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The Corticotropin-Releasing Hormone Stimulation Test: A Possible Aid in the Evaluation of Patients with Adrenal Insufficiency*

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ABSTRACT. Ten patients with adrenal insufficiency receiving chronic glucocorticoid therapy were studied. All had subnormal plasma cortisol responses to ovine corticotropin-releasing hormone (CRH) (1 μg/kg as an iv bolus) 12–60 h after discontinuation of steroid treatment. Plasma ACTH responses to CRH fell into three different patterns. The first three patients with primary adrenal insufficiency had high basal ACTH levels and augmented ACTH responses to CRH. A fourth such patient, however, treated with pharmacologic doses of prednisone, had a low normal ACTH response. Patients with secondary adrenal insufficiency had either low basal ACTH levels and diminished responses to CRH or low basal ACTH values but prolonged and augmented plasma ACTH responses to CRH with a delayed peak. We postulate that the group of patients with the former pattern have pituitary gland destruction whereas the patients with the latter pattern have hypothalamic CRH deficiency. Thus, CRH may be useful in differentiating between hypothalamic and pituitary causes of adrenal insufficiency. (*J Clin Endocrinol Metab* **58:** 1064, 1984)

CORTICOTROPIN-releasing hormone (CRH), a 41 amino acid peptide first isolated from ovine hypothalami, stimulates pituitary ACTH and β-endorphin secretion when given to rodents, subhuman primates, and man (1–9). It provides a safe means for studying patients with aberrant ACTH secretion (10). The purpose of this report is to present preliminary studies with CRH in patients with various forms of adrenal insufficiency and to determine its use as a diagnostic test in the evaluation of this condition.

Subjects and Methods

Subjects

Ten patients with adrenal insufficiency and 15 normal subjects (3 women, 12 men, ages 18–30 yr) were studied. The clinical profiles of the patients are summarized in Table 1. All were receiving chronic glucocorticoid therapy which was discontinued 12–60 h before testing. All patients and normal subjects were studied at the Clinical Center of the National Institutes of Health. Testing with CRH was performed after obtaining informed consent using a protocol approved by the NIH institutional review board and the National Center for Drugs and Biologics (protocol 82CH45, IND 19802).

CRH stimulation test

Ovine synthetic CRH was obtained from Bachem Co. (Torrance, CA). The initial preparation was purified by high pressure liquid chromatography, dissolved in water with 5% mannitol, sterilized by filtration (0.22 μ, Millipore, Bedford, MA), lyophilized, and placed into sterile vials under vacuum. The CRH content of each lot was verified by high pressure liquid chromatography and specific RIA. The vials were refrigerated at 4 C. Sterile water was used as diluent and was added immediately before administration.

CRH was given as an iv bolus injection of 1 μg/kg at 2000 h. No subject experienced changes in heart rate or blood pressure during the test. Blood was drawn at −15, 0, 5, 15, 30, 60, 90, 120, 150, and 180 min for measurement of ACTH, cortisol, and CRH.

Blood for ACTH and CRH determination was collected in prechilled glass tubes containing EDTA. The samples were immediately placed on ice. Plasma was separated from red cells within 3 h of collection. Blood for cortisol determination was collected in heparinized glass tubes, centrifuged at the end of

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TABLE 1. Patient clinical profiles

Patient	Age (yr)	Sex	AI	Diagnosis	Comments	Off Rx (h)
1	29	M	P	Congenital adrenal hypoplasia	Hydrocortisone (40 mg/day)	34
2	26	M	P	Adrenomyeloneuropathy	Hydrocortisone (24 mg/day)	27
3	19	M	P	Adrenomyeloneuropathy	Hydrocortisone (30 mg/day)	24
4	36	M	P	MAE type I	Prednisone (30 mg/day)	12
5	26	F	S	Pituitary adenoma, post resection	Hydrocortisone (20 mg/day)	34
6	36	F	S	Pituitary-hypothalamic sarcoidosis	Prednisone (10 mg/day, 20 mg/day alternate days)	60
7	33	F	S	Pituitary adenoma, post resection	Hydrocortisone (30 mg/day)	24
8	23	F	S	Hypothalamic dermoid cyst, post resection	Hydrocortisone (25 mg/day)	12
9	56	M	S	Suprasellar chromophobe adenoma, post resection, post CR 3900 rad	Hydrocortisone (25 mg/day)	12
10	33	M	S	Sellar and supra sellar germinoma, post resection	Hydrocortisone (27.5 mg/day)	12

AI, Adrenal insufficiency; P, primary; S, secondary; MAE, multiple autoimmune endocrinopathy; CR, cranial radiation.

the test, and plasma was separated from red cells the following morning. All plasma samples were placed in capped polypropylene vials and frozen at −20 C until assayed.

