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Modulation of endothelial prostaglandin synthesis by corticotropin releasing factor and antagonists

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Abstract

Corticotropin releasing factor (CRF) is a hypothalamic hormone that also displays autocrine/paracrine roles at peripheral sites. High concentrations of CRF have been identified in endothelial cells and other inflammatory tissues. We investigated the effects of CRF and antagonists in the regulation of prostaglandin synthesis in bovine aortic endothelial cells, and also characterized the binding of CRF in these cells. Interleukin-1α increased prostacyclin (prostaglandin I_2) synthesis in endothelial cells and this response to interleukin-1α was abolished by simultaneous exposure to CRF. The effect of CRF on interleukin-1α-induced prostaglandin synthesis was antagonised by the CRF receptor antagonist α-helical CRF-(9–41). In addition, this as well as another CRF receptor antagonist, namely [D-Phe^{12}]CRF-(12–41), when applied alone at low concentrations inhibited the interleukin-1α-induced prostaglandin synthesis similarly to CRF, suggesting partial agonistic action. Binding of [^{125}I]-labeled CRF in endothelial cells was saturable and fitted a two sites model. K_d for the higher-affinity class of receptors was 0.2 ± 0.02 nM, and B_{max} 0.79 ± 0.095 fmol/mg protein. The lower-affinity class of receptors had a K_d of 1.77 ± 0.14 μM and B_{max} 0.97 ± 0.12 fmol/mg protein. These findings suggest a direct role for CRF in the local regulation of inflammation.

Keywords: CRF (corticotropin releasing factor); CRF receptor; CRF-(9–41), α-helical; [D-Phe^{12}]CRF-(12–41); Interleukin-1; Endothelium

1. Introduction

Recent years have witnessed extensive exploration of possible communications between the neuroendocrine and the immune systems. Thus, many neuropeptides have been demonstrated to exhibit immunomodulatory as well as neuromodulatory actions. Peptides, hormones and receptors common to both systems appear to mediate a reciprocal regulation of immune and neuroendocrine functions (Pert et al., 1985). Corticotropin releasing factor (CRF), a 41-amino acid peptide, is well characterised as the predominant regulator of the neuroendocrine, autonomic and behavioral responses to stress. CRF stimulates the synthesis and release of pro-opiomelanocortin (POMC)-derived peptides such as adrenocorticotropic hormone (ACTH) and β endorphin from the pituitary (Vale et al., 1981). The target of circulating ACTH is the adrenal cortex, where it induces the biosynthesis and secretion of glucocorticoids. The latter exert, among other things, anti-inflammatory effects that are attributed in part to their interference with prostaglandin synthesis (Goppelt-Struebe, 1997).

Recent studies have suggested that not only glucocorticoids, but also CRF, might be directly involved in the local regulation of inflammation. The presence of CRF has been documented in the gastrointestinal tract (Kawahito et al., 1994), lungs (Dieterich et al., 1994), adrenal gland (Dave et al., 1985) and placenta (Petraglia et al., 1987). Importantly, CRF mRNA and immunoreactivity have been demonstrated in lymphoid tissues such as the thymus and spleen (Webster and De Souza, 1983; Redei, 1992), in leukocytes and in T and B lymphocytes (Karalis et al., 1991). Furthermore, peripheral CRF expression was enhanced under inflammatory conditions such as carrageenin-induced inflammation, where concentrations of CRF were much higher in inflamed tissue fibroblasts and vascular endothelial cells than in non-inflamed tissues (Karalis et al., 1991).

Prostaglandins are major component of the inflammatory response and they also modulate many actions of the inflammatory cytokines. Earlier work has shown that CRF

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stimulates prostaglandin output from the human placenta, decidua and fetal membranes (Jones and Challis, 1989). On the other hand, the prostaglandin E_2-response to interleukin-1β in rat astrocyte cultures was suppressed by simultaneous addition of CRF (Katsuura et al., 1989). In our recent study (Fleisher-Berkovich and Danon, 1995) we showed that CRF blocked the interleukin-1α-stimulated prostaglandin synthesis in endothelial cells. The present study was designed to characterise the receptor mediation of this effect. Two strategies were employed, namely CRF binding assays and the use of CRF receptor antagonists. The results indicate involvement of more than one type of CRF receptors, resulting in anomalous antagonist behavior.

Part of this work was presented at the Fourth International Conference on Essential Fatty Acids and Eicosanoids in Edinburgh (Fleisher-Berkovich et al., 1997).

