

# Autoradiographic Localization of CRF<sub>1</sub> and CRF<sub>2</sub> Binding Sites in Adult Rat Brain

Renee J. Primus, Ph.D., Eileen Yevich, B.S., Carolyn Baltazar, B.S., and Dorothy W. Gallager, Ph.D.

The regional distribution of corticotropin-releasing factor<sub>1</sub>  $(CRF_1)$  and  $CRF_2$  binding sites was assessed autoradiographically in adult rat brain. The differential pharmacological profiles of the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes were used for the discrimination of the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes in rat brain. Pharmacological characterization at the human CRF<sub>1</sub> receptor subtype, expressed in baculovirus-infected Sf9 cells, showed high affinity binding ( $Ki \leq 10.0$  nM) for the peptide agonists sauvagine, urotensin I, rat/human CRF, and ovine CRF. *Pharmacological characterization at the rat CRF*<sub>2</sub> *receptor* subtype expressed in CHO cells showed a rank order affinity for the peptide agonists such that sauvagine, urotensin I and rat/human CRF showed high affinity binding whereas ovine CRF had a Ki value of 300 nM. Based on this differential binding affinity for ovine CRF, [<sup>125</sup>I]sauvagine binding in the presence of increasing

concentrations of ovine CRF was used to discriminate CRF<sub>1</sub> from  $CRF_2$  receptor subtypes in rat brain. The  $CRF_1$ receptor subtype was found to be localized to various regions of the cerebellum, as well as to several cortical areas. *The* CRF<sub>2</sub> *receptor subtype was shown to be localized to the* lateral septal nucleus, entorhinal cortex, and to amygdaloid and hypothalamic regions. The present autoradiographic findings provide evidence that each subtype has a distinct regional distribution, thus strengthening the suggestion that CRF<sub>1</sub> and CRF<sub>2</sub> receptors serve different roles in mediating CRF function. Such data suggest that the development of CRF receptor subtype selective antagonists should help to delineate the role of CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes in central nervous system function. [Neuropsychopharmacology 17:308–316, 1997] © 1997 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

## KEY WORDS: Corticotropin-releasing factor; [<sup>125</sup>I]sauvagine; Receptor subtype; Stress

Corticotropin-releasing factor (CRF), a 41 amino acid peptide synthesized and released in the central nervous system, plays a primary role in the regulation of the hypothalamic-pituitary-adrenal axis (HPA). Several behavioral and clinical studies have suggested that CRF is involved in the pathophysiology of depression, stressrelated disorders, and feeding disorders (Owens and Nemeroff 1991). Using molecular biological techniques,

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655 Avenue of the Americas, New York, NY 10010

two distinct CRF receptor subtypes,  $CRF_1$  and  $CRF_2$ , have been identified in rat (Potter et al. 1994; Lovenberg et al. 1995a, 1995b) and human (Chen et al. 1993; Liaw et al. 1996) brain. The influence that each receptor subtype has on mediating CRF function remains to be determined.

Functional studies using expressed CRF<sub>1</sub> receptors have shown that the CRF-related peptides, urotensin I, sauvagine, ovine CRF, and rat/human CRF, are all potent activators of cAMP production (Lovenberg et al. 1995a). In contrast, at expressed CRF<sub>2</sub> receptors, sauvagine and urotensin I are considerably more potent than are ovine CRF and rat/human CRF in activating cAMP production (Lovenberg et al. 1995a). Localization studies have shown that the anatomical distribution of mRNA also differs markedly for the two receptor subtypes (Potter et al. 1994; Chalmers et al. 1995). CRF<sub>1</sub> receptor mRNA is abundant in cerebellum, pituitary, ce-

From the Neurogen Corporation, Branford, CT.

Address correspondence to: Dr. Renee Primus, Neurogen Corporation, 35 N.E. Industrial Rd., Branford, CT 06405.

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rebral cortex, and olfactory bulb, whereas  $CRF_2$  receptor mRNA is prominently expressed in the ventromedial nucleus of the hypothalamus, entorhinal cortex, amygdala, and lateral septal nucleus (Potter et al. 1994; Chalmers et al. 1995). This differential brain localization and pharmacological profile for the  $CRF_1$  and  $CRF_2$  receptor subtypes may suggest distinct functional roles for each subtype.

