Abnormal Responsiveness to Dexamethasone-Suppressed CRH Test in Patients With Bilateral Adrenal Incidentalomas

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Context: The bilateral formation of nodules indicates that the pathogenesis of bilateral adrenal incidentalomas (AI) may differ from that of unilateral AI. A possible role of hypothalamo-pituitary-adrenal (HPA) axis dysregulation in their formation has not been investigated.

Objective: The objective of the study was to evaluate the presence of altered feedback regulation of HPA axis in patients with bilateral AI.

Design: The dexamethasone (DEX) suppression-CRH test was used to assess ACTH and cortisol responses in controls and patients with unilateral and bilateral AI.

Setting: The study was conducted at endocrine departments of two tertiary centers.

Patients: We studied 24 controls and 39 patients with unilateral and 46 with bilateral AI.

Interventions: All subjects underwent standard low-dose dexamethasone suppression followed by iv bolus administration of human CRH (100 μg).

Results: Bilateral AI had higher levels of ACTH and cortisol after the DEX-CRH challenge compared with both controls ($P < .01$ for ACTH and $P < .001$ for cortisol) and unilateral AI ($P < .01$ for ACTH and cortisol). A positive response, defined as peak ACTH greater than 10 pg/mL at 15 and/or 30 minutes followed by a significant rise in cortisol levels, was noted in 41.3% of bilateral vs 2.6% in unilateral AI ($P < .001$). Bilateral responders did not differ from nonresponders in demographic or hormonal characteristics, but they had larger total adrenal size compared with nonresponders.

Conclusions: A significant proportion of patients with bilateral AI demonstrate positive responses to the DEX-CRH test compared with unilateral AI, providing ground for potential involvement of HPA axis dysregulation in the pathogenesis, in at least a subgroup, