

Comparison of Adrenocorticotropin and Adrenal Steroid Responses to Corticotropin-Releasing Hormone *Versus* Metyrapone Testing in Patients with Hypopituitarism

LINDA RIDDICK, GEORGE P. CHROUSOS, SUSAN JEFFRIES, AND SONGYA PANG

Department of Pediatrics, University of Illinois, College of Medicine at Chicago, Chicago, Illinois 60612 [L.R., S.J., S.P.], and National Institutes of Health, Developmental Endocrinology Branch, Bethesda, Maryland 20892 [G.P.C.]

ABSTRACT

We compared the responses of ACTH and cortisol (F) to corticotropin-releasing hormone (CRH) administration (ovine 1 $\mu\text{g}/\text{kg}$ i.v. bolus) with the responses of urinary 17-OH corticosteroids (17-OHCS) and serum deoxycorticosterone (DOC) to metyrapone administration (450 mg/ m^2 /dose every 4 h \times seven doses) in 16 hypopituitary patients. Glucocorticoid therapy for these patients was withheld for a minimum of 3 wk before testing. The CRH test was performed 3 d before or 3 wk after the metyrapone test was used to diagnose the ACTH reserve status. In nine ACTH-intact hypopituitary patients (postmetyrapone 17-OHCS $>$ 12.2 $\mu\text{mol}/\text{m}^2/\text{d}$; DOC \geq 11.5 nmol/L), the peak F (497–773 nmol/L) and ACTH (5.2–22 pmol/L) responses to CRH stimulation were similar to those of normal subjects (F peak = 554–993 nmol/L and ACTH peak = 6–25 pmol/L at 15–60 min). In one patient with partial ACTH deficiency (postmetyrapone 17-OHCS = 10.5 $\mu\text{mol}/\text{m}^2/\text{d}$; DOC = 6 nmol/L), the peak F response was low and delayed (246 nmol/L at 180 min) and the peak ACTH response was normal (7 pmol/L). Six severely ACTH-deficient patients (postmetyrapone 17-OHCS $<$ 5.4 $\mu\text{mol}/\text{m}^2/\text{d}$; DOC \leq 3.4 nmol/L) had a low F

response at 15–90 min in all, with a delayed rise in three at 120–180 min in response to CRH administration, whereas ACTH responses were variable: absent or low, normal, delayed, or persistently exaggerated. In conclusion, the CRH-stimulated F response pattern in hypopituitary patients was comparable to the urinary 17-OHCS and serum DOC response to metyrapone administration. Thus, F response pattern to CRH was useful in the evaluation of ACTH reserve in hypopituitary patients. ACTH response to CRH in ACTH-deficient patients was not consistently useful for ACTH reserve evaluation because of the variable response possibly resulting from a different etiology (hypothalamus *versus* pituitary) of ACTH deficiency. (*Pediatr Res* 36: 215–220, 1994)

Abbreviations

CRH, corticotropin-releasing hormone
F, cortisol
17-OHCS, 17-hydroxycorticosteroid
DOC, deoxycorticosterone
DDAVP, desmopressin

Distinct patterns of hypothalamic-pituitary-adrenal axes function during CRH stimulation have been characterized in Cushing's disease or syndrome and in primary adrenal insufficiency (1–6). Hyperresponse of ACTH and F in Cushing's disease and low or minimal response of ACTH and F in Cushing's syndrome to CRH administration assisted in the differentiation of etiology (1–5). The CRH stimulation test has also been proposed as a means of distinguishing either

primary or secondary adrenal insufficiency due to hypothalamic or pituitary dysfunction (6–10). However, no comparison has been reported between the ACTH and adrenocortical steroid response to the CRH stimulation test *versus* a standard ACTH provocative test, via metyrapone administration, in patients with hypopituitarism.

The aim of this study was to investigate whether the CRH stimulation test would accurately reflect pituitary-adrenal function in the evaluation of ACTH reserve compared with metyrapone testing in patients with one or more pituitary hormone deficiencies. The responses of plasma ACTH and F to CRH were therefore compared with hormonal responses to oral metyrapone administration in patients with hypopituitarism.

Received September 8, 1993; accepted March 18, 1994.

Correspondence and reprint requests: Dr. Songya Pang, University of Illinois, Department of Pediatrics (M/C 856), 840 South Wood St., Chicago, IL 60612.

Supported in part by USPHS Grant ROI HD 24360, a grant from Washington Square Health Foundation at Chicago, and a biomedical research grant from the University of Illinois College of Medicine, Chicago.

METHODS

All studies were performed under institutionally approved protocols after informed consent was obtained from normal volunteer subjects, patients, their parents or legal guardians, and, whenever possible, from the minors if they understood the test purpose.

Patients. Sixteen patients with proven single or multiple pituitary hormone deficiencies were studied (Table 1). The hypopituitary subjects, eight females and eight males, ranged in chronologic age from 2 to 18 y. Five patients had isolated growth hormone deficiency, and 11 had multiple hormone deficiencies, including growth hormone, TSH, LH, FSH, and antidiuretic hormone. The treatment status of hypopituitarism in these patients is indicated in Table 1. Eight patients were receiving growth hormone therapy at the time of testing. Two patients with antidiuretic hormone deficiency were treated with DDAVP (5–10 µg/d) in doses that resulted in normal electrolyte and water balance. Eight patients received L-thyroxine (75–150 µg/d) and were euthyroid at the time of testing. Four patients received occasional hydrocortisone therapy only for stressful events; however, no hydrocortisone was given at least 8 wk before testing in these patients. One patient received maintenance hydrocortisone therapy (25 mg/d), which was discontinued 3 wk before testing. All patients with hypopituitarism had metyrapone and CRH stimulation tests. Control subjects were six healthy female and three male volunteers, aged 15–45 y, who underwent CRH stimulation testing only.

Metyrapone provocative test. ACTH reserve secretory capacity was determined by the response of urinary 17-OHCS excretion and serum DOC to oral metyrapone

administration (450 mg/m²/dose every 4 h for seven doses) (Table 1). Urine samples were collected for 24 h preceding, during, and for 24 h after metyrapone administration for the determination of urinary 17-OHCS excretion. Blood samples were obtained for DOC in the morning of the baseline day and again in the morning after the 7th dose of metyrapone.

Definition of ACTH reserve status. The patients whose urinary 17-OHCS excretion rose to greater than 2.5 times the baseline levels, demonstrated a marked rise in serum DOC (11.5–85.3 nmol/L), or both after seven doses of metyrapone were defined as having adequate ACTH reserve using a normal response reported previously (cases 1–9, Table 1) (11, 12). The patients whose urinary 17-OHCS and serum DOC (<0.3–3.4 nmol/L) responses to metyrapone were minimal or absent were classified as having severe ACTH deficiency (cases 11–16, Table 1). Partial ACTH deficiency was diagnosed in one patient (case 10, Table 1) based on the modest rise in urinary 17-OHCS and serum DOC (6 nmol/L) levels, which were greater than those in the severe ACTH deficiency patients but lower than in ACTH-intact patients.

CRH stimulation test. The test was conducted at 1300–1600 h after a half-hour rest in the recumbent position and at least 3 d before or 3 wk after metyrapone testing. A slow i.v. bolus of 1 µg/kg of ovine CRH was given over a few minutes. Two subjects experienced short-lasting hot flashes within a few minutes after CRH administration. Blood samples were drawn at –15, 0, 15, 30, 60, 90, 120, 150, and 180 min for measurement of serum ACTH and F. In young children, blood samples were collected less frequently. Blood samples for measurement of

Table 1. Clinical and hormonal data in patients with hypopituitarism at ACTH reserve evaluation*