The lack of high affinity, subtype-selective ligands for the  $CRF_1$  and  $CRF_2$  receptor subtypes has restricted detailed pharmacological receptor characterization in brain tissue. In the present study, pharmacological characterization of the recombinant human  $CRF_1$  and rat  $CRF_2$  receptor subtype was performed. The distinct pharmacological profile obtained for the  $CRF_1$  and  $CRF_2$ receptor subtypes was then used to discriminate the two receptor subtypes in rat brain. Based on pharmacologically-defined differences between the two receptor subtypes, the distribution of  $CRF_1$  and  $CRF_2$  receptor proteins in rat brain was assessed using receptor autoradiographic techniques.

#### **METHODS**

#### Molecular Cloning

The cloning of the human CRF<sub>1</sub> receptor was conducted as described in Ross et al. (1995). The rat CRF<sub>2</sub> receptor gene was isolated from cDNA made from polyA mRNA prepared from rat hypothalamus. CRF<sub>2</sub> receptor gene oligonucleotides were designed from the DNA sequence obtained from Genbank. The 5' primer was a 32mer with a 2 basepair mismatch centrally located to allow for the incorporation of an XHOI site. The 3' primer was a 35mer containing 2 mismatches to incorporate a SacI site. Following PCR, the DNA was extracted, subcloned into pBacpak 8, and sequenced.

### **Homogenate Membrane Preparation**

Recombinant human CRF<sub>1</sub> receptors expressed in baculovirus-infected Sf9 cells and recombinant rat CRF<sub>2</sub> receptors expressed in CHO cells were harvested 72 hours post infection and immediately washed in PBS. Pellets were homogenized in wash buffer containing 50 mM Tris-HCl, pH7.7; 2 mM EGTA; and 10 mM MgCl<sub>2</sub> (Polytron, setting 4), followed by centrifugation at 48,0000 × g for 10 minutes. Pellets were rinsed in wash buffer and centrifuged, as described above, two additional times. Membranes were diluted in assay buffer (50 mM Tris-HCl, pH7.7; 2 mM EGTA; 10 mM MgCl<sub>2</sub>; 0.1% BSA; 0.2% bacitracin; and 0.01% aprotinin) to a protein concentration of 150 µg/mL for expressed human CRF<sub>1</sub> receptors, and 250 µg/mL for expressed rat CRF<sub>2</sub> receptors, and analyzed for receptor binding immediately.

#### Homogenate [125]Sauvagine Binding

Using polypropylene assay tubes, 0.100 mL of diluted membrane preparation was added to tubes containing [<sup>125</sup>I]sauvagine (100 pM for competition analysis; 10 pM-1.2 nM for saturation analysis), displacers and assay buffer (50 mM Tris, pH 7.7; 10 mM MgCl<sub>2</sub>; 2 mM EGTA; 0.1% BSA; 0.2% bacitracin; and 0.01% aprotinin) to yield a final volume of 0.250 mL. Competition analysis was performed at concentrations ranging from  $10^{-10}$ M to  $10^{-5}$  M. Nonspecific binding was defined by the addition of 2 µM rat/human CRF. Following a 2 hour incubation at room temperature, the reaction was terminated by rapid vacuum filtration through GF/C Whatman filters pretreated with 1.0% PEI. Filters were rinsed twice with ice-cold 50 mM Tris-HCl, pH 7.7. Filters were air-dried and then counted using a gamma counter. Specific binding ranged from 85% to 90%. To estimate the Bmax, Kd, and Ki, the results of binding experiments were analyzed using SigmaPlot (Jandel).

#### **Receptor Autoradiography**

Animals were deeply anesthetized with sodium pentobarbital and then sacrificed by intracardial perfusion with ice-cold buffered saline followed by 0.1% buffered paraformaldehyde solution. Brains were removed, frozen on powdered dry ice, and stored at -80°C. Twentymicron frozen sections were cut on a Frigocut cryostat and thaw mounted onto gelatin-coated slides. Tissue sections were stored at -80°C for no more than 48 hours before the assay. On the day of assay, slidemounted tissue sections were preincubated  $2 \times 15$  minutes at room temperature in buffer containing 50 mM Tris (pH 7.7), 10 mM NaCl, and 2 mM EGTA to remove endogenous inhibitors of binding. Following preincubation, slides were incubated at room temperature in assay buffer (50 mM Tris, pH 7.7; 10 mM NaCl; 2 mM EGTA; 0.1% BSA; 0.1 mM bacitracin; 0.01% aprotinin) containing 150-200 pM [125I]Sauvagine for 2 hours. Competition analysis of CRF peptides was performed at concentrations ranging from  $3 \times 10^{-9}$  M to  $3 \times 10^{-7}$ M. Nonspecific binding was defined by the addition of  $2 \,\mu\text{M}$  rat/human CRF. Sections were rinsed  $2 \times 5$  minutes in ice-cold PBS with 1% BSA, followed by a brief dip in ice-cold distilled water. Sections were dried under a stream of cool air, apposed to Hyperfilm, and exposed in X-ray cassettes for 3-5 days at room temperature. Films were analyzed with the MCID-M4 imaging system (Imaging Research, St. Catherines, Ontario).

