Immunoreactive Corticotropin-releasing Factor in Human Plasma

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Abstract

Plasma immunoreactive corticotropin-releasing factor (I-CRF) levels were determined by using a human CRF radioimmunoassay and an immunoaffinity procedure. The basal plasma I-CRF level in normal subjects was 6 ± 0.5 pg/ml (mean±SD). We found that most plasma I-CRF levels were affected by stress, negative feedback, and circadian rhythm. Basal I-CRF levels were high in patients with Addison's disease, Nelson's syndrome, hypopituitarism stemming from pituitary macroadenoma, and CRF- and adrenocorticotropic hormone-producing tumors. A very low, but significant, amount of I-CRF was detected (1–3 pg/ml) in patients with Cushing's syndrome, in corticosteroid-treated patients, and in a patient with hypothalamic hypopituitarism. These results suggest that a major component of plasma I-CRF is of hypothalamic origin, however, other extrahypothalamic tissues cannot be ruled out as a minor source of plasma I-CRF.

Introduction

The release of hypothalamic corticotropin-releasing factor (CRF)[1] is affected by many factors, such as stress, circadian rhythm, and negative feedback. This CRF has been sequenced from ovine (1), rat (2), and human (3) hypothalamic tissues. CRF is mainly localized in the paraventricular nucleus of the hypothalamus (4, 5), and is released into the hypophyseal portal blood (6) to stimulate synthesis and secretion of proopiomelanocortin-related peptides, such as adrenocorticotropic hormone (ACTH), lipotropins, and β-endorphin (1, 7, 8). In earlier research, we detected the presence of immunoreactive CRF (I-CRF) in human cerebrospinal fluid (9, 10), the cerebral cortex, cerebellum, and other tissues outside the brain, such as the lungs, pancreas, stomach, duodenum, and adrenal glands, as well as in the hypothalamus (11, 12). I-CRF in the extrahypothalamic tissues was indistinguishable from the hypothalamic CRF on

1. *Abbreviations used in this paper:* ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; HPLC, high performance liquid chromatography; I-ACTH and I-CRF, immunoreactive ACTH and CRF; ITT, insulin tolerance test; RIA, radioimmunoassay.

high performance liquid chromatography (HPLC). In the present study, we have examined I-CRF levels in plasma from normal human subjects and patients with hypothalamic-pituitary-adrenal disorders.

Methods

Study subjects. All subjects were examined after they gave their informed consent. Normal subjects were between 24 and 38 yr old. Basal levels (8:30–9:30 a.m.) were determined under fasting conditions. Steroid administration was discontinued for 48 h prior to the study in patients with Addison's disease, Nelson's syndrome, and in patients with systemic lupus erythematosus who had been treated with prednisolone for 5 yr. An insulin tolerance test (ITT, 0.1 U/kg i.v.) and a single-dose metyrapone test (1.5 g of metyrapone, oral) were started at 9:00 a.m. In these tests, blood samples were obtained from an indwelling scalp vein needle inserted into the forearm. The samples were then transferred into ice-cold 10-ml plastic tubes containing a small amount of heparin, and immediately centrifuged at 4°C. Plasma samples were stored frozen until assayed.

Immunoaffinity chromatography. Human CRF antiserum was coupled with activated CH-Sepharose 4B as previously described (13). 10 ml of plasma was diluted with 20 ml of 0.05 M phosphate buffer, pH 7.4, containing 0.25% human serum albumin. These materials were percolated through an immunoaffinity column (0.3 ml) of gel immobilized human CRF antiserum; then an additional 5 ml of phosphate buffer was passed through the column and discarded. The retained I-CRF was eluted with 5 ml of 0.5 M acetic acid containing 0.25% human serum albumin. The acid eluates were lyophilized and reconstituted with CRF radioimmunoassay (RIA) buffer. Recoveries of 1 pmol of human CRF added to plasma averaged 90%.

Gel filtration. 100 ml of pooled plasma from normal subjects was subjected to the immunoaffinity procedure. The lyophilized material was reconstituted in 0.1 N HCl, and applied to a Sephadex G-75 Superfine column, (Pharmacia, Uppsala, Sweden) which was eluted with 0.1 N HCl. Fractions were collected, divided into two portions for RIA and HPLC, and lyophilized.