Case	Sex	Age (y)	Etiology	Hormone deficiency	Treatment	Urinary 17-OHCS (µmol/m ² /24 h)		Serum DOC (nmol/L)	
						Baseline	Postmetyrapone	Baseline	Postmetyrapone
1	F	2	Postremoval craniopharyngioma	GH	GH	0.14	18.9		30.9
2	F	11	Idiopathic	GH	GH	1.9	14.6	0.4	19.7
3	M	14	Idiopathic	GH	GH	4.6	19.7	0.7	47.9
4	F	7.3	Idiopathic	GH	GH	4.1	39.7	0.5	23.4
5	F	13.5	Idiopathic	GH, ADH	GH	10.5	41.9	3.1	85.3
6	F	12	Idiopathic	GH	GH	11.6	26.2	0.7	33.6
7	M	11	Septooptic dysplasia	GH, TSH	GH, T ₄	9.7		0.8	17.4
8	M	13	Idiopathic	GH, TSH, LH, FSH	T ₄	5.9	13	0.06	11.5
9	M	14	Idiopathic	GH, LH, FSH	NONE	5.7	13.5		
10	F	14	Postremoval craniopharyngioma	GH, TSH, LH, FSH	T ₄	4.6	10.5	0.2	6.0
11	F	12	Septooptic dysplasia	GH, TSH, ADH	GH, T ₄ , DDAVP	7.3	4.9	<0.3	<0.3
12	F	17	Postremoval craniopharyngioma	GH, TSH, ADH, LH, FSH	T ₄ , DDAVP	3.5	2.7	0.06	1.5
13	M	14	Idiopathic	GH, TSH, LH, FSH	T ₄	0.8	1.4	0.24	3.4
14	M	14	Idiopathic	GH, TSH, LH, FSH	T ₄	2.2	2.7	0.06	1.7
15	M	18	Postremoval craniopharyngioma	GH, TSH, LH, FSH	T ₄	3.2	5.1	0.06	0.7
16	M	18	Idiopathic	GH, TSH, LH, FSH	T ₄	3.5	3.5	0.06	0.4

* GH, growth hormone; ADH, antidiuretic hormone; T₄, L-thyroxine. To convert 17-OHCS from µmol/24 h to mg/24 h, multiply by 0.362; to convert DOC from nmol/L to ng/dL, multiply by 33.3. Cases 1–9 have intact ACTH reserve; case 10 has partial ACTH deficiency; cases 11–16 have severe ACTH deficiency.

ACTH were collected in chilled glass tubes containing EDTA. Blood samples were immediately centrifuged for separation of plasma and serum, placed into polypropylene vials, and frozen at -20°C until assayed.

Laboratory methods. Samples from an individual were analyzed simultaneously. Serum DOC and F were determined by RIA after chromatographic purification of the sera (12–14). RIA for ACTH was performed using previously described RIA methods (3). The lowest limit of detection was 0.8 nmol/L for F RIA and <0.06 nmol/L for DOC RIA. The intraassay and interassay coefficients of variation for F and DOC assays were 5–10% and 8–15%, respectively. The lowest limit of detection for the ACTH assay was 1.1 pmol/L, and the intraassay and interassay coefficients of variation were 3% and 5%, respectively. Urinary 17-OHCS was measured by a colorimetric method.

RESULTS

Responses of serum F and plasma ACTH to CRH administration in control normal subjects are shown in Figures 1 and 2, respectively. The mean baseline F level (-15 and 0 min) was 359 nmol/L (range: 110–497 nmol/L) in normal subjects and rose to a mean peak of 773 nmol/L (range: 554–993 nmol/L) between 30 and 60 min after CRH administration (Fig. 1A). The mean baseline ACTH level (-15 and 0 min) was 5.6 pmol/L (range: 3.4–10.2 pmol/L) and rose to a mean peak of 12.6 pmol/L

(range: 6.3–25.2 pmol/L) between 15 and 60 min after CRH administration (Fig. 2A).

In nine hypopituitary patients with intact ACTH reserve, as determined by the metyrapone test (cases 1–9, Table 1), the baseline F and ACTH levels were within the normal range (Figs. 1B and 2B). The mean baseline F level was 304 nmol/L (range: 110–607 nmol/L) and rose to a mean peak of 607 nmol/L (range: 497–773 nmol/L) between 30 and 60 min in most patients, which was similar to the response in normal subjects (Fig. 1A and B). The mean baseline ACTH level was 6.1 pmol/L (range: 4.3–10.1 pmol/L) and rose to a mean peak response of 11.7 pmol/L (range: 5.2–22 pmol/L) between 15 and 60 min in most patients. ACTH response to CRH in all except one patient with intact ACTH secretion was essentially in the normal range (Fig. 2B). One patient's ACTH response was below the normal range between 30 and 180 min; however, the peak response at 15 min was in the normal range.