#### RESULTS

Saturation analysis at the recombinant human CRF<sub>1</sub> receptor expressed in baculovirus-infected Sf9 cells using



**Figure 1.** Representive saturation curve for [<sup>125</sup>I]sauvagine binding to membranes prepared from Sf9 insect cells expressing the human CRF<sub>1</sub> receptor. Each concentration was tested in triplicate using 8–10 concentrations of [<sup>125</sup>I]sauvagine (0.010 to 0.700 nM). Saturations were repeated three times. Saturation analysis at the recombinant human CRF<sub>1</sub> receptor showed two distinct binding sites (Kd1: 0.066  $\pm$  0.009 nM: Bmax1: 220.5  $\pm$  2.5 fmole/mg protein; Kd2: 0.46  $\pm$  0.014 nM; Bmax2: 1251.5  $\pm$  224.8 fmole/mg protein). The inset shows the corresponding linear Rosenthal plot of the data.

 $[^{125}I]$ sauvagine showed two distinct binding sites (Kd1: 0.066 ± 0.009; Bmax1: 220.5 ± 2.5 fmole/mg protein; Kd2: 0.46 ± 0.014; Bmax2: 1251.5 ± 224.8); (Figure 1). The significance of the presence of the lower affinity, higher capacity CRF<sub>1</sub> binding site in the Sf9 cell is unclear. We have observed that  $[^{125}I]$ sauvagine binding at the CRF<sub>1</sub> receptor is modulated by guanine nucleotides, suggesting regulation by G-proteins of CRF<sub>1</sub> receptors expressed in Sf9 cells (data not shown). Therefore, the existence of both high and low affinity sites at the CRF<sub>1</sub> receptor to G-proteins endogenously expressed in Sf9 cells.

Pharmacological characterization of the human CRF<sub>1</sub> receptor showed a pharmacological profile similar to studies published previously using COS-M6 cells (Chen et al. 1993) or LtK<sup>-</sup> (Grigoriadis et al. 1996) cells. High affinity binding to the CRF<sub>1</sub> receptor was shown for the peptide agonists, urotensin I, sauvagine, ovine CRF, and rat/human CRF (Ki values:  $0.68 \pm 0.4$  mM,  $1.5 \pm 0.8$  nM,  $6.7 \pm 2.3$  mM and  $11.1 \pm 2.3$  nM, respectively) (Table 1). The peptide antagonists alpha-helical CRF<sub>(9-41)</sub> and D-Phe alpha-helical CRF<sub>(12-41)</sub> showed binding affinities of  $88.04 \pm 24.5$  nM, and  $88.6 \pm 2.9$  nM, respectively, at the CRF<sub>1</sub> receptor.

Saturation analysis of [<sup>125</sup>I]sauvagine binding to recombinant rat CRF<sub>2</sub> receptors expressed in CHO cells showed high-affinity, saturation binding that was best fit using a one-site model (Kd: 0.27  $\pm$  0.055 nM; Bmax: 413.3  $\pm$  46.0 fmole/mg protein) (Figure 2). Pharmacological characterization showed a rank order affinity for the peptide agonists such that sauvagine > urotensin I  $\geq$ rat/human CRF > ovine CRF (Ki values: 3.6  $\pm$  0.6 nM,

**Table 1.** Comparison of Receptor Binding Affinities forRecombinant Human CRF1 Receptors and Rat CRF2Receptors

	Ki (nM)	
	Human CRF <sub>1</sub>	Rat CRF <sub>2</sub>
Urotensin I	$0.7 \pm 0.4$	22.9 ± 1.7
Sauvagine	$1.5\pm0.8$	$3.6 \pm 0.6$
Ovine CRF	$6.7 \pm 2.3$	$300.5 \pm 83.3$
Rat/human CRF	$11.1 \pm 2.3$	$24.6 \pm 4.0$
$\alpha$ -helical CRF <sub>(9-41)</sub>	$88.0 \pm 24.5$	$4.6 \pm 2.9$
D-Phe $\alpha$ -helical $CRF_{(12-41)}$	$88.62\pm2.9$	$3.5\pm0.7$

Each data point represents N = 2-3.

