Continuous Administration of Synthetic Ovine Corticotropin-releasing Factor in Man

Physiological and Pathophysiological Implications

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Abstract

The continuous 24-h infusion of a maximally stimulating dose (1 μg/kg per h) of ovine corticotropin-releasing factor (CRF) in man caused a modest elevation of plasma cortisol (17.2±1.4 μg/dl) and urinary-free cortisol (173±43 μg/24 h) concentrations, which was far less than that seen with a maximally stimulating dose of ACTH (50.4±2.2 μg/dl and 1,200±94 μg/24 h, respectively). The circadian rhythms of plasma ACTH and cortisol were preserved during CRF administration. An intravenous bolus injection of 1 μg/kg of ovine CRF given to normal volunteers under basal conditions resulted in elevated plasma ACTH and cortisol peak levels (28±6 pg/ml and 15.0±1.0 μg/dl, respectively). However, no plasma ACTH and cortisol responses were observed when an identical CRF stimulation test was given at the end of the continuous infusion. These findings suggest that the stimulatory activity of exogenous CRF on the ACTH-secreting cells of the pituitary gland is restrained by the negative feedback of cortisol. The persistent circadian rhythm of ACTH, despite a constant level of plasma CRF during the infusion, suggests that the circadian variation in the activity of the hypothalamic-pituitary-adrenal axis cannot be explained solely by circadian periodicity of the endogenous CRF stimulus.

Introduction

Corticotropin-releasing factor (CRF)[1] is a 41 amino acid peptide that was first isolated from ovine hypothalami (1). This hypothalamic hormone has greater corticotropin (ACTH)-releasing potency than any previously identified endogenous or synthetic peptide. Although recent studies indicate that arginine vasopressin, oxytocin, angiotensin II, and the catecholamines may have corticotropin-releasing activity or may modulate the ACTH response to CRF, CRF is thought to play the dominant role in pituitary-adrenal regulation (2–9). Thus, the 7–9 daily ACTH and cortisol secretory episodes that occur in the average, nonstressed individual are generally attributed to an equal number of CRF pulses released into the hypophyseal portal blood (10, 11).

Similarly, the relative aggregation of these ACTH and cortisol pulses in the early morning hours, which accounts for the characteristic circadian surge of these hormones (see Fig. 1 *C*, shaded area), is thought to reflect the temporal organization of CRF secretory activity as it responds to inputs from one or more central circadian pacemakers (10, 11). It has been postulated, moreover, that increased or relatively continuous pulsing of the CRF neuron translates psychological or somatic stress into increased ACTH and cortisol secretion and, possibly, into illnesses such as Cushing's disease or the pseudo-Cushing's states (e.g., alcoholism or depression).

We report here studies that utilized synthetic ovine CRF to test the premise that CRF plays a dominant role in the circadian organization of ACTH and cortisol secretion, and that increased CRF stimulation can explain the hypercortisolism of illnesses such as Cushing's disease or depression. We determined the 24-h pattern of cortisol secretion in a group of healthy control subjects and compared this pattern to that obtained in volunteers given 24-h infusions of either CRF or ACTH at pharmacologic doses. We asked three questions: (1) Is the circadian rhythm of ACTH and cortisol abolished during continuous CRF stimulation of the corticotroph, as expected, or is it preserved (2)? Does the increase in the magnitude of ACTH and cortisol during continuous CRF administration resemble that seen during stress, Cushing's disease, or depression (3)? Are there differences in the pattern of cortisol response during continuous CRF and ACTH administration, and if so, do these differences help explain the pathophysiology of the different hypercortisolemic states?

In an additional study we administered an intravenous bolus of 1 μg/kg of CRF at 0800 to four of the volunteers at the end of their continuous CRF infusion and compared the ACTH and cortisol responses to those obtained in volunteers given an identical CRF stimulation test under basal conditions. We had previously observed that, compared with normal controls, the ACTH responses to a CRF stimulation test were exaggerated in Cushing's disease and were blunted in depression (12–14). Hence, we wished to see if the response to a CRF stimulation test given after a continuous 24-h CRF infusion would resemble that seen in either Cushing's disease or depression, since such an observation would indirectly suggest the presence of excess endogenous CRF production in that particular disorder.

