NOTE

Responses of Plasma Adrenocorticotropin and Cortisol to Intravenous Injection of Synthetic Ovine Corticotropin Releasing Factor in the Morning and Early Evening in Normal Human Subjects

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Abstract

This study was designed to compare the responsiveness of adrenocorticotropic (ACTH) and cortisol secretion to corticotropin-releasing factor (CRF) in the morning and early evening in normal human subjects. Synthetic ovine CRF (1.0 μg/kg) or normal saline, was administered as an i.v. bolus injection to six normal males at 900 h and 1700 h. Blood samples were obtained before and 15, 30, 60, 90 and 120 min after CRF or saline injection. Significant increases in plasma ACTH and cortisol levels were observed in all subjects at both the testing time after CRF injection. The net increments in the areas under the concentration curve (areas in the CRF experiment minus those in the saline control experiment) were not statistically different for both ACTH (mean ±SEM: 41.0 ± 10.6 pg/ml h in the morning; 51.1 ± 8.9 pg/ml h in the evening) and cortisol (mean ±SEM: 28.5 ± 5.0 μg/dl h in the morning; 36.2 ± 4.0 μg/dl h in the evening). Also no significant difference was observed in net increment, peak level and the ratio of peak level to the basal level of ACTH and cortisol after CRF injection. There were no appreciable changes in plasma concentrations of growth hormone, thyroid-stimulating hormone or prolactin, although slight but statistically significant rises in plasma levels of luteinizing hormone and follicle-stimulating hormone were observed.

These results suggest that there is no significant difference in responsiveness of the pituitary-adrenal axis to CRF in the morning (900 h) and early evening (1700 h), and thus the time of day will not necessarily have to be considered when CRF is used between these times in a clinical test to evaluate pituitary ACTH reserve.

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The existence of a circadian periodicity of adrenocorticotropin (ACTH) and cortisol secretion in man has been well established (Orth et al., 1967; Weitzman et al., 1971; Gallagher et al., 1973). In addition, a diurnal variation in the pituitary-adrenal response to several stimuli such as insul-
induced hypoglycemia (Ichikawa et al., 1972) or pyrogen (Takebe et al., 1966) has been reported. These stressful stimuli are considered to increase ACTH secretion by acting not directly upon the pituitary but upon the hypothalamus to stimulate secretion of corticotropin-releasing factor (CRF). CRF has recently been isolated from ovine hypothalami and its primary structure determined (Vale et al., 1981; Spiess et al., 1981). Synthetic replicate of the CRF has been shown to be active in stimulating ACTH release by acting directly upon the pituitary in several species including human subjects (Vale et al., 1981; Chan et al., 1982; Suda et al., 1983; Lamberts et al., 1984). Thus it is now possible to evaluate the diurnal variation in responsiveness of the pituitary itself in secreting ACTH by using synthetic CRF. The previous study by Tsukada et al. (1984) has suggested no significant difference at 0900 h and 2200 h, in secretion of ACTH by CRF. However, others (Schulte et al., 1985) have recently reported that the response of the pituitary-adrenal axis to CRF is greater at 2000 h than at 0900 h. To clinically evaluate the pituitary function in secreting ACTH, it may not be convenient to perform a CRF-test so late in the evening, even if the response of the pituitary to CRF is greater at that time than in the morning. Therefore, it would be also worthwhile from the clinical as well as physiological point of view to define the difference in the responsiveness of the pituitary-adrenal axis to CRF in the morning and in the early evening, rather than in the late evening, as in previous reports (Tsukada et al., 1983; Schulte et al., 1985). The present experiment was designed to determine whether or not the responsiveness of the pituitary-adrenal axis to CRF is different at 0900 h and 1700 h in normal human subjects. In addition, the effects of CRF on the other anterior pituitary hormones at these two times were also studied.

