Measurement of circulating corticotrophin-releasing factor in man

D. Cunnah*, D. S. Jessop, G. M. Besser* and L. H. Rees

Departments of Chemical Endocrinology and *Endocrinology, St Bartholomew’s Centre for Clinical
St Bartholomew’s Hospital, LONDON EC1A 7BE

(Requests for offprints should be addressed to D. S. Jessop)

RECEIVED 18 July 1986

ABSTRACT

A radioimmunoassay was developed to measure corticotrophin-releasing factor (CRF-41) extracted from human plasma using Vycor glass. Assay sensitivity was 20 ng/l and intra- and interassay coefficients of variation were 10-2 and 11.4% respectively. The normal range of plasma CRF-41 was <20–110ng/l (n = 46). Plasma concentrations of CRF-41 in patients with Cushing’s disease, Nelson’s syndrome and Addison’s disease were within the normal range. No correlation was found between CRF-41 and ACTH in these syndromes. Two patients with the ectopic ACTH syndrome had increased plasma concentrations of CRF-41. In normal subjects no changes in plasma CRF-41 occurred after insulin-induced hypoglycaemia, treatment with dexamethasone or feeding, and changes in the concentrations of CRF-41 did not reflect circadian changes in plasma concentrations of cortisol. Concentrations of immunoreactive CRF in plasma of women in the third trimester of pregnancy were increased (550–9300 ng/l) and gel filtration chromatography showed that this comprised CRF-41 and a higher molecular weight form. Reversed-phase high-performance liquid chromatography also revealed multiple peaks of immunoreactive CRF in extracts of plasma and placenta.


INTRODUCTION

Corticotrophin-releasing factor (CRF-41) is a 41 amino acid peptide which was first purified from ovine hypothalami (Vale, Spiess, Rivier & Rivier, 1981) and specifically stimulates the release of adrenocorticotrophin (ACTH) in man (Grossman, Perry, Schally et al. 1982). Screening of a human gene library with an ovine complementary DNA (cDNA) probe for CRF located the gene for the human CRF-41 precursor, from which the sequence of human CRF-41 was deduced and found to contain seven amino acid changes compared with the ovine peptide (Shibahara, Morimoto, Furutani et al. 1983). Secretion of CRF-41 into the hypophysial portal system is thought to be a major influence in the initiation and control of the stress response and circadian rhythm of the hypothalomo-pituitary-adrenal (HPA) axis. In the rat the concentration of CRF-41 in hypophysial portal blood is increased fourfold over that in peripheral plasma (Gibbs & Vale, 1982) and high concentrations of CRF-41 have been found in the human hypothalamaus (Suda, Tomori, Tozawa et al. 1984a; Ackland, Ratter, Bourne & Rees, 1986) and pituitary stalk (Coates, 1985) through which capillaries of the portal system extend to the anterior pituitary.

Corticotrophin-releasing factor has also been located in lesser quantities in extrahypothalamic tissues such as the adrenal cortex and medulla, the central nervous system, posterior pituitary, lung and liver (Suda et al. 1984a) and is widespread in the gastrointestinal tract (Nieuwenhuyzen Kruseman, Linton, Rees et al. 1982; Petrusz, Merchenthaler, Maderdrut et al. 1983). Although this peripheral CRF-41 appears to be immunologically and chromatographically similar to hypothalamic CRF-41, it is not known whether it is secreted into the circulation and whether it plays any role in the control of ACTH secretion.

Three reports measuring the concentrations of CRF-41 in human plasma have appeared (Linton & Lowry, 1985; Suda, Tomori, Yajima et al. 1985; Charlton, Leake, Ferrier et al. 1986). In one (Charlton et al. 1986) there was no evidence of a circadian rhythm or correlation between CRF-41 and cortisol after administration of dexamethasone. In contrast, Suda et al. (1985) reported an increase in concentrations of plasma CRF-41 during hypoglycaemia and
an apparent circadian rhythm in normal subjects together with increased concentrations in patients with Addison’s disease not receiving glucocorticoid therapy.