2. Materials and methods

2.1. Materials

The following materials were used: aprotinin (Trasylol, Bayer, Leverkusen, Germany), human/rat CRF (Sigma), α-helical CRF-(9–41) (Sigma), [D-Phe^{12},Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF-(12–41) ([D-Phe^{12}]CRF-(12–41); kindly provided by Dr. Jean Rivier) and recombinant human interleukin-1α (a generous gift from Hoffmann–La Roche).

2.2. Cell cultures

Cultures of bovine aortic endothelial cells were kindly provided by Prof. Vlodavsky (Hebrew University, Jerusalem, Israel) and treated as previously described (Einhorn et al., 1985). Cells, used between passages 6–17, were laid in 24-well tissue culture plates (Greiner, Frickenhausen, Germany) and incubated at 37°C in humidified 8% CO_2-air atmosphere. Each 16 mm well contained 1 ml Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Beth Haemek, Israel), supplemented with 10% calf serum (Sigma, St. Louis, MO), 2 mM L-glutamine and penicillin/streptomycin (Biological Industries) at concentrations of 100 U/ml and 100 μg/ml, respectively. During the subconfluent stages 2 ng/ml fibroblast growth factor (Sigma) were added to the cells every other day until the cultures became confluent. Cells were used 10 days after reaching confluency. Experiments were initiated by washing twice with serum-free DMEM (SFM). The cells were incubated in 1 ml SFM for 24 h and then the test agents were added for the length of time indicated in each experiment.

2.3. Determination of 6-keto prostaglandin $F_{1\alpha}$

Experiments were terminated by transferring the media to test tubes containing indomethacin (final concentration 100 nM). 6-Keto prostaglandin $F_{1\alpha}$, the stable dehydration product of prostacyclin, that accumulated in the media, was measured in unextracted samples of DMEM by single antibody radioimmunoassay with dextran-coated charcoal precipitation. DMEM did not interfere with the assay. The assay was performed in duplicate for each sample. Rabbit antiserum to 6-keto prostaglandin $F_{1\alpha}$ was obtained from Bio-Makor (Rehovot, Israel) and tritium-labeled 6-keto prostaglandin $F_{1\alpha}$ (175 Ci/mmol) was supplied by Amersham (Buckinghamshire, England). The sensitivity of the assay was 0.15 ng/ml and the assayable range, 0.15–5 ng/ml. The 6-keto prostaglandin $F_{1\alpha}$ antiserum cross-reacted with other prostaglandins as follows: prostaglandin E_1, 22%; prostaglandin E_2, 10%; prostaglandin $F_{1\alpha}$, 16%; prostaglandin $F_{2\alpha}$, 10%; other prostaglandins $< 1\%$. The coefficient of variation of the assay was 7.5% and 6.4% at concentrations of 0.3 and 1.2 ng/ml, respectively.

2.4. CRF receptor binding assay

(2-[^{125}I] iodohistidyl^{32}) human CRF (2000 Ci/mmol) was obtained from Amersham. Cells were dispersed with 0.25% trypsin for 2 min. After centrifugation at 2500 rpm for 5 min, the dispersed cells pellet was incubated for 1 h with 1 nM [^{125}I]human CRF in the presence of increasing concentrations of unlabeled rat/human CRF at 37°C in a binding buffer containing 20 mM Tris, 3 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM EDTA, 140 mM NaCl, aprotinin 10^4 U/ml and 2.5% bovine serum albumin, pH 7.4. The reaction was terminated by centrifugation at 4°C for 10 min at 6000 rpm. The resulting pellets were gently washed twice with 1 ml ice-cold incubation buffer and were monitored for radioactivity in a γ-counter (Savyon, Israel) at 90% efficiency. Nonspecific binding, determined in the presence of 5 μM unlabeled peptide, was 0.18 of the total.

2.5. Determination of protein content

After removing the medium, the attached cells in each well were dissolved in 1 ml of 10 mM NaOH and transferred into test tubes. Protein concentrations were determined by the Bradford reaction, using the Bio-Rad Protein Assay (acidic solution of Coomassie Blue that shifts from 465 nm to 595 nm when bound to protein). Bovine serum albumin (Sigma) was used as standard.

2.6. Data analysis

Results are expressed as means ± S.E.M. for each experiment. Statistical analysis of the results was performed using two-tailed Student's *t*-test, and $P < 0.05$ was considered significant. Radioligand binding data were ana-

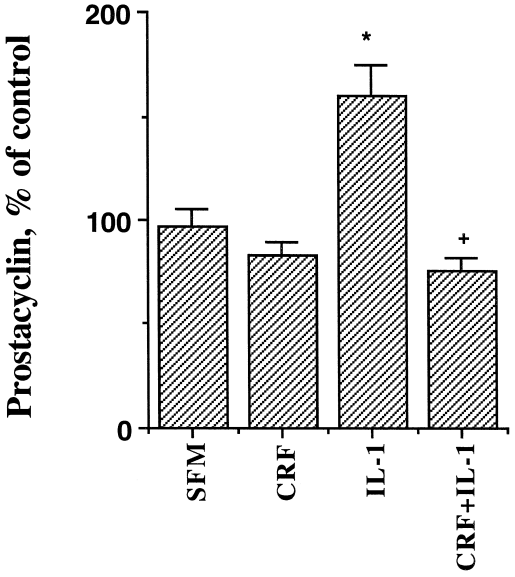

Fig. 1. Effect of corticotropin releasing factor on interleukin-1α-induced prostacyclin production. Bovine aortic endothelial cells were incubated with interleukin-1α (IL-1)(100 U/ml, equivalent to 5.55×10^{-14} M) and CRF (2.5×10^{-8} M), alone or in combination, for 24 h. Thereafter, the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$ (the stable dehydration product of prostacyclin). Results are means ± S.E.M. of $n=24$. $*$ $P<0.05$ compared with SFM control, $^{+}P<0.05$ vs. interleukin-1α.

lyzed by nonlinear curve fitting which is part of Kaleidagraph software for MacIntosh, version 3.04.

3. Results

As shown in Fig. 1, exposure (24 h) of bovine aortic endothelial cells to CRF did not affect basal levels of prostacyclin, the major prostaglandin that is synthesized in these cells, but significantly suppressed the interleukin-1α-induced synthesis of this prostaglandin. To characterise receptor involvement in CRF's action on prostaglandin

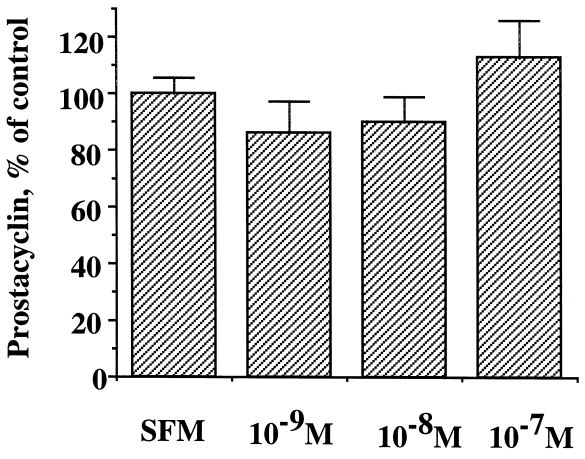

Fig. 2. Effect of α-helical CRF-(9–41) on prostacyclin production. Bovine aortic endothelial cells were incubated with α-helical CRF-(9–41) ($10^{-9}-10^{-7}$ M) for 24 h. Then the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$. Results are means ± S.E.M. of $n=6$.

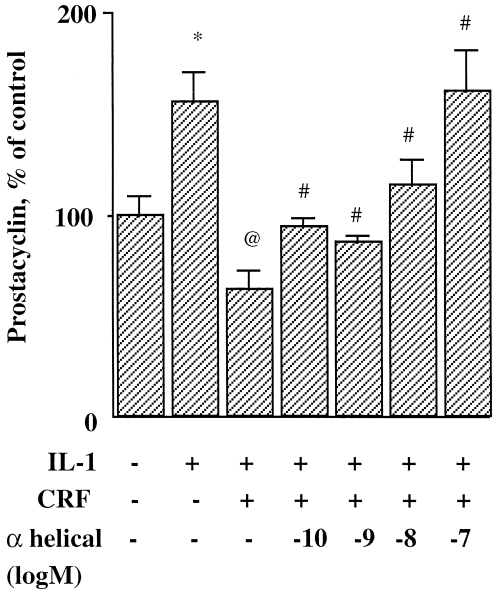

Fig. 3. Effects of corticotropin releasing factor and α helical CRF-(9–41) on interleukin-1α-induced prostacyclin production. Bovine aortic endothelial cells were incubated with CRF (2.5×10^{-8} M), interleukin-1α (IL-1) (100 U/ml) or both in the presence of α-helical CRF-(9–41) at increasing concentrations ($10^{-10}-10^{-7}$ M) for 24 h. Thereafter, the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$. Results are means ± S.E.M. of $n=6$. $*$ $P<0.05$ compared with SFM control; $^{@}P<0.05$ vs. interleukin-1α; $^{\#}P<0.05$ vs. interleukin-1α + CRF.