[<sup>125</sup>I]Sauvagine at 0.1 nM used for pharmacological characterization.



# [<sup>125</sup>I]Sauvagine Binding at Rat CRF<sub>2</sub> Receptors Stably Expressed in CHO Cells

**Figure 2.** Representative saturation curve for [<sup>125</sup>I]sauvagine binding to membranes prepared from CHO cells stably expressing the rat CRF<sub>2</sub> receptor. Each concentration was tested in triplicate using 8–10 concentrations of [<sup>125</sup>I]sauvagine (0.010 to 1.200 nM). The average K<sub>d</sub> and Bmax values, as determined by computer analysis of the saturation isotherm data from three independent experiments, were  $0.27 \pm 0.05$  nM and  $413.3 \pm 46.0$  fmol/mg protein, respectively. The inset shows the corresponding linear Rosenthal plot of the data.

22.9 ± 1.7 nM, 24.6 ± 4.0 nM, and 300.5 ± 83.3 nM, respectively) (Table 1), a finding consistent with that reported by Grigoriadis et al. (1996). The peptide antagonists alpha-helical CRF<sub>(9-41)</sub> and D-Phe alpha-helical CRF<sub>(12-41)</sub> showed high affinity binding at the CRF<sub>2</sub> receptor (Ki values: 4.6 ± 2.9 nM and 3.5 ± 0.7 nM, respectively).

Based on the distinct pharmacological profiles for the  $CRF_1$  and  $CRF_2$  receptor subtypes (Table 1), ovine CRF was used to discriminate the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes in rat brain. Urotensin I at 100 nM, a saturating concentration at both receptor subtypes, fully displaced [<sup>125</sup>I]sauvagine binding in rat brain sections (Figures 3A and 3B). In contrast, ovine CRF at 100 nM, a saturating concentration only at the CRF1 receptor subtype, displaced [125]sauvagine binding in cerebellar regions, and in frontoparietal and temporal cortical regions, but showed incomplete displacement in the lateral septal nucleus (LTS), entorhinal cortex (ETC), and portions of the amygdala (Amg) and hypothalamus (Hyp) (Figures 4A-4C and 5A-5C). At 300 nM, ovine CRF displaced residual [125I]sauvagine binding in rat brain LTS, ETC, Amg, and Hyp, consistent with the

pharmacology of the CRF<sub>2</sub> receptor subtype (Figures 4D-4E and 5D-5E). The ability of ovine CRF to discriminate CRF<sub>2</sub> receptors is demonstrated by the similarity between autoradiographic images in Figures 4B and 5B (actual) and Figures 4F and 5F (subtraction). Table 2 shows data from quantitative analysis of [<sup>125</sup>I]sauvagine binding to rat brain tissue.

#### DISCUSSION

The CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes have been shown to differ both functionally (Lovenberg et al. 1995a) and in the regional distribution of mRNA expression (Chalmers et al. 1995). The present study further establishes distinct pharmacological profiles for expressed human CRF<sub>1</sub> and rat CRF<sub>2</sub> receptor subtypes. The lack of commercially available high affinity, subtype-selective ligands for the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes makes further pharmacological characterization in brain tissue difficult. However, defined differences in CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtype pharmacology provide a tool with which to distinguish the two receptor subtypes in brain.



**Figure 3.** Representative autoradiogram of total (**A**) and nonspecific (**B**) [<sup>125</sup>I]sauvagine binding in rat brain. Nonspecific binding was determined in the presence of 100 nM Urotensin I. Serial sections from two to three separate animals were analyzed for [<sup>125</sup>I]sauvagine binding using quantitative autoradiographic techniques. *Abbreviations:* Amg, amygdala; Cbm, cerebellum; Ctx, cortex; ETC, entorhinal cortex.