To investigate further the role and origin of circulating CRF-41, we have developed a method for the measurement of CRF-41 by radioimmunoassay (RIA) after extraction from plasma using Vycor glass. Normal volunteers were subjected to a variety of pharmacological and physiological tests of the HPA axis, including hypoglycaemia, dexamethasone suppression, and circadian and food studies, during the course of which plasma concentrations of CRF-41 were measured. The circulating concentrations of CRF-41 in patients with Cushing’s and Nelson’s syndromes and adrenal insufficiency were also assessed. Increased circulating concentrations of CRF-41 in the third trimester of pregnancy have been reported (Sasaki, Liotta, Luckey et al. 1984b; Stalla, Hartwimmer, von Werder & Muller, 1984; Linton, Campbell, Wolfe et al. 1986). Corticotrophin-releasing factor has been found in significant amounts in human placenta (Sasaki, Liotta & Krieger, 1984a) which may be the source of the circulating material. We have subjected pregnancy plasma and placental tissue to chromatographic analysis and include a comparative study of CRF immunoreactivity in this report.

MATERIALS AND METHODS

Measurement of CRF-41

Collection of plasma
Venous blood samples (10 ml) were collected into lithium heparin tubes containing 0.5 ml Trasylol (10 000 KIU; Bayer UK Ltd, Haywards Heath, Sussex). Plasma was separated immediately by centrifugation at 4 °C, flash-frozen and stored at -20 °C. Samples were taken at 09.00 h from fasted normal volunteers unless otherwise stated.

Plasma extraction
The method utilizing Vycor glass was a modification of that employed by Rees, Cook, Kendall et al. (1971) for ACTH. Plasma samples were rotated for 1 h with 150 mg Vycor glass (Corning Glass International, New York, NY, U.S.A.) activated by heating at 650 °C for 3 h. Vycor pellets were washed sequentially with H2O and HCl (1 mol/l) and rotated in 1 ml 60% (v/v) aqueous acetone for 30 min. The acetone was evaporated under N2 at 60 °C. To determine recovery, synthetic human CRF-41 (Bachem, Torrance, CA U.S.A.) was extracted from plasma previously stripped of endogenous CRF-41 by Vycor glass ('zero' plasma).

Radioimmunoassay
Residues after evaporation of acetone were reconstituted in 0.5 ml assay buffer (50 mmol phosphate/l, pH 7.4, containing 5% (v/v) Trasylol, 0.25% (w/v) human serum albumin, 0.1% (v/v) 2-mercaptoethanol, 0.1% (v/v) Triton X-100 and 0.02% (w/v) sodium azide) and serial double dilutions (200 μl) prepared for assay. Assay standard was synthetic human CRF-41. Antibodies were raised against synthetic human CRF-41 conjugated to bovine thyroglobulin with carbodiimide (Skowisky & Fisher, 1972) and injected into New Zealand White rabbits (Ackland et al. 1986). Antiserum (50 μl) was added to each assay tube (final dilution 1:48 000) and incubated for 24 h at 4 °C. Synthetic Tyr-CRF-41 (Bachem) was iodinated with 125I by the chloramine T method (Hunter & Greenwood, 1962) and purified on an octadecylsilica column using a gradient of aqueous I-propanol containing 1% (v/v) trifluoroacetic acid (TFA). Specific activity was approximately 110 μCi/μg. Tracer (50 μl) was added to each tube and incubated for a further 48 h at 4 °C. Antibody-bound tracer was separated from unbound tracer by the addition to each tube of 50 μl sheep anti-rabbit serum (final dilution 1:350) and normal rabbit serum (1:3500) followed by 0.5 ml 4% (w/v) aqueous polyethylene glycol (Edwards, 1983). After incubation for 2 h at 4 °C, tubes were centrifuged at 2000g for 1 h and pellets counted for radioactivity.