Hormone assays

Plasma cortisol was measured by RIA as previously described (11). The detection limit was 0.2 μg/dl and the intra- and interassay variability were 4.6% and 6.0%, respectively. Plasma for measurement of ACTH was extracted and concentrated on Octa-DecaSilicaGel cartridges (Sep-PAK C 18, Waters Assoc., Milford, MA), lyophilized, and reconstituted in assay buffer (0.063 Na_2HPO_4, 0.013 M Na_2EDTA, pH 7.4, containing 0.02%, NaN_3, 0.1% Triton X-100, and 250 kallikrein inhibiting units/ml Trasylol). The antibody was obtained from IgG Corporation, Nashville, TN (IgG ACTH-1). The reconstituted samples were then measured by RIA as previously described (12). The detection limit for this assay ranged between 3–5 pg/ml. Intra- and interassay variability were 4.4% and 19.7%, respectively.

Plasma CRH was measured as follows: CRH was iodinated with the chloramine-T method (13). The SA did not exceed 120 μCi/μg. ^{125}I-CRH was separated from free ^{125}I on a Sephadex G50-column (Pharmacia, Piscataway, NJ). Antisera were generated against CRH in female New Zealand white rabbits using CRH conjugated to BSA by the carbodiimide reaction (14). The assay mixture contained antiserum HS 20 at a final dilution of 1:180,000 in assay buffer (0.063 M Na_2HPO_4, 0.013 M Na_2EDTA, pH 7.4, containing 0.02% NaN_3, 0.1% Triton X-100, and 250 KIU/ml Trasylol). The antibody was first allowed to react with the unknown plasma samples (0.1 ml) for 24 h and then ^{125}I-CRH was added. Antibody-bound and free CRH were separated by a second antibody method using goat anti-rabbit antiserum at 1:20 dilution. The detection limit for this assay ranged between 5–7 pg/ml when plasma was extracted and concentrated as mentioned above for ACTH. The intra- and interassay coefficients of variation were 5% and 13%, respectively.

Statistical methods

Individual patient responses are plotted. The response curve from normal subjects is expressed by the area included by the range or by the mean ± 1 SD. The RIA data were analyzed by a computerized best fit logit log analysis (15).

Results

All 10 patients studied had low basal plasma cortisol levels and diminished plasma cortisol responses to CRH (Fig. 1). Three patients with primary adrenal insufficiency (Table 1) had high baseline plasma ACTH concentrations and exaggerated ACTH responses to CRH (Fig. 1, *left*). Another patient with primary adrenal insufficiency (patient 4), who was receiving pharmacologic doses of prednisone (Table 1), had low baseline ACTH levels and a low ACTH response (Fig. 1, *left*).

Patients with secondary adrenal insufficiency (Table 1) fell into two groups: those having poor ACTH responses to CRH and those having prolonged and augmented ACTH responses associated with a delayed peak (Fig. 1, *right*).

All patients had immunoreactive CRH (IR-CRH) disappearance curves from plasma similar to those of normal subjects. The results of patients with secondary adrenal insufficiency are shown in Fig. 2.

Discussion

Stimulation with CRH produced three patterns of plasma ACTH response when given to patients with adrenal insufficiency. Patients with primary adrenal insufficiency, except one who was receiving high dose prednisone therapy, had high basal plasma ACTH concentrations and augmented responses to CRH. Lack of cortisol negative feedback, and possibly hypertrophy or hyperplasia of the corticotrophs may account for these findings. Patients with secondary adrenal insufficiency, on the other hand, had either diminished plasma ACTH responses or prolonged and augmented responses to CRH with a delayed peak. The different ACTH response pat-