synthesis, we used the CRF receptor antagonists α helical CRF-(9–41) and [D-Phe[12]]CRF-(12–41). Fig. 2 shows that α helical CRF-(9–41), at all concentrations that were used, failed to affect basal levels of prostacyclin. However, as shown in Fig. 3, CRF effectively blocked the interleukin-

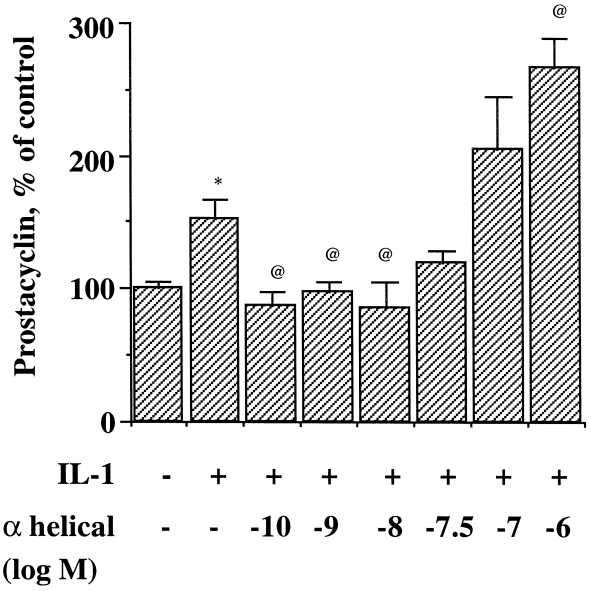

Fig. 4. Effect of α helical CRF-(9–41) on interleukin-1α-induced prostacyclin production. Bovine aortic endothelial cells were incubated with interleukin-1α (IL-1) (100 U/ml) and α-helical CRF-(9–41) ($10^{-10}-10^{-6}$ M) for 24 h. Thereafter, the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$. Results are means ± S.E.M. of $n=6$. $*$ $P<0.05$ compared with SFM control; $^{@}P<0.05$ vs. interleukin-1α.

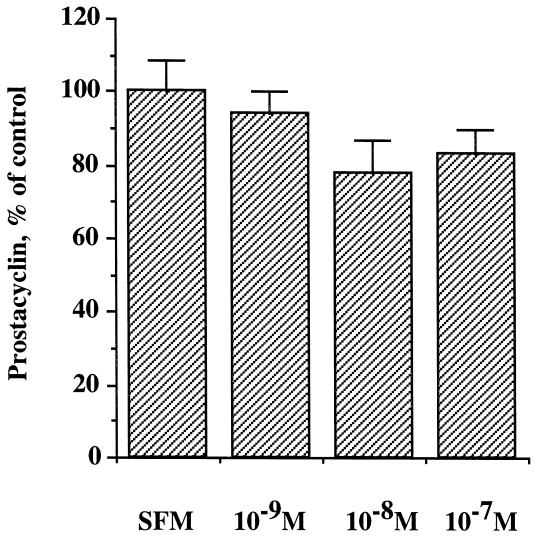

Fig. 5. Effect of [D-Phe^{12}]CRF-(12–41) on prostacyclin production. Bovine aortic endothelial cells were incubated with [D-Phe^{12}]CRF-(12–41) ($10^{-9} - 10^{-7}$ M) for 24 h. Then the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$. Results are means ± S.E.M. of $n = 6$.

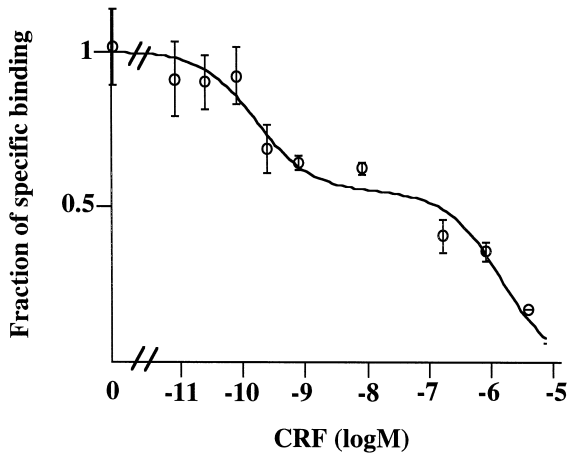

Fig. 7. Competitive displacement of ^{125}I-CRF by unlabeled human CRF. Dispersed endothelial cells were incubated with 1 nM [^{125}I]human CRF in the presence of increasing concentrations of unlabeled rat/human CRF at 37°C for 1 h. The reaction was terminated by centrifugation at 4°C for 10 min. The resulting pellets were washed and monitored for radioactivity as described in Section 2. Data points represent means ± S.E.M. of three experiments.