Measurements of ACTH and cortisol
Adrenocorticotropic was measured by the method of Rees et al. (1971). Cortisol was measured in unextracted serum (sample volume 50 μl). Tracer and antibody were diluted in phosphate buffer (50 mmol/l, pH 7.4, containing 0.1% Triton X-100). The assay used sheep antiserum raised to a conjugate of cortisol-3-carboxymethylxime and keyhole limpet haemocyanin (100 μl at 1:10 000 final dilution, also containing rabbit anti-sheep serum at 1:20 initial dilution) and a tracer of 125I-labelled cortisol prepared by the mixed anhydride method (100 μl; 10 000 c.p.m., containing 8-anilino-naphthalene-sulphonic acid (12 mg/ml)). After incubation for 1.5 h at room temperature, separation of the antibody-bound and free hormone was achieved by the addition of 5% (w/v) polyethylene glycol (1 ml) and centrifugation at 2600g for 30 min. The supernatant was aspirated to waste and the radioactivity in the pellet recorded.

Blood sugar was measured by the neocuproine technique.

Chromatography

Gel filtration
Plasma samples were extracted and chromatographed on a Sephadex G-75 Superfine column (100 × 1.5 cm;
Pharmacia, Uppsala, Sweden) in 1% (v/v) aqueous formic acid containing 0-1% (w/v) Polypep (Sigma, St Louis, MO, U.S.A.) at 4 °C and a flow rate of 3 ml/h. Fractions (1-2 ml) were collected, desiccated and reconstituted in assay buffer for RIA.

Reversed-phase high-performance liquid chromatography (RP-HPLC)

The RP-HPLC system (Altex, Berkeley, CA, U.S.A.) comprised two 100A pumps controlled by a 421 controller, a 210 loop injector and an Ultrapore RPSC C3 column of pore size 30 nm (0-46 cm internal diameter × 7-5 cm). Water was distilled, deionized and further purified through a Milli-Q four-cartridge system (Millipore, Waters Chromatographic Div., Harrow, Middx). All other reagents were HPLC grade. Solvent A was aqueous 0-1% (v/v) TFA and solvent B was 1-propanol (Rathburn Chemicals, Walkerburn, Borders) containing 0-1% TFA. Plasma samples were extracted and reconstituted in solvent A containing 0-1% (v/v) Triton X-100. Samples were eluted at a flow rate of 1 ml/min. Fractions (1 ml) were collected with a Gilson FC-220 collector and measured for CRF-41 by RIA.

Tissue extraction

A human placenta (obtained within 1 h of delivery) was washed in ice-cold phosphate-buffered saline and divided into pieces for freezing on dry ice and storage at −70 °C. Tissue samples were homogenized in ice-cold HCl (0-1 mol/l) containing ascorbic acid (1 mg/ml) and heated at 85 °C for 10 min. Homogenates were centrifuged at 3600 g for 30 min at 4 °C and supernatants stored at −20 °C.

Bioactivity

Bioactivity of plasma samples was determined using the isolated rat anterior pituitary cell column method of Gillies & Lowry (1978). Samples were extracted by Vycor and reconstituted in Earle’s medium for bioassay. The ratio of biological to immunological activity for CRF-41 was calculated by measuring the amount of CRF-41 in the sample perfused through the cell column and the amount of ACTH released in response to the CRF-41, and dividing the amount of ACTH by the amount of CRF-41.

Studies in normal volunteers and patients

All studies were approved by the Ethical Committee of the City and Hackney District Health Authority and informed consent from all subjects was obtained.

Insulin induced hypoglycaemia

Six males (age range 22–38 years) were studied on two occasions separated by 1 week. In single blind random order, each fasted volunteer had either insulin (Actrapid; 0-15 units/kg; Novo Laboratories Ltd, Basingstoke, Hants) in 10 ml saline, or 10 ml saline only, 45 min after cannulation. Blood was taken at 5-min intervals for the first 30 min of each study and thereafter less frequently for a further 90 min.