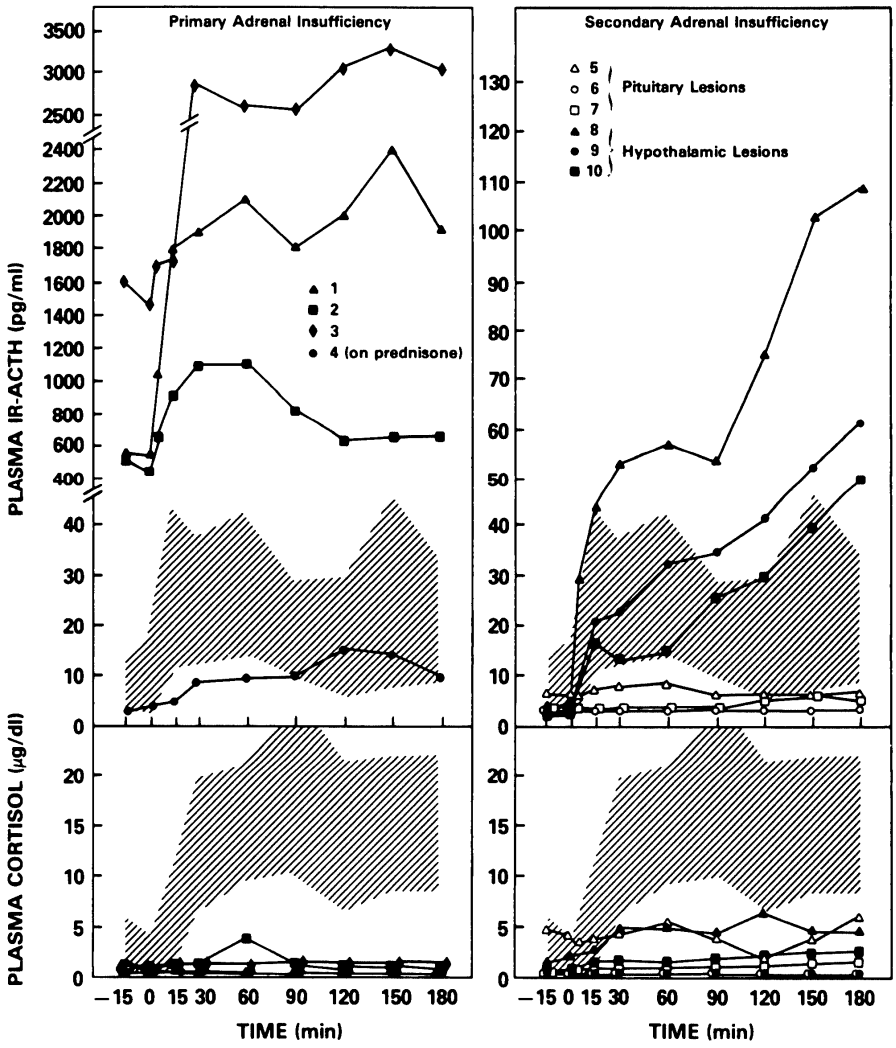

FIG. 1. Plasma ACTH (*top*) and cortisol (*bottom*) responses to CRH in subjects with primary adrenal insufficiency (*left*) or secondary adrenal insufficiency (*right*). Patients with hypothalamic lesions had clearly distinct ACTH responses to CRH, different from those in three patients with pituitary adrenal insufficiency. *Shaded area*: Absolute range from 15 normal subjects.

terns to CRH cannot be explained by different clearance rates of CRH in view of the similar IR-CRH plasma disappearance curves.

The two patterns of anterior pituitary hormone response to a releasing factor in patients with secondary endocrine insufficiency were reported previously in patients with secondary hypothyroidism or hypogonadism who were given TRH or GnRH, respectively. Characteristically, in these disorders a diminished or low normal response was associated with a pituitary lesion, whereas a normal or delayed and augmented response was associated with hypothalamic or pituitary stalk damage (16–18). However, the discriminatory power of a single iv bolus dose of TRH or GnRH has been poor, and these tests have been useful only when stimulation with the releasing hormone was performed repetitively to permit priming of the pituitary response (16, 19).

The anatomical categorization of the patients with secondary adrenal insufficiency is somewhat arbitrary since tumors in the hypothalamic-pituitary region could have damaged both structures in patients 6 and 10. The ability of CRH to stimulate ACTH release in patient 10 suggests that adrenal insufficiency in this patient cannot be explained on the basis of pituitary destruction, and thus, must have arisen predominantly from hypothalamic damage. In patient 6 the absence of a response to CRH might be explained by pituitary corticotroph destruction or by corticotroph cell atrophy and lack of priming from hypothalamic dysfunction. Pituitary destruction seems the most likely explanation in view of the brisk ACTH response to a single CRH bolus in the other patients who had secondary adrenal insufficiency associated with hypothalamic lesions.

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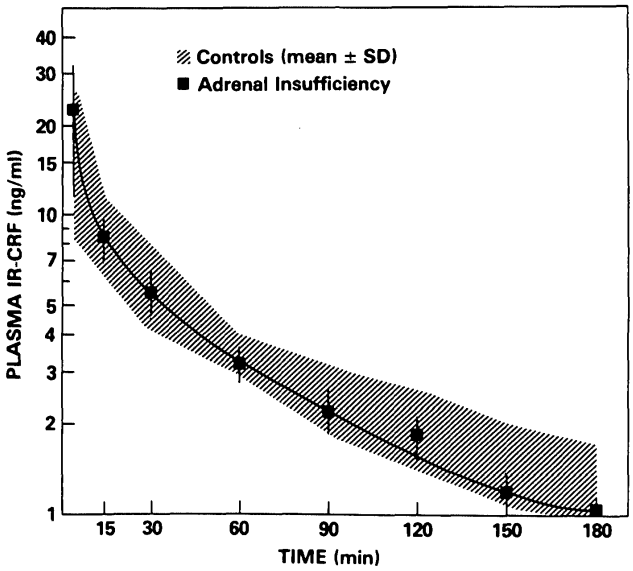

FIG. 2. Disappearance *curve* of immunoreactive CRH from plasma in the patients with secondary adrenal insufficiency (mean ± SE). Shaded area, mean ± 1 SD from 10 normal subjects.

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