EVIDENCE FOR THE INTERNAL FEEDBACK PHENOMENON IN HUMAN SUBJECTS: EFFECTS OF ACTH ON PLASMA CRF

By

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ABSTRACT

Corticotrophin (ACTH) was administered to three patients with lipomatous diabetes (LD), one hypophysectomized LD patient and one control subject in an effort to simulate the internal feedback mechanism as measured by plasma corticotrophin releasing factor (CRF) activity. Plasma cortisol was determined at zero, five and eight hours after the beginning of ACTH infusion. Every LD patient showed a lack of adrenal response to ACTH as evidenced by the absence of significant increments in plasma cortisol levels. The control subject had a normal adrenal response to ACTH. Three of the four LD patients showed a reduction in plasma CRF levels after ACTH. The control subject had no detectable plasma CRF before or after ACTH. The reduction in plasma CRF accompanied by constant levels of plasma cortisol after ACTH administration suggests a direct regulatory effect of ACTH on CRF secretion. These data provide strong supportive evidence for the existence of an internal or 'short-loop' feedback system in human subjects in which the levels of plasma CRF appear to be regulated directly by blood levels of ACTH – presumably at the hypothalamic level.

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The internal or 'short-loop' feedback phenomenon (the ability of an anterior pituitary hormone to affect its respective hypothalamic releasing factor (RF) in the absence of peripheral target organ hormone intervention) has been adequately demonstrated in animals (Brodish & Long 1962; Chowers et al. 1967; Corbin et al. 1970; Martini et al. 1968; Seiden & Brodish 1971). Previous studies have demonstrated the absence of detectable levels of RF in plasma of intact animals (Brodish & Long 1962; Chowers et al. 1967; Corbin et al. 1970; Martini et al. 1968; Seiden & Brodish 1971), and humans (Corbin et al. 1971; Mabry et al., 1973; Upton et al., 1973) in contrast to elevated plasma levels found in hypophysectomized animals (Brodish & Long 1962; Chowers et al. 1967; Seiden & Brodish 1971) and hypophysectomized humans (Corbin et al. 1971; Mabry et al. 1973; Upton et al. 1973).

Lipoatrophic diabetes (LD) is a rare, genetically determined disease characterized by the loss of subcutaneous and other body fat, skeletal muscle overgrowth, hepatomegaly, genital enlargement, hyperlipaemia, hyperpigmentation with acanthosis nigricans and insulin-resistant hyperglycaemia. It has recently been shown that LD also presents as one of its characteristics, chronic hypersecretion of RF's (corticotrophin releasing factor (CRF) and follicle stimulating hormone releasing factor (FRF) (Mabry et al. 1973; Upton et al. 1973). The endocrine pathophysiology of patients with the LD syndrome presented us with an unusual opportunity to test the existence of the internal feedback phenomenon. This study was designed to determine whether the internal feedback mechanism existed in human subjects with LD in terms of corticotrophin (ACTH) control of CRF secretion.

**Materials and Methods**

Forty units of ACTH in 500 ml of isotonic saline was administered to three LD patients, one LD patient who had been hypophysectomized twelve months earlier and one control subject. The infusions were begun at 8 a.m. and continued for eight hours. Plasma samples were drawn at zero, five and eight hours during ACTH infusion for cortisol determination. Plasma cortisol was estimated using a fluorescence technique (Van der Vies 1961). Forextractions of CRF-like peptides, 30 ml of heparinized plasma was acidified with 1/10 volume of glacial acetic acid Trasylol (plasma inhibitor – 400 units/ml plasma) were added to each sample. The plasma specimens were stored frozen until extracted for CRF-like peptides as outlined in Fig. 1. Material that was not adsorbed to oxycellulose was pooled, desalted on Amberlite CG-50 (Dixon 1959) and lyophilized. The dried lyophilized extract was dissolved in 5 per cent acetic acid and subjected to successive Sephadex gel filtration. The eluted fractions were scanned by bioassay (vide infra) for CRF-like activity. The CRF-like material was found consistently in the area of small molecular size originally described by Guillemin et al. (1957), Schally et al. (1962), and Schally & Bowers (1964). The fractions to be assayed were dissolved in albumin (0.1 per cent) and saline (0.8 per cent) solution acidified with 0.1 n hydrochloric acid to pH 2 just before administration.
EXTRACTION OF PLASMA

EXTRACTION PROCEDURE FOR RF-ACTIVE SUBSTANCES IN PLASMA

Fig. 1.
Extraction procedure for RF-active substances in plasma.

Dose-response curves were performed on each sample at 25 µg, 50 µg and 75 µg. A minimum of 5 rats per dose was used with 2–3 replications, CRF assays were performed in pharmacologically-blocked rats by the method of Arimura et al. (1969), but modified by twelve-minute sampling and by estimation of adrenal content of corticosterone rather than peripheral corticosterone levels (Upton & Amatruda 1971), CRF is reported in milliunits (mU) per 100 ml. We have arbitrarily defined our CRF units in direct relation to ACTH-releasing activity – that is, 1 unit of CRF is that amount necessary to cause the effect of 1 unit of ACTH (100 units/milligram).