The recombinant human  $CRF_1$  receptor subtype showed high affinity ( $\leq 10.0$  nM) binding for the peptide agonists including: sauvagine, urotensin I, rat/human CRF, and ovine CRF. Pharmacological characterization at the CRF<sub>2</sub> receptor subtype showed high affinity binding for all peptide agonists analyzed except for ovine CRF, which showed lower affinity binding at this subtype (Ki value of 300 nM at CRF<sub>2</sub> receptors). This differential pharmacological profile for the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes is consistent with reported differences measured functionally at the two receptor subtypes (Lovenberg et al. 1995a). Interestingly, the peptide antagonists alpha-helical CRF<sub>(9-41)</sub> and D-Phe alpha-helical CRF<sub>(12-41)</sub> showed only moderate affinity at the human  $CRF_1$  receptor subtype but high affinity at the rat  $CRF_2$  receptor subtype. Overall, the distinct pharmacological profile for the  $CRF_1$  and  $CRF_2$  receptor subtypes may suggest that  $CRF_1$  and  $CRF_2$  receptor subtypes serve different roles in mediating CRF function.

The differential pharmacological profile for the human CRF<sub>1</sub> and rat CRF<sub>2</sub> receptor subtypes was used to discriminate the two receptor subtypes autoradiographically in rat brain. Since sauvagine shows high affinity for both CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes, [<sup>125</sup>I]sauvagine was used to label both subtypes in rat brain. Ovine CRF, on the other hand, shows a Ki of <10.0 nM at CRF<sub>1</sub> receptors, but a Ki of 300 nM at CRF<sub>2</sub> receptors. Therefore, ovine CRF was used as the displacing agent for characterizing CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes in rat brain. In the present study, ovine CRF at a concentration of 100 nM or 300 nM was used to displace [<sup>125</sup>I]sauvagine binding from CRF<sub>1</sub> and CRF<sub>2</sub> receptors, respectively.

As demonstrated autoradiographically, the regional distribution of CRF<sub>2</sub> binding sites is distinct from that of CRF<sub>1</sub> sites and is consistent with the reported distribution of CRF receptor mRNA expression (Chalmers et al. 1995; Lovenberg et al. 1995a; Potter et al. 1994). CRF<sub>1</sub> binding sites, defined using 100 nM ovine CRF, were shown to be most abundant in the cerebellum and in frontoparietal and temporal cortical regions. However, at 100 nM CRF, [125I]sauvagine binding in limbic/hypothalamic regions was still present. When ovine CRF was used at a concentration of 300 nM, [125I]sauvagine binding in these limbic/hypothalamic regions was considerably reduced. Therefore, CRF<sub>2</sub> receptors, defined using 300 nM ovine CRF, could be localized to limbic/hypothalamic regions including the lateral septal nucleus, entorhinal cortex, amygdala and hypothalamus. The localization of CRF<sub>2</sub> receptor binding to these areas in rat brain using 300 nM ovine CRF is in agreement with reports describing the localization of CRF<sub>2</sub> receptor mRNA (Chalmers et al. 1995; Lovenberg et al. 1995a). Hence, the method used in the present study, which is based on pharmacological differences as defined at expressed human  $CRF_1$  and rat  $CRF_2$  receptor subtypes, can be used to localize populations of CRF<sub>2</sub> receptors in rat brain. However, an accurate quantitative analysis of the localization of CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes to discrete nuclei of the hypothalamus and amygdala will require the development of more selective radiolabeled ligands for the CRF receptor subtypes.

CRF<sub>2</sub> receptors were also present in rat frontal cortex and cerebellum. The localization of CRF<sub>2</sub> binding sites in rat frontal cortex and cerebellum is at discrepancy with the reported localization of the messenger RNA for the CRF<sub>2</sub> receptor (see Lovenberg et al. 1995 and Chalmers et al. 1995). These reports show that CRF<sub>2</sub> mRNA is found almost exclusively in nuclei of the amygdala, lateral septum, entorhinal cortex, hippocam-



Figure 4. See legend to Figure 5.

pal regions, and specific nuclei of the hypothalamus. Our studies show  $CRF_2$  receptor density in the frontal cortex and cerebellum comparable in amount to the density of  $CRF_2$  binding sites measured in hypothala-

mus. The reason for this discrepancy between the above-mentioned mRNA studies and our present binding results is unclear. One possibility for this discrepancy is a mismatch between the  $CRF_2$  mRNA localiza-

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**Figure 5.** Figures 4 and 5 display sagittal sections from rat brain showing the distribution of total [<sup>125</sup>I]sauvagine binding (**A**, **D**), nonspecific [<sup>125</sup>I]sauvagine binding using ovine CRF at either 100 nM (**B**) or 300 nM (**E**), and specific [<sup>125</sup>I]sauvagine binding for CRF<sub>1</sub> (**C**) and CRF<sub>2</sub> (**F**) receptors. Serial sections from two to three separate animals were analyzed for [<sup>125</sup>I]sauvagine binding. *Abbreviations:* Amg, amygdala; Cbm, cerebellum; Ctx, cortex; ENT, entorhinal cortex; LTS, lateral septal nucleus.

