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Early Adrenal Hypofunction in Patients with Organ-Specific Autoantibodies and No Clinical Adrenal Insufficiency

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ABSTRACT

Idiopathic Addison's disease occurs frequently in association with other organ-specific autoimmune diseases, and autoantibodies to adrenal cortex are markers of this condition. A variable asymptomatic period with subtle adrenal dysfunction may precede the onset of clinical manifestations. We studied the pituitary-adrenal axis by measuring plasma ACTH, cortisol, and 17α-hydroxyprogesterone after ovine CRH (100 μg as an iv bolus) stimulation in 19 patients with organ-specific autoimmune disease and adrenal autoantibodies, in whom adrenal steroids were normal under baseline conditions and normally responsive to a standard ACTH stimulation test (250 μg). In all subjects, oCRH produced a normal increase in plasma ACTH. Plasma cortisol, which was normoresponsive in 11 subjects, showed little or no increase in 8 subjects. Two of these patients developed overt adrenal failure after 1 yr. The 17α-hydroxyprogesterone response to oCRH, tested in 10 of 19 patients, paralleled that of plasma cortisol, excluding a steroidogenic block at the 21-hydroxylase site. Our data demonstrate the existence of a very early phase of Addison's disease in which adrenal function shows an impaired response to ovine CRH-stimulated ACTH. (*J Clin Endocrinol Metab* **79:** 452–455, 1994)

INITIAL adrenocortical insufficiency presents with very few, fluctuating, and nonspecific symptoms. In this subclinical stage, hormonal abnormalities can be recognized only by an accurate endocrinological evaluation, with periodic testing of cortisol reserve (1–8). This is of particular importance in organ-specific autoimmune diseases, in which a variable subclinical period is marked only by the presence of specific autoantibodies (9). In asymptomatic patients with adrenal autoantibodies (AA), a subtle adrenal impairment may precede the onset of clinical manifestations (10–14). It has been clearly proved that the ACTH response to an ovine (o) CRH stimulation test is influenced by basal cortisol levels and their subsequent increase; *i.e.* in the absence of cortisol feedback inhibition, it should be abnormally high (15, 16). Only scarce data (16–18) have been published on plasma ACTH and cortisol responses to oCRH in patients with Addison's disease, and to our knowledge, no data are available in patients at the earliest phase of adrenal dysfunction. A child with type 1 autoimmune polyglandular syndrome, AA, and no clinical evidence of adrenal failure had an increased ACTH response to oCRH in the face of an attenuated cortisol response (19). However, the cortisol response to ACTH in this patient was subnormal, indicating partial adrenal insufficiency. To investigate this issue, we examined the adrenal response to both standard ACTH and oCRH stimulation tests in patients with AA and no signs of adrenal insufficiency. In the light of recent reports indicating the 21-hydroxylase complex as the major antigen recognized by autoantibodies in Addison's disease (20, 21), plasma 17α-hydroxyprogesterone was measured to verify whether AA may cause an enzymatic block of adrenal steroidogenesis.

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Subjects and Methods

Nineteen patients (all female; age range, 23–56 yr) affected by organ-specific autoimmune disease and with AA were studied. Ten patients with hypothyroidism due to Hashimoto's thyroiditis (HT), 2 with insulin-dependent diabetes mellitus (IDDM), 2 with hypothyroidism due to HT and IDDM, and 1 with hypothyroidism due to HT and premature ovarian failure were tested during replacement therapy. Three patients with hyperthyroidism due to Graves' disease were receiving treatment with methimazole. One patient had vitiligo, which required no treatment. All patients were in biochemical and hormonal balance at the time of the study. In particular, patients with thyroid dysfunction had been euthyroid with treatment for at least 4 months. No signs or symptoms of adrenal dysfunction were observed at clinical examination at the time of the study. Routine chemistry, including sodium and potassium values, was normal in all cases, as were baseline PRA, ACTH, cortisol, aldosterone, and 17α-hydroxyprogesterone. Patients with menses were tested in the follicular phase. Ten healthy subjects, negative for AA and matched for age and sex, were selected as normal controls. Patients were divided in two groups, *i.e.* responders and nonresponders, according to cortisol response to oCRH. In individual patients no response was defined as a cortisol increase at a peak level less than 20% with respect to the 0 min value. This was based on the minimum percent increment in cortisol at the peak level in normal subjects (at least 50% of baseline). According to this strict criterion for responsiveness, 8 patients were classified as nonresponders. Among the other 11 patients classified as responders, 9 had a cortisol increase above 50%, and 2 had a cortisol increase ranging from 20–50%. Informed consent was obtained from all subjects.