RESULTS

Table 1 shows the plasma cortisol values determined at zero, five and eight hours during ACTH infusion. The control subject showed approximately an eight-fold increase in plasma cortisol at the end of the infusion. The intact lipoatrophic diabetics showed no significant rise in plasma cortisol during or at the end of the infusion. The hypophysectomized LD (J. W.) showed a slight increase in plasma cortisol at 5-hours and a subsequent decrease at eight hours. Since J. W. had been given her usual supplementary therapy at 25 mg of cortisone at 8 a.m. and again at 4 p.m., the slight increment in plasma cortisol is interpreted as a reflection of the metabolism of the administered steroid rather than endogenous cortisol secretion resulting from adrenal stimulation.

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Table 1.
Plasma cortisol levels before, during and after 8-hour ACTH* infusion.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Condition</th>
<th>Cortisol levels (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Base-line</td>
</tr>
<tr>
<td>Control</td>
<td>Post-pubertal</td>
<td>13</td>
<td>Normal</td>
</tr>
<tr>
<td>L. F.</td>
<td>Pre-pubertal</td>
<td>6</td>
<td>Generalized LD</td>
</tr>
<tr>
<td>A. F.</td>
<td>Post-menopausal</td>
<td>58</td>
<td>Generalized LD</td>
</tr>
<tr>
<td>L. B.</td>
<td>Post-menopausal</td>
<td>60</td>
<td>Generalized LD</td>
</tr>
<tr>
<td>J. W.**</td>
<td>Pre-pubertal</td>
<td>13</td>
<td>Generalized LD-Hypophysectomized</td>
</tr>
</tbody>
</table>

* ACTH 40 units in 500 ml of isotonic saline.
** Supplementary cortisone therapy (25 mg) administered at 8 a.m. and 4 p.m.

Fig. 2 (upper portion) depicts the plasma cortisol levels before, during and after ACTH administration in relationship to plasma CRF levels (lower portion) of the same subjects. It can be readily seen from comparison of both upper and lower portions of Fig. 2 that under the influence of 40 units of ACTH administered intravenously over an 8 hour period, the plasma CRF levels of the LD patients were significantly reduced with concomitant absence of any significant fluctuation in plasma cortisol. The plasma CRF values are shown in Fig. 2 (lower portion). One LD child (L. F.) showed a complete reduction of plasma CRF activity to non-detectable levels after ACTH administration. One LD patient (A. F., age 58) showed no effect, while another patient (L. B., age 60) showed a 65% reduction in CRF values. The child with LD (J. W.) who had been hypophysectomized one year earlier, showed only a 50% reduction in CRF levels. The control subject had no detectable levels of plasma CRF before or after ACTH.

DISCUSSION

The method for plasma CRF extraction removes all other potentially interfering substances such as vasopressin and ACTH, in addition to the other pituitary hormones. Using an extract purified in this manner, the assay method is rendered specific for CRF-like substances. Synthetic luteinizing hormone releasing factor and thyrotrophin releasing factor are not active in this assay nor do they have any direct adrenal-stimulating ability in agreement with the
Comparison of the plasma cortisol levels with plasma CRF levels before, during and after the 8-hour infusion of 40 units of ACTH. The upper portion of this figure shows the plasma cortisol values (μg per 100 ml) before, during (5-hour) and after ACTH infusion (8-hour) of the LD patients (L. F., A. F., L. B.), the hypophysectomized LD patient (J. W.) and the control subject. The lower half of this figure depicts the plasma levels of CRF activity (milliunits per 100 ml) in the same LD patients and control as shown in the upper portion of this figure, determined simultaneously, before, and after the ACTH infusion. The number in parenthesis refers to the number of separate CRF assays and the horizontal bars at each point represent the standard error of the mean.

results of Koch et al. (1972). Based on the above information, we can be fairly certain that the material isolated from the plasma of these LD patients is a CRF-like substance. It is well-recognized that the plasma of these patients is hyperlipaemic. Again, the extraction method is so complete that it precludes interfering substances of this nature.

We feel it necessary to emphasize the point that the milliunits of CRF assigned to each plasma sample does not imply that these patients had equivalent amounts of ACTH. This assignation is arbitrary and emphasizes a potential biological potency. These patients have a pathophysiological state that has enabled us to demonstrate a basic theoretical concept that would not otherwise have been possible if their hypothalamic-pituitary-adrenal axis had been normal.
The data uniquely demonstrate that ACTH administered exogenously can directly affect the plasma levels of CRF. It remains an enigma that exogenous hormone could reduce the plasma RF levels in LD patients but that the existing levels of ACTH as indirectly indicated by the “high-normal” cortisol levels observed were ineffective. Since the six-year-old LD patient (L. F.) showed the greatest reduction in plasma CRF activity after ACTH, the question is raised as to whether those patients who have had the disease for longer periods become refractory to the feedback mechanism.

The constant secretion of CRF in lipoatrophic diabetes is not affected by an internal feedback control unless the pituitary hormone is administered exogenously in pharmacological amounts. It is possible that the existing ACTH levels present in LD patients may not be elevated because of the external feedback component exerted by “high normal” cortisol levels seen in these patients. The absence of adrenal response to exogenous ACTH would suggest that either the adrenal was insensitive to exogenous ACTH or that it was maximally stimulated at that time.

Since the levels of cortisol remained essentially the same in the LD’s throughout the ACTH infusion, the observed reduction in plasma CRF levels is interpreted as a direct effect of exogenously administered ACTH. These data provide strong supportive evidence for the existence of the internal feedback mechanism in human subjects in terms of ACTH control of CRF secretion. It is speculated that this control is exerted at the hypothalamic level.

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REFERENCES


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