1α-induced prostacyclin synthesis in endothelial cells, and this effect was apparently abrogated, in a concentration-dependent manner, by the CRF receptor antagonist α-helical CRF-(9–41). Fig. 4 indicates that the receptor antagonist itself, at lower concentrations ($10^{-10} - 10^{-8}$ M), in the absence of CRF, inhibited the effect of interleukin-1α similarly to CRF. However, this inhibition was lost at higher concentrations ($3 \times 10^{-8} - 10^{-6}$ M) of the antagonist and moreover, prostacyclin production was enhanced by these concentrations of α-helical CRF-(9 − 41) beyond that induced by interleukin-1α. Similar inhibition of interleukin-1α-induced prostacyclin production was obtained with α-helical CRF-(9–41) from an alternative source (Cambridge Research Biochemicals, England, data not shown).

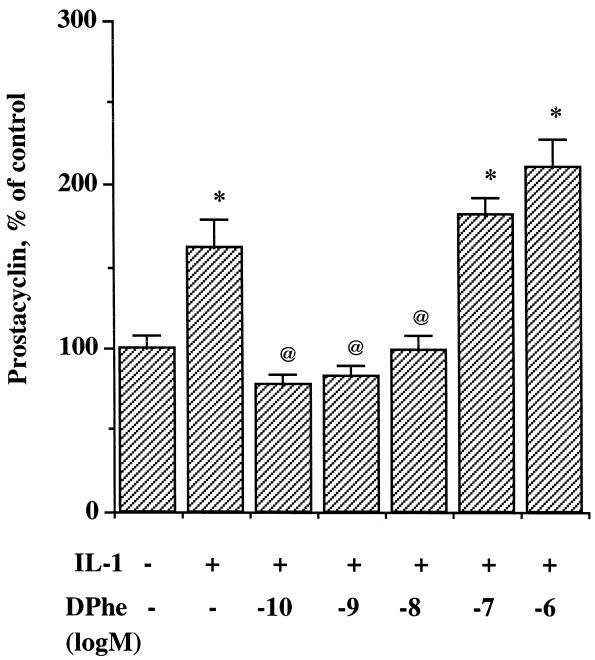

Fig. 6. Effect of [D-Phe^{12}]CRF-(12–41) on interleukin-1α-induced prostacyclin production. Bovine aortic endothelial cells were incubated with interleukin-1α (IL-1) (100 U/ml) and [D-Phe^{12}]CRF-(12–41) ($10^{-10} - 10^{-6}$ M) for 24 h. Then the media were collected and assayed for 6-keto prostaglandin $F_{1\alpha}$. Results are means ± S.E.M. of $n = 12$. $*\ P < 0.05$ compared with SFM control; $^{@}P < 0.05$ vs. interleukin-1α.

To corroborate the results obtained with α helical CRF-(9–41), another CRF receptor antagonist, namely [D-Phe^{12}] CRF-(12–41) was tested. As shown in Fig. 5, [D-Phe^{12}] CRF-(12–41) itself, at all concentrations used, did not significantly affect the basal levels of prostacyclin. In interleukin-1α-stimulated cells, the effects of [D-Phe^{12}]-CRF-(12–41) were similar to those obtained with α-helical CRF-(9–41). At lower concentrations ($10^{-10} - 10^{-8}$ M), in the absence of CRF, [D-Phe^{12}]-CRF-(12–41) inhibited the effect of interleukin-1α, and this inhibition was lost at higher concentrations ($10^{-7} - 10^{-6}$ M) of the receptor antagonist (Fig. 6).

Fig. 7 shows competitive displacement by unlabeled human CRF of [^{125}I]human CRF. Nonlinear curve fitting analysis yielded a significantly better fit for the two sites model than for single site model ($F = 7.00$, $P = 0.049$). Binding parameters were $K_{d1} = 0.2 \pm 0.02$ nM and B_{max1} 0.79 ± 0.095 fmol/mg protein, and $K_{d2} = 1.77 \pm 0.14$ μM and B_{max2} 0.97 ± 0.12 fmol/mg protein.