In six severely ACTH-deficient hypopituitary subjects (cases 11–16, Table 1), the baseline serum F level was low (mean/range: 3.3/2.8–80 nmol/L) compared with the level in control and ACTH-intact hypopituitary patients. The F response to CRH administration in this group was lower during the first 15 to 60 min in all subjects and showed a delay in increase at 90–180 min in three subjects compared with the response in normal subjects and ACTH-intact hypopituitary patients (Fig. 1C). ACTH

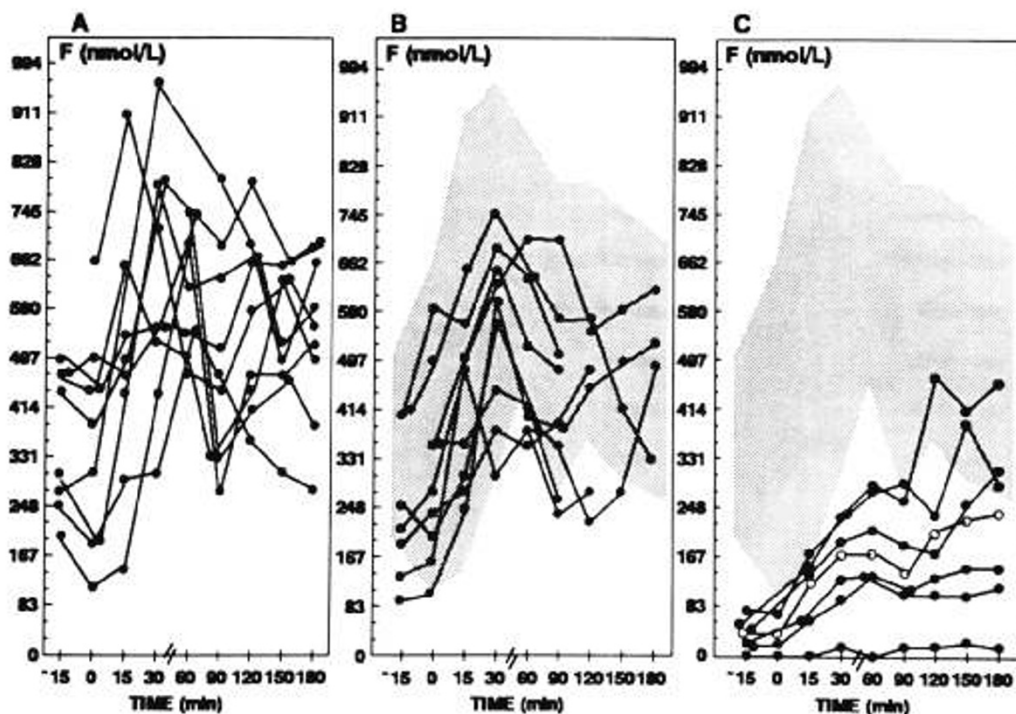


Figure 1. Serum F response to CRH stimulation in normal subjects and hypopituitary patients. *A* represents the response in normal subjects; *B* represents the response in ACTH-intact hypopituitary patients; and *C* represents the response in ACTH-deficient patients. The shaded area in *B* and *C* represents the response in normal subjects. Filled circles indicate severely ACTH-deficient patients, and open circles indicate a patient with partial ACTH deficiency. State of ACTH reserve was determined by a standard metyrapone stimulation test. The lines connect individual values. To convert F levels from nmol/L to $\mu\text{g/dL}$, divide by 27.6.