Immunological study

Organ-specific autoantibodies (thyroid microsomal, thyroglobulin, gastric parietal cell, islet cell, adrenal and steroid-producing cell) were determined using the standard indirect immunofluorescence technique

on an unfixed cryostat section of normal human tissues or by passive hemoagglutination, as described previously (22). Positive sera were titrated by doubling dilution to the end point.

Functional adrenal study

Tests were performed in the morning at 0900 h, with subjects maintaining a supine position after overnight fasting. Plasma samples were collected for cortisol, aldosterone, and 17α-hydroxyprogesterone measurements under basal conditions and 60 min after the administration of ACTH-(1–24) (Synacthen, Ciba, Basel, Switzerland; 250 μg as an iv bolus). On a different day, ACTH, cortisol, and 17α-hydroxyprogesterone were tested at 15 min before and 0, 15, 30, 45, 60, 90, and 120 min after treatment with oCRH (Novachem, Läufelfingen, Switzerland; 100 μg as an iv bolus). 17α-Hydroxyprogesterone was measured in 10 of 19 patients and in 10 normal subjects. All patients are still being followed, with complete immunological and hormonal evaluation every 6 months.

Immunoassays

Plasma ACTH was measured by a two-site immunoradiometric assay, supplied by Euro-Diagnostic [Amsterdam, Holland; normal values, 4–18 pmol/L; intra- and interassay coefficients of variation (CVs), 4.8% and 8.8%, respectively]. The lower limit of sensitivity of this assay is 2.0 pmol/L. Plasma cortisol was measured by a RIA kit from Diagnostic Products Corp. (Los Angeles, CA; normal values, 138–550 nmol/L; intra- and interassay CVs, 4.1% and 5.0%, respectively). Plasma aldosterone was measured with a RIA kit supplied by Sorin (Vercelli, Italy; normal supine values, 80–280 pmol/L; intra- and interassay CVs, 7.9% and 10%, respectively]. Plasma 17α-hydroxyprogesterone was determined by a RIA kit from Diagnostic Systems Laboratories (Webster, TX; normal values, 1.5–9.6 nmol/L; intra- and interassay CVs, 5.9% and 5.5%, respectively).

Statistical analysis

The results are given as the mean ± SE. In the same group of subjects (responders, nonresponders, and normal subjects), the response of each variable at subsequent time points with respect to the baseline level (0 min) was evaluated by paired Student's t test. The responses of each parameter to oCRH were compared between groups using two-way analysis of variance (ANOVA) with repeated measures, followed by the Newman-Keuls test for pairwise comparisons and unpaired Student's t test. $P < 0.05$ was considered significant.