HPLC. A Tracor Associates system (Tracor, Inc., Austin, TX) was used. The fractions containing I-CRF from gel filtration were reconstituted in 35% acetonitrile, applied to a Nucleosil C_{18} reverse-phase column (5 μm, 4 × 250 mm), and eluted with a linear gradient of 35–70% acetonitrile in 0.065% trifluoroacetic acid for 30 min at 1 ml/min. The fraction volume was 0.2 ml. Fractions were lyophilized and then reconstituted with CRF RIA buffer. Column recoveries averaged 90%.

RIA. Details of the human CRF and ACTH RIAs were previously given (11, 12). Synthetic human CRF (Peninsula Laboratories, Belmont, CA) and ACTH were used as tracer and for standards. The useful range of the CRF standard curve is 2–100 pg/tube. The minimum detectable level of plasma I-CRF is 0.5 pg/ml. The intraassay coefficient of variation was 6.2% and the interassay coefficient of variation was 13.1% in these immunoaffinity methods. The ACTH RIA kit was supplied by the National Hormone and Pituitary Program. The plasma ACTH RIA was performed using the silicic acid extraction method as previously described (14). Plasma cortisol RIA was performed using the RIA kit (Eiken ICL, Tokyo, Japan).
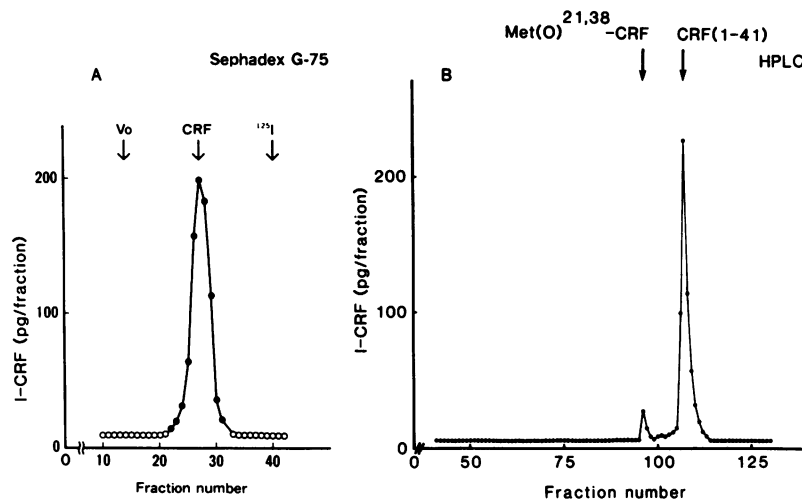
Figure 1. Gel filtration chromatography (*A*) of I-CRF from pooled plasma and HPLC analysis (*B*) of I-CRF peaks from (*A*). Met(O), methionine sulfoxide; V_o; void volume.

Results

I-CRF in plasma. Dilution curves of plasma I-CRF were parallel to that of the standard. Gel filtration of pooled plasma revealed that I-CRF was eluted in the position of synthetic human CRF (Fig. 1 *A*). This I-CRF was analyzed in a reverse-phase HPLC system. The main peak was eluted in the position of synthetic human CRF, and a small peak was found in the position of the methionine sulfoxide [Met(O)] form of human CRF (Fig. 1 *B*). In Fig. 2, basal plasma I-CRF levels in normal subjects were 6±0.5 pg/ml (mean±SD, n = 12). In patients with Addison's disease, plasma I-CRF levels were high (28±9 pg/ml, n = 6), and such high levels of I-CRF decreased to normal levels after hydrocortisone replacement (8 and 6 pg/ml) in these two patients. In patients with Cushing's syndrome with adrenal adenomas, Cushing's disease with pituitary adenomas, and in patients with systemic lupus erythematosus, I-CRF levels were low, but sig-

nificant amounts of I-CRF were detected (1–3 pg/ml). I-CRF levels were high in patients with Nelson's syndrome (11, 14, and 17 pg/ml, respectively, n = 3) and in patients with CRF- and ACTH-producing lung cancer (22 and 14 pg/ml, respectively n = 2). In hypopituitarism, the I-CRF level was high (22 pg/ml) in a patient with a pituitary nonfunctioning macroadenoma without suprasellar extension, and low (2 pg/ml) in another patient with hypothalamic craniopharyngioma. In patients with pheochromocytoma, I-CRF levels were within normal ranges (7±1 pg/ml, n = 4). In our four normal subjects, plasma I-CRF levels were significantly ($P < 0.05$) higher in the morning (6±0.8 pg/ml) than in the evening (4.4±0.7).

