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Functional characterization of corticotropin-releasing factor type 1 receptor endogenously expressed in human embryonic kidney 293 cells

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Abstract

The endogenous expression in human embryonic kidney 293 (HEK293) cells of corticotropin-releasing factor (CRF) receptors was detected. High-affinity binding sites for human CRF ($K_i = 3.6$ nM), ovine CRF ($K_i = 4.6$ nM), rat urocortin ($K_i = 2.2$ nM), sauvagine ($K_i = 2.4$ nM) and astressin ($K_i = 4.3$ nM) with the pharmacological characteristics for CRF type 1 (CRF_1) receptors and B_{max} values of ~ 30 fmol/mg protein were determined. The four CRF receptor agonists nonselectively stimulated cAMP production in HEK293 cells at low agonist concentrations, whereas the antagonist astressin shifted the dose–response curve for ovine CRF significantly rightward. Transfection of the pcDNA3 vector into HEK293 cells strongly reduced the expression of the endogenous CRF receptor. Northern blot analysis revealed the expression of a CRF_1 transcript in human neuronal tissues, HEK293, human NTera-2 (NT2) carcinoma, Y-79 retinoblastoma and African green monkey kidney (COS-7) cells. Neither by Northern blot analysis nor by reverse transcriptase PCR (RT-PCR), the expression of CRF_2 could be detected. In cAMP stimulation experiments, functional CRF receptors were detected in these cell lines. These data show that HEK293 and other cell lines endogenously express CRF_1 receptors.

Keywords: Receptor expression; Ligand binding; cAMP; mRNA detection

1. Introduction

Corticotropin-releasing factor (CRF) is the main integrator of the stress response and is mainly expressed in the central nervous system (Dunn and Berridge, 1990; Arborelius et al., 1999). Besides CRF, another member of the CRF family, urocortin is expressed in mammals (Vaughan et al., 1995; Donaldson et al., 1996). Urocortin, like CRF, is mainly expressed in the central nervous system and only limited data is available for its expression in the periphery (reviewed in Turnbull and Rivier, 1997).

CRF and urocortin mediate their effects through receptors, which belong to the large family of G-protein-coupled receptors. Two major subtypes of the CRF receptor have been identified: CRF receptor type 1 (CRF_1) and type 2 (CRF_2). Both receptors have been cloned from a variety of species (reviewed in Chalmers et al., 1996). Three functional CRF_2 splice variants, α, β and γ, which differ from each other in their N-terminal sequences and are 70% identical to CRF_1, have been isolated from humans (Liaw et al., 1996; Valdenaire et al., 1997; Kostich et al., 1998). Besides their high degree of sequence homology, CRF_1 and CRF_2 differ markedly from each other in their pharmacological properties and tissue distribution.

Mammalian CRF_1 is nonselective for CRF isolated from different species, urocortin, the fish urocortin homologue urotensin I (Lederis et al., 1982) and amphibian sauvagine (Montecucchi and Henschen, 1981). These peptides are bound with similar affinity and are equipotent in their ability to stimulate cAMP accumulation in cells transfected with the mammalian CRF_1 cDNA (Vaughan et al., 1995; Donaldson et al., 1996; Dautzenberg et al., 1997, 1999; Palchaudhuri et al., 1998). CRF_2, in contrast to mammalian CRF_1, displays a different substrate specificity (Donaldson et al., 1996; Dautzenberg et al., 1997, 1999; Ardati et al., 1999; Palchaudhuri et al., 1999). CRF isolated from different species is bound with significantly lower affinity than urocortin, urotensin I and sauvagine.

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CRF_1 is widely expressed in the brain and in the pituitary (Chalmers et al., 1995; Palchaudhuri et al., 1998). However, in peripheral organs, CRF_1 mRNA is only expressed at low levels in a limited number of organs (Palchaudhuri et al., 1998). Thus, the receptor is generally regarded as the brain pituitary CRF receptor. In contrast to CRF_1, CRF_2 expression in the brain, is very discrete, occurring only in loci, such as hypothalamus, lateral septum, and hippocampus, which are important for the neuroendocrine stress response or memory acquisition (Chalmers et al., 1995; Palchaudhuri et al., 1999; Sanchez et al., 1999). In peripheral organs, CRF_2 mRNA has been detected in heart and skeletal muscle (see Chalmers et al., 1996). In the kidney, the expression of CRF_1 or CRF_2 has not been shown yet.