Results

As indicated above, patients were divided into 2 groups, consisting of 11 responders and 8 nonresponders, according to the cortisol response to oCRH. Baseline plasma cortisol, aldosterone, and 17α-hydroxyprogesterone levels were in the normal range and normally responded in both groups after a standard ACTH-(1–24) stimulation test (Table 1). In both nonresponders and responders, oCRH produced a maximal increase in plasma ACTH within 15 min (from 4.3 ± 0.5 pmol/L to a peak value of 19.2 ± 5.1 pmol/L, and from 7.1 ± 1.7 pmol/L to a peak value of 18.7 ± 6.1 pmol/L, respectively; $P < 0.001$). There were no significant differences between the global plasma ACTH responses of the 2 groups of patients and that observed in 10 control subjects (Fig. 1; responders *vs.* normal subjects, by ANOVA: $F_{1,7} = 0.08$; P = NS; nonresponders *vs.* normal subjects, by ANOVA: $F_{1,7} = 0.50$; P = NS; responders *vs.* nonresponders, by ANOVA: $F_{1,7} = 0.36$; P = NS). Plasma cortisol, which was normally responsive in 11 patients (from 350 ± 35 nmol/L to a peak value of 695 ± 74 nmol/L at 45 min; $P < 0.001$), showed little or no increase in the other 8 (from 419 ± 49 nmol/L to a peak value of 460 ± 44 nmol/L at 60 min; P = NS). The global response curve of plasma cortisol obtained in the group of nonresponders was significantly different from that in the group of normal subjects (by ANOVA, $F_{1,7} = 9.74$; $P < 0.0001$) or the responders (by ANOVA, $F_{1,7} = 8.97$; $P < 0.0001$; Fig. 1). No difference was observed between the cortisol responses of the normal subjects and responders (by ANOVA, $F_{1,7} = 0.27$; P = NS). Both baseline plasma ACTH and cortisol levels in the nonresponders were in the normal range and slightly higher than those in the responders and control group (Fig. 1). The peak cortisol level after oCRH treatment was markedly higher in responders than in nonresponders ($P < 0.001$). The plasma 17α-hydroxyprogesterone response to oCRH, tested in 6 responders and 4 nonresponders, paralleled that of plasma cortisol in all cases [from 1.33 ± 0.44 nmol/L to a peak value of 3.81 ± 0.26 nmol/L in responders ($P < 0.01$) and from 1.31 ± 0.35 nmol/L to a peak value of 1.65 ± 0.42 nmol/L in nonresponders (P = NS), respectively]. After 1 yr, 2 nonresponders (1 with hypothyroidism due to HT and 1 with IDDM) progressed toward overt Addison's disease and required gluco- and mineralocorticoid replacement therapy. At 1 yr follow-up, these 2 patients showed hyperpigmentation of the skin and complained of fatigue and malaise. Serum potassium levels were in the upper normal range. Baseline ACTH levels were increased (46 and 58 pmol/L), whereas cortisol was below normal and not responsive to ACTH-(1–24) (from 110 to 114 nmol/L and from 132 to 140 nmol/L). Because of already elevated ACTH levels, the oCRH test was not repeated. During follow-up, serum AA persisted with fluctuating titers in all patients.

Discussion

Our findings identified a lack of cortisol response to oCRH in a group of patients with AA, termed nonresponders, before

TABLE 1. Plasma cortisol, aldosterone and 17α-hydroxyprogesterone (17-OHP) responses to ACTH-(1-24) (250-μg iv bolus) in the three study groups (normal subjects, responders to oCRH, and nonresponders to oCRH)

	Cortisol (nmol/L)		Aldosterone (pmol/L)		17-OHP (nmol/L)	
	Baseline	60 min	Baseline	60 min	Baseline	60 min
Normal subjects (n = 10)	361 ± 35	980 ± 78[a]	130 ± 16	332 ± 30[a]	2.4 ± 0.3	6.2 ± 0.7[a]
Responders (n = 11)	358 ± 25	993 ± 66[a]	122 ± 14	314 ± 32[a]	2.0 ± 0.3	6.0 ± 0.5[a]
Nonresponders (n = 8)	413 ± 44	863 ± 88[a]	108 ± 21	248 ± 33[a]	2.1 ± 0.3	5.7 ± 0.9[a]

[a] $P < 0.01$, 60 min *vs.* baseline.
There were no significant differences between groups by Student's t test.

