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European Journal of Pharmacology 371 (1999) 205–211

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In vitro pharmacological profile of nonpeptide CRF_1 receptor antagonists, CRA1000 and CRA1001

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Received 15 October 1998; received in revised form 13 January 1999; accepted 19 February 1999

Abstract

We investigated pharmacological properties of CRA1000 (2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine) and CRA1001 (2-(*N*-(2-bromo-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine), novel and selective antagonists for the corticotropin-releasing factor$_1$ (CRF_1) receptor. Both CRA1000 and CRA1001 inhibited [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing the rat CRF_1 receptor with IC_{50} values of 30 and 38 nM, respectively, without affecting [^{125}I]sauvagine binding to membranes of COS-7 cells expressing the rat $CRF_{2\alpha}$ receptor. CRF elicited intracellular cyclic AMP (cAMP) accumulation in AtT-20 cells which express the CRF_1 receptor but not the CRF_2 receptor, and COS-7 cells expressing CRF_1 or $CRF_{2\alpha}$ receptors. The CRF-induced cAMP accumulation was inhibited by both CRA1000 and CRA1001, concentration-dependently, in AtT-20 cells and COS-7 cells expressing the CRF_1 receptor, while these compounds did not attenuate the CRF response in COS-7 cells expressing the $CRF_{2\alpha}$ receptor. CRF increased adrenocorticotropin (ACTH) secretion from AtT-20 cells, and CRA1000 and CRA1001 inhibited CRF-induced ACTH secretion, concentration-dependently, as did other CRF_1 receptor antagonists. These results show that both CRA1000 and CRA1001 are potent and selective CRF_1 receptor antagonists.

Keywords: CRA1000; CRA1001; CRF_1 receptor antagonist; AtT-20 cell; cAMP formation

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid peptide originally isolated from ovine hypothalamus as the primary hypothalamic factor driving stress-induced adrenocorticotropin (ACTH) secretion from the anterior pituitary (Vale et al., 1981; Rivier and Plotsky, 1986). Triggering the secretion of ACTH from the pituitary, hypothalamic CRF is the major physiological regulator of the hypothalamic–pituitary–adrenal axis. In addition, CRF may also function as a neurotransmitter or neuromodulator to coordinate stress-induced neural responses in extrahypothalamic areas in the central nervous system.

There are several lines of evidence that CRF is involved in depression and anxiety. Intracerebroventricular administration of CRF to laboratory animals produces behavioral effects similar to those observed in depression (Dunn and Berridge, 1990; Owens and Nemeroff, 1991), and brain CRF has been demonstrated to be implicated in anxiety, in animal models (Menzachi et al., 1994; Skutella et al., 1994a,b: Sarnyai et al., 1995). CRF transgenic mice overexpressing CRF gene show increased anxiety (Stenzel-Poore et al., 1994). Moreover, patients with major depression have increased CRF levels in the cerebrospinal fluid (Nemeroff et al., 1984). CRF-induced decrease in exploratory behavior was observed in hypophysectomized mice (Berridge and Dunn, 1989), which suggests that CRF elicits action in the brain distinct from its potential to activate the hypothalamic–pituitary–adrenal axis. Thus, hypersecretion of CRF into the brain may underlie some of the symptomatology seen in affective and anxiety-related

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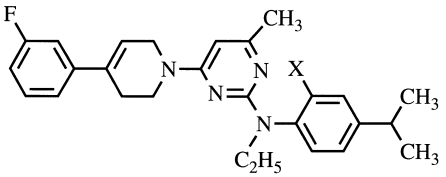
PII: S0014-2999(99)00120-X



X = CH_3S : CRA1000
X = Br : CRA1001

Fig. 1. Chemical structure of CRA1000 and CRA1001.

disorders. CRF receptor antagonists may be effective for treating patients with these disorders.