Administration of dexamethasone

Six males (age range 24–35 years) received 0-5 mg dexamethasone at intervals of 6 h for 48 h.

Food study

Six male volunteers (age range 25–38 years) were studied on two occasions separated by 1 week. In single blind random order, each fasted subject had either a standard lunch at 12.00 h (0-98 g protein/kg body weight, 0-74 g carbohydrate/kg and 0-53 g fat/kg; calorific content 11-5 cal/kg) or no lunch. Blood was taken at 5-min intervals for the first 30 min of each study and thereafter less frequently for the next 60 min.

Circadian study

Two male volunteers (ages 32 and 35 years) were studied over a 24-h period in a quiet room. Both individuals were fully accustomed to the environment and allowed freedom of movement.

RESULTS

Plasma extraction

Measurement of plasma CRF-41 requires an extraction step before RIA, as plasma caused gross inhibition of antibody binding to tracer.

Reversed-phase HPLC of synthetic CRF-41 after incubation in undiluted plasma for 24 h at 4 °C did not reveal any peaks of degraded CRF-41 and all CRF-41 was fully recovered, so that inhibition of binding in unextracted plasma must be due to an effect other than proteolysis. Recovery of CRF-41 extracted from plasma varied between assays (30–55%) but not within assays and was independent of plasma volume (2–5 ml), amount of CRF-41 (20–2000 pg) or plasma source (n = 6). The amount of Vycor glass required for reproducible intra-assay recovery was 150 mg. ‘Zero’ plasma obtained by the prior rotation of human plasma with Vycor showed no change in osmolality or protein concentration (Biuret test) compared with unstripped plasma. There was no difference in recovery of CRF-41 added to unadulterated plasma or Vycor-stripped plasma using 150 mg Vycor glass. The extracted CRF-41 diluted in parallel to the standard curve (Fig. 1). Assay sensitivity was 16 pg/tube which was 2 s.d. from the zero standard (n = 10), giving a lower limit of detection of...
20 ng/l in a 5 ml plasma sample with 40% recovery. Intra-assay coefficients of variation were assessed by adding CRF-41 to Vycor-stripped plasma at concentrations of 60 (n = 7) and 500 (n = 10) ng/l; the variations calculated were 10 and 6-6% respectively. The coefficient of variation for endogenous plasma CRF-41 (mean concentration 52 ng/l, n = 10) was 10.2%. Interassay coefficient of variation was assessed by the addition of CRF-41 to Vycor-stripped plasma at a concentration of 500 ng/l and was 11.4% (n = 51 samples in 28 assays). Extraction of plasma with Sep-pak C18 cartridges gave a low recovery of CRF-41 which further decreased with increasing amounts of standard CRF-41; this method was therefore considered unsuitable for use.

Radioimmunoassay of CRF-41

The antiserum would appear to be directed towards the mid-portion of CRF-41 as it did not cross-react with fragments of synthetic human CRF(1-20) and (36-41). The antiserum did not cross-react with any other relevant human peptide examined (gastrin-releasing peptide(14-27), growth hormone-releasing factor(1-40), luteinizing hormone-releasing hormone, neuromedin B, insulin, β-endorphin, γ- and α-melanocyte-stimulating hormone, ACTH(4-10) and (1-39), β-lipotrophin, Met-enkephalin, thyroglobulin, somatostatin, corticotrophin-like intermediate lobe peptide, vasopressin, vasoactive intestinal peptide and pancreatic polypeptide). Cross-reactivity of antiserum with the Tyr-CRF-41 used as tracer was 100%.

