Comparison of an Agonist, Urocortin, and an Antagonist, Astressin, as Radioligands for Characterization of Corticotropin-Releasing Factor Receptors

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ABSTRACT

The characteristics of a high-affinity antagonist radioligand are compared with those of a high-affinity agonist in binding to the cloned corticotropin-releasing factor receptor type 1 (CRF-R1) and type 2 (CRF-R2) and to the native receptors that exist in rat cerebellum and brain stem. The relative potencies of CRF antagonists and agonists to the two types of cloned CRF receptors overexpressed stably in Chinese hamster ovary cells are determined using the antagonist radioligand [125I]-DTyr1astressin (Ast*), and the agonist radioligand, [125I]-[Tyr9]rat urocortin (Ucn*). The inhibitory binding constants (K) of astressin and urocortin are 1 to 2 nM for all receptors and are independent of which radioligand is employed. Astressin binds with high affinity to the native cerebellar/brain stem receptor and relative potencies of selected CRF analogs determined with Ast* on the native receptor are similar to those obtained for the cloned CRF-R1. The specific binding of Ast* to endogenous brain receptors is greater than that of Ucn*, resulting in more sites being detected by the antagonist than by the agonist. In contrast to another CRF agonist, the binding of Ucn* to the cloned receptors is relatively insensitive to guanyl nucleotides at both 20°C and 37°C; however, its binding to the native receptor is displaced by guanyl nucleotides at 37°C and, to a lesser degree, at 20°C. As expected, the binding of the antagonist Ast* is not affected by guanyl nucleotides. Because it is a high-affinity, specific CRF antagonist, astressin is eminently suitable as a ligand for detection and characterization of both endogenous and cloned CRF receptors.

Corticotropin-releasing factor (CRF) is a key modulator of the hypothalamic-pituitary-adrenal axis and has a broad diversity of actions including those on the cardiovascular and immune systems. CRF exerts its actions by binding to specific cell surface receptors on target tissues. Two CRF receptors, encoded by different genes and existing in multiple forms as splice variants, have been cloned: CRF-R1 (Chang et al., 1993; Chen et al., 1993; Vita et al., 1993) and CRF-R2 (Kishimoto et al., 1995; Lovenberg et al., 1995; Perrin et al., 1995; Stenzel et al., 1995; Kostich et al., 1998). CRF-R1 is the predominant receptor type in the pituitary (Potter et al., 1994; Chalmers et al., 1995) and is also widely distributed throughout the central nervous system. In the rat, one of the splice variants, CRF receptor type 2α (CRF-R2α), is found mainly in a restricted localization in the brain (Chalmers et al., 1995). The second splice variant, CRF receptor type 2β (CRF-R2β), is expressed not only in the rat brain, but also in peripheral tissues such as the heart, gastrointestinal tract, and epididymis (Perrin et al., 1995).

The CRF receptors belong to the 7-transmembrane domain receptor family that is coupled to adenylate cyclase via GTP-binding proteins (G proteins). The actions of CRF are inhibited by specific antagonists. A new antagonist, astressin, has been shown to be more potent than other previous antagonists, such as α-helCRF(9–41) or [DPhe12,Nle38]rat CRF (rCRF), at inhibiting CRF-stimulated adrenocorticotropin (ACTH) release in cultured rat anterior pituitary cells (Gulyas et al., 1995), a CRF-R1-mediated event. Recently, another CRF-like peptide, urocortin (Ucn), cloned from rat brain (Vaughan et al., 1995) has been shown to be ~8 times more potent than CRF at stimulating ACTH release from anterior pituitary cells, and 10 times more potent than CRF at stimulating cAMP accumulation in cells stably expressing CRF-R2 (Vaughan et al., 1995; Donaldson et al., 1996).

Receptors that are coupled to G proteins are characterized by agonist states of high and low affinity, which interconvert via GDP/GTP exchange on the G protein (Conklin and Bourne, 1993). The apparent affinity of an agonist depends

ABBREVIATIONS: CRF, corticotropin-releasing factor; CRF-R1, corticotropin-releasing factor receptor type 1; CRF-R2α, corticotropin-releasing factor receptor type 2α; CRF-R2β, corticotropin-releasing factor receptor type 2β; G protein, GTP-binding proteins; CHO, Chinese hamster ovary; HPLC, high-performance liquid chromatography; Ucn, urocortin; Ast, astressin.
on the degree of coupling of the receptor to the G protein, whereas the affinity of an antagonist is independent of such coupling. As a consequence, the binding of agonists, but not that of antagonists, is modulated by guanyl nucleotides. Accordingly, we have shown that GTP analogs inhibit the binding of \(^{{25}}\)I-[Nle\(^{21},\) Tyr\(^{32}\)]ovine CRF (oCRF\(^{b}\)) to the pituitary receptor and convert the high-affinity state into one of lower affinity (Perrin et al., 1986).