Three CRF receptor cDNAs, designated CRF_1, $CRF_{2\alpha}$ and $CRF_{2\beta}$, have been cloned, and characterized in terms of their pharmacological specificity and regional localization (Chen et al., 1993; Lovenberg et al., 1995a; Perrin et al., 1995). In situ hybridization studies revealed relative distributions of CRF_1 and $CRF_{2\alpha}$ receptors (Chalmers et al., 1995). CRF_1 receptor mRNA is present in discrete regions of the central nervous system including pituitary, neo-, olfactory and hippocampal cortices, cerebellum, septum, amygdala and brainstem sensory relay structures with only low levels in thalamic and hypothalamic nuclei. In contrast, the expression of $CRF_{2\alpha}$ receptor is more confined to subcortical structures such as the lateral sepatal nucleus, ventromedial hypothalamic nucleus, amygdala and entorhinal cortex. The $CRF_{2\beta}$ receptor is localized in nonneuronal elements such as the choroid plexus and cerebral arterioles as well as having been identified heart and skeletal muscle (Lovenberg et al., 1995b).

The discrete distributions of CRF_1 and CRF_2 receptors imply that they subserve distinct physiological functions, which is supported by dissimilar pharmacologies of these receptor subtypes (Lovenberg et al., 1995a).

Urocortin with higher affinity for the CRF_2 receptor is more potent than CRF in suppressing feeding behavior, but is less potent than CRF in producing anxiety-like effects (Spina et al., 1996). By contrast, chronic infusion of a CRF_1 receptor antisense oligodeoxynucleotide into the central amygdaloid nucleus reduced anxiety-related behavior in socially defeated rats (Liebsch et al., 1995). Therefore, the CRF_2 receptor may be involved in appetite while the CRF_1 receptor may be related to anxiety and depression. This hypothesis is supported by recent findings that selective CRF_1 receptor antagonists such as CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-d]-pyrimidin-4-yl]-ethylamine) and antalarmin have antidepressant-like and anxiolytic-like effects, in experimental animals (Lundkvist et al., 1996: Schulz et al., 1996: Webster et al., 1996: Mansbach et al., 1997). Recently, we have identified novel CRF_1 receptor specific antagonists, CRA1000 and CRA1001, and demonstrated that both CRA1000 and CRA1001 have antidepressant-like and anxiolytic-like effect (Okuyama et al., submitted). We report here the in vitro pharmacological characteristics of novel and selective nonpeptide antagonists for the CRF_1 receptor.

2. Materials and methods

2.1. Materials

CRA1000 (2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine, Fig. 1), CRA1001 (2-(*N*-(2-romo-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophe-yl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine, ig. 1), CP-154,526 and SC241 ([3-(2-bromo-4-isopropylphenyl)-5-methyl-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-bis-(2-methoxy-ethyl)-amine) were synthesized in Taisho Pharmaceutical laboratories. [^{125}I]ovine CRF (specific radioactivity: 81.4 TBq/mmol) and [^{125}I]sauvagine (specific radioactivity: 81.4 TBq/mmol) were purchased from Du Pont/New England Nuclear (Wilmington, DE, USA). The cAMP assay system was purchased from Amersham International (Buckinghamshire, England). The ACTH IRMA system was purchased from Mitsubishi Kagaku (Tokyo, Japan). COS-7 cells and AtT-20 cells were purchased from American Type Culture Collection (Rocksville, MD, USA). Ovine CRF and sauvagine were purchased from Peninsula Laboratories (Belmont, CA, USA). All other chemicals used in this study were obtained commercially, and of the highest purity available. The test compounds such as CRA1000, CRA1001, CP-154,526 and SC241 were dissolved in 0.1% dimethylsulfoxide. Dimethylsulfoxide (0.1%) itself did not affect both the binding assay and cAMP level.

2.2. Methods

2.2.1. CRF receptor expression constructs

Male Wistar rats (Japan SLC, Hamamatsu, Japan; 200–250 g) were decapitated, and the frontal cortex and hypothalamus were dissected according to Glowinski and Iversen (1966). CRF_1 and $CRF_{2\alpha}$ receptor cDNAs were isolated from the frontal cortex and hypo-thalamus, respec-

Table 1
Competition by CRA compounds and CRF_1 receptor antagonists for [^{125}I]ovine CRF binding to membranes of cells expressing CRF_1 or $CRF_{2\alpha}$ receptors

Compounds	(IC_{50}: nM)	
	COS-7 cells (CRF_1)	COS-7 cells ($CRF_{2\alpha}$)
CRA1000	30 ± 3.1	> 10000
CRA1001	38 ± 1.0	> 10000
CP-154,526	5 ± 0.5	> 10000
SC241	36 ± 1.8	> 10000

Data represent means ± S.E. from three to four determinations, each done in duplicate.

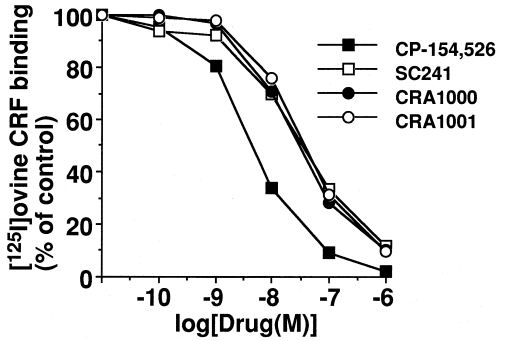


Fig. 2. Effect of CRA compounds and CRF_1 receptor antagonists on [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing the rat CRF_1 receptor. The membranes of COS-7 cells expressing the rat CRF_1 receptor was incubated with increasing concentrations of compounds in the presence of 0.2 nM [^{125}I]ovine CRF for 2 h at 25°C. The results given are the mean values of three separate experiments, each done in duplicate. Key: CP-154,526 (■), SC241 (□), CRA1000 (●) and CRA1001 (○).

tively, by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was prepared from the rat frontal cortex and hypothalamus by means of the acid guanidine-phenol/chloroform method described by Chomczynski and Sacchi (1987), and cDNA was synthesized using reverse transcriptase (Superscript II, GIBCO BRL, Gaithersburg, MD, USA). The oligonucleotide primers 5'AATTGAATTCCTCTGGGATGTCGGAGCGATCCAGGCATCC3' (sense) and 5'AATTGGTACCGTCAGTGAGCTTGCATCATCTCCCCGGCCT3' (antisense) was used for the CRF_1 receptor, and 5'ATATGAATTCCAACGCGCGCGGCTCCGGAGCGCAATG3' (sense) and 5'TTTTGGTACCAGGGAAGGCTGTGAAGAATGAGGAA3' (antisense) were used for the $CRF_{2\alpha}$ receptor. The PCR conditions for both CRF_1 and $CRF_{2\alpha}$ receptors were 1 min at 95°C, 1 min at 60°C and 2 min at 72°C for 35 cycles. cDNAs were cloned into the expression vector pcDLΔPE derived from pcDLSRα296 (Takebe et al., 1988). In this plasmid, the *Pst*I site of pcDLSRα296 was converted to *Eco*RI sites by ligation of an *Eco*RI linker to its blunting termini, and the *Pst*I–*Eco*RI short segment was deleted.

2.2.2. Cell cultures and transfection

COS-7 cells and AtT-20 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 incubator at 37°C. The rat CRF_1 and $CRF_{2\alpha}$ receptor cDNAs inserted into pcDLΔPE were separately transfected into COS-7 cells using lipofectin (GIBCO BRL) according to the protocol provided by manufacturer (Felgner et al., 1987). At 72 h after transfection, COS-7 cells expressing CRF_1 or $CRF_{2\alpha}$ receptor were used for pharmacological experiments.

2.2.3. Membrane preparations

COS-7 cells expressing the CRF_1 or $CRF_{2\alpha}$ receptor were washed with PBS, scraped and pelleted by centrifugation. Cell pellets were homogenized with 50 mM Tris–HCl buffer (pH 7.0) containing 10 mM $MgCl_2$ and 2 mM EDTA, and centrifuged at 48,000 $\times$ *g* for 20 min at 4°C. The pellet was washed twice with the buffer, and the final pellet was suspended in the assay buffer (50 mM Tris–HCl buffer, pH 7.0, containing 10 mM $MgCl_2$, 2 mM EDTA, 0.1% BSA and 100 kU/ml aprotinin), and used as crude membrane preparations for binding studies. Protein concentration was determined according to Bradford (1976).

2.2.4. Binding studies

Binding assays for [^{125}I]ovine CRF and [^{125}I]sauvagine were done according to reported method (De Souza, 1987; Grigoriadis et al., 1996) but with slight modification. The reaction was initiated by incubating 0.5 ml of membrane preparations with 0.2 nM [^{125}I]ovine CRF or 0.2 nM [^{125}I]sauvagine. The reaction mixture was incubated for 2 h at 25°C (for [^{125}I]ovine CRF binding) or at 23°C (for [^{125}I]sauvagine binding), and terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 ml of PBS containing 0.01% Triton X-100. The radioactivity was quantified in a

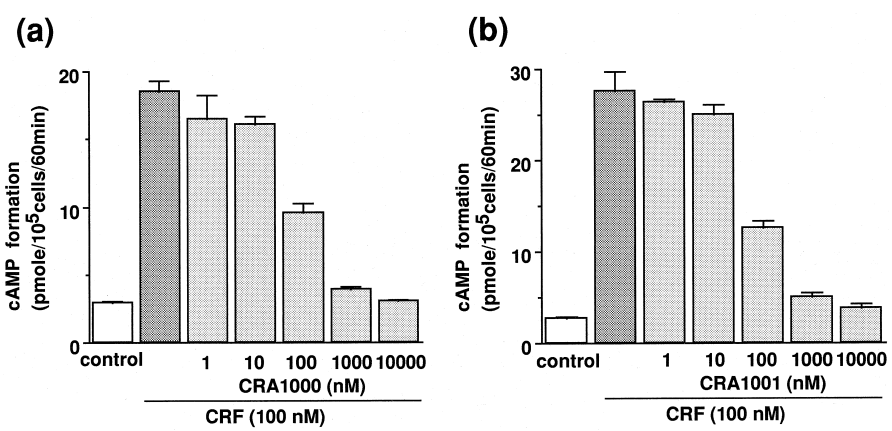


Fig. 3. Antagonism of CRF-induced increase in cAMP formation in COS-7 cells transiently expressing the rat CRF_1 receptor by CRA1000 (a) and CRA1001 (b). Cells were incubated with 100 nM CRF and/or increasing concentrations of the compounds for 60 min at 37°C, and measurement of cAMP produced in the cells was done as described in Section 2. Data represent the means $\pm$ S.E. of three determinations.

Table 2
Effect of CRA compounds and CRF_1 receptor antagonists on CRF-induced increase in cAMP formation and ACTH secretion

Compounds	cAMP formation (IC_{50}: nM)			ACTH secretion (IC_{50}: nM) (AtT-20 cells)
	COS-7 cells (CRF_1)	AtT-20 cells	COS-7 cells ($CRF_{2\alpha}$)	
CRA1000	79	377	> 10000	142
CRA1001	68	187	> 10000	363
CP-154,526	19	42	> 10000	86
SC241	ND	92	> 10000	131

ND: not determined.
Data represent means from three determinations.

γ-counter. Nonspecific bindings were determined in the presence of unlabeled 1 μM ovine CRF (for [^{125}I]ovine CRF binding) or 1 μM sauvagine (for [^{125}I]sauvagine binding). Specific binding was determined by subtracting nonspecific binding from total binding. In the competition binding assay, concentration of the test compound that caused 50% inhibition of specific radiolabeled ligand binding (IC_{50} values) was determined from each concentration–response curve. IC_{50} values were determined by the Marquardt–Levenberg nonlinear least-squares curve-fitting procedure of the MicroCal ORIGIN program (MicroCal, Northampton, MA, USA) running on a Microsoft Windows 3.1.

2.2.5. Determination of cAMP

COS-7 cells expressing CRF_1 or $CRF_{2\alpha}$ receptors, or AtT-20 cells grown in a six-well plate were used. The culture medium was removed, the cells were washed with PBS, and 1 ml of DMEM containing 1 mM isobutylmethylxanthine, a phosphodiesterase inhibitor, was added. The cells were incubated with CRF and/or compound for 1 h at 37°C. The culture medium was then aspirated, and the cells were washed with PBS. Some 2 ml of ice-cold 65% EtOH was added, and the cells were scraped from the wells. The supernatant was collected by centrifugation at 15,000 rpm for 15 min at 4°C. cAMP formed in the cells was determined using a commercially available cAMP EIA system.

2.2.6. Determination of ACTH

The culture medium of AtT-20 cells grown in a six-well plate was removed, and the cells were washed with PBS. Some 2 ml of DMEM containing 0.1% BSA and 100 kU/ml aprotinin was then added, and the cells were incubated with CRF and/or compound for 1 h at 37°C. An aliquot of the medium was removed and stored at −80°C until use. ACTH content was determined using a commercially available ACTH IRMA system.

3. Results

3.1. Affinity of CRA1000 and CRA1001 for CRF receptors

CRA1000 and CRA1001 inhibited [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing rat CRF_1 receptor (Table 1; Fig. 2). Competition curves of CRA1000 and CRA1001 for all preparations were monophasic, and Hill coefficients were approximately 1.0. Affinity of CRA compounds for the CRF_1 receptor was practically the same as that of SC241, and approximately ten times lower than

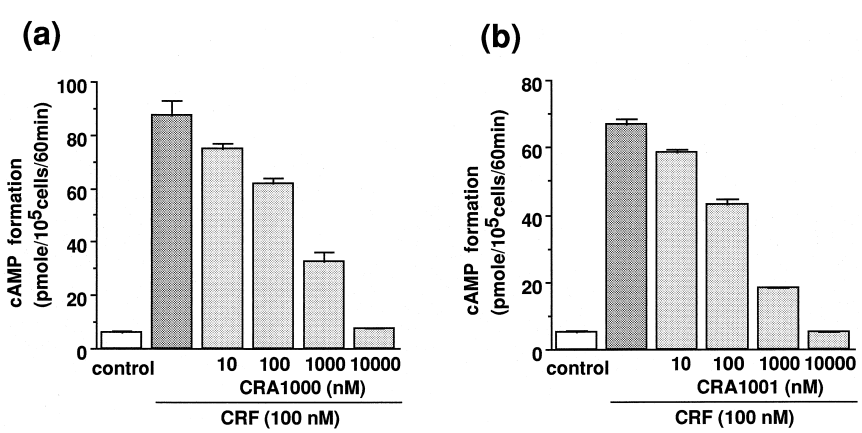


Fig. 4. Antagonism of CRF-induced increase in cAMP formation in AtT-20 cells by CRA1000 (a) and CRA1001 (b). Cells were incubated with 100 nM CRF and/or increasing concentrations of the compounds for 60 min at 37°C, and measurement of cAMP produced in the cells was done as described in Section 2. Data represent the means ± S.E. of three determinations.

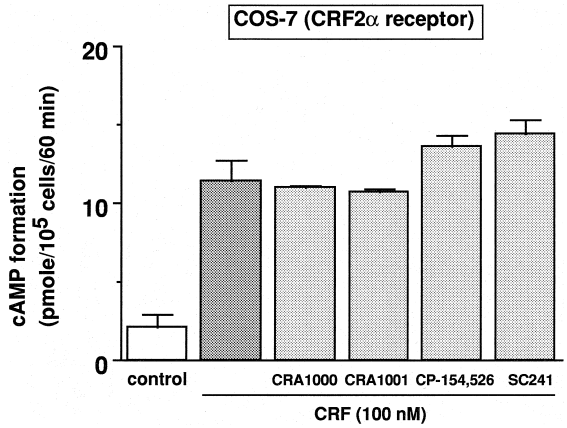


Fig. 5. Effect of CRA compounds and CRF_1 receptor antagonists on CRF-induced increase in cAMP formation in COS-7 cells transiently expressing the rat $CRF_{2\alpha}$ receptor. Cells were incubated with 100 nM CRF and/or increasing concentrations of the compounds for 60 min at 37°C, and measurement of cAMP produced in the cells was done as described in Section 2. Data represent the means ± S.E. of three experiments.

CP-154,526. Neither CRA1000 nor CRA1001 inhibited [^{125}I]sauvagine binding to membranes of COS-7 cells expressing the rat $CRF_{2\alpha}$ receptor, even at 10 μM, as did other CRF_1 receptor antagonist (Table 1).

3.2. Blockade of CRF-induced cAMP formation

Ovine CRF stimulated cAMP formation in AtT-20 cells and COS-7 cells expressing CRF_1 or $CRF_{2\alpha}$ receptor, in a concentration-dependent manner (data not shown). CRA compounds and other CRF_1 receptor antagonists concentration-dependently attenuated cAMP accumulation induced by 100 nM CRF in COS-7 cells expressing the rat CRF_1 receptor (Fig. 3; Table 2). IC_{50} values were as follows: 79 nM for CRA1000; 68 nM for CRA1001; and 19 nM for CP-154,526. These compounds also concentration-dependently diminished the 100 nM CRF-induced cAMP formation in AtT-20 cells (Fig. 4; Table 2), which was seen to express only the CRF_1 receptor among CRF receptor subtypes, determined using RT-PCR using specific primers for each subtype (data not shown). IC_{50} values were as follows: 377 nM for CRA1000; 188 nM for CRA1001; 42 nM for CP-154,526; and 92 nM for SC241, which is in good agreement of affinities of these compounds for the CRF_1 receptor. In contrast, CRA1000, CRA1001 and CRF_1 receptor antagonists were without effect on CRF (100 nM)-induced cAMP formation in COS-7 cells expressing the rat $CRF_{2\alpha}$ receptor (Fig. 5; Table 2). These compounds, per se, had no effect on basal cAMP levels in both AtT-20 cells and COS-7 cells expressing CRF_1 or $CRF_{2\alpha}$ receptor (data not shown).

3.3. Blockade of the CRF-induced ACTH secretion

CRF was reported to stimulate ACTH secretion from the pituitary, and activate hypothalamic–pituitary–adrenal axis (Rivier and Plotsky, 1986). Since AtT-20 cells were derived from the mouse pituitary tumor, AtT-20 cells have been widely used as a model for functions mediated through the CRF receptor (Hook et al., 1982; Schwartz et al., 1991). CRF concentration-dependently induced ACTH secretion in AtT-20 cells (data not shown), and CRA1000, CRA1001 and CRF_1 receptor antagonists inhibited the CRF-induced ACTH secretion, in a concentration-dependent manner (Fig. 6; Table 2). These compounds, per se, had no effect on basal ACTH secretion in AtT-20 cells (data not shown).

4. Discussion

We obtained evidence that CRA1000 and CRA1001 are potent and selective antagonists for the CRF_1 receptor.

CRA1000 and CRA1001 were identified from screening in which [^{125}I]ovine CRF binding to membranes of rat

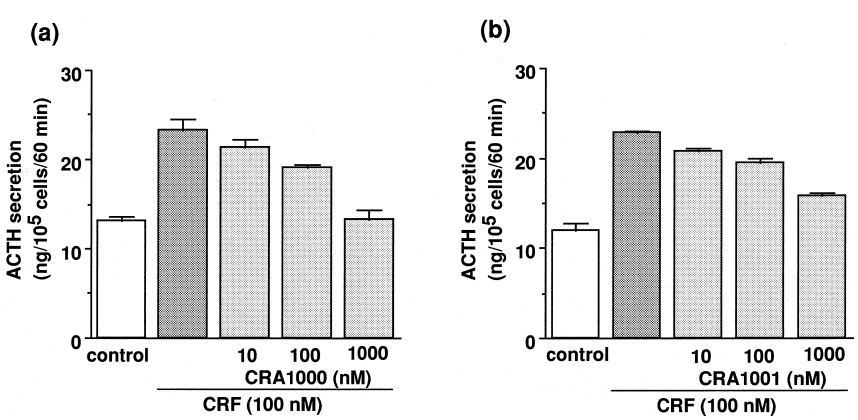


Fig. 6. Antagonism of CRF-induced increase in ACTH release from AtT-20 cells by CRA1000 (a) and CRA1001 (b). Cells were incubated with 100 nM CRF and/or increasing concentrations of the compounds for 60 min at 37°C, and measurement of ACTH release in the conditioned medium was done as described in Section 2. Data represent the means ± S.E. of three experiments.

frontal cortex was used to determine the affinity for CRF_1, and found that both CRA1000 and CRA1001 inhibited [^{125}I]ovine CRF binding in the pituitary, but not [^{125}I]sauvagine binding in the heart (Okuyama et al., submitted). The CRF receptor has been divided into two major subtypes, termed CRF_1 and CRF_2, based on molecular cloning techniques (Chen et al., 1993; Lovenberg et al., 1995a). Moreover, CRF_2 has isoforms, $CRF_{2\alpha}$ and $CRF_{2\beta}$ (Perrin et al., 1995), in which sequences of the N-terminus differ. The distributions and pharmacological properties of these subtypes are distinct. Among CRF related peptides such as sauvagine, urotensin I and urocortin, CRF_1 has a higher affinity for CRF than for related peptides, while CRF_2 has a higher affinity for related peptides than for CRF itself (Chen et al., 1993; Lovenberg et al., 1995a; Perrin et al., 1995). In the rat brain, CRF_1 is diffusely distributed and is abundant in cortical areas and the pituitary (Chalmers et al., 1995). In contrast, $CRF_{2\beta}$ has been reported to be absent in the rat brain, but abundant in the rat heart and skeletal muscle (Lovenberg et al., 1995b). Therefore, it is suggested that both CRA1000 and CRA1001 are CRF_1 receptor antagonists.

To confirm the affinity and specificity of the compounds for CRF_1 receptor, we established cell lines transiently expressing either rat CRF_1 or rat $CRF_{2\alpha}$ receptors. Both CRA1000 and CRA1001 inhibited [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing CRF_1 receptor, although these compounds were without effect on [^{125}I]sauvagine binding to membranes of COS-7 cells expressing the $CRF_{2\alpha}$ receptor. These results, accompanied with the evidence that CRA1000 and CRA1001 did not inhibit [^{125}I]sauvagine binding in the rat heart in which $CRF_{2\beta}$ is abundant, strongly indicate that both CRA1000 and CRA1001 are CRF_1 receptor specific ligands and that these compounds had no affinity for both $CRF_{2\alpha}$ and $CRF_{2\beta}$.

CRF receptors have been reported to be coupled to GTP binding protein (Gs), and activate adenylate cyclase activity, which leads to accumulation of cAMP in targeted cells (Bilezikjian and Vale, 1983). Thus, consequences of CRA compounds binding to CRF receptors were determined using in vitro activation of adenylate cyclase by CRF in both COS-7 cells transiently expressing CRF_1 or $CRF_{2\alpha}$ receptor, and AtT-20 cells.

Both CRA1000 and CRA1001 concentration-dependently attenuated CRF-induced increment in cAMP in AtT-20 cells and COS-7 cells expressing the CRF_1 receptor. In contrast, these compounds did not affect CRF-induced augmentation of cAMP formation in COS-7 cells expressing the $CRF_{2\alpha}$ receptor. In a preliminary study, AtT-20 cells expressed only CRF_1 mRNA by RT-PCR among CRF receptor subtypes. Therefore, both CRA1000 and CRA1001 function as antagonists at the CRF_1 receptor.

CRF is involved in alteration of neuroendocrine systems responding to stress through activation of the hypothalamic–pituitary–adrenal axis (Rivier and Plotsky, 1986). Secretion of ACTH from the pituitary has been thought to be the trigger eliciting the physiological functions mediated by CRF (Rivier and Plotsky, 1986), although it was also suggested that CRF acts in the brain, independent of activation of the hypothalamic–pituitary–adrenal axis (Berridge and Dunn, 1989). AtT-20 cells, a cell line derived from a pituitary tumor, induces ACTH secretion by several hormones, and is a useful model to investigate activity of the hypothalamic–pituitary–adrenal axis (Hook et al., 1982; Schwartz et al., 1991). To examine the functional significance of CRA compounds, we investigated the effect of CRA compounds on ACTH secretion from AtT-20 cells. Both CRA1000 and CRA1001 attenuated CRF-induced ACTH secretion from AtT-20 cells, in a concentration-dependent fashion. Thus, these CRA compounds seem to attenuate functionally the responses mediated by CRF.

It has been suggested that CRF_1 mediates physiological responses related to stress such as anxiety and depression, while CRF_2 mediates feeding behavior (Liebsch et al., 1995; Spina et al., 1996). We found that both CRA1000 and CRA1001 attenuated stress-induced anxiogenic effects and showed antidepressant-like activities in experimental animals, and that these compounds attenuated the CRF-induced firing rate of locus coeruleus neurons (Okuyama et al., submitted) as did other nonpeptide CRF_1 receptor specific antagonists such as CP-154,526 and antalarmin (Lundkvist et al., 1996; Schulz et al., 1996; Mansbach et al., 1997). Moreover, anxiolytic-like effects and antidepressant-like effects of CRA1000 and CRA1001 were more potent than those of CP-154,526 when administered orally (Okuyama et al., submitted).

Taken together, both CRA1000 and CRA1001 are apparently potent and selective CRF_1 receptor antagonists, which may prove effective for treating subjects with excess CRF-related secretion disorders such as depression or anxiety.

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