4. Discussion

The present study confirms the modulatory effect of CRF on interleukin-1α-induced prostaglandin synthesis in endothelium as we reported previously (Fleisher-Berkovich and Danon, 1995). We also identified in bovine aortic endothelial cells specific CRF binding sites, which proba-

bly mediate CRF's modulation of the interleukin-1α-induced prostacyclin production. Moreover, the action of CRF was suppressed by the CRF receptor antagonist α helical CRF-(9–41). The present findings, along with data indicating the presence of high concentrations of CRF in inflamed endothelium (Karalis et al., 1991), strongly suggest a significant role for CRF in the regulation of endothelial inflammatory response. Moreover, this regulatory pathway may not be confined to the endothelium, as similar results were also observed in fibroblasts (Fleisher-Berkovich et al., 1997).

It has been shown that CRF exhibits specific binding to rabbit aorta and that binding was diminished after removal of the endothelium (Dashwood et al., 1987). However, to the best of our knowledge, this is the first report showing direct binding of CRF to endothelial cells. It is of interest that the K_d for the higher-affinity class of endothelial receptors in the present study (0.2 ± 0.02 nM) is very similar to values reported for CRF receptors in the pituitary (Hauger and Aguilera, 1993; Perrin et al., 1986), spleen (Dave et al., 1985) and testis (Dufau et al., 1993), where a single high-affinity binding site ($K_d = 0.2$ nM) was reported. Likewise, the K_d value for the lower affinity binding sites revealed in this study (1.77 ± 0.14 μM) is of the same order of magnitude as reported for adrenal cortical (1 μM) and chromaffin cells (0.25 μM) (Dave et al., 1985).

Cloning of CRF receptors has been recently accomplished, and two classes with two subtypes each were identified, namely $CRFR_{1\alpha}$, $CRFR_{1\beta}$, $CRFR_{2\alpha}$ and $CRFR_{2\beta}$. $CRFR_{1\beta}$ is identical to the $CRF_{1\alpha}$ receptor, except it contains a 29-amino acid insert in its first cytoplasmic loop (Chang et al., 1993; Chen et al., 1993). mRNA for the CRF_1 receptor was identified in the pituitary, cerebellum and cerebral cortex. $CRFR_1$ binds CRF with high affinity ($K_d = 0.2$ nM). Among the CRF_2 class of receptors $CRFR_{2\alpha}$, which consists of 411 amino acids, was identified primarily in the CNS, and appears to be the predominant $CRFR_2$ isoform expressed in neuronal tissue (Lovenberg et al., 1995b). The second subtype ($CRFR_{2\beta}$), comprising 431 amino acids, binds CRF with high affinity ($K_d = 0.57$ nM) and is distributed predominantly in peripheral tissues, with the highest abundance reported in the heart and skeletal muscle (Kishimoto et al., 1995; Perrin et al., 1995) and also in the lung, epididymis, kidney, intestine and mesenteric small arteries (Lovenberg et al., 1995a; Rohde et al., 1996). Clearly, our results fall short of identifying the actual CRFR subtypes involved in the effects shown in the present study.

The effects of the CRF receptor antagonists in this study are complex. On one hand α helical CRF-(9–41), apparently abrogated the action of CRF, supporting the conclusion that CRF's action on interleukin-1α-induced prostaglandin synthesis is indeed mediated by CRF receptor activation. On the other hand, in the absence of CRF, both antagonists at lower concentrations ($10^{-10} - 10^{-8}$ M), inhibited the effect of interleukin-1α on prostaglandin synthesis similarly to CRF, compatible with a partial agonistic action of these receptor antagonists. Surprisingly, this effect was lost at the higher concentrations of the antagonists ($3 \times 10^{-8} - 10^{-6}$ M), possibly reflecting interaction with another type of CRF receptors. A similar phenomenon was also observed in experiments that were carried out with human dermal fibroblasts, where the same two antagonists exerted agonistic or antagonistic effects at different concentrations (Fleisher-Berkovich et al., 1997).

These data are hard to reconcile with simple receptor antagonism. However, critical review of the literature provides ample evidence for complex actions of the available CRF receptor antagonists. Thus, α helical CRF-(9–41) inhibits CRF-induced ACTH release both in vitro and in vivo, but can also increase ACTH release similarly to CRF (Rivier et al., 1984). Also, Rainnie et al. (1992) showed that α helical CRF-(9–41) concentration-dependently blocked CRF action, but when administered alone it acted similarly to CRF, suggesting partial agonistic activity. In the same study, unexpectedly, the partial agonistic activity of α helical CRF-(9–41) was abolished at a higher concentration. The latter findings are compatible with our present results, in which α helical CRF-(9–41) concentration-dependently blocked CRF action, but in the absence of CRF, low antagonist concentrations exhibited partial agonistic action, which was reversed at higher antagonist concentrations.