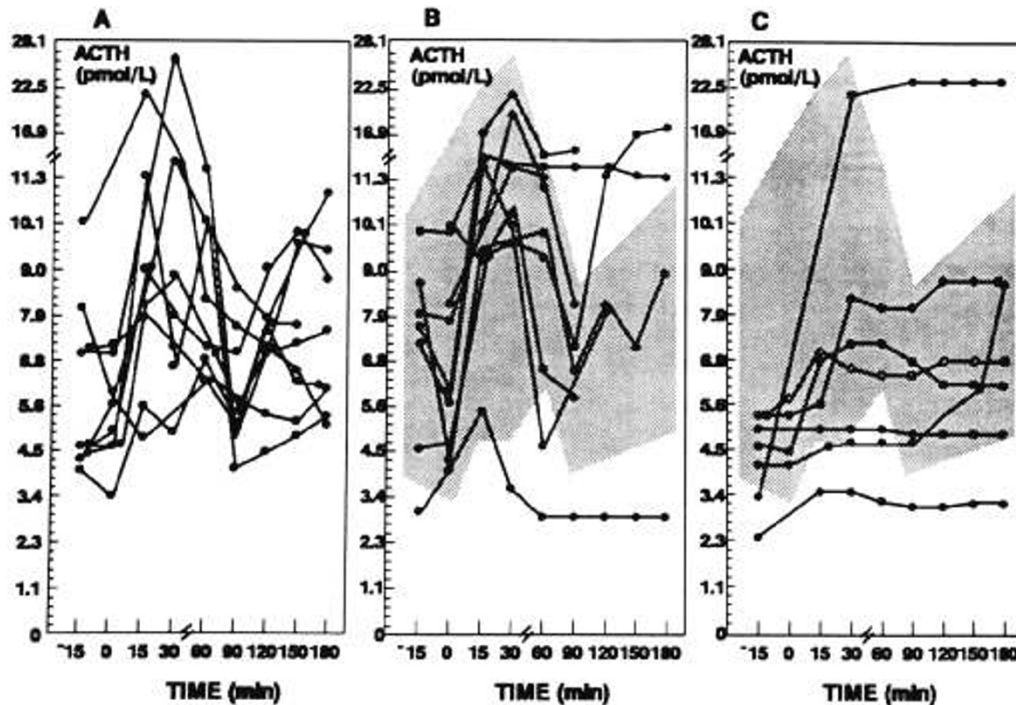


Figure 2. Plasma ACTH response to CRH stimulation in normal subjects and hypopituitary patients. *A* represents the response in normal subjects; *B* represents the response in ACTH-intact hypopituitary patients; and *C* represents the response in ACTH-deficient patients. The shaded area in *B* and *C* represents the response in normal subjects. Filled circles indicate severely ACTH-deficient patients, and open circles indicate a patient with partial ACTH deficiency. State of ACTH reserve was determined by a standard metyrapone stimulation test. The lines connect individual values. To convert ACTH levels from pmol/L to pg/mL, multiply by 4.5.

levels in these patients rose from a mean baseline of 3.8 pmol/L (range: 2.5–5.4 pmol/L) to a mean peak of 9.5 pmol/L (range: 3.4–24.3 pmol/L at 15–180 min) in response to CRH (Fig. 2C). In these subjects, peak ACTH responses were either noticeably lower (cases 13 and 15), normal (cases 14 and 16), delayed (case 12), or persistently elevated (case 11) compared with ACTH-intact hypopituitary patients and normal subjects.

In one patient (case 10, Table 1) with partial ACTH deficiency, the baseline F level was lower (39 nmol/L) than the levels in ACTH-intact hypopituitary subjects and normal controls; peak F response to CRH was also low and delayed (246 nmol/L at 180 min) (Fig. 2C). In this patient, both the peak ACTH response (7 pmol/L) and the overall ACTH response to CRF were normal (Fig. 2C).

DISCUSSION

Accurate assessment of the integrity of the hypothalamic-pituitary-adrenal axes requires a test that is sensitive, safe, and practical. No one test as yet fulfills this requirement (15). Often, multiple tests are necessary to unequivocally determine ACTH reserve status. This is of particular concern for children in whom multiple testing is not always practical or safe. In this study, we examined the usefulness of the CRH stimulation test for evaluation of the integrity of the hypothalamic-pituitary-adrenal axes. Based on F response, we found that the CRH stimulation test is a safe and efficient method of determining the ACTH reserve in children with hypopituitarism.