The human embryonic kidney 293 line (HEK293) is often used to study the heterologous expression of many G-protein-coupled receptors, including those for neuropeptides and neurotransmitters (McHale et al., 1994; Valiquette et al., 1996; Bot et al., 1998; Hiltscher et la., 1998; Kaupmann et al., 1998; Koch et al., 1998; Thomas et al., 1998). HEK293 cells are fast-growing cells, can be transfected easily and produce recombinant proteins in sufficient amounts. Since HEK293 cells are derived from an embryonic kidney tumor (Graham et al., 1977), it seems unlikely that they endogenously express neuropeptide or neurotransmitter G-protein-coupled receptors. Thus, it is generally believed that HEK293 cells are an ideal tool to study the pharmacology of recombinant G-protein-coupled receptors.

During several studies to characterize the pharmacological profile of recombinant CRF_1 and CRF_2 receptors (Dautzenberg et al., 1998, 1999; Palchaudhuri et al., 1999; Wille et al., 1999), we obtained evidence for the expression of CRF receptors endogenous to HEK293 cells. Here we report on the pharmacological and molecular characterization of endogenously expressed CRF_1 receptors from HEK293, human Ntera 2 (hNT2) and African green monkey kidney (COS-7) cells.

2. Experimental procedures

2.1. Materials, peptides and reagents

All cell culture reagents were purchased from Gibco/BRL. Aprotinin was obtained from Roche Diagnostics (Mannheim, Germany). CRF peptides were from Bachem (Bubendorf, Switzerland; purity greater than 98%).

2.2. Cell culture and transfections

HEK293, COS-7 (American Type Cell Culture, Rockville, MD) and hNT2 cells (Stratagene, La Jolla, CA) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and the culture medium was exchanged every 2–3 days. Y-79 cells were grown as described previously (Hauger et al., 1997). All cells were used between passages 5 and 25, and their viability (which was routinely $>98\%$) was assessed before each experiment by staining with trypan blue. Five micrograms of the control pcDNA3 vector (Invitrogen, San Diego, CA) was stably transfected into HEK293 cells with the SuperFect reagent (Qiagen) as described previously (Palchaudhuri et al., 1998, 1999).

2.3. RNA isolation

Poly(A)$^+$ RNA was isolated from human Y-79, HEK293, hNT2 and COS-7 cells using the FAST Track system (Invitrogen) as described previously (Dautzenberg et al., 1997). Poly(A)$^+$ RNA from various human tissues was purchased from Clontech (Palo Alto, CA).

2.4. Northern blot analysis for CRF_1 mRNA

Poly(A)$^+$ RNA from a selection of human tissues and cell lines (1 μg each) was immobilized and hybridized at 42°C with 50% formamide with a ^{32}P-labeled 724 bp *Pst*I/*Bcl*I fragment of the $hCRF_1$ cDNA (accession number L23332). The blot was washed at room temperature in 2× saline-sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS) followed by two successive washes at 65°C with 0.1× SSC, 0.1% SDS for 30 min, respectively.

2.5. Reverse transcriptase PCR (RT-PCR)

First strand cDNA was synthesized from 500 ng poly(A)$^+$ RNA using random primers (Dautzenberg et al., 1997). Human hypothalamus Marathon-Ready™ cDNA was from Clontech. CRF_1 cDNA was amplified from 10 (control HEK293 cells) to 25 ng (pcDNA3-transfected HEK293 cells) HEK293 cell cDNA using primers CRF1A (5′-CATGTGACCAACTTCTTCTGGATG-3′, position 632–655 of $hCRF_1$), CRF1B (5′-GTAGAACACAGACACAAAGAAGCCC-3′, position 1126–1102 of $hCRF_1$) and 1 U Taq DNA polymerase (Roche Diagnostics) for 35 cycles (94°C for 10 s, 65°C for 20 s and 72°C for 1 min). CRF_2 cDNA was amplified with primers CRF2A (5′-GGAGAATGGGACGTGGGCCTCAAAG-3′, position 252–276 of $hCRF_{2\alpha}$) and CRF2B (5′-CTCATAGTAGAGCTTGCC GATGGC-3′, position 750–727 of $hCRF_{2\alpha}$). Amplification was performed for 35 cycles (94°C for 10 s, 60°C for 20 s and 72°C for 1 min) with 1 U Taq DNA polymerase and ~2 ng (hypothalamus), 10 ng (cortex) or 50 ng (HEK293, hNT2, COS-7 and Y-79 cells) cDNA. Human β-actin was amplified using primers act1 and act2 as described previously (Palchaudhuri et al., 1998, 1999).

2.6. CRF receptor binding assay

Membranes from nontransfected HEK293 cells or HEK293 cells transfected with the pcDNA3 vector were

prepared as described previously (Dautzenberg et al., 1997). Scatchard analysis using 100 pM [^{125}I]Tyr^0-sauvagine (~100,000 cpm) (Amersham, Little Chalfont, UK) was performed with 100 μg protein as described (Dautzenberg et al., 1997; Hauger et al., 1997). Nonspecific binding was always defined as residual [^{125}I]Tyr^0-sauvagine binding in the presence of 10 μM unlabeled CRF peptides. The dissociation constant, K_d, and the inhibition constant, K_i, were calculated using the LIGAND program (Munson and Rodbard, 1980). In the displacement binding assays, specific binding (~2400 cpm) contributed to ~40% of total binding (~6000 cpm).

2.7. cAMP assay

HEK293, hNT2, COS-7 and Y-79 cells were plated at 5×10^5 cells per well into 24-well dishes. Stimulation for 30 min (37°C, 5% CO_2) with increasing concentrations of peptides in stimulation buffer (2 mM 3-isobutyl-1-methyl xanthine, 1 mg/ml bovine serum albumin in Dulbecco's modified Eagle's medium) and intracellular cAMP assays were performed as described (Dautzenberg et al., 1997, 1998). Statistical analysis was performed by two-way analysis of variance (ANOVA). Significant differences between groups were determined by post hoc analysis using Dunnett's test.

3. Results

3.1. Binding of CRF analogs to HEK293 membranes

Membranes from HEK293 cells were prepared and tested for their ability to bind CRF analogs. Saturation binding experiments using [^{125}I]Tyr^0-sauvagine revealed a high-affinity binding site (K_d ~ 100 pM) and a maximal binding capacity of ~30 fmol/mg protein (Fig. 1A). Scatchard analysis favored a one-site model (inset to Fig. 1A). In displacement binding assays a CRF_1-like pharmacology was observed (Fig. 1B, Table 1). The five CRF analogs ovine CRF ($K_i = 4.6 \pm 1.1$ nM), human CRF ($K_i = 3.6 \pm 1.1$ nM), rat urocortin ($K_i = 2.2 \pm 0.5$ nM), sauvagine ($K_i = 2.4 \pm 0.5$ nM) and astressin (4.3 ± 0.9 nM) were bound with comparable high affinity by HEK293 membranes. The peptides did not show any significant difference in their binding affinities [$F(4,5) = 1.584$, $p = 0.3096$]. B_{max} values of 34 ± 11 fmol/mg (ovine CRF), 33 ± 6 fmol/mg (human CRF), 27 ± 7 fmol/mg (rat urocortin), 30 ± 10 fmol/mg (sauvagine) and 36 ± 4 fmol/mg (astressin), respectively, were calculated for the five CRF peptides (Table 1). No significant differences in maximal binding for human CRF, ovine CRF, rat urocortin, sauvagine and astressin were observed [$F(4,5) = 0.194$, $p = 0.9313$]. When HEK293 cells were transfected

Table 1
Binding of different CRF ligands to membranes from HEK293 cells
The data are the mean ± S.D. of two different binding experiments.

Peptide	K_i (nM)	B_{max} (fmol/mg protein)
Ovine CRF	4.6 ± 1.1	34 ± 11
Human CRF	3.6 ± 1.1	33 ± 6
Rat urocortin	2.2 ± 0.5	27 ± 7
Sauvagine	2.4 ± 0.5	30 ± 10
Astressin	4.3 ± 0.9	36 ± 4

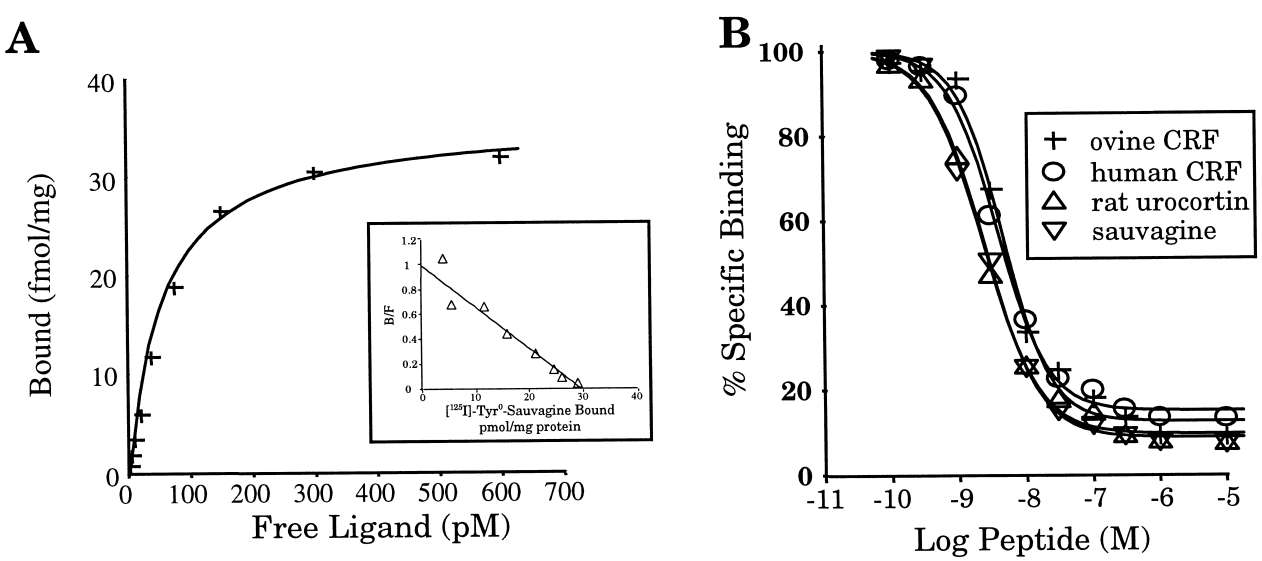

Fig. 1. Binding of CRF peptides to HEK293 membranes. (A) Saturation binding was performed using increasing concentrations (25 pM–1 nM) [^{125}I]Tyr^0-sauvagine. Inset: Scatchard analysis of the saturation binding. (B) Competitive binding was performed using 100 pM [^{125}I]Tyr^0-sauvagine and increasing concentrations (0.1 nM to 1 μM) of unlabeled human CRF, ovine CRF, rat urocortin or sauvagine. Data represent duplicates from one representative experiment.

Table 2
Effect of several CRF peptides on the stimulation of cAMP accumulation in HEK293 cells
The data are the mean ± S.D. of three independent stimulations.

Peptide	EC_{50} (nM)	E_{max} (pmol/well)
Ovine CRF	$2.4 \pm 0.3^{a,b}$	9.6 ± 0.05
Ovine CRF (+100 nM astressin)	92 ± 14^{c}	9.9 ± 0.11
Human CRF	1.8 ± 0.1	9.5 ± 0.05
Rat urocortin	$2.9 \pm 0.4^{a,b}$	9.2 ± 0.08
Sauvagine	1.5 ± 0.1	9.6 ± 0.05

[a] Statistically significant differences: $p < 0.001$ vs. human CRF.
[b] Statistically significant differences: $p < 0.0001$ vs. sauvagine.
[c] Statistically significant differences: $p < 0.001$ vs. ovine CRF.

with the pcDNA3 vector, specific CRF binding was completely abolished (not shown).

4. Stimulation of cAMP accumulation in HEK293 cells

The ability of the CRF receptor agonists to stimulate cAMP accumulation in nontransfected HEK293 cells was tested. When human CRF, ovine CRF, rat urocortin or sauvagine were applied to HEK cells, cAMP production was stimulated ~8-fold over basal levels (Fig. 3, Table 2). The four CRF analogs stimulated cAMP production with similar potencies [$F(3,8) = 3.53$, $p = 0.068$] at low peptide concentrations (Table 2). The EC_{50} values for human CRF ($EC_{50} = 1.8 \pm 0.1$ nM), ovine CRF ($EC_{50} = 2.4 \pm 0.3$ nM), rat urocortin ($EC_{50} = 2.9 \pm 0.4$ nM) and sauvagine ($EC_{50} = 1.5 \pm 0.1$ nM) differed only minimally from one another (Fig 2A, Table 2). In the presence of the CRF antagonist astressin (100 nM), the potency of ovine CRF (92 ± 14 nM) to stimulate cAMP production was shifted significantly [$F(1,4) = 83.24$, $p < 0.001$] to the right (Fig 2B).

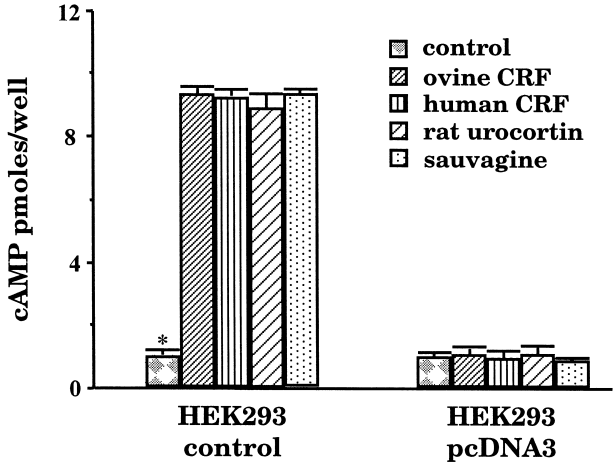

Fig. 3. Stimulation of cAMP production by various agonists in HEK293 control cells and HEK293 cells stably transfected with the pcDNA3 vector. Stimulations were performed with saturation concentrations of the various agonists. Statistically significant differences: [a] $p < 0.0001$ vs. control.

In HEK293 cells stably transfected with the pcDNA3 vector, the CRF peptides were unable to stimulate cAMP production. No significant differences compared to basal cAMP levels were measured (Fig. 3).

4.1. Detection of CRF_1 mRNA in HEK293 cells and other cell lines

Northern blot analysis with mRNA from human tissues, HEK293 cells, hNT2, COS-7 and Y-79 retinoblastoma cells was performed to determine the expression of CRF receptors. Using a $hCRF_1$ cDNA probe, a single mRNA band of ~2.7 kb in size, was detected in retina, amygdala, brainstem, cerebellum, hippocampus, pituitary, retina,

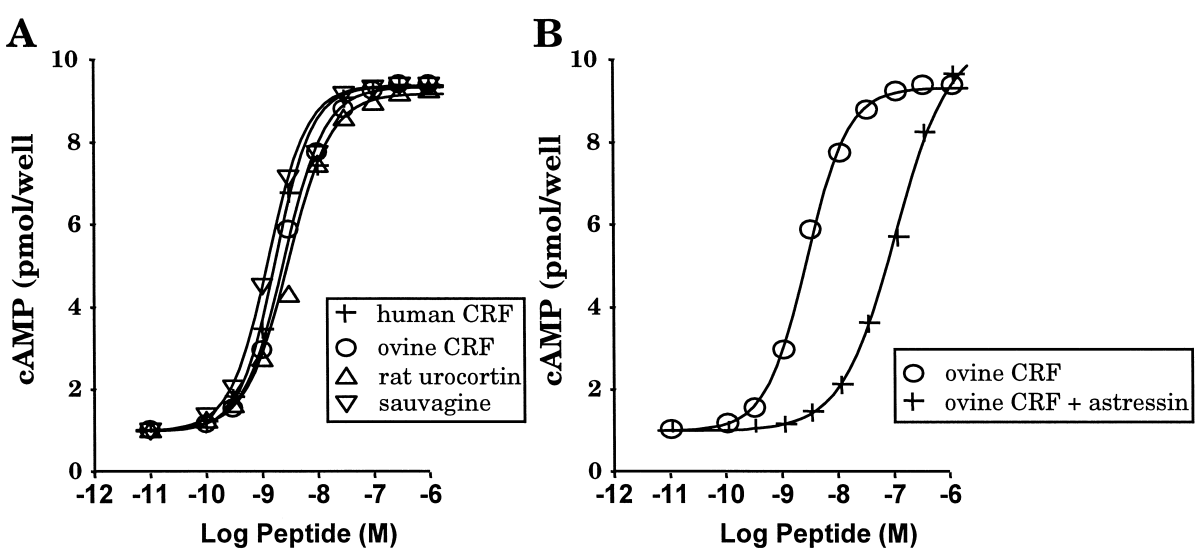

Fig. 2. Stimulation of cAMP in HEK293 cells by CRF analogs. (A) HEK293 cells (5×10^5 cells/well) were incubated with increasing concentrations of CRF analogs for 30 min at 37°C. (B) Rightward shift of the dose–response curve of ovine CRF in the presence of CRF antagonist astressin (100 nM). The cAMP level was determined as described in Experimental procedures. The results are representative of three independent stimulations.

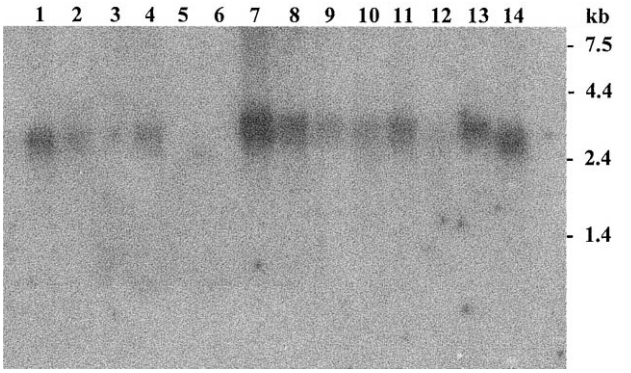

Fig. 4. Expression of CRF_1 mRNA in various cell lines and native human tissues. A blot immobilized with poly(A)$^+$ RNA from human tissues or cell lines (1 μg per lane) was hybridized to a $hCRF_1$-specific cDNA fragment. Lanes 1 and 14 = Y-79 cells, 2 = retina, 3 = lymphocytes, 4 = brainstem, 5 = heart, 6 = kidney, 7 = cerebellum, 8 = amygdala, 9 = hippocampus, 10 = pituitary, 11 = hNT2 cells, 12 = COS-7 cells, 13 = HEK293 cells.

hNT2 neurons, HEK293, Y-79 and in COS-7 cells. No signal was detected in human heart, kidney and lymphocytes (Fig. 4). Reprobing of the same blot with a human CRF_2 probe failed to produce a hybridization signal (not shown). Similar results were obtained in a RT-PCR approach. Amplification of cDNA prepared from HEK293, hNT2, COS-7 and Y-79 cells failed to produce a positive signal for CRF_2. In control reactions, a strong CRF_2 band was amplified from human cortex and hypothalamus cDNA, although 5- to 25-fold lower cDNA amounts had been used for the PCR reaction (Fig. 5).

To measure the effect of the pcDNA3 transfection on the CRF_1 mRNA content in HEK293 cells, PCR amplifications were performed. In contrast to the strong amplification of CRF_1 in control HEK293 cells, only a weak band was observed in pcDNA3-transfected cells (Fig. 6). Control amplifications of β-actin revealed only minor reductions of its mRNA in pcDNA3-transfected HEK293 cells when compared with control cells (Fig. 6).

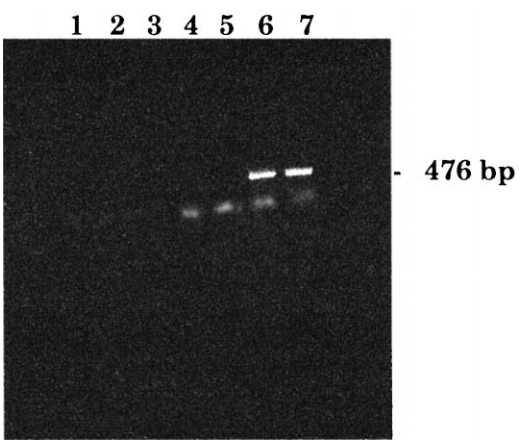

Fig. 5. RT-PCR amplification of CRF_2 from human cortex and hypothalamus but not from HEK293, Y-79, hNT2 and COS-7 cells. Aliquots of the cDNA corresponding to ~2 ng (hypothalamus), 10 ng (cortex) or 50 ng (cell lines) poly(A)$^+$ RNA were amplified with primers CRF2A and CRF2B for 35 cycles (see Experimental procedures). PCR products were separated on a 1.5% agarose gel. Lanes 1 = no DNA, 2 = Y-79 cells, 3 = hNT2 cells, 4 = COS-7 cells, 5 = HEK293 cells, 6 = hypothalamus, 7 = cortex.

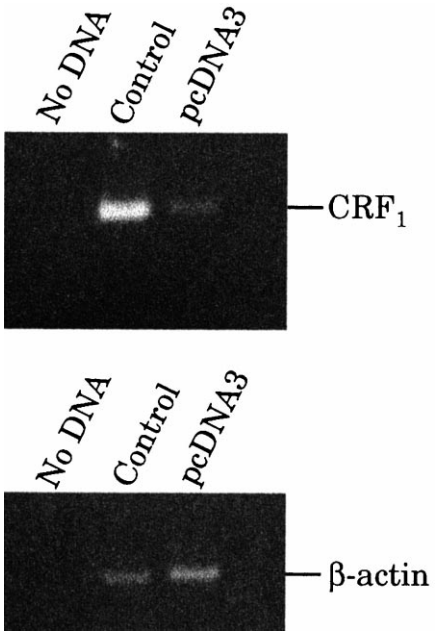

Fig. 6. RT-PCR amplification of CRF_1 and β-actin from control HEK293 cells and HEK293 cells stably transfected with the pcDNA3 vector. CRF_1 and β-actin were amplified from 10 ng (control HEK293 cells) and 25 ng (pcDNA3-transfected HEK293 cells) cDNA as described in Experimental procedures. PCR products were separated on 1.2% agarose gels.

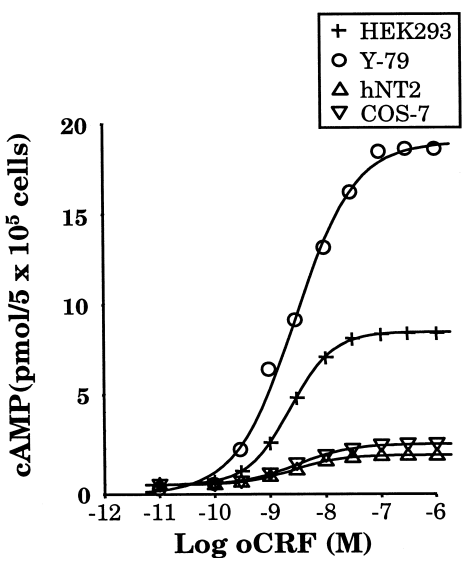

Fig. 7. Stimulation of cAMP accumulation in HEK293, Y-79, hNT2 and COS-7 cells by ovine CRF. The cell lines (5×10^5 cells/well) were incubated with increasing concentrations of ovine CRF as described in Experimental procedures. The results are representative of two independent stimulations.

4.2. Stimulation of cAMP production in hNT2 and COS-7 cells by ovine CRF

Because of the positive Northern blot hybridization with the CRF_1 probe (Fig. 5), the ability of ovine CRF to stimulate cAMP production in hNT2 and COS-7 cells was tested. As controls, HEK293 and Y-79 cells were also stimulated with ovine CRF (Fig. 7).

Although ovine CRF was equally efficacious at HEK293 ($EC_{50} = 2.4 \pm 0.3$ nM), Y-79 ($EC_{50} = 3.0 \pm 0.2$ nM), hNT2 ($EC_{50} = 2.7 \pm 0.2$ nM) or COS-7 cells ($EC_{50} = 3.1 \pm 0.1$ nM), there were marked differences in the maximal cAMP production in the various cell lines. In contrast to the ~25-fold and ~8-fold stimulation of intracellular cAMP content in Y-79 and HEK293 cells by maximal ovine CRF concentrations, the peptide produced only a modest increase of cAMP accumulation in hNT2 (~1.9-fold) and COS-7 cells (~2.1-fold).

5. Discussion

In the current study, we detected functional receptors for CRF being endogenously expressed in HEK293, hNT2 and COS-7 cells. Membranes prepared from HEK293 cells bound the CRF analogs human and ovine CRF, rat urocortin, sauvagine and astressin nonselectively with high affinity, as reported for recombinant CRF_1 proteins (Donaldson et al., 1996; Dautzenberg et al., 1997, 1999; Palchaudhuri et al., 1998). B_{max} values of ~30 fmol/mg protein were determined for the five CRF peptides. Thus, the B_{max} values were similar to that observed for undifferentiated IMR-32 neuroblastoma cells (Dieterich and De Souza, 1996), but lower than those obtained for small cell lung carcinomas (Dieterich et al., 1994), differentiated IMR-32 (Hogg et al., 1996), and Y-79 retinoblastoma cells (Hauger et al., 1997). It was further established that human and ovine CRF, rat urocortin, and sauvagine were almost equally potent and efficacious in their ability to stimulate cAMP production in HEK293 cells. Only minor differences between human CRF and sauvagine versus ovine CRF and rat urocortin were detected. Furthermore, as an indication for the presence of functional CRF receptors in HEK293 cells, the CRF antagonist astressin significantly shifted the dose–response curve for ovine CRF to the right.

Human CRF_1 ($hCRF_1$) has been reported to nonselectively bind CRF isolated from various species, urotensin I, human and rat urocortin, and sauvagine (Donaldson et al., 1996; Dautzenberg et al., 1997, 1999; Palchaudhuri et al., 1998; Wille et al., 1999). In contrast to $hCRF_1$, human CRF_2 splice variants display a strict ligand selective profile. Urocortin, urotensin I, and sauvagine, in contrast to CRF, are preferentially bound by CRF_2 (Kostich et al., 1998; Ardati et al., 1999). Thus, the pharmacological profile for the agonists human CRF, ovine CRF, rat urocortin and sauvagine and the antagonist astressin matched the ligand specificity of recombinant mammalian CRF_1, but differed strongly from that reported for CRF_2. Although the ability of CRF to stimulate cAMP production in native HEK293 cells had been reported recently (Antoni et al., 1995), neither the nature nor the expression rate of the HEK cell CRF receptor had been determined. Therefore, this is the first study to pharmacologically identify CRF_1 as the CRF receptor being endogenously expressed in HEK293 cells.

Consequently, in a Northern blot hybridization, we detected a 2.7 kb CRF_1 mRNA band in HEK cells. Furthermore, in human neuronal tissues such as amygdala, brainstem, cerebellum, hippocampus, pituitary and retina but not in heart, kidney and lymphocytes, the same signal was detected. In addition to HEK293 cells and neuronal tissues, the CRF_1 mRNA was detected in Y-79, hNT2 and COS-7 cells. Since hNT2 and Y-79 cells are of neuronal origin (Andrews, 1984; Kyritsis et al., 1984), it seems plausible that they can express CRF receptors. Furthermore, CRF_1 mRNA expression in Y-79 cells has been reported previously (Hauger et al., 1997). However, the detection of CRF_1 mRNA in COS-7 cells was surprising. CRF_1 is predominantly a neuronal gene (Chalmers et al., 1995; Palchaudhuri et al., 1998) and only low expression levels of its mRNA have been found in a few peripheral organs (see Chalmers et al., 1996; Palchaudhuri et al., 1998). COS-7 and HEK293 cells are derived from the kidney, a tissue that does not express CRF_1 mRNA (see Results and Palchaudhuri et al., 1998). Both HEK293 and COS-7 cells have been immortalized by viral transformation (Graham et al., 1977; Mellon et al., 1981). Thus, it is probable that the viral transformation may have initiated the expression of this gene.

To further establish the presence of functional CRF_1 receptors in hNT2 neurons and COS-7 cells, we performed cAMP stimulation experiments using ovine CRF. Indeed, ovine CRF was able to stimulate cAMP production in hNT2 and COS-7 cells, although at low cAMP levels, when compared to HEK293 and Y-79 cells.

In agreement with the CRF_1-like pharmacological profile, no evidence for the expression of CRF_2 in HEK293 cells could be obtained. Neither by Northern blot hybridization nor by RT-PCR, even with very high cDNA concentrations, was CRF_2 mRNA expression detectable in HEK293 cells. It was concluded that HEK293 cells express only a homogenous population of CRF_1 receptors. Similarly, COS-7, hNT2 and Y-79 cells did not express CRF_2 mRNA. Thus, except human A-431 epidermoid cells, which contain $CRF_{2\beta}$ mRNA (Kiang et al., 1998), there is no information on a cell line endogenously expressing CRF_2. Interestingly, in A-431 cells, there is no evidence for the signaling of $CRF_{2\beta}$ through cAMP (our unpublished results).

The characterization of functional CRF_1 receptors in HEK293 cells opens the possibility to study the regulation

of this receptor in a peripheral cell line. Although CRF_1 is only expressed in a limited number of peripheral organs (Chalmers et al., 1996; Palchaudhuri et al., 1998), it is important to study the regulation of this receptor in cells derived from tissues other than pituitary or the neuronal system. To date, the regulation of CRF_1 mRNA or protein has been studied in the pituitary and in neuronal cells (reviewed in Hauger and Dautzenberg, 1999). From these studies, it became evident that, depending on the nature of the cell type, there are marked differences in the desensitization and/or downregulation of the CRF_1 mRNA and protein (Reisine and Hoffmann, 1983; Pozzoli et al., 1996; Hauger et al., 1997). However, nothing is known on the regulation of CRF_1 in peripheral organs. In the future, the authors plan to extend their studies on CRF_1 regulation to peripheral cells. Thus, HEK293 cells provide an important tool for such studies because they express CRF_1 at reasonable amounts.

The expression of CRF_1 protein in native HEK293 cells does not reflect an artificial expression of neuronal genes per se, since we could not detect the expression of two opioid-like receptors. Using saturation binding assays, no evidence for the expression of the human μ-opioid receptor (Mestek et al., 1995) or the ORL1 receptor (Mollereau et al., 1994) could be obtained (not shown).

Furthermore, the detection of CRF_1 mRNA and protein in HEK293 and COS-7 cells, does not discredit these cell lines for further usage. As shown in Results, the transfection of the empty pcDNA3 vector completely abolished CRF binding to HEK293 membranes or abolished the ability of CRF agonists to stimulate cAMP production in these cells. In agreement with the binding and cAMP results, a strong reduction of CRF_1 mRNA was observed in HEK293 cells stably transfected with the pcDNA3 vector. In contrast to the results obtained with the CRF_1 expression, pcDNA3 transfection only minimally reduced the level of β-actin mRNA. Thus, the overexpression of recombinant genes seems to strongly influence the expression rate of the endogenous CRF_1 mRNA. The utility of HEK293 cells for biochemical studies on CRF receptors is further supported by the amounts of CRF_1 and CRF_2 proteins obtained after transfection (Dautzenberg et al., 1998; Palchaudhuri et al., 1998, 1999; Ardati et al., 1999; Wille et al., 1999). The expression rates of the recombinant receptors are usually 40- to 400-fold higher than those calculated for native HEK cells (see Results). Thus, it is very unlikely that the endogenously expressed CRF_1 in HEK293 cells plays an important role for studies of recombinant CRF_1 and CRF_2 receptors.

The characterization of specific CRF_1 receptors in HEK293 cells further extends the list of G-protein-coupled receptors being endogenously expressed in this cell line. Using either pharmacological approaches or cloning studies, the expression of somatostatin type 2, bradykinin, sphingosine-1-phospate, β_2 adrenoceptor, P2Y or muscarinic acetylcholine receptors endogenous to HEK293 cells has been reported (Law et al., 1993; Anderson et al., 1995; van Koppen et al., 1996; Schachter et al., 1997; Daaka et al., 1998; Lin et al., 1999). Future studies may identify neuropeptide G-protein-coupled receptors additional to CRF_1 in HEK293 cells.

In conclusion, we have characterized the endogenous CRF receptor in HEK293 cells as CRF_1. Using molecular and biochemical approaches, we have also identified CRF_1 expression in hNT2 and COS-7 cells. No evidence for the expression of CRF_2 could be obtained.

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