The complex pharmacological effects of CRF receptor antagonists shown in the present study go beyond the conclusion of a partial agonistic action. Historically, data of a similar nature had been obtained with several opiate antagonists, where the term 'agonist–antagonist' was coined (Chrubasik, 1990; Chiara and North, 1992). Thus, drugs such as pentazocine, nalorphine and cyclazocine are competitive antagonists of the μ receptor. Yet they appear to exert partial agonistic actions at other opioid receptors, including the δ and κ receptors. We hypothesize that the complex effects of CRF receptor antagonists which were displayed in the present study might be related to the involvement of multiple CRF receptors possessing differential affinities for each of the receptor antagonists. In fact, Kishimoto et al. (1995) reported that α helical CRF-(9–41) is a much more effective receptor antagonist at the $CRFR_2$ than at $CRFR_1$. Our binding results confirm the presence of at least two different subtypes of CRF receptors in endothelial cells. Thus, depending upon the nature of the receptors that are occupied, the compounds under discussion may reveal either agonistic or antagonistic characteristics. Moreover, interaction with different receptors may result in different signal transduction pathways and differential cross talking. Thus, CRF has been reported to stimulate the adenylyl cyclase–cAMP system in the pituitary, adrenal (Dave et al., 1985), lung (Dieterich et al., 1994) and heart (Perrin et al., 1995). On the other hand, in the testis CRF was reported to stimulate protein kinase C

rather than cAMP dependent protein kinase A (Dufau et al., 1993).

The interaction of mediators of inflammation, such as the cytokines, with the classical neuroendocrine control systems is an exciting concept that could explain many physiological and clinical observations. The precise position of immune CRF in the inflammatory cascade cannot yet be discerned, especially in view of conflicting evidence produced by several groups. Thus, Karalis et al. (1991) has suggested that CRF exerts proinflammatory actions. On the other hand, CRF exhibited anti-inflammatory effects in other models (Wei and Thomas, 1993). Based on our results, one might envisage apparently opposing actions of CRF emanating from activation of different CRF receptor types, depending on the concentration of the peptide.

Acknowledgements

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References

Chang, C.P., Pearse, R.I., O'Connell, S., Rosenfeld, M.G., 1993. Identification of a seven transmembrane helix receptor for corticotropin releasing factor and sauvagine in mammalian brain. Neuron 11, 1187–1195.

Chen, R., Lewis, K.A., Perrin, M.H., Vale, W., 1993. Expression cloning of a human corticotropin releasing factor receptor. Proc. Natl. Acad. Sci. USA 90, 8967–8971.

Chiara, G.D., North, R., 1992. Neurobiology of opiate abuse. Trends Pharmacol. Sci. 13, 185–193.

Chrubasik, J., 1990. Opiates and peptides. Adv. Pain Res. Ther. 13, 349–363.

Dashwood, M.R., Andrews, H.E., Wei, E.T., 1987. Binding of [^{125}I]Tyr–corticotropin releasing factor to rabbit aorta is reduced by removal of the endothelium. Eur. J. Pharmacol. 135, 111–112.

Dave, J.R., Eiden, L.E., Eskay, R.L., 1985. Corticotropin releasing factor binding to peripheral tissue and activation of the adenylate cyclase–adenosine 3′,5′-monophosphate system. Endocrinology 116, 2152–2159.

Dieterich, K.D., Grigoriadis, D.E., De Souza, E.B., 1994. Corticotropin releasing factor receptors in human small cell lung carcinoma cells: radioligand binding, second messenger, and Northern blot analysis data. Endocrinology 1351, 1551–1558.

Dufau, M.L., Tinajero, J.C., Fabbri, A., 1993. Corticotropin releasing factor: an antireproductive hormone of the testis. FASEB J. 7, 299–307.

Einhorn, S., Eldor, A., Vlodavsky, I., Panet, A., 1985. Production and characterization of interferon from endothelial cells. J. Cell. Physiol. 12, 200–204.

Fleisher-Berkovich, S., Danon, A., 1995. Effect of corticotropin releasing factor on prostaglandin synthesis in endothelial cells and fibroblasts. Endocrinology 136, 4068–4072.

Fleisher-Berkovich, S., Rimon, G., Danon, A., 1997. Modulation of prostaglandin synthesis in endothelial cells and fibroblasts by corticotropin releasing factor and antagonists. Prostaglandins Leukotrienes Essent. Fatty Acids 57, 197.

Goppelt-Struebe, M., 1997. Molecular mechanisms involved in the regulation of prostaglandin biosynthesis by glucocorticoids. Biochem. Pharmacol. 53, 1389–1395.

Hauger, R.L., Aguilera, G., 1993. Regulation of pituitary corticotropin releasing hormone receptors by CRH: interaction with vasopressin. Endocrinology 133, 1708–1713.

Jones, S.A., Challis, J.R.G., 1989. Local stimulation of prostaglandin production by corticotropin releasing hormone in human fetal membranes and placenta. Biochem. Biophys. Res. Commun. 159, 192–199.

Karalis, K., Sano, H., Redwine, J., Listwak, S., Wilder, R.L., Chrousos, G.P., 1991. Autocrine or paracrine inflammatory actions of corticotropin releasing hormone in vivo. Science 254, 421–423.

Katsuura, G., Gottschall, P.E., Dahl, R.R., Arimura, A., 1989. Interleukin-1 beta increases prostaglandin E_2 in rat astrocyte cultures: effect of neuropeptides. Endocrinology 124, 3125–3127.

Kawahito, Y., Sano, H., Kawata, M., Yuri, K., Mukai, S., Yamammura, Y., Kato, H., Chrousos, G.P., 1994. Local secretion of corticotropin releasing hormone by enterochromaffin cells in human colon. Gastroenterology 106, 859–863.

Kishimoto, T., Pearse, R.V., Lin, C.R., Rosenfeld, M.G., 1995. A sauvagine/corticotropin releasing factor receptor expressed in heart and skeletal muscle. Proc. Natl. Acad. Sci. USA 92, 1108–1112.

Lovenberg, T.W., Chalmers, D.T., Liu, C., De Souza, E.B., 1995a. $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136, 4139–4142.

Lovenberg, T.W., Liaw, C.W., Grigoriadis, D.E., Clevenger, W., Chalmers, D.T., De Souza, E.B., Oltersdorf, T., 1995b. Cloning and characterization of a functionally distinct corticotropin releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. USA 92, 836–840.

Perrin, M.H., Haas, Y., Rivier, J.E., Vale, W., 1986. Corticotropin releasing factor binding to the anterior pituitary receptor is modulated by divalent cations and guanyl nucleotides. Endocrinology 118, 1171–1179.

Perrin, M.H., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., Sawchenko, P., Vale, W., 1995. Identification of a second CRF receptor gene and characterization of a cDNA expressed in heart. Proc. Natl. Acad. Sci. USA 92, 2969–2973.

Pert, C.B., Ruff, M.R., Weber, R.J., Herkenham, M., 1985. Neuropeptides and their receptors: a psychosomatic network. J. Immunol. 135, 820–826.

Petraglia, F., Sawchenko, P.E., Rivier, J., Vale, W., 1987. Evidence for local stimulation of ACTH secretion by corticotropin releasing factor in human placenta. Nature 328, 717–719.

Rainnie, D.G., Fernhout, B.J., Shinnick-Gallagher, P., 1992. Differential actions of corticotropin releasing factor on basolateral and central amygdaloid neurones, in vitro. J. Pharmacol. Exp. Ther. 263, 846–858.

Redei, E., 1992. Immuno-reactive and bioactive corticotropin releasing factor in rat thymus. Neuroendocrinology 55, 115–118.

Rivier, J., Rivier, C., Vale, W., 1984. Synthetic competitive antagonists of corticotropin releasing factor: effect on ACTH secretion in the rat. Science 224, 889–891.

Rohde, E., Furket, J., Fechner, K., Beyermann, M., Mulvany, M.J., Richter, R., Denef, C., Bienert, M., Berger, H., 1996. Corticotropin releasing hormone (CRH) receptors in the mesenteric small arteries of rats resemble the (2)-subtype. Biochem. Pharmacol. 52, 829–833.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41 residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213, 1394–1397.

Webster, E.L., De Souza, E.B., 1983. CRF receptors in mouse spleen: identification, localization and regulation by divalent cations and guanine nucleotides. Endocrinology 122, 609–617.

Wei, E.T., Thomas, H.A., 1993. Anti-inflammatory peptide agonists. Annu. Rev. Pharmacol. Toxicol. 33, 91–108.