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1. *Abbreviations used in this paper:* CRF, corticotropin-releasing factor; HPA, hypothalamic-pituitary-adrenal; HPLC, high performance liquid chromatography; IR, immunoreactive; urinary free cortisol.

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Methods

Subjects. Four separate groups of young, healthy volunteers (a total of 47 subjects, 19–30 yr, 27 male and 20 female) participated in the continuous CRF and ACTH infusion studies and provided normative data for the circadian pattern of cortisol and for the plasma ACTH and cortisol responses to an intravenous bolus of CRF. All subjects were admitted to the National Institutes of Health Clinical Center after giving informed consent. The protocol for CRF infusion studies was approved under an investigational exemption for a new drug by the National Center for Drugs and Biologics, U. S. Public Health Service and by the National Institute of Child Health and Human Development Committee for the protection of human subjects (protocol 82-CH-45, Investigational New Drug 19802). Pregnancies in female volunteers were excluded before infusions by rapid HCG determinations.

Corticotropin-releasing factor preparation. Ovine synthetic CRF was obtained from Bachem Co. (Torrance, CA). The initial preparation was purified by high performance liquid chromatography, dissolved in water with 5% mannitol, sterilized by filtration (0.22 μm, Millipore, Bedford, MA), lyophilized, and placed into sterile vials under vacuum. The CRF content of each lot was verified by high performance liquid chromatography and a specific radioimmunoassay (RIA). The vials were kept refrigerated at 4°C. Sterile water was added immediately before human administration.

Protocol. An intravenous needle was inserted in the antecubital vein of both arms and kept open with normal saline. CRF, ACTH, or normal saline was infused at constant rates via an automatic pump (Harvard model 975, Harvard Apparatus, Milles, MA). The infusate was kept at 4°C. Blood was drawn from the opposite arm at 0, 30, and 60 min, and every 30 or 60 min up to 24 h for measurements of ACTH, CRF, and cortisol. During the ACTH infusion studies, blood was drawn at 0, 10, 30, and 130 min, and at 4, 6, 8, and 24 h. Urine was collected throughout the test for measurement of urinary-free cortisol. Blood for ACTH and CRF determination was collected in prechilled glass tubes containing EDTA. Blood samples were immediately placed on ice and centrifuged within 3 h of collection followed by immediate separation of plasma. Blood for the remaining assays was collected into heparinized glass tubes, centrifuged at the end of the test, and the plasma was separated the following morning. Plasma for all assays was placed into capped polypropylene vials and frozen at −20°C until assayed. Aliquots of urine were kept frozen at −20°C until assayed, total volumes were recorded.

Six subjects received continuous infusions of CRF at a constant rate of 1 μg/kg per h (total dose of 1,800 μg); 26 subjects received ACTH 1-24 (Cortrosyn Organon Inc., West Orange, NJ) at a constant rate of 0.5 μg/kg per h for 24 h and normal saline was administered under same conditions to 11 subjects. A bolus intravenous injection of 1 μg/kg of CRF was given to four subjects at 0800 a.m. under basal conditions and to four of the subjects after the end of the continuous CRF infusion.

Hormone assays. Immunoreactive (IR) CRF, ACTH, cortisol, and urinary free cortisol (UFC) were measured by RIAs that have been previously described (12, 15–17). The detection limit of the plasma CRF, ACTH, and cortisol assays were 5–7 pg/ml, 3–5 pg/ml, and 0.1–0.2 μg/dl, respectively. The within and between assay variabilities were 4.4 and 19.7% for ACTH, 4.6 and 6.0% for cortisol, and 5 and 13% for CRF. All samples of each individual subject were assayed in a single assay.

Statistical analysis. The results are expressed as the mean±SE. Differences between groups were examined with a two-tailed *t* test. The RIA data were analyzed by a computer program that performed a best fit logit-log analysis (18).

