 1020–1024.
40. LOVENBERG, T.W., D.T. CHALMERS, C. LIU *et al.* 1995. CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology **136:** 4139–4142.
41. HELDWEIN, K.A., D.L. REDICK, M.B. RITTENBERG *et al.* 1996. Corticotropin-releasing hormone receptor expression and functional coupling in neonatal cardiac myocytes and AT-1 cells. Endocrinology **137:** 3631–3639.
42. ROHDE, E., J. FURKERT, K. FECHNER *et al.* 1996. Corticotropin-releasing hormone (CRH) receptors in the mesenteric small arteries of rats resemble the (2)-subtype. Biochem. Pharmacol. **52:** 829–833.
43. OKAWARA, Y., S.D. MORLEY, L.O. BURZIO *et al.* 1988. Cloning and sequence analysis of cDNA for corticotropin-releasing factor precursor from the teleost fish Catostomus commersoni. Proc. Natl. Acad. Sci. USA **85:** 8439–8443.
44. STENZEL-POORE, M.P., K.A. HELDWEIN, P. STENZEL *et al.* 1992. Characterization of the genomic corticotropin-releasing factor (CRF) gene from *Xenopus laevis*: two members of the CRF family exist in amphibians. Mol. Endocrinol. **6:** 1716–1724.
45. VAUGHAN, J., C. DONALDSON, J. BITTENCOURT *et al.* 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature **378:** 287–292.
46. DONALDSON, C.J., S.W. SUTTON, M.H. PERRIN *et al.* 1996. Cloning and characterization of human urocortin. Endocrinology **137:** 2167–2170.
47. KOZICZ, T., H. YANAIHARA & A. ARIMURA. 1998. Distribution of urocortin-like immunoreactivity in the central nervous system of the rat. J. Comp. Neurol. **391:** 1–10.
48. YAMAMOTO, H., T. MAEDA, M. FUJIMURA *et al.* 1998. Urocortin-like immunoreactivity in the substantia nigra, ventral tegmental area and Edinger-Westphal nucleus of rat. Neurosci. Lett. **243:** 21–24.
49. OKI, Y., M. IWABUCHI, M. MASUZAWA *et al.* 1998. Distribution and concentration of urocortin, and effect of adrenalectomy on its content in rat hypothalamus. Life Sci. **62:** 807–812.
50. TAKAHASHI, K., K. TOTSUNE, M. SONE *et al.* 1998. Regional distribution of urocortin-like immunoreactivity and expression of urocortin mRNA in the human brain. Peptides **19:** 643–647.
51. WONG, M.L., A. AL-SHEKHLEE, P.B. BONGIORNO *et al.* 1996. Localization of urocortin messenger RNA in rat brain and pituitary. Mol. Psychiatry **1:** 307–312.
52. IINO, K., H. SASANO, Y. OKI *et al.* 1997. Urocortin expression in human pituitary gland and pituitary adenoma. J. Clin. Endocrinol. Metab. **82:** 3842–3850.
53. IINO, K., H. SASANO, T. SUZUKI *et al.* 1998. Urocortin expression in mucosal inflammatory cells of human gastrointestinal tract. (Abst. # P2-561). *In* Endocrine Soc. Meeting, June 1988, New Orleans, Louisiana.

54. PETRAGLIA, F., P. FLORIO, R. GALLO *et al.* 1996. Human placenta and fetal membranes express human urocortin mRNA and peptide. J. Clin. Endocrinol. Metab. **81:** 3807–3810.
55. OKOSI, A., B.K. BRAR, M. CHAN *et al.* 1998. Expression and protective effects of urocortin in cardiac myocytes. Neuropeptides **32:** 167–171.
56. BAMBERGER, C.M., M. WALD, A.M. BAMBERGER *et al.* 1998. Human lymphocytes produce urocortin, but not corticotropin-releasing hormone. J. Clin. Endocrinol. Metab. **83:** 708–711.