Radioimmunoassay of cortisol

The assay range was 50-2000 nmol/l. Cross-reactivity of the antiserum with related steroids was: prednisolone, 19%; 11-deoxycortisol, 0.45% and all other steroids and steroid conjugates, <0.05%. Interassay precision at 100, 300 and 1000 nmol/l was 13, 7.4 and 7% respectively.

Plasma concentrations of CRF-41

The normal range of CRF-41 (46 subjects, age range 20-50 years) was <20-110 ng/l. In plasma from a normal subject extracted and subjected to RPHPLC, a single peak of CRF-41 was detected which co-
eluted with synthetic CRF-41 (Fig. 2). Plasma concentrations of CRF-41 in patients with Cushing's syndrome (n=27), Nelson's syndrome (n=7) and adrenal insufficiency (n=11) were all within the normal range with the exception of two patients, suffering from a phaeochromocytoma and a metastatic pancreatic carcinoid tumour both associated with Cushing's syndrome, who had increased plasma concentrations of CRF-41 (Fig. 3). The plasma from both of these patients was chromatographed on RPHPLC and, in each sample, a single peak of CRF-41 co-eluted with synthetic CRF-41. In six patients with Addison's disease, sampled before their morning hydrocortisone therapy, no correlation between CRF-41 and simultaneously measured ACTH was demonstrated, nor was any correlation found between ACTH and CRF-41 in patients with Cushing's disease or Nelson's syndrome (data not shown).

In normal subjects with insulin-induced hypoglycaemia there was a sequential rise in plasma concentrations of ACTH and cortisol associated with neuroglycopenia but no change in plasma concentrations of CRF-41, despite rapid sampling at 5-min intervals (Fig. 4). There was considerable variation in CRF-41 concentrations with time in individual subjects but this was not related to the period of hypoglycaemia (Fig. 5). There was no decrease in plasma concentrations of CRF-41 following administration of low doses of dexamethasone, despite suppression.
of ACTH and cortisol (Fig. 6) and there was no change in plasma CRF-41 after a meal, despite a rise in cortisol (Fig. 7). There was no apparent variation in plasma CRF-41 related to the clear circadian changes of cortisol concentrations in two normal volunteers (data not shown).

Plasma concentrations of immunoreactive (ir) CRF from the third trimester of pregnancy were grossly increased (550–9300 ng/l). When pregnancy plasma extracts were chromatographed on Sephadex G-75, two peaks of irCRF were detected, one of which co-eluted with synthetic CRF-41 and the other in a position consistent with a higher molecular weight form (Fig. 8). Synthetic CRF-41 added to plasma, extracted and chromatographed on Sephadex G-75 eluted in the position expected for CRF-41, rendering unlikely the possibilities that the larger form of irCRF was CRF-41 binding to plasma proteins or was the result of aggregation. Reversed-phase HPLC of pregnancy plasma revealed three peaks of irCRF, one of which co-eluted with CRF-41 (Fig. 9). The extra peaks did not result from degradation or aggregation of CRF-41 during extraction, since CRF-41 added to

Figure 6. Effect of dexamethasone (0.5 mg at 6-h intervals) on circulating concentrations of cortisol, ACTH and corticotropin-releasing factor (CRF-41) in six men. Values are means ± S.E.M. at 09:00 h.

Figure 7. Effect of a standard meal (●) compared with a control day without food (○) on circulating concentrations of cortisol, ACTH and corticotropin-releasing factor (CRF-41) in six men. The meal was begun at 12:00 h and continued throughout the period shown by the hatched bar. Values are means ± S.E.M.

Figure 8. Sephadex G-75 chromatogram of immunoreactive corticotropin-releasing factor (CRF) detected by radiomunoassay in a human placental extract (stippled area) and in plasma from the third trimester of pregnancy (open area). The void volume (V₀), salt peak (Vₛ) and elution positions of human pituitary LH and synthetic human CRF-41 are arrowed.