Results

Continuous intravenous infusion of CRF at the dose of 1 μg/kg per h increased plasma CRF levels rapidly and achieved a steady state supraphysiologic concentration within 4–5 h (Fig. 1 *A*). 24-h integrated plasma cortisol concentrations were significantly higher ($P < 0.005$) in normal volunteers who received continuous CRF infusion (307.75±17.4 μg/dl·24 h, $n = 6$) than in normal volunteers receiving normal saline (166.96±17.76 μg/dl·24 h, $n = 11$) (Fig. 1 *C*). Both plasma IR-ACTH and cortisol concentrations retained a clear circadian variation during continuous CRF administration when mean grouped zenith (mean of three values between 12.00 and 14.00 for each subject) and nadir (mean of three values between 24.00 and 20.00 for each subject) values were compared ($P < 0.01$). The plasma cortisol curve obtained under this experimental condition had higher levels but was otherwise virtually superimposable upon the curve obtained during the administration of normal saline (Fig. 1 *C*).

The plasma concentrations of cortisol during the continuous infusion of 0.5 μg/kg per h of ACTH were ~3 times higher than those observed during the continuous CRF infusion and 10 times greater than the corresponding level of cortisol

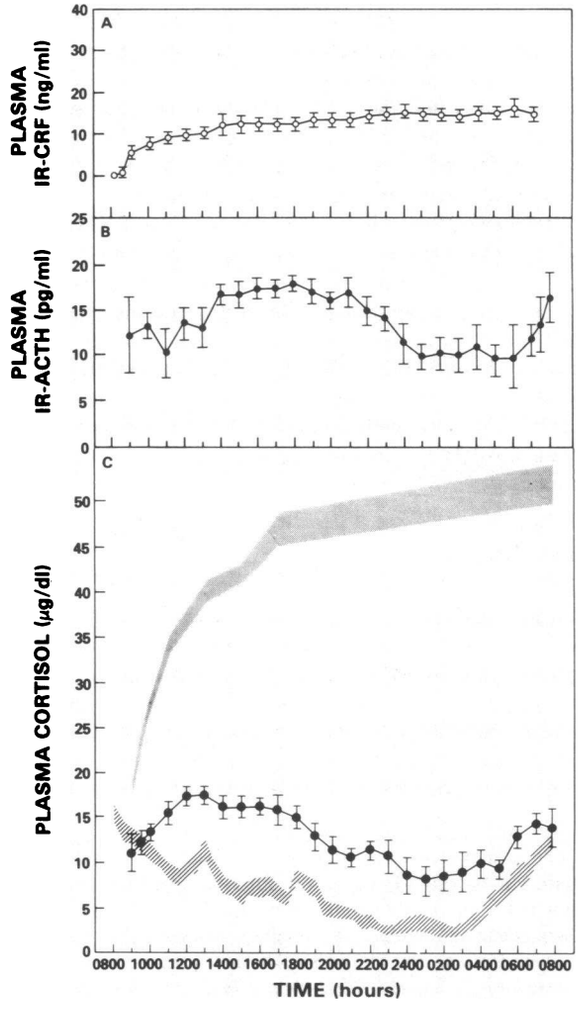

Figure 1. Plasma concentrations (mean±SE) of IR-CRF (*A*), IR-ACTH (*B*), and cortisol (*C*) during a 24-h continuous infusion of CRF at a dose of 1 μg/kg per h. Plasma concentrations of cortisol (mean±SE) during a 24-h infusion of ACTH 1-24 (▨) or normal saline (▩) are shown in *C*. □, ACTH infusion, 0.5 μg/kg per h ($n = 26$); ●, CRF infusion, 1 μg/kg per h ($n = 6$); ▩, normal saline infusion ($n = 11$).

obtained under basal conditions (Fig. 1 *C*). The plasma cortisol levels rose rapidly after initiation of the continuous ACTH infusion, plateaued out ~8 h into the infusion, and continued to rise slowly for the remainder of the procedure. The circadian pattern of cortisol secretion was disrupted by the continuous ACTH infusion.

UFC excretion was much higher during the 24-h ACTH infusion (1,200±94 μg/24 h) than during the CRF infusion (173±43 μg/24 h) or in normal subjects who were studied under basal conditions (48±5 μg/24 h). UFC excretion was higher during the CRF infusion than in the unstimulated state ($P < 0.01$).