57. POLIAK, S., F. MOR, P. CONLON *et al.* 1997. Stress and autoimmunity: the neuropeptides corticotropin-releasing factor and urocortin suppress encephalomyelitis via effects on both the hypothalamic-pituitary-adrenal axis and the immune system. J. Immunol. **158:** 5751–5756.
58. TURNBULL, A.V., W. VALE & C. RIVIER 1996. Urocortin, a corticotropin-releasing factor-related mammalian peptide, inhibits edema due to thermal injury in rats. Eur. J. Pharmacol. **15:** 213–216.
59. SMAGIN, G.N., L.A. HOWELL, D.H. RYAN *et al.* 1998. the role of CRF2 receptors in corticotropin-releasing factor- and urocortin-induced anorexia. Neuroreport **9:** 1601–1606.
60. PERRIN, M.H., S.W. SUTTON, L. CERVINI *et al.* 1999. Comparison of an agonist, urocortin, and an antagonist, astressin, as radioligands for characterization of CRF receptors. J. Pharm. Exp. Ther. **288:** 729–734.
61. GULYAS, J., C. RIVIER, M. PERRIN *et al.* 1995. Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor. Proc. Natl. Acad. Sci. USA **92:** 10575–10579.
62. PERRIN, M.H., S. SUTTON, D.B. BAIN *et al.* 1998. The first extracellular domain of corticotropin releasing factor-R1 contains major binding determinants for urocortin and astressin. Endocrinology **139:** 566–570.
63. MATHEWS, L. & W. VALE. 1991. Expression cloning of an activin receptor, a predicted transmembrane serine kinase. Cell **65:** 973–982.
64. SYDOW, S., J. RADULOVIC, F.M. DAUTZENBERG *et al.* 1997. Structure-function relationship of different domains of the rat corticotropin-releasing factor receptor. Brain Res. Mol. Brain Res. **52:** 182–193.
65. QI, L.J., A.T. LEUNG, Y. XIONG *et al.* 1997. Extracellular cysteines of the corticotropin-releasing factor receptor are critical for ligand interaction. Biochemistry **36:** 12442–12448.
66. LIAW, C.W., D.E. GRIGORIADIS, T.W. LOVENBERG *et al.* 1997. Localization of ligand-binding domains of human corticotropin-releasing factor receptor: a chimeric receptor approach. Mol. Endocrinol. **11:** 980–985.
67. LIAW, C.W., D.E. GRIGORIADIS, M.T. LORANG *et al.* 1997. Localization of agonist- and antagonist-binding domains of human corticotropin-releasing factor receptors. Mol. Endocrinol. **11:** 2048–2053.
68. DAUTZENBERG, F.M., S. WILLE, R. LOHMANN *et al.* 1998. Mapping of the ligand-selective domain of the *Xenopus laevis* corticotropin-releasing factor receptor 1: Implications for the ligand-binding site. Proc. Natl. Acad. Sci. **95:** 4941–4946.
69. CHEN, C., J.R. DAGNINO, E.B. DESOUZA *et al.* 1996. Design and synthesis of a series of non-peptide high-affinity human corticotropin-releasing factor$_1$ receptor antagonists. J. Med. Chem. **39:** 4358–4360.
70. WHITTEN, J.P., Y.F. XIE, P.E. ERICKSON *et al.* 1996. Rapid microscale synthesis, a new method for lead optimization using robotics and solution phase chemistry: application to the synthesis and optimization of corticotropin-releasing factor$_1$ receptor antagonists. J. Med. Chem. **39:** 4354–4357.

71. SCHULZ, D.W., R.S. MANSBACH, J. SPROUSE *et al.* 1996. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc. Natl. Acad. Sci. **93:** 10477–10482.
72. WEBSTER, W.L., D.B. LEWIS, D.J. TORPY *et al.* 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:** 5747–5750.