Up to now, studies characterizing CRF receptors have used various agonist analogs as radioligands, such as oCRF\(^{b}\) (Perrin et al., 1986) and \(^{125}\)I-[Tyr\(^{3}\)]oCRF (Grigoriadi and De Souza, 1989). Recently, \(^{125}\)I-sauvagine has been used successfully to map the CRF-R2 binding sites in rat brain (Rominger et al., 1998). Identification and characterization of other receptors, e.g., beta adrenergic, have been facilitated by the use of antagonist radioligands (Maguire et al., 1977; Leffkowitz, 1978). Data on the binding of Substance P to the natural killer cell (NK)-1 receptor (Gether et al., 1978) and more generally on other peptide hormones (Schwartz et al., 1980) suggest that the binding determinants of agonists may differ from those of antagonists. The purpose of this work was to determine whether a radioligand based on the potent antagonist astressin is more useful for the detection and characterization of both cloned and native CRF receptors than radioligands based on the agonists.

Materials and Methods

Membrane Fractions. Crude membrane fractions were prepared from tissues and stably transfected Chinese hamster ovary (CHO) cells as previously described (Perrin et al., 1986; Chen et al., 1993) and stored in 10% sucrose at \(-80^\circ\)C until use. Clonal cell lines for each receptor type were established as previously described (Sutton et al., 1995). Protein concentrations were determined with the Bio-Rad assay kit (Bio-Rad, Hercules, CA) using gamma globulin as standard.

Peptide Iodination. To a 1-mg/ml solution in 10 mM acetic acid of peptides [DTyr\(^{1}\)]astressin or [Tyr\(^{3}\)]rUcn was added 2.1 equivalents of a 0.7-mM sodium iodide solution (0.05 N sodium phosphate buffer at pH 7) while stirring at 4°C for [DTyr\(^{1}\)]astressin or 22°C for [Tyr\(^{3}\)]rUcn. This was followed by dropwise addition of 1 to 1.1 equivalents of chloramine T (Sigma Chemical Co., St. Louis, MO) (0.4 mM in phosphate buffer) over 5 min. The reaction was quenched after an additional 1 min (29 min total reaction time for [Tyr\(^{3}\)]rUcn) with 7 to 8 equivalents of sodium metabisulfite solution in phosphate buffer. The reaction products consisted of unlabeled, mono- and bisiodinated peptides in the approximate ratios of 1:1.2:1 and 2.6:1.7:1 for [Tyr\(^{3}\)]rUcn and [DTyr\(^{1}\)]astressin, respectively. The crude mixtures were then purified by reversed-phase high-performance liquid chromatography (HPLC) (Hoeger et al., 1987) to \(>97\%\) purity as determined by capillary zone electrophoresis and quantitative HPLC. Characterization by mass spectroscopy confirmed the identity of the moniodinated species of both peptides by agreement between the observed and calculated mass values.

Peptide Radioiodination. The peptides [Nle\(^{21},\)Tyr\(^{32}\)]oCRF, [DTyr\(^{1}\)]astressin, and [Tyr\(^{3}\)]rUcn were radioiodinated using the chloramine T (Sigma) method and purified by HPLC, as previously described (Perrin et al., 1986). Moniodinated and monoradio-iodinated peptides were shown to coelute on reversed-phase HPLC and were separated from the noniodinated or di-iodinated peptides.

Receptor Binding Assays. CRF binding to recombinant human CRF (r/hCRF) receptor was performed in a manner similar to that described (Perrin et al., 1986). Briefly, crude membrane fractions were combined with 50,000 to 120,000 cpm of oCRF\(^{b}\), Ucn\(^{a}\), or Ast\(^{a}\) (\(-0.1–0.3\) nM; 2200 Ci/mmol) and peptide competitors in assay buffer (20 nM HEPES 10 mM MgSO\(_4\), 0.075% BSA, 7.5% sucrose, and 1.75 mM EGTA), for 2 h at 20°C, or for 30 min at 37°C. Assays of recombinant receptors used 5 to 20 µg of total membrane protein per well, whereas 60 to 100 µg of total membrane protein were used for native receptors. Reactions were performed in 96-well Multi-Screen plates (Millipore, Bedford, MA). Binding was terminated by aspiration through the plate, followed by a 0.2-mL wash with assay buffer. Binding to cloned receptors was performed in plates with GF/C filters (prewetted with 0.1% polyethyleneimine) when using Ast\(^{a}\) and oCRF\(^{b}\), whereas reactions with Ucn\(^{a}\) were performed in plates with 0.22-m Durapore filters, wetted with assay buffer. Binding to native membranes was performed in plates with GF/C filters for all radioligands. All assays contained tubes for nonspecific binding, which was taken to be the counts per minute remaining in the presence of 100 to 200 nM unlabeled ligand. K\(_{d}\) values were determined by pooling data from at least three independent assays using the LIGAND computer program (Munson and Rodbard, 1980).

Results

Binding to Cloned CRF Receptors. Ast\(^{a}\) and Ucn\(^{a}\) bind with high affinity to CRF-R1 stably expressed in CHO cells (Fig. 1). The affinities for a selected group of CRF analogs for CRF-R1 are listed in Table 1. For Ast\(^{a}\), the total binding was \(~20~\)to \(50\%\) and the specific binding was \(~15~\)to \(30\%\); for Ucn\(^{a}\), the total binding was 10 to 20% and the specific binding was 5 to 15%. The binding affinity of astressin for CRF-R1 is greater than that of two other antagonists, a-helCRF(9–41) and [DPhe\(^{12},\)Nle\(^{21},\)Tyr\(^{38}\)]rCRF(12–41); however, the increase in affinity is much less than the observed increase in biological activity in vitro (Gulyas et al., 1995). On the cloned receptors, the number of binding sites detected by Ast\(^{a}\) is greater than that detected by Ucn\(^{a}\) (Table 2).

To calculate the affinities and receptor densities using the LIGAND program, it was necessary to determine the affinities of Ast\(^{a}\) and Ucn\(^{a}\). To do this, the nonradioactive compounds, moniodo-[DTyr\(^{1}\)]astressin and moniodo-[Tyr\(^{3}\)]rUcn were synthesized and their affinities were measured by competitive displacement. It was found that the K\(_{d}\) values of the nonradioactive iodinated analogs were not significantly different from those of astressin and urocortin, as was found previously for the affinity of oCRF\(^{b}\) (Perrin et al., 1986). Therefore, the affinities of Ast\(^{a}\) and Ucn\(^{a}\) are the same as those for astressin and urocortin, and these latter compounds were used as standards in all competitive displacement assays.

Both Ast\(^{a}\) and Ucn\(^{a}\) bind with high affinity to either splice variant of the cloned CRF-R2. As shown in Table 1, the K\(_{i}\) of astressin is 1 to 3 nM for both CRF-R2\(_{a}\) and CRF-R2\(_{b}\), regardless of which radioligand was used. The K\(_{i}\) of a-helCRF(9–41) was \(~1~\)nM for CRF-R2\(_{a}\) and \(~5~\)nM for CRF-R2\(_{b}\). The K\(_{i}\) for Ucn\(^{a}\) binding to type 2 receptors was \(~1~\)nM using either Ucn\(^{a}\) or Ast\(^{a}\). For all the cloned receptors, the K\(_{i}\) for r/hCRF as determined with Ast\(^{a}\) or Ucn\(^{a}\) was greater than that determined with the radioligand oCRF\(^{b}\) (Perrin et al., 1995; Vaughan et al., 1995). In addition, the K\(_{i}\) values for urotensin and for sauvagine binding to CRF-R1 were also higher when determined with Ast\(^{a}\) and Ucn\(^{a}\) than those determined with oCRF\(^{b}\) (Vaughan et al., 1995).

Binding to Native Receptors. Both Ast\(^{a}\) and Ucn\(^{a}\) bound with high affinity to membrane fractions from mouse and rat cerebellum/brain stem, rich sources of CRF-R1. The specific binding of both astressin and Ucn was greater to...
mouse tissue compared with rat tissue. On the native rat receptor, Ast* detected more binding sites than did Ucn* (Table 2); the $K_i$ (based on displacement of Ast*) for r/hCRF, Ucn, and astressin are given in Table 3. Astressin and Ucn had the same $K_i$s on the native receptor (1–3 nM), whereas the $K_i$ for r/hCRF was ~10 times higher as determined with Ast* as radioligand. These data were similar to those for the cloned CRF-R1 (Table 1). The antagonist, [DPhe$^{12}$,Nle$^{21,38}$]r/hCRF(12–41), had a lower $K_i$ on the native receptor compared with that on the cloned CRF-R1, whereas that for a-helCRF(9–41) was similar to that found on CRF-R1.

**Guanyl Nucleotide Effects.** Guanyl nucleotides did not affect the binding of Ast* to any of the cloned receptors at either 20 or 37°C. An unanticipated observation was that there was very little effect of guanyl nucleotides on the binding of the agonist, Ucn*. Increasing concentrations of 5'-guanylylimidodiphosphate [Gpp(NH)p] had a barely detectable effect on the binding of Ucn* to the cloned receptors at either 20° or 37°C; in the same assays, Gpp(NH)p exhibited the expected displacement of bound oCRF* (another CRF agonist) (Figs. 2 and 3). For the native receptor, the effects of guanyl nucleotides on Ucn* binding were more pronounced at 37°C than at 20°C. In Fig. 4, we show the effects of guanyl nucleotides on Ucn* binding to cerebellum/brain stem; we show the guanyl nucleotide sensitivity of Ucn* binding to the mouse brain at both 20° and 37°C because the specific binding of Ucn* to mouse tissue is greater than that to rat tissue at 20°C. The dissociation rate of Ucn* bound to CHO-R1 was much smaller than that of Ast*: There was ~20% Ucn* dissociated after 3 h, whereas for Ast* there was ~75% dissociated after 2 h at 20°C.

**Discussion**

Labeled antagonists have advantages over agonists for the characterization of G protein-coupled receptors if, for example, the total number of binding sites is of interest, because the binding of antagonists is independent of the fraction of receptors coupled to the GTP-binding proteins (DeLean et al., 1980). For example, in the beta adrenergic receptor system, high-affinity antagonists were used in receptor characterization (Brown et al., 1976).

All previously reported CRF antagonists were of too low an affinity to be used as radioligands for characterizing CRF-R1 receptors. With astressin (Gulyas et al., 1995), we now have an antagonist of sufficient affinity to make it suitable as a radioligand for the detection and characterization of both cloned and endogenous CRF receptors. Using autoradiographic techniques and radioreceptor assays, brain receptors have also been detected with oCRF* (De Souza et al., 1984) and $^{125}$I-labeled [Tyr$^0$]oCRF (Grigoriadis and De Souza, 1988; Webster et al., 1996).

It is noteworthy that Ast* detects more native receptors than does Ucn* even though their $K_i$s are similar. This observation is consistent with the predictions of the model for G protein-coupled receptors in which an agonist binds with high affinity to only the fraction of receptors associated with the G protein, whereas an antagonist recognizes uncoupled receptors as well. Recently, tritium-labeled urocortin has been used to detect cerebellar receptors with the number of sites detected found to be 9 fmol/mg (Gottowick et al., 1997). The number of cerebellar receptors that we detect is 9 or 800 fmol/mg of protein using labeled urocortin or astressin, respectively.

The binding data in Table 1 show that both astressin and urocortin have nearly the same affinity for the three cloned CRF receptors. Furthermore, the data show that either Ast* or Ucn* can be used to determine the relative potencies of CRF analogs, but the absolute values of the $K_i$s may depend on the radioligand. In particular, the $K_i$ values for r/hCRF determined for both receptors with both radioligands appear to be much higher than the values determined with the oCRF* radioligand (Perrin et al., 1995; Vaughan et al., 1995). A similar difference was found for sauvagine for CRF-R1. The reasons for these discrepancies are currently not understood.

The antagonist, a-helCRF(9–41), has a significantly higher affinity for type 2 receptors than for type 1. Indeed, the affinities of a-helCRF(9–41) and astressin are nearly the same on CRF-R2B. A similar difference in potency for a-helCRF(9–41) on CRF-R2$^\beta$ compared with CRF-R1 was shown in the inhibition of CRF-stimulated cAMP (Kishimoto et al., 1985). This difference is consistent with the experimental observation that a-helCRF(9–41) is less potent...
at inhibiting CRF-stimulated ACTH release than at reversing CRF inhibition of edema (Turnbull et al., 1996). The pituitary effects of CRF are probably mediated by CRF-R1, based on the observation from in situ data that there is little, if any, CRF-R2 in that tissue (Chalmers et al., 1995) and that mice null for CRF-R1 exhibit low hypothalamic-pituitary-adrenal activation under basal and stressful conditions (Smith et al., 1998). In the rat, CRF-2β has been found not only in cerebral blood vessels, but also in the periphery, including in blood vessels, heart, epididymis, and gastrointestinal tract (Perrin et al., 1995), so that the effect of CRF on edema may be mediated by CRF-R2β.

Differential labeling of receptors by agonists and antagonists has been found for the cloned 5-hydroxytryptamine 2A receptor expressed in NIH 3T3 cells. In this system, a labeled antagonist detected significantly more receptor sites than a labeled agonist and the apparent affinities of antagonists were independent of the radiolabel, but agonists had a higher

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TABLE 1

Inhibitory binding constants for CRF and related compounds

The 95% confidence limits are given in parentheses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>hCRF-R1 (Ast*)</th>
<th>Ucn*</th>
<th>rCRF-R2α (Ast*)</th>
<th>Ucn*</th>
<th>mCRF-R2β (Ast*)</th>
<th>Ucn*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astressin</td>
<td>2.0 nM</td>
<td>1.6</td>
<td>1.5 nM</td>
<td>2.8</td>
<td>1.0 nM</td>
<td>0.87</td>
</tr>
<tr>
<td>α-helical CRF(9–41)</td>
<td>17 nM</td>
<td>49</td>
<td>5.0 nM</td>
<td>4.1</td>
<td>0.97 nM</td>
<td>0.81</td>
</tr>
<tr>
<td>[DPhex12,Nle21,38]r/hCRF(12–41)</td>
<td>56 nM</td>
<td>75</td>
<td>5.2 nM</td>
<td>31</td>
<td>8.4 nM</td>
<td>6.9</td>
</tr>
<tr>
<td>r/hCRF(12–41)</td>
<td>11 nM</td>
<td>5.2</td>
<td>44 nM</td>
<td>13</td>
<td>38 nM</td>
<td>17</td>
</tr>
<tr>
<td>r/hCRF</td>
<td>(8.4–15)</td>
<td>(2.9–9.3)</td>
<td>(26–75)</td>
<td>(7.2–22)</td>
<td>(21–67)</td>
<td>(10–29)</td>
</tr>
<tr>
<td>Ucn</td>
<td>1.3 nM</td>
<td>0.79</td>
<td>1.5 nM</td>
<td>0.56</td>
<td>0.97 nM</td>
<td>0.41</td>
</tr>
<tr>
<td>sfUrotensin I</td>
<td>3.1 nM</td>
<td>2.8</td>
<td>9.8 nM</td>
<td>3.4</td>
<td>6.4 nM</td>
<td>3.0</td>
</tr>
<tr>
<td>(2.4–4.0)</td>
<td>(2.4–3.3)</td>
<td></td>
<td>(5.5–17)</td>
<td>(2.4–4.4)</td>
<td>(3.8–11)</td>
<td>(1.8–4.8)</td>
</tr>
<tr>
<td>Sauvagine</td>
<td>9.4 nM</td>
<td>11</td>
<td>9.9 nM</td>
<td>1.4</td>
<td>3.8 nM</td>
<td>2.0</td>
</tr>
<tr>
<td>(7–13)</td>
<td>(8.8–13)</td>
<td></td>
<td>(4.4–22)</td>
<td>(1.1–1.8)</td>
<td>(2.3–6.2)</td>
<td>(1.1–3.6)</td>
</tr>
</tbody>
</table>

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TABLE 2

Number of binding sites (pmol/mg) detected by Ucn* and Ast*

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ucn*</th>
<th>Ast*</th>
<th>No. Ucn sites/No. Ast sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-CRF-R1</td>
<td>4.4</td>
<td>19</td>
<td>0.23</td>
</tr>
<tr>
<td>CHO-CRF-R2α</td>
<td>1.0</td>
<td>4.2</td>
<td>0.24</td>
</tr>
<tr>
<td>CHO-CRF-R2β</td>
<td>2.0</td>
<td>24</td>
<td>0.05</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.009</td>
<td>0.8</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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TABLE 3

Inhibitory binding constants of CRF and analogs for binding to rat cerebellum/brain stem using Ast*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kᵡ</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Astressin</td>
<td>0.91 nM</td>
<td>(0.58–1.4)</td>
</tr>
<tr>
<td>α-helical CRF(9–41)</td>
<td>30 nM</td>
<td>(18–50)</td>
</tr>
<tr>
<td>[DPhex12,Nle21,38]r/hCRF(12–41)</td>
<td>9.6 nM</td>
<td>(4.2–22)</td>
</tr>
<tr>
<td>r/hCRF</td>
<td>20 nM</td>
<td>(9.6–46)</td>
</tr>
<tr>
<td>rUcn</td>
<td>2.9 nM</td>
<td>(1.1–7.2)</td>
</tr>
</tbody>
</table>

---

Fig. 2. Displacement at 20°C by 0 or 100 mM Gpp(NH)p of CRF*, Ast*, or Ucn* bound to CRF-R1 (□), CRF-R2α (□), and CRF-R2β (■). Data are from representative assays replicated at least twice. B = cpm bound; T = total cpm.
apparent affinity for receptors labeled with an agonist compared with those labeled with an antagonist (Sleight et al., 1996). Data from mutational analysis of endothelin receptors have suggested different determinants for binding of agonists and antagonists to the endothelin receptors ETA and ETB (Becker et al., 1994). For the tachykinin receptors, it was shown that mutations in the second transmembrane domain of the natural killer cell-1 receptor eliminate Substance P binding when assayed with a nonpeptide antagonist radioligand, but these same mutations do not impair radiolabeled Substance P binding (Rosenkilde et al., 1994). It may be, however, that some of these differences result from different binding determinants for nonpeptide antagonists, compared with peptide antagonists.

For most G protein-coupled receptors, the binding of agonists is modulated by guanyl nucleotides. Previously we showed that for bovine pituitary membranes the binding of oCRF* is specifically inhibited in a dose-dependent manner by guanyl nucleotides (Perrin et al., 1986). We found similar effects for the oCRF* when bound to the cloned receptors at either 20° or 37°C. In the case of Ucn*, the effects of guanyl nucleotides on the binding to the cloned type 1 receptor are minimal at both temperatures. Interestingly, the binding of Ucn* to the cerebellar receptor is modulated by guanyl nucleotides, with their effects increasing as the temperature is raised from 20° to 37°C. The temperature dependence of these guanyl nucleotide effects is reminiscent of that found for GnRH binding to bovine pituitary membranes: guanyl nucleotides did not modulate the binding of a GnRH agonist at 4° or 20°C, but only at 37°C (Perrin et al., 1989). For the liver hepatic alpha-1 adrenergic receptor, the effect of guanyl nucleotides was absent when the temperature was lowered from 25 to 2°C (Lynch et al., 1985). In that case, it was speculated that the conversion from the high- to low-affinity form of the receptor might involve a temperature-dependent energy requiring process or molecular diffusion in the plane of the plasma membrane.

The difference in effects of guanyl nucleotide on Ucn* binding to cloned and native receptors may reflect a difference in their interaction with the G proteins in the two different membrane environments or may reflect a different ratio of receptor to G protein in the overexpression system (Kenakin, 1997). Additionally, other cell-specific components may influence the coupling of the receptor/G proteins. For example, a heat-sensitive, possibly proteinaceous, membrane component of PC-12 cells directly activates a G protein and increases the agonist-stimulated response of transfected alpha adrenergic receptors (Sato et al., 1995).
Not only does the membrane environment appear to affect the receptor/G protein interaction, but the nature of the agonist also plays a role. When the agonist oCRF\textsuperscript{a} is bound to the cloned receptor, the interaction with the G protein is more sensitive to guanyl nucleotides than when the agonist Ucn\textsuperscript{a} is bound. It is possible that CRF and Ucn induce different conformational changes in the receptor that then result in differences in their interaction with G proteins. In this regard, it is interesting that the off-rate of Ucn\textsuperscript{a} bound to CHO-R1 is much smaller than that of Ast\textsuperscript{a} and also smaller than that of oCRF\textsuperscript{a} (Perrin et al., 1986). It is possible that these two phenomena, insensitivity to guanyl nucleotides and long dissociation times, are just two aspects of the same phenomenon reflecting a different receptor/G protein interaction when urocortin is bound to the receptor.

In summary, we found that there is a difference in the interaction of the two agonists, oCRF\textsuperscript{a} and Ucn\textsuperscript{a}, with the cloned receptor expressed in CHO cells in terms of the modulation of their binding by guanyl nucleotides. Furthermore, we found that for characterization of cloned receptors, either Ast\textsuperscript{a} or Ucn\textsuperscript{a} can be used as the radioligand to determine relative affinities. For the native receptors in the brain, Ast\textsuperscript{a} appears to detect more receptors in the radioreceptor assay than does the agonist Ucn\textsuperscript{a}. An added advantage is that astressin binds with very low affinity to the CRF binding protein, so that in native tissues only the receptors will be detected.

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References


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