When a bolus of 1 μg/kg of CRF was given at 0800 to four male volunteers under basal conditions, plasma ACTH reached peak levels of 28±6 pg/ml at 15–30 min. Plasma cortisol rose to 15±1 μg/dl at 60 min. In contrast to the usual response observed under basal conditions, no plasma cortisol and ACTH responses were observed when an identical CRF stimulation test was given at the end of the continuous CRF infusion (Fig. 2, *a, b,* and *c*).

Discussion

We cannot explain the persistence of the circadian rhythm of ACTH during continuous CRF administration. The levels of plasma CRF that were achieved are known to produce maximal stimulation of the corticotroph cell when CRF is given as an intravenous bolus in man and have been shown to be severalfold higher than hypophyseal portal levels in the anesthetized rat (19–21). We should note, however, that levels of CRF in the human portal hypophyseal system are not known as yet and may be different from those in the rat. There are several possible explanations for the persistence of the circadian rhythm during continuous CRF infusion. First, the endogenous secretion of CRF may persist during the continuous infusion of the peptide, so that the observed rhythms of ACTH and cortisol reflect the endogenous secretory pattern superimposed on the continuously administered exogenous stimulus. We think this is unlikely, however, since we have shown that an intravenous bolus of CRF is unable to produce any discernible ACTH or cortisol response at the end of the continuous infusion. Moreover, high levels of cortisol have been observed to suppress plasma ACTH, presumably via suppression of the corticotroph cell and/or the CRF-neuron (12, 13).

A second possibility is that there is an intrinsic circadian variation in the sensitivity of the pituitary corticotroph cell to CRF. Our previous studies exploring the response to CRF at two time points (0900 and 2000) do not support this hypothesis (22). A third possibility is the presence of an unknown modulating factor that could sensitize the corticotroph cell to CRF in the morning or desensitize it in the evening, or the presence of a separate stimulatory or inhibitory factor that influences the circadian pattern of ACTH. None of these latter possibilities can be ruled out, nor do they seem mutually exclusive. We conclude that the weight of available evidence suggests that a factor(s) other than CRF contributes to the circadian rhythm of the hypothalamic-pituitary-adrenal (HPA) axis. Such factors may be arginine vasopressin, oxytocin, angiotensin II, the catecholamines or others as yet unknown (2–9).

The elevations of plasma cortisol and UFC concentrations noted during the continuous administration of CRF were much lower than those observed during continuous ACTH infusion. This disparity in the hormonal responses between continuous CRF and ACTH administration is compatible with current concepts concerning the physiology of the HPA axis. Thus, during continuous CRF administration to experimental animals, there is evidence of a modest desensitization of the pituitary corticotroph cell to the effects of CRF (23–25). In addition, the cortisol secretion secondary to CRF-induced ACTH secretion would be expected to restrain further CRF-induced ACTH secretion through negative feedback.

The pattern and magnitude of the cortisol responses to continuous administration of CRF challenge the idea that CRF is the sole mediator of stress-induced ACTH secretion or of the hypercortisolism of Cushing's disease. The levels of