ITT and metyrapone tests in normal subjects (Fig. 3). Effective hypoglycemia (a decrease to <50% of basal glucose levels) resulted in parallel rises in plasma I-CRF and immunoreactive ACTH (I-ACTH) levels. Peak levels of both hormones were obtained 60 min after insulin injection. I-ACTH levels were 28±4 pg/ml at 0 time and 152±19 pg/ml at 60 min. I-CRF levels were 7±0.6 pg/ml at 0 time and 12±0.8 pg/ml at 60 min. Plasma cortisol levels also increased from 11±3 µg/dl at 0 time to 28±4
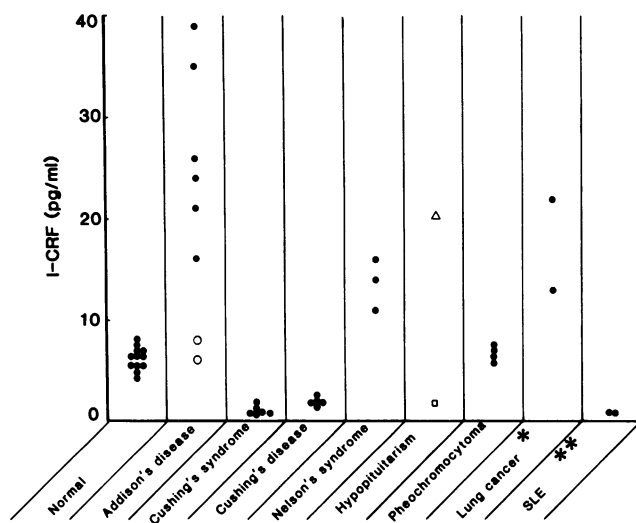

Figure 2. I-CRF levels in plasma from patients with hypothalamic-pituitary-adrenal disorders. SLE, systemic lupus erythematosus. (•) CRF- and ACTH-producing lung cancer; (••) under steroid treatment; (○) with steroid replacement; (□) hypothalamic craniopharyngioma; (△) pituitary macroadenoma.
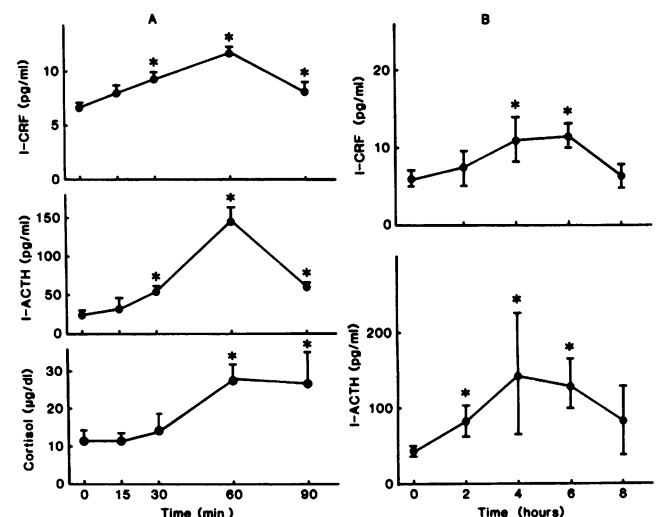

Figure 3. Plasma I-CRF, I-ACTH, and cortisol responses to ITT (*A*) and a metyrapone test (*B*) in normal subjects (mean±SD).
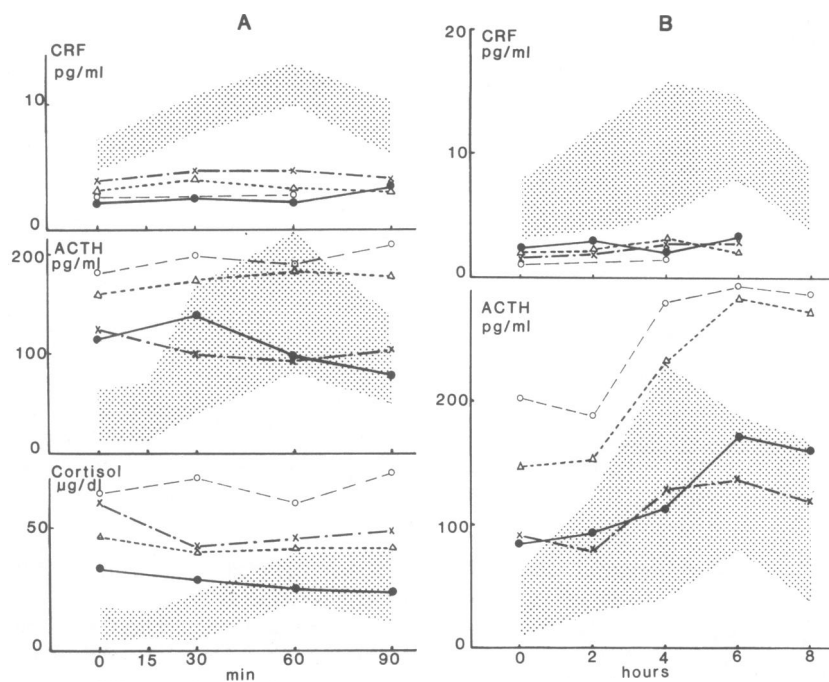
Figure 4. Plasma I-CRF, I-ACTH, and cortisol responses to ITT (*A*) and a metyrapone test (*B*) in four patients with Cushing's disease. The shaded areas indicate the mean±2 SD for normal subjects.