DISCUSSION

Using a specific and sensitive RIA to measure circulating CRF-41, we found that the concentrations of CRF-41 in normal subjects were higher than those reported by two other groups which used a direct immunoradiometric assay (Linton & Lowry, 1985) and an RIA after prior extraction of CRF-41 by an immunoaffinity procedure (Suda et al. 1985). The reasons for this discrepancy, notwithstanding the absence of an internationally recognized reference standard for CRF-41, may be methodological. When we incubated CRF-41 with antibody in the presence of plasma, little binding was obtained compared with that in assay buffer. Reversed-phase HPLC showed that this was not due to degradation of CRF-41 by plasma proteases, leaving the possibility that the binding of CRF-41 to antibody was being inhibited by plasma. It is entirely conceivable that both of the above techniques which employ incubation of CRF-41 with antibody, albeit in excess, in the presence of plasma may be affected by the same inhibition. It is possible that some of the irCRF which our assay measures in plasma may represent molecular forms other than CRF-41, but chromatography of plasma samples shows that the main component of the immunoreactive material is intact CRF-41, so it is unlikely that this accounts for differences in reported levels.

Overall, our results do not support the hypothesis that circulating CRF-41 is predominantly of hypothalamic origin. Insulin-induced hypoglycaemia resulted in the expected sequential rise in ACTH and cortisol but did not produce any change in peripheral plasma CRF-41 concentrations despite a sampling frequency of 5 min. Within this rapid sampling period, marked fluctuations in the concentrations of CRF-41 occurred, possibly representing pulsatile secretion. Suda et al. (1985) reported an increase in circulating CRF-41 following insulin-induced hypoglycaemia, with peak concentrations at 1 h coinciding with, but not before, changes in plasma concentrations of ACTH. They did not report either the number of subjects tested or the results of control studies and it is possible that they simply monitored random fluctuations in CRF-41 concentrations. We have shown that low doses of dexamethasone do not suppress circulating concentrations of CRF-41, despite evidence that dexamethasone acts at the hypothalamus to decrease CRF-41 content (Suda, Tomori, Tozawa et al. 1984b). In two subjects, no circadian rhythm of CRF-41 synchronous with that of cortisol was detected. These results are consistent with those of Charlton et al. (1986). It has been demonstrated previously that there is a mid-day surge in plasma cortisol after eating (Quigley & Yen, 1979).

Placental concentrations of irCRF

Immunoreactive CRF was detected in extracts from eight out of nine sites of a placenta in concentrations up to 326 ng/g tissue. An extract chromatographed by gel filtration and RPHPLC showed similar multiple peaks of irCRF to those found in plasma (Figs 8 and 9).

Bioactivity

The index of precision (λ) of the assay was 0·11 ± 0·05 (mean ± s.d., n = 10). The ratio of synthetic CRF-41 bioactivity to immunoreactivity was 0·9 ± 0·4 (n = 12 samples). The cells released ACTH in response to CRF-41 in a dose-dependent manner. An extract of pregnancy plasma which contained 7·1 ng irCRF released 4·4 ng ACTH. An extract of plasma with undetectable irCRF released 0·4 ng ACTH. When this background stimulus was subtracted, the bioactivity to immunoreactivity ratio for irCRF in pregnancy plasma was 0·6. The pregnancy plasma irCRF was perfused at two dilutions and this limited dose–response curve was parallel to that of CRF-41.

FIGURE 9. Reversed-phase high-performance liquid chromatogram of immunoreactive corticotrophin-releasing factor (CRF) detected by radioimmunoassay in human placental extract (stippled area) and in plasma from the third trimester of pregnancy (open area). The broken line represents the elution gradient of 1-propanol and the elution position of synthetic human CRF-41 is arrowed.

a plasma sample, extracted and subjected to RPHPLC eluted as a single peak.
but this does not appear to be related to circulating CRF-41.