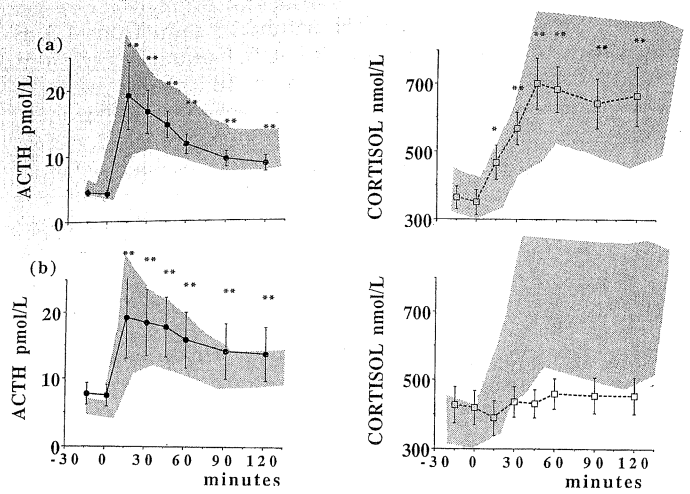

FIG. 1. oCRH test in patients with AA. Responders (a) and nonresponders (b) were classified according to individual cortisol responses, as indicated in the text. *Shaded areas*, ACTH and cortisol responses as the mean ± 1 SD of values from 10 normal subjects who were used as controls. *, $P < 0.05$; **, $P < 0.01$ (post-oCRH *vs.* baseline values; see between-group differences by ANOVA in the text).

any clinical evidence of adrenocortical insufficiency. The absence of a cortisol response to oCRH, reported in overt primary adrenal insufficiency (16–18), might be unexpected in our subjects, because this steroid was normal in baseline conditions and normally responsive to a standard ACTH stimulation test. It has been recently stressed that the rapid ACTH test is able to detect the presence of primary adrenal insufficiency with high sensitivity (8). However, caution in interpreting this test when exogenous ACTH-(1–24) is employed at high doses (250 μg) has been suggested by others (23, 24). ACTH stimulation testing at supraphysiological doses may, in fact, overdrive minimal abnormalities of adrenal function, masking an initial adrenocortical insufficiency. Our data demonstrate the existence of an early subclinical phase of Addison's disease in which the ACTH response to oCRH is still normal, whereas plasma cortisol shows an impaired secretion. Baseline ACTH levels, although in the normal range, were slightly higher in nonresponders than in responders. This difference is in agreement with previous findings of a relatively heightened pituitary function in asymptomatic patients with AA, directed to compensate an impairment of adrenal reserve and maintain normal cortisol secretion (13). However, baseline cortisol levels were higher in our nonresponder patients than in responders. In this respect, it cannot be excluded that lymphocytes or monocytes infiltrating the adrenal cortex in patients with this disease (25) may secrete ACTH or other active compounds (*i.e.* lymphokines) able to enhance, at least temporarily, adrenal steroidogenesis by a paracrine mode (26–30).

Progression of two of eight nonresponders toward overt Addison's disease over a period of 1 yr reveals a high risk of clinical disease in this group of patients. This confirms a common event in organ-specific autoimmune disease, where the presence of circulating antibodies precedes for a variable length of time the onset of clinical manifestations (9). During the period of observation, the titers of adrenal autoantibodies were found to fluctuate, but none of these patients lost seropositivity, as reported by others (31).

The role of AA in producing adrenal failure by blocking adrenal ACTH receptors is still controversial (32, 33). Based on recent reports that the 21-hydroxylase complex is a major antigen for autoantibodies in Addisonian patients (20, 21), a role for this enzyme in the pathogenesis of adrenal insufficiency could be postulated. The mechanisms by which adrenal enzymes are involved in autoimmune response could be similar to those described in patients with autoimmune thyroid disease. Thyroid microsomal antibody against thyroid peroxidase in patients with autoimmune thyroid disease not only binds, but also inhibits, thyroid peroxidase enzymatic activity and thyroid function (34). However, in our patients, normal baseline 17α-hydroxyprogesterone and its responsiveness to stimulation tests, parallel to those of plasma cortisol, do not support a steroidogenetic block at the 21-hydroxylase site.

In conclusion, more physiological amounts of exogenous ACTH or stimulation of endogenous ACTH by oCRH are recommended to evaluate pituitary-adrenal function and to unmask a possible adrenocortical impairment in subjects with increased risk of adrenal failure.

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