As a standard for comparison, a multiple-dose metyrapone test using urinary 17-OHCS excretion and serum DOC response was used to accurately assess ACTH reserve (11, 16). The shorter, more widely used single-dose metyrapone test (17) was not used in this study because very mild or subtle forms of ACTH deficiency may not be detected by this test (15) and reported data of normal subjects were limited to adults (18, 19). The longer, multiple-dose metyrapone test would allow a more definitive assessment of ACTH reserve status for comparison with CRH testing. CRH testing was performed during the afternoon hours, because it has been established that the greatest incremental F response to CRH occurs during the afternoon hours (20, 21). The normal and low baseline F levels in hypopituitary patients corroborated with the ACTH reserve status determined by metyrapone test as being an ACTH-intact or -deficient state, respectively. Thus, the baseline 1300-h F levels seem to be useful for assessing ACTH reserve status. The CRH-stimulated F level in patients with an intact ACTH reserve, as determined by the metyrapone test, was similar to that of normal controls and corroborated previous reports of CRH stimulation in normal subjects (21). The peak F response in both normal and ACTH-intact hypopituitary patients occurred between 30 and 60 min after CRH administration. In all ACTH-deficient subjects, including severely and partially ACTH-deficient patients, the F response to CRH was below the normal range within the 60-min response and a

few subjects had delayed peaks between 90 and 180 min. This F response was comparable to the urinary 17-OHCS and serum DOC responses to metyrapone administration in these patients. Thus, F response evaluation during the first hour (at 30 and 60 min) after CRH administration is useful in distinguishing ACTH deficiency from ACTH-intact hypopituitarism.

The plasma ACTH response to CRH in hypopituitary patients was variable and did not correspond with the urinary 17-OHCS or serum DOC response to metyrapone administration in any ACTH reserve status group. In ACTH-intact hypopituitary subjects, the peak ACTH response to CRH was observed at 15–60 min and was similar to that of controls. Several investigators have reported a similar plasma ACTH response to CRH in normal subjects (20, 22). The variable ACTH response to CRH in either partially or severely ACTH-deficient hypopituitary subjects may reflect the various pathologic sites of ACTH deficiency. Poor ACTH response to CRH stimulation in subjects with secondary adrenal insufficiency has been associated with primary pituitary dysfunction (6), and persistently elevated or delayed ACTH responses to CRH in ACTH-deficient subjects have been interpreted as “primary CRH deficiency” (4, 6, 7). A low ACTH response to CRH in two ACTH-deficient subjects with either resected craniopharyngioma or idiopathic panhypopituitarism in our study suggests primary pituitary failure. Persistently elevated, normal, or delayed ACTH response to CRH in five patients with idiopathic hypopituitarism, septooptic dysplasia, or resected craniopharyngioma was suggestive of partial CRH deficiency. Synergism between vasopressin and CRH has been documented (23, 24). Two of the patients with ACTH deficiency received maintenance DDAVP treatment at the time of CRH testing; their ACTH response was elevated or delayed, although their F response was low. ACTH response to CRH in these patients was not likely influenced by DDAVP, because DDAVP does not bind to the receptor for vasopressin and does not stimulate ACTH release (25).

Neither the multiple-dose nor the single-dose metyrapone test distinguishes a hypothalamic *versus* pituitary cause of ACTH deficiency, and either dose may cause symptoms of adrenal insufficiency during the test. Furthermore, the conventional multiple-dose metyrapone test is cumbersome, requiring urine collections and hospitalization. The insulin provocative test entails risk of severe hypoglycemia in hypopituitary patients. The adrenal stimulation test by exogenous ACTH administration is a simple, practical method of screening for adrenal insufficiency. It is, however, at best an indirect method of assessing hypothalamic-pituitary-adrenal axis function, and patients with mild or early-stage ACTH deficiency may respond normally to the exogenous ACTH stimulation (15, 26).

Simultaneous evaluation of F and ACTH response during the first hour of the CRH stimulation test may be advantageous in the assessment of ACTH reserve as well

as differentiation of hypothalamic *versus* pituitary causes of ACTH deficiency. This differentiation may be important, inasmuch as restoration of normal F secretion was possible in corticotropin-sparing CRH-deficient patients by administration of CRH (27, 28), and the potential for a CRH analog as a therapeutic agent exists for CRH-deficient hypopituitary patients. The outpatient testing method and safety of the CRH test are advantageous in its use in children for evaluation of ACTH reserve. Thus, we conclude that evaluation of F response to CRH is a useful and safe screening test for ACTH reserve in addition to providing information regarding the hypothalamic *versus* pituitary etiology for ACTH deficiency.

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