μg/dl at 60 min. In a metyrapone test, plasma I-CRF and I-ACTH levels increased and reached peak levels 4–6 h after single-dose metyrapone administration. I-ACTH levels were 43±7 pg/ml at 0 time, 144±72 pg/ml at 4 h, and 132±33 pg/ml at 6 h. I-CRF levels were 6±2 pg/ml at 0 time, 11±3 pg/ml at 4 h, and 12±2 pg/ml at 6 h. There is a good correlation between I-ACTH and I-CRF levels in plasma from normal subjects in the ITT and the metyrapone test results ($r = 0.5935$, $y = 6.7095x + 0.0282$, $P < 0.00001$).

Cushing's disease. An ITT and a single-dose metyrapone test were performed in four patients with Cushing's disease who had pituitary adenomas (Fig. 4). In the ITT, high levels of plasma I-ACTH and cortisol, and low levels of I-CRF were not changed by effective hypoglycemia. In contrast to the ITT, plasma I-ACTH levels were increased by a metyrapone test, but I-CRF levels were not changed in these patients.

Discussion

In the present study, a small amount of I-CRF was detected in normal human plasma. Plasma I-CRF is immunologically and chromatographically similar to authentic human CRF. Plasma I-CRF levels were high in patients with Addison's disease, Nelson's syndrome, and hypopituitarism due to pituitary macroadenoma, and low in patients with Cushing's syndrome due to adrenal or pituitary adenomas and SLE under treatment with prednisolone. In patients with Addison's disease, high levels of plasma I-CRF decreased to normal levels after steroid replacement. These results suggest that plasma I-CRF levels were decreased by increased plasma cortisol levels except in ACTH- and CRF-producing tumors. In addition, plasma I-CRF levels increased after a single dose of metyrapone administered in normal subjects. From these results, it is suggested that plasma I-CRF is affected by a negative feedback mechanism. These results also agree with the previous reports that I-CRF levels in the hypothalamic median eminence of rats were increased after

adrenalectomy and were decreased by dexamethasone administration (15, 16).

In normal subjects, plasma I-CRF levels were higher in the morning than in the evening, and increased after insulin hypoglycemia. These findings suggest that plasma I-CRF affected by circadian rhythm and stress, and this suggests that a major part of plasma I-CRF is released from tissue which is affected by circadian rhythm, negative feedback, and stress. We speculate that a major component of plasma I-CRF is of hypothalamic origin. Other tissues, however, such as those of the gastrointestinal tract, pancreas, and lungs cannot be ruled out as a minor source of plasma I-CRF (12). This is because a very low, but significant, amount of I-CRF was detected in plasma taken from patients with Cushing's syndrome, in steroid-treated patients, and in a patient with hypothalamic hypopituitarism. To examine the pathophysiology of Cushing's disease, an ITT and a metyrapone test were performed in our patients with Cushing's disease who had pituitary adenomas. Usually, in patients with Cushing's disease, plasma ACTH does not respond to an ITT; however, it does respond to a metyrapone test (17). We have not been able to answer the question of which tissues respond to a metyrapone test: pituitary, hypothalamus, or both. In the present study, plasma I-ACTH levels were high and were increased by a metyrapone test, but were not by an ITT. Plasma I-CRF levels were low and did not respond to these tests. This result raises the possibility that, in these patients, hypothalamic CRF release is inhibited by high plasma cortisol levels, and pituitary adenomas directly respond to a decrease of plasma cortisol levels caused by metyrapone administration. Therefore, the determination of plasma I-CRF levels is important for understanding the pathophysiology of hypothalamic-pituitary-adrenal disorders.

Acknowledgments

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