Materials and Methods

Synthetic ovine CRF was purchased from The Peptide Institute, Osaka, Japan. The CRF was prepared for injection as described previously (Tanaka et al., 1983), and stored at 4°C until used. Biological activity of CRF preparation has been shown to be satisfactory by in vitro assay using monolayer culture of rat anterior pituitary cells (Tanaka et al., 1983).

Six normal males, aged 25 to 43 (mean 31.0) years old and weighing 50 to 80 (mean 56.8) kg were the subject of this study. Written consent was obtained from each subject after informing him of the purpose and the nature of the experiments. The subjects remained active supine for 45 min prior to the injection of CRF throughout the experiment. A vial which contained 100 μg of lyophilized CRF was dissolved in 2.0 ml of sterile normal saline and a dose of 1.0 μg/kg was administered as an intravenous bolus injection at 0900 h and at 1700 h after an overnight fast and a more than 4-hour fast, respectively. All subjects were given the same amount of normal saline as in control experiments at the same time of the day on different occasions. Each experiment was performed at intervals of at least 7 days. Blood samples were obtained into test tubes containing EDTA from an antecubital vein immediately before and at 15, 30, 60, 90, and 120 min after the injection of CRF. The plasma were separated by centrifugation at 4°C and stored frozen at −20°C for the later determination of ACTH, other anterior pituitary hormones and cortisol.

Plasma ACTH was measured by radioimmunoassay (RIA) after extraction with Sep-Pak C_{18} cartridge (Waters Associates, Milford, Mass., USA) according to a modification of the method of Chrousos et al. (1984). The cartridges were first washed with 5.0 ml acetonitrile followed by 10 ml of distilled water. 1.0 ml plasma samples were then applied and the cartridges were washed again with 5.0 ml of distilled water, followed by elution with 3.5 ml of TEAF buffer (1 percent formic acid, pH adjusted to 3.2 with triethylamine) and acetonitrile (4: 6 by volume). The eluent was lyophilized and reconstituted in assay buffer (0.02 M Veronal buffer containing 0.1 percent human serum albumin, 0.4 percent mercaptoethanol, and 500 KIU of Trasylol per ml). An antiserum which specifically recognize the 12 to
18 amino acid sequences of $^{1-39}$hACTH was used. Synthetic $^{1-39}$hACTH provided by NIAMDD was used as standard and for labelling with $^{125}$I by the chloramine-T method. Recovery of standard ACTH added to ACTH-free plasma by this extraction method was 77.2±6.2 (mean±SEM) percent. Intra and inter assay coefficients of variation for ACTH-RIA were 8.4 and 10.8 percent, respectively, for the mean concentration of 100.1 pg/ml. Plasma cortisol, growth hormone (GH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined by RIA as described previously (Tanaka et al., 1984).

Analysis of variance for two-way analysis of multiple responses to baseline values were used. The paired Student’s t-test was used to analyze the statistical difference between dose response and basal values, and between response in the morning and evening. Results were expressed as the mean±SEM, unless otherwise indicated.

Fig. 1. Effect of synthetic ovine CRF on plasma ACTH level in the morning and early evening in six normal male subjects. CRF and control in this figure represent the ACTH levels after CRF and normal saline injection, respectively. Each point and bracket represent the mean and SEM, respectively. The asterisks designate those points that are significantly different from the corresponding basal value as in the following figures.

Fig. 2. Effect of synthetic ovine’CRF on plasma cortisol levels determined simultaneously in the experiments shown in Fig. 1.
Results