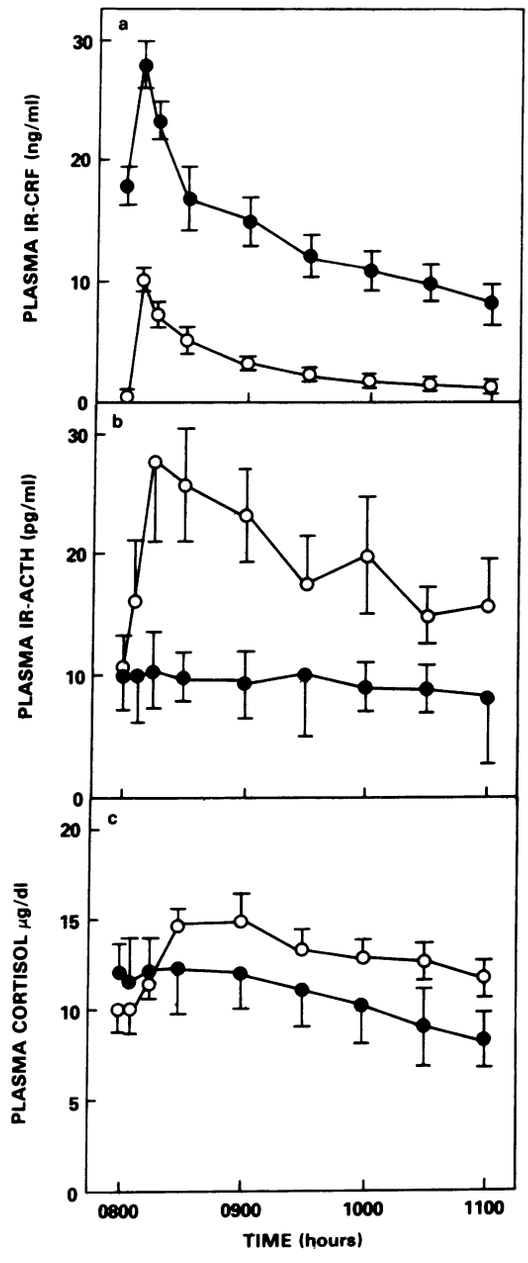

Figure 2. Plasma IR-CRF (*a*), IR-ACTH (*b*), and cortisol (*c*) responses (mean±SE) to an intravenous bolus of CRF (1 μg/kg) after a 24-h continuous CRF infusion (●) and under base-line conditions (○).

ACTH and cortisol achieved during continuous pharmacologic CRF administration are not as high as the elevations in these hormones that can be observed during periods of major physical stress (26–28). Synergy with other factors, as suggested by other authors (2–9), or an augmented ACTH response to pulsed rather than continuous endogenous CRF secretion may account for the higher levels seen in stress. The latter question is not testable with ovine CRF in man due to its long plasma half-life (29, 15), but can be tested with human CRF, which has a short plasma half-life in man (30).

The plasma cortisol and ACTH concentrations during continuous CRF infusions are lower than those seen in most cases of Cushing's disease (12, 13, 28). Moreover, the characteristic circadian organization of the HPA axis is usually abolished in subjects with Cushing's disease (31). Thus, the cortisol levels characteristic of this condition more closely resemble those obtained during continuous administration of ACTH, a situation that is physiologically analogous to the relatively continuous secretion of ACTH by an autonomous pituitary microadenoma that is partially resistant to the negative feedback effects of cortisol (32). Such a model for Cushing's disease is supported by our finding that patients with this illness generally respond to exogenous CRF administration with an exaggerated ACTH response despite high circulating cortisol levels, which suggests that the ACTH secretion in Cushing's disease originates in an adenoma that is relatively unresponsive to inhibition by corticosteroids (12, 13).

In contrast to Cushing's disease, the pathophysiology of the hypercortisolism of depression seems most likely to represent an excess secretion of endogenous CRF. The hypercortisolism of depression resembles both quantitatively and qualitatively what we see experimentally during the continuous administration of exogenous CRF to normal volunteers (33–35). The blunted ACTH responses to exogenous CRF, which we have observed in depression, supports a model in which there is excess endogenous CRF secretion in the setting of a normal pituitary gland restrained by the negative feedback effects of cortisol (14). These blunted ACTH responses to CRF can be likened to the markedly blunted ACTH responses to the bolus of CRF given to normal volunteers at the end of the continuous infusion of exogenous CRF.

In summary, these studies suggest that CRF is not the sole mediator of the circadian pattern of the HPA axis. Continuous 24-h CRF infusion provides a 24-h cortisol secretory pattern similar to that in depression (both quality and magnitude) or mild Cushing's syndrome (magnitude). The inability of exogenous CRF to cause marked ACTH and cortisol secretion after a prolonged continuous CRF infusion suggests that excessive CRF secretion does not cause severe Cushing's disease and that the blunted response observed in depression may be explained by increased CRF secretion in this condition. The fact that 24-h infusions were employed rather than more chronic administration may limit the significance of the data to acute rather than chronic situations. For instance, chronic hypersecretion of CRF might lead to development of pituitary corticotroph hyperplasia or corticotropinomas manifest as classic Cushing's disease.

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