No correlation between circulating CRF-41 and ACTH was evident in untreated patients with Addison's disease, Cushing's syndrome or Nelson's syndrome, and plasma concentrations of CRF-41 in patients with these diseases were within the normal range. This does not rule out the possible involvement of CRF-41 alone, or as a component in a complex, in controlling the secretion of ACTH in these disorders, since changes in hypothalamic secretion of CRF-41 may be too subtle to be detected in peripheral plasma due to the large dilution. Although the measurement of plasma CRF-41 does not allow an evaluation of disorders of the HPA axis, it is of value in the detection of ectopic Cushing's syndrome associated with excess CRF-41 production (Jessop, Cunnah, Millar et al. 1987). Increased circulating concentrations of bioactive CRF-41 were detected in two patients with this syndrome, and now that more advanced biochemical methods of analysis are available, CRF-41 may prove to be more commonly involved in the ectopic Cushing's syndrome than has been believed previously.

In common with other workers (Sasaki et al. 1984b; Stalla et al. 1984; Linton et al. 1986) we found increased concentrations of irCRF in plasma from the third trimester of pregnancy and in placenta. A high molecular weight form of irCRF from placenta has been reported (Sasaki et al. 1984a) but this is the first report of irCRF of similar size in pregnancy plasma. This material eluted in a position similar to that of luteinizing hormone (28 000 daltons) on Sephadex G-75. Although no direct evidence exists for the synthesis of CRF-41 in placenta, the similar chromatographic profile of placental and plasma irCRF in pregnancy suggests that the increased circulating concentrations are sustained by placental synthesis and secretion of several forms of irCRF. Adrenocorticotrophin has been located in the placenta and it has been proposed that this is the source of the rise in circulating ACTH during gestation (Rees, Burke, Chard et al. 1975). This rise, together with increasing urinary concentrations of cortisol, was resistant to dexamethasone suppression. Plasma concentrations of free cortisol are also reported to be increased in the second and third trimesters (Abou-Samra, Pugeat, Dechaud et al. 1984). Despite these findings, and a single case report implicating pregnancy as a cause of Cushing's syndrome (Wieland, Shaffer & Glove, 1971), women do not develop Cushing's syndrome in late pregnancy. To explain this phenomenon there may be a reduction in pituitary responsiveness to CRF-41 in pregnancy, perhaps related to glucocorticoid feedback. The pituitary, however, responds normally to stress during labour, with large increases in plasma concentrations of ACTH (Genazzani, Fraioli, Hurlimann et al. 1975), suggesting that the bioactivity of plasma irCRF which is near normal in vitro may be inhibited in pregnancy. At present, the paradox of why concentrations of plasma irCRF should be so high in pregnancy with so little apparent effect remains unexplained.

We have demonstrated that circulating CRF-41 does not appear to be influenced by physiological or pharmacological manipulation designed to stimulate or suppress the hypothalamus. The concentrations we have determined are of similar magnitude to those of other hypothalamic hormones with widespread distribution and function (Penman, Wass, Lund et al. 1979; Penny, Penman, Price et al. 1984). There is increasing evidence for a physiological role involving CRF-41 other than at the pituitary. High-affinity receptors for CRF-41 have been reported in the human cerebral cortex (De Souza, Whitehouse, Kuhar et al. 1986) and in the primate sympathetic nervous system (Udelsman, Harwood, Millan et al. 1986), and CRF-41 has been shown to stimulate the release of pancreatic polypeptide in man (Lytras, Grossman, Rees et al. 1984). We propose, therefore, that tissues other than the hypothalamus may synthesize and secrete some of the CRF-41 found in human plasma and that this peptide may have a biological function in peripheral tissues.

Acknowledgements

D. C. is an MRC training fellow and D.S.J. is supported by a grant from the MRC. We are indebted to Mr M. Setchell for pregnancy plasma, Mr L. Perry and Ms D. Pendlebury for cortisol assays and Miss T. Capy for typing the manuscript.

References