The responses of plasma ACTH and cortisol after the injection of CRF or saline are shown in Fig. 1 and 2, respectively. The mean plasma ACTH level increased significantly ($p<0.01$) from $13.4 \pm 1.9 \text{ pg/ml}$ (basal) to $39.0 \pm 10.8 \text{ pg/ml}$ (peak) in the morning and from $11.4 \pm 3.0 \text{ pg/ml}$ (basal) to $47.2 \pm 9.4 \text{ pg/ml}$ (peak) in the evening, at 30 min in both cases, after the administration of CRF. The mean plasma cortisol concentration also rose significantly ($p<0.01$) after CRF injection, from $14.6 \pm 1.7 \text{ µg/dl}$ (basal) to $28.4 \pm 2.0 \text{ µg/dl}$ (peak) at 60 min in the morning and from $8.9 \pm 1.7 \text{ µg/dl}$ (basal) to $27.9 \pm 2.7 \text{ µg/dl}$ (peak) at 60 min in the evening. In the control experiments, no significant changes in plasma ACTH levels was observed, although a slight but significant ($p<0.01$) decrease in plasma cortisol was observed during the two test time periods.

Four parameters were calculated from the data shown in Fig. 1 and 2, in order to compare the magnitude of the response of plasma ACTH and cortisol to CRF and the results are summarized in Fig. 3. The “Net area” in Fig. 3 was arbitrarily defined as the area under the concentration curves after CRF injection minus those after saline injection. The net increment represents the maximum concentration after CRF injection subtracted by the corresponding basal concentration. The peak concentration shown in Fig. 3 represents peak levels of ACTH and cortisol after CRF injection. “Peak/Basal” in Fig. 3 indicates the ratio of the peak concentration after CRF injection to the basal concentration of each hormone. There was no statistically significant difference between the “Net areas” of ACTH and cortisol. “Net increment” and “Peak” for ACTH and cortisol was also not significantly different in the morning and evening experiments. The “Peak/Basal” of ACTH and cortisol were 1.75 and 1.98 times greater, respectively, in the evening than in the morning, although the differences were not statistically significant.

No significant changes were observed at both times of testing by CRF in plasma concentrations of GH, TSH and PRL (Fig. 4). A slight but significant ($p<0.05$) rise in plasma LH and FSH levels was observed after CRF injection in the evening and
morning experiments, respectively. However, changes in plasma LH and FSH concentration after CRF injection in the morning and evening, respectively, were not statistically significant (Fig. 4).

Discussion

The magnitude and time course of the response of plasma ACTH and cortisol to 1.0 µg/kg CRF observed in this study were comparable to those in previous reports by others (Grossman et al., 1982; Müller et al., 1982; Orth et al., 1983; Nakahara et al., 1983; Chrousos et al., 1984; Lytras et al., 1984) and us (Tanaka et al., 1983) in which the same or a similar dose of CRF was used. However, for both ACTH and cortisol, no significant differences between the 0900 h and 1700 h studies were detected in any of the parameters such as “Net area”,

Fig. 4. Effects of synthetic ovine CRF on plasma levels of GH, TSH, PRL, LH and FSH in the morning and evening.
"Net increment", "Peak" and "Peak/Basal" which would reflect the resposiveness of the pituitary-adrenal axis to CRF. Since it is unlikely that the metabolic clearance rate of ACTH and cortisol is different in the morning and evening, the present observation suggests that there are no significant differences in the amount of ACTH and cortisol secreted to CRF in the morning and early evening. The present observations are in good agreement with the previous report by Tsukada et al. (1983). They have performed similar experiments at 0900 h and 2200 h and found no difference in the response of the pituitary-adrenal axis to 100 μg synthetic ovine CRF. In contrast, Schulte et al. (1985) have recently reported significantly greater responses of ACTH and cortisol, to the same dose of CRF as used in the present study, at 0900 h than at 2200 h. The discrepancy among these findings could not be explained by the dose of CRF, because no significant difference in responsiveness is expected at a dose between 1.0 μg/kg and 100 μg/subject, based upon the previous dose-response study by Orth et al. (1983) and Schürmeyer et al. (1984). The most probable reason for this discrepancy may be the difference in clock time selected for the study and the relationship between the time of study and the sleep-wake cycle of the subjects studied. The activity of the hypothalamic-pituitary-adrenal axis is thought to depend upon the sleep-wake schedule of each individual rather than clock time, and this activity is greatest at the time of or shortly after awaking, and lowest at around retiring time (Orth et al., 1967; Tanaka et al., 1978). Therefore, a clearer difference may be observed if responsiveness is compared at times closer to awaking and retiring of each subject.