**Table 2.** Regional Distribution of and Specific $[^{125}I]$ sauvagine Binding (fmole/mg Protein TissueEquivalence) to CRF1 and CRF2 Receptor Subtypesin Adult Rat Brain Sections

Brain region	CRF <sub>1</sub>	CRF <sub>2</sub>
Amygdala	$0.32 \pm 0.03$	$0.89 \pm 0.1$
Cerebellum	$2.57 \pm 0.18$	$0.4 \pm 0.02$
Entorhinal cortex	$0.68\pm0.05$	$1.1 \pm 0.09$
Frontal cortex	$0.95\pm0.05$	$0.68 \pm 0.04$
Hippocampus	$0.07\pm0.02$	$0.01\pm0.01$
Hypothalamus	$0.23 \pm 0.15$	$0.37 \pm 0.025$
Lateral septum	$0.44\pm0.05$	$0.83 \pm 0.06$
Nucleus accumbens	$0.1 \pm 0.01$	$0.08\pm0.01$
Occipital cortex	$0.84\pm0.07$	$0.83\pm0.07$

Serial sections from two to three separate animals were analyzed for [ $^{125}$ I]sauvagine binding using quantitative autoradiographic techniques. Results reported as mean  $\pm$  SEM.

tion and the location of CRF<sub>2</sub> protein expression. That is, the protein can be transported and expressed at a location different from the site of mRNA expression. A second possibility may be that the appearance of CRF<sub>2</sub> binding in frontal cortex could arise from an artifact inherent in the subtraction binding technique used in this autoradiographic study (for review, see Boast et al. 1986). However, the results obtained in this study were reproducible as demonstrated in subsequent experiments. Furthermore, the distribution in the brain (other than the cortex and cerebellum) of the CRF2 receptor correlates well with the CRF2 mRNA localization. Therefore, it seems unlikely that the cortical localization of CRF<sub>2</sub> binding sites is solely due to technical artifacts. Such discrepancies should be resolved by the development of a selective radiolabeled CRF<sub>2</sub> receptor ligand.

Research has indicated that brain corticotropin releasing factor (CRF) plays a role in several neuropsychiatric and feeding disorders, including anxiety, depression, and anorexia nervosa (Dunn and Berridge 1990; Coplan et al. 1996). The localization of CRF<sub>2</sub> receptors in limbic/hypothalamic brain regions may suggest an involvement of this receptor subtype in the regulation of emotional behavior. The recent identification of urocortin, a mammalian neuropeptide that binds with high affinity to CRF<sub>2</sub> receptors and has appetite-suppressing effects in rats, further suggests a role for the CRF<sub>2</sub> receptor in behavioral regulation (Spina et al. 1996; Vaughan et al. 1995).

Thus far, two pharmacologically distinct CRF receptor subtypes, CRF<sub>1</sub> and CRF<sub>2</sub>, have been identified. In addition, two forms of the CRF<sub>2</sub> receptor have been identified in rat brain, CRF<sub>2</sub> and CRF<sub>2</sub> (Lovenberg et al. 1995b). The identification of a CRF-binding protein (Chamlers et al. 1996; Potter et al. 1992), which displays a pharmacological profile distinct from either the CRF<sub>1</sub> or CRF<sub>2</sub> receptors, suggests further complexity in this system. The present study provides a method that per-

mits the detection of CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes endogenously expressed in rat brain. Because of the discrete localization of the CRF2 receptor subtype and low density of this receptor in rat brain, detection of the CRF<sub>2</sub> receptor using currently available ligands and conventional homogenate binding techniques is difficult if not impossible. The method described in this study circumvents these problems, and therefore, provides a tool to distinguish and examine endogenously expressed CRF receptor subtypes. Overall, the distinct pharmacological profile and regional distribution for the CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes may suggest that CRF<sub>1</sub> and CRF<sub>2</sub> receptor subtypes serve different roles in mediating CRF function. The development of CRF receptor subtype selective antagonists should help to delineate the role  $CRF_1$  and  $CRF_2$  receptor subtypes play in behavioral and physiological regulation as well as in psychiatric illness.

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