Corticotrophin-releasing factor as a mediator of the acute-phase response in rats, mice and rabbits

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RECEIVED 9 June 1992

ABSTRACT

The acute-phase response involves a number of separate physiological components, including induction of acute-phase protein synthesis by the liver. This response can be induced in vivo by administration of the endogenous leucocytic mediator interleukin-1β. A number of in-vivo effects of interleukin-1β have been reported to be mediated by corticotrophin-releasing factor (CRF), including activation of the hypothalamo-pituitary-adrenal axis and induction of fever, and in this report we have examined a possible involvement of CRF in mediating interleukin-1β-induced acute-phase protein synthesis.

Interleukin-1β stimulated the elevation of species-specific plasma acute-phase proteins in rats, mice and rabbits. Co-injection of interleukin-1β with the specific CRF receptor antagonist α-helical-CRF$_{9,41}$NH$_2$ abolished or attenuated acute-phase protein synthesis induced by interleukin-1β in all three species for up to 12 h after injection. The inhibitory effect of α-helical-CRF$_{9,41}$NH$_2$ was reduced or absent 24 h after injection. Neutralizing anti-CRF antisera had no effect on acute-phase protein synthesis in the mouse and, paradoxically, potentiated acute-phase protein synthesis induced by interleukin-1β in the rat. These results indicate a possible mediatory role for CRF in regulation of acute-phase protein synthesis, and suggest that CRF may mediate induction of acute-phase protein synthesis by a different mechanism from that involved in regulation of corticotrophin secretion.


INTRODUCTION

The acute-phase response is a generalized defence reaction to injury, infection or inflammation, and involves a number of physiological responses including fever, alterations in plasma ion levels, activation of the hypothalamo-pituitary-adrenal axis and induction of hepatic acute-phase protein synthesis (Kushner, 1988). The monocyte/macrophage-derived inflammatory cytokine interleukin-1 is now recognized as an important endogenous mediator of many or all aspects of the acute-phase response (Dinarello, 1984), acting either directly, or indirectly through induction of another inflammatory monocyte-derived cytokine, interleukin-6 (Heinrich et al. 1990).

It is now well-established that a complex, bi-directional system of interactions exists between these inflammatory mediators of the acute-phase/immune responses and the hypothalamo-pituitary-adrenal axis (Blalock, 1989). These interactions include: the immunosuppressive actions of glucocorticoids (Snyder & Unanue, 1982; Lee et al. 1988); immunomodulatory actions of corticotrophin-releasing factor (CRF) (Irwin et al. 1990; Jain et al. 1991; Hagan et al. 1992); and stimulation of corticotrophin secretion by both interleukin-1 and interleukin-6, mediated by an increase in hypothalamic CRF (Sapolsky et al. 1987; Naitoh et al. 1988). It has also been reported that central CRF mediates interleukin-1-induced fever in the rat, since the febrile response is blocked by intracerebroventricular injections of either neutralizing CRF antibodies or a specific CRF receptor antagonist, α-helical CRF$_{9,41}$NH$_2$ (αh-CRF$_{9,41}$NH$_2$; Rothwell, 1989). In view of these studies, we have examined the effects of CRF antagonists on another major cytokine-mediated aspect of the acute-phase response, the induction of hepatic acute-phase protein synthesis.

Acute-phase proteins are circulating plasma proteins produced in the liver which undergo changes in concentration during the acute-phase response (Kushner, 1988) and are classed as negative or
positive, showing decreases or increases in concentration respectively. The major positive acute-phase proteins are normally either entirely absent, or present at very low concentrations in plasma and show up to several hundred-fold increases in concentration during the acute-phase response. These proteins vary between species. In the rat, the major acute-phase proteins are α-1-acid glycoprotein and α-2-macroglobulin, in the mouse it is serum amyloid-P and in the rabbit (and in man) it is C-reactive protein. Acute-phase proteins are also classed as primary, increasing early in the acute-phase response, or secondary, increasing later. In the present study we report the effects of interleukin-1 on the major acute-phase proteins in rats (α1-acid glycoprotein and α2-macroglobulin), mice (serum amyloid-P) and rabbits (C-reactive protein), and the inhibitory effects in all three species of the specific CRF receptor antagonist αh-CRF$_{9-41}$NH$_2$ on acute-phase protein synthesis induced by interleukin-1, suggesting a role for CRF in mediating this response. We also describe potentiating effects in the rat of a neutralizing CRF antiserum, suggesting that CRF may mediate the acute-phase by a mechanism distinct from its regulation of corticotrophin secretion.

MATERIALS AND METHODS

Materials

Human recombinant interleukin-1β was a kind gift of Dr A. Shaw, Glaxo IMB, Geneva, Switzerland. Endotoxin was derived from E. coli, and was the World Health Organization preparation 84/650. α-Helical-CRF$_{9-41}$NH$_2$ was synthesized using 9-fluorenylemethoxycarbonyl chemistry on a Biolyx peptide synthesizer (Novabiochem, UK Instrument Division, Cambridge, Cambs, U.K.). Amino acids were coupled as the pentafluorophenyl active esters, with the exception of threonine and serine, which were coupled as the 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine active esters, and arginine, which was coupled using benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate.

Following synthesis the peptide was cleaved from the resin and deprotected using 95% trifluoroacetic acid (TFA)/5% ethane dithiol (2 h), and purified to >95% homogeneity by reverse-phase chromatography on ODS-RSII (Bio-Rad, Hemel Hempstead, Herts, U.K.), using a linear gradient of 36-48% acetonitrile in 0-1% TFA. Identity was confirmed by analytical high-performance liquid chromatography, using commercially available αh-CRF$_{9-41}$NH$_2$ as a standard.

Antisera to CRF were prepared by immunizing rabbits with CRF conjugated to ovalbumin, using either carbodiimide or glutaraldehyde as a cross-linking reagent. Antisera to each conjugate were raised in separate animals by primary immunization at multiple intradermal sites in Freund’s complete adjuvant (50 μg CRF/animal), and boosting at 12, 16 and 20 weeks by intramuscular injection in Freund’s incomplete adjuvant (30 μg CRF/animal). Each antiserum had a titre (20–30% binding of $^{125I}$O-Tyr-CRF) of 1/48 000. A mixture of the two antisera was immunoneutralizing in the following experiment: rats, treated with interleukin-1β (5 μg/kg, i.p.) showed an increase in plasma corticotrophin from a mean basal level of 15 pg/ml to 175 pg/ml 90 min after injection. Pretreatment of the animals 18 h and 1 h before the experiment with 0-25 ml/animal of each of the above antisera inhibited the interleukin-1β-induced increase in plasma corticotrophin (mean levels 25 pg/ml) at 90 min. For immunoneutralization experiments in the present studies, animals were treated with a mixture of 0-25 ml of each antiserum as described in the figure legends.

Interleukin-1β, αh-CRF$_{9-41}$NH$_2$, anti-CRF, and all buffers and diluents for these reagents were prepared and maintained in pyrogen-free (<0-125 endotoxin units/ml) conditions, and regularly monitored using the limulus amoebocyte lysate test (European Pharmacopoeia, 1987).

In-vivo experiments with αh-CRF$_{9-41}$NH$_2$

Interleukin-1β was dissolved in sterile, pyrogen-free 0-9% (w/v) NaCl (sterile saline). αh-CRF$_{9-41}$NH$_2$ was dissolved in sterile saline containing NaOH (0-001 mol/l). Concentrations are given in the figure legends.

Male Sprague-Dawley rats (200 g) were injected i.p. with 0-5 ml of either interleukin-1β in solution or vehicle and immediately after, where appropriate, with 0-5 ml of either αh-CRF$_{9-41}$NH$_2$ in solution or vehicle. At the times specified in the figures, animals were decapitated and trunk blood was collected into citrate. Plasma was prepared within 20 min of collection, and stored at −40 °C until assayed for acute-phase proteins.

Male C57/BL6 mice (20–30 g) were injected i.p. with 0-2 ml of either interleukin-1β in solution or vehicle and immediately after, where appropriate, with 0-2 ml of either αh-CRF$_{9-41}$NH$_2$ in solution or vehicle. At the times specified in the figures, blood samples were withdrawn from the retro-orbital sinus into heparinized capillaries. Plasma was treated in the same way as the rat samples.

Female Dutch × Lop cross-bred rabbits (1.5 kg) were injected i.v. with 1-0 ml of either interleukin-1β in solution or vehicle and immediately after, where appropriate, with 1-0 ml of either αh-CRF$_{9-41}$NH$_2$ in solution or vehicle. At the times specified in the
figures, blood samples were withdrawn from the marginal ear vein into citrate, and treated in the same way as the rat samples.

**In-vivo experiments with anti-CRF antisera**

Male Sprague-Dawley rats (200 g) were injected with anti-CRF or normal rabbit serum (0.5 ml/animal, i.p.). The animals were injected 18 h later with either anti-CRF or normal rabbit serum (0.5 ml/animal, i.p.) and, after a further 1 h, with interleukin-1β or vehicle (0.5 ml/animal, i.p.). Blood samples were collected at the times specified in the figures, as described above. Male C57/BL6 mice (20–30 g) were treated in the same way, except that the injection volume for each treatment was 0.2 ml (i.p.). Blood samples were collected as described above.

**Acute-phase protein assays**

Rat α1-acid glycoprotein was measured by radioimmunoassay. Rabbits were immunized with α1-acid glycoprotein (aαgy) and a2-macroglobulin (a2M) levels were determined by immunoassay as described. Data points are means ± s.e.m. (n = 5).

**In-vivo experiments with anti-CRF antisera**

Male Sprague-Dawley rats (200 g) were injected with anti-CRF or normal rabbit serum (0.5 ml/animal, i.p.). The animals were injected 18 h later with either anti-CRF or normal rabbit serum (0.5 ml/animal, i.p.) and, after a further 1 h, with interleukin-1β or vehicle (0.5 ml/animal, i.p.). Blood samples were collected at the times specified in the figures, as described above. Male C57/BL6 mice (20–30 g) were treated in the same way, except that the injection volume for each treatment was 0.2 ml (i.p.). Blood samples were collected as described above.
glycoprotein obtained from Sigma Chemical Co. (Poole, Dorset, U.K.; 50 µg/animal in Freund’s complete adjuvant, multiple intradermal sites), boosted after 12, 16 and 20 weeks (30 µg in Freund’s incomplete adjuvant, i.m.), and serum was collected 2 weeks after the last boost. α1-Acid glycoprotein was radioiodinated using a chloramine T procedure (2 µg protein/10 µl sodium phosphate (0·1 mol/l) pH 7·0, 7·4 MBq Na125I (2 µl), 0·6 mg chloramine T/ml (10 µl), sodium phosphate (0·4 mol/l) pH 7·4 (10 µl), for 30 s), the reaction being terminated with sodium metabisulphite (0·6 mg/ml, 25 µl), and the tracer purified by chromatography on Sephadex G-75 in phosphate-buffered saline containing 0·5% bovine serum albumin. The radioimmunoassay was performed in plastic tubes (LPS; Luckhams Ltd, Burgess Hill, Sussex, U.K.) in phosphate (0·1 mol/l)-buffered saline containing 0·5% bovine serum albumin. Radioimmunoassay tubes contained: 100 µl/tube test (rat plasma diluted 1/10^5) or standard; 100 µl tracer/tube (10 000 c.p.m.), and 100 µl/tube rabbit anti-α1-acid glycoprotein (diluted 1/15 000). Assay tubes were incubated overnight at 4°C, and bound tracer separated using cellulose-linked second antibody (Sac-Cel, IDS Ltd, Boldon, Tyne and Wear, U.K.) according to the manufacturer’s instructions. The assay had a working range of 1–100 ng/ml, and within- and between-assay coefficients of variation of 6·5 and 10·6% respectively.

Rat α2-macroglobulin was measured by radioimmunoassay. Rabbit anti-rat α2-macroglobulin was a generous gift of Professor P. C. Heinrich, Institut für Bichemie der RWTH, Aachen, F.R.G. For the preparation of tracer and assay standards, α2-macroglobulin was purified from acute-phase rat plasma, by affinity chromatography on Sepharose-blue and gel-filtration, essentially as described by Andus et al. (1983). The purified protein was >90% pure by sodium dodecyl sulphate (SDS)-gel electrophoresis, co-migrated with commercially available human α2-macroglobulin and showed complete cross-reactivity with the antiserum provided by Professor Heinrich. Purified α2-macroglobulin was radioiodinated using the procedure described for α1-acid glycoprotein except that the tracer was purified on Sepharose-C1 4B. Radioimmunoassay tubes contained: 100 µl/tube test (rat plasma diluted 1/100) or standard; 100 µl/tube tracer (10 000 c.p.m./tube) and 100 µl/tube rabbit-anti-rat α2-macroglobulin (diluted 1/32 000). The radioimmunoassay procedure was that described for α1-acid glycoprotein. The working range was 10–500 ng/ml, and within- and between-assay coefficients of variation were 7·97 and 11·06% respectively.

Rabbit C-reactive protein was measured by radioimmunoassay. C-reactive protein was purified from acute-phase rabbit plasma by affinity chromatography on immobilized phosphoryl choline. Briefly, 25 mg amino-phenyl phosphoryl choline (Sigma) was coupled to 10 ml Affi-gel-10 (Bio-Rad) in 10 ml methanol, and equilibrated in Tris–HCl (0·02 mol/l)/NaCl (0·15 mol/l)/CaCl2 (0·002 mol/l), pH 7·4. Acute-phase plasma was obtained from rabbits 24 h after injection with turpentine (2 ml/animal, i.m.), and CaCl2 was added to a concentration of 0·002 mol/l. Plasma (10 ml) was incubated with the affinity matrix for 30 min, washed exhaustively with the equilibration buffer, and bound proteins eluted with equilibration buffer containing phosphoryl choline (0·01 mol/l, Sigma). The purified protein had an apparent molecular weight of 26 000–28 000 on SDS-gel electrophoresis, and showed complete cross-reactivity with a goat anti-rabbit C-reactive protein antiserum kindly provided by Dr D. T. Liu, Food and Drugs Agency, Bethesda, MD, U.S.A. Anti-rabbit C-reactive protein antiserum was raised in guinea-pigs. Briefly, guinea-pigs were immunized by primary intramuscular injection of C-reactive protein (50 µg/animal, Freund’s complete adjuvant), with boosts (50 µg, Freund’s incomplete adjuvant, i.m.) being given at 8 and 12 weeks. Serum was collected at 14 weeks. Radioiodinated C-reactive protein was prepared by the procedure described for rat α1-acid glycoprotein, using 7·4 MBq Na125I/3 µg protein, and purifying the tracer on Ultragel ACA-54 (IBF-biotechnics, Villeneuve-la-Garenne, France). Radioimmunoassay tubes contained: 100 µl/tube test (rabbit plasma diluted 1/1000) or standard; 100 µl/tube tracer (10 000 c.p.m.), and 100 µl/tube guinea-pig anti-rabbit C-reactive protein, diluted 1/20 000. The radioimmunoassay procedure was as described for rat α1-acid glycoprotein. The working range of the assay was 20–1000 ng/ml, and within- and between-assay coefficients of variation were 4·05 and 9·44% respectively.

Mouse serum amyloid-P was measured using the microtitre plate enzyme-linked immunosorbent assay described by Taktak & Stenning (1992), using sheep anti-serum amyloid-P as the capture antibody, and rabbit anti-serum amyloid-P followed by peroxidase-labelled anti-rabbit-IgG as the detection system.

Statistical treatment of data was performed by unpaired Student’s t-test, making the comparisons described in the figure legends.

RESULTS

Stimulation of acute-phase protein synthesis by interleukin-1β in rats, mice and rabbits is shown in Figs 1–3. In rats, interleukin-1β (5 and 25 µg/kg) stimulated a dose-dependent increase in plasma levels of the
primary acute-phase protein, \( \alpha_1 \)-acid glycoprotein with significant elevation of plasma levels 6 h after injection and maximum values being seen at 24 h (Fig. 1a). The maximum stimulation seen in these studies was approximately 15-fold (25 \( \mu \)g interleukin-1\( \beta \)/kg, 24 h). The secondary acute-phase protein \( \alpha_2 \)-macroglobulin (Fig. 1b) showed a similar dose-dependent increase in response to interleukin-1\( \beta \), with a maximum elevation of approximately 20-fold over basal. The response was later than that for \( \alpha_1 \)-acid glycoprotein, with peak values being seen 48 h after injection, although a significant increase in levels was seen as early as 6 h. In mice (Fig. 2), the increase in plasma serum amyloid-P reached peak values (tenfold-stimulation over basal) 24 h after injection of interleukin-1\( \beta \) (2.5 and 10 \( \mu \)g/kg), although the magnitude of the response appeared to be approaching maximum at the 1 \( \mu \)g/kg dose. As in the rat, significant elevation over basal was seen at the earliest time-point studied (6 h).

It is also notable that in the mouse there was an increase in basal levels during the course of the experiment; this may have been stress-related. In the rabbit (Fig. 3), maximum plasma C-reactive protein levels in response to interleukin-1\( \beta \) (1 and 5 \( \mu \)g/kg) were seen at 24 h. The magnitude of the response was again approximately tenfold over basal, and a significant increase was seen after 6 h at the higher dose of interleukin-1\( \beta \). There was again an increase in basal levels throughout the experiment; this may also have been stress-related since in these experiment the same rabbits were bled repeatedly.

**Figure 3.** Interleukin-1\( \beta \)-induced C-reactive protein in rabbits. Rabbits were injected with interleukin-1\( \beta \) (1 \( \mu \)g/kg, ■; 5 \( \mu \)g/kg, ▲) or saline (●), plasma samples obtained and plasma C-reactive protein determined by immunoassay as described in Materials and Methods. Data points are means ± S.E.M. (n = 4).

**Figure 4.** \( \alpha \)-Helical-corticotrophin releasing factor \( \alpha_4 \)-NH\(_2\) (\( \alpha \)-CRF\(_4 \)-NH\(_2\))-inhibition of acute-phase proteins: \( \alpha_1 \)-acid glycoprotein in rats. Rats were injected with vehicle (A), \( \alpha \)-CRF\(_4 \)-NH\(_2\) (750 \( \mu \)g/kg; B), interleukin-1\( \beta \) (5 \( \mu \)g/kg; C), or interleukin-1\( \beta \) plus \( \alpha \)-CRF\(_4 \)-NH\(_2\) (D), as described in Materials and Methods. Plasma samples were obtained after (a) 4 h, (b) 12 h and (c) 24 h, and \( \alpha_1 \)-acid glycoprotein was determined by immunoassay. Data points are means ± S.E.M. (n = 5). *P<0.05, **P<0.01, ***P<0.001 compared with saline-injected animals; †P<0.05, ‡P<0.01 compared with animals injected with interleukin-1\( \beta \) (Student's \( t \)-test).
The effects of co-injection with the CRF receptor antagonist αh-CRF(9-41)NH₂ on acute-phase protein synthesis induced by interleukin-1β in the three species studied are shown in Figs 4–7. In rats, interleukin-1β (5 μg/kg) stimulated an increase in plasma α1-acid glycoprotein levels which showed a progressive increase at 4, 12 and 24 h after injection (Fig. 4). Co-injection with αh-CRF(9-41)NH₂ (750 μg/kg) almost completely inhibited the production of α1-acid glycoprotein production induced by interleukin-1β (5 μg/kg) at 4 and 12 h after injection. At 24 h after injection, plasma α1-acid glycoprotein levels in animals treated with interleukin-1β plus αh-CRF(9-41)NH₂ were not different from those of animals injected with interleukin-1β alone. The CRF antagonist had no significant effect on the basal level of α1-acid glycoprotein, although there was a progressive increase in the basal α1-acid glycoprotein level throughout the course of the experiment. Similar results were obtained for the secondary acute-phase protein, α2-macroglobulin in rats (Fig. 5). Interleukin-1β (5 μg/kg) caused a progressive increase in plasma α2-macroglobulin levels, which was significantly and almost completely attenuated by the antagonist at 12 h after injection. The same trend was apparent at 4 h, although the results did not reach statistical significance. At 24 h, levels of α2-macroglobulin in animals treated with interleukin-1β plus αh-CRF(9-41)NH₂ were slightly lower than those in animals treated with interleukin-1β alone, although once again the degree of inhibition was much less than at earlier times. The CRF antagonist had no significant effect on basal levels of α2-macroglobulin.

In mice, the production of plasma serum amyloid-P stimulated by interleukin-1β (2.5 μg/kg) at 12 h was significantly, although not completely, inhibited by co-injection with αh-CRF(9-41)NH₂ (Fig. 6). The same trend was apparent at 4 h, although the results did not reach significance because of the low level of acute-phase protein synthesis induced by interleukin-1β in the mouse at this early time. Once again, the inhibitory effect of αh-CRF(9-41)NH₂ was no longer significant at 24 h. The CRF antagonist also completely inhibited production of C-reactive protein induced by interleukin-1β (1 μg/kg) at both 4 and 12 h after injection in the rabbit, and also in this species at 24 h (Fig. 7). It is notable, however, that the basal level of acute-phase protein synthesis was significantly elevated at 24 h in the rabbit, which was unaffected by co-injection with αh-CRF(9-41)NH₂.
The effect of passive immunoneutralization with anti-CRF on acute-phase protein synthesis induced by interleukin-1β in the rat is shown in Fig. 8. Basal levels of α1-acid glycoprotein in animals injected with either normal rabbit serum or with anti-CRF were approximately threefold higher than those normally seen (e.g., Fig. 1), and continued to rise throughout the experiment, probably indicating some stimulation of the acute-phase response by the injection of a large quantity of foreign protein. Significant further stimulation of plasma α1-acid glycoprotein levels by interleukin-1β was seen only at 24 h; however, at this time-point, rather than inhibiting the response, the neutralizing anti-CRF antiserum caused a significant potentiation of interleukin-1β-induced plasma α1-acid glycoprotein levels (Fig. 8a). Plasma α2-macroglobulin levels were not significantly increased by preinjection with heterologous serum (Fig. 8b) and significant stimulation by interleukin-1β was seen at both 12 and 24 h. Once again, however, the response to interleukin-1β was potentiated by the specific anti-CRF serum; slightly but significantly at 12 h and markedly at 24 h. The same antiserum treatment had no effect on acute-phase protein synthesis induced by interleukin-1β in mice (results not shown), and was not tested in rabbits.

DISCUSSION

The primary mechanism regulating acute-phase protein synthesis is generally thought to be the peripheral action of the inflammatory cytokine interleukin-6 on the liver, regulating both acute-phase protein synthesis and acute-phase protein gene expression (Heinrich et al. 1990). Interleukin-1 has only limited effects in vitro on hepatic acute-phase protein synthesis and its stimulatory effect in vivo is usually attributed to interleukin-6 production induced by interleukin-1 from circulating cells of the monocyte-macrophage lineage (Heinrich et al. 1990). It does seem, however, that the central nervous system may play some role in regulating acute-phase protein synthesis. It is known, for instance, that direct injections into the brain of inflammatory cytokines is a potent stimulus for acute-phase protein production (Blatteis et al. 1984).

The studies we report indicate that blocking the action of endogeneous CRF with a single injection of the specific CRF receptor antagonist αh-CRF9-41NH2 partially or completely inhibited acute-phase protein synthesis induced by interleukin-1β in the three species of animal studied, for at least 12 h. The mechanism of this inhibition is unclear. In-vivo administration of αh-CRF9-41NH2 has been used
widely to study the involvement of CRF in physiological responses other than the hypothalamic-pituitary-adrenal axis, for instance in regulating gastric acid secretion (Stephens et al. 1988), growth hormone secretion (Rivier & Vale, 1985) and behavioural effects (Krahn et al. 1986). Although few data are available on the pharmacokinetic properties of αh-CRF₉₄₄-NH₂, its half-life is considerably shorter than the effect at 12 h seen in these studies, since the inhibitory action of the antagonist on adrenocorticotropic hormone (ACTH) secretion lasts for only 2 h (Rivier et al. 1984). Interleukin-1β, however, also has a short (3 min) half-life in rats (Poole et al. 1990), despite having effects on acute-phase protein synthesis which may last for more than 48 h. Interleukin-1β probably has a rapid, transient action which results in a persistent stimulation of acute-phase protein synthesis, and it is this action which is inhibited by αh-CRF₉₄₄-NH₂. In all three species, the inhibitory effect of αh-CRF₉₄₄-NH₂ on acute-phase protein synthesis was diminished or absent at 24 h, suggesting that the event triggered by interleukin-1β persists longer than the inhibitory action of the CRF antagonist, and that the site of action of the antagonist is at a step subsequent to the early action of interleukin-1.

The antagonistic activity of αh-CRF₉₄₄-NH₂ has been shown to vary widely in different in-vivo systems (Fisher et al. 1991), suggesting that different CRF receptor subtypes may exist, or that extrahypothalamic, or 'tissue' CRF may be inhibited by in-vivo administration of the antagonist. In our studies the dose of CRF antagonist employed was equivalent to that which inhibited ACTH release in vivo (Rivier et al. 1984). Passive immunoneutralization of CRF activity, using the antisera and treatment regime described in these studies, completely inhibits the in-vivo corticotrophin-releasing activity of interleukin-1β in the rat as described in Materials and Methods, an action which is mediated by hypothalamic CRF acting on the pituitary (Sapolsky et al. 1987). The same antisera and treatment regime actually potentiated acute-phase protein synthesis in the rat. It seems possible therefore that the mechanism whereby endogenous CRF mediates increased synthesis of acute-phase proteins induced by interleukin-1β may be distinct from the mechanism whereby CRF mediates the in-vivo corticotrophin-releasing activity of interleukin-1β. It is not possible to say whether such a mechanism is within the central nervous system, where antibodies might have reduced activity due

![Figure 7](https://example.com/figure7.png)

**Figure 7.** α-Helical-corticotrophin releasing factor₉₄₄-NH₂ (αh-CRF₉₄₄-NH₂) inhibition of acute-phase proteins: C-reactive protein in rabbits. Rabbits were injected with vehicle (A), αh-CRF₉₄₄-NH₂ (750 μg/kg, B), interleukin-1β (1 μg/kg, C), or interleukin-1β plus αh-CRF₉₄₄-NH₂ (D), as described in Materials and Methods. Plasma samples were obtained after (a) 4 h, (b) 12 h and (c) 24 h, and serum amyloid-P was determined by immunoassay. Data points are means ± S.E.M. (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline-injected animals; ††P < 0.01, †††P < 0.001 compared with animals injected with interleukin-1β (Student's t-test).
to limited access or reduced ‘on-time’, or in the periphery, where the apparent potentiating action of anti-CRF in the rat might be due to increased half-life. It should also be noted that preinjection of the animals with the heterologous immunoglobulin does itself stimulate acute-phase protein synthesis. Under the conditions used, any mechanism involving CRF would already have occurred, limiting the possibilities for any inhibitory actions of the antisera. Results of the passive immunoneutralization experiments should therefore be interpreted with caution. It is interesting to note, however, that an entirely extrahypothalamic αh-CRF9-41, NH2-sensitive CRF/ACTH system, which responds to interleukin-1, has recently been described in the adrenal medulla (Andreas et al. 1991). The lack of effect of CRF immunoneutralization in the mouse may also be explained by a failure of the anti-rat/human CRF to cross-react appropriately with the mouse CRF molecule.

These studies suggest that the elevation of acute-phase protein synthesis in response to the specific stimulus interleukin-1β is mediated in some way by CRF. It is not clear whether acute-phase protein synthesis is mediated by CRF under all circumstances. In these studies, increases in the level of acute-phase protein synthesis induced by interleukin-1β were 10 to 20-fold. Responses to potent in-vivo inflammatory stimuli such as turpentine or endotoxin are many fold higher (results not shown), and involve the complete network of inflammatory mediators, and the effect of αh-CRF9-41NH2 has not been studied under these circumstances. It is also notable that the CRF antagonist had no effect on the basal secretion of acute-phase proteins in any of the species studied. The results do, however, extend the number of interactions that have been reported between mediators of the immune system and the hypothalamo-pituitary-adrenal axis.

ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr A. Shaw for the gift of interleukin-1β, and to Professor P. C. Heinrich and Dr D. T. Liu for gifts of anti-acute-phase protein antisera.

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