It has been suggested that the response of ACTH to stressful stimuli such as insulin induced-hypoglycemia (Ichikawa et al., 1972) and pyrogen (Takebe et al., 1966) is greater in the evening than in the morning. These stimuli are thought to increase the secretion of CRF from the hypothalamus, leading to stimulation of ACTH release from the pituitary. Thus, these observations suggest that secretion of CRF to the stressful stimuli is greater in the evening, when combined with our present findings and the results of Tsukada et al. (1983). Pyrogen and insulin-induced hypoglycemia may stimulate, in addition to secretion of endogenous CRF, the secretion of a substance(s), other than CRF, which modulates the release of CRF or acts directly on the pituitary to modulate the action of CRF, or may inhibit CRF-antagonist(s), since these stimuli are not considered to be specific to CRF. For these reasons, it would be very difficult and imprecise to evaluate the response of the hypothalamus to release CRF to these stimuli with ACTH as a parameter. Recently, the primary structure of human CRF has been elucidated by deduction from the DNA sequence of the CRF genome (Shibahara et al., 1983). An RIA system has been developed using synthetic CRF, demonstrating the existence of immunoreactive CRF in blood (Gibbs and Vale, 1982; Plotsky and Vale, 1984) and cerebrospinal fluid (Suda et al., 1983; Tomori et al., 1983). Thus, direct assessment of CRF in the circulation after these stimuli may shed light on the more exact nature of diurnal variation in the secretion of CRF to the stress.

Several reports have indicated that synthetic ovine CRF is very specific to ACTH and does not affect the plasma concentrations of other anterior pituitary hormones (Grossman et al., 1982; Müller et al., 1982; Orth et al., 1983; Schulte et al., 1985). In the present study, no appreciable changes in the concentration of plasma GH, TSH and PRL was observed during either time of study, but the present data differ from these previous reports in that plasma LH and FSH levels rose following CRF injection. This discrepancy between the present observation and the others is difficult to explain.
The secretion of LH and FSH may be stimulated by non-specific stress, and thus the rise in the plasma concentration of these hormones in our subjects may possibly be due to such stress. However, plasma levels of GH which can also be elevated by stress (Greenwood and Landon, 1966; Copinschi et al., 1967; Best et al., 1968), did not change at all during the experiments, suggesting that rises in plasma LH and FSH levels observed in our experiments are unlikely to be due to stress. In other mammalian species such as rat, significant suppression by CRF of LH and FSH has been reported (Sirinathsinghjin et al., 1983; Rivier and Vale, 1984). Thus, synthetic ovine CRF may have some effects on LH and FSH secretion in man. However, the increases in plasma levels of gonadotropins were relatively small and not consistent phenomena in the morning and evening and these changes may be well within the range of spontaneous fluctuation which occurs in normal men (Boyar et al., 1972). Thus, the present data do not allow a conclusion to be drawn on the effect of synthetic ovine CRF on plasma gonadotropin levels, and further studies will be required to clarify whether or not the CRF is strictly specific to ACTH.

The number of subjects studied in this as well as previous reports is rather small and, as mentioned earlier in this paper, the time of the study is not adjusted to the sleep-wake cycle of each individual. Thus, further investigations will be required to establish a more definitive conclusion on the diurnal variation in responsiveness of pituitary to CRF, as well as that of the hypothalamic-pituitary-adrenal axis as a whole.

In summary, the present observations suggest that the amount of ACTH secreted in response to CRF in normal subjects is not significantly different in the morning from that in the early evening, and that the time of the day is not critical when CRF is used between 900 h and 1700 h in a clinical test of hypothalamic-pituitary-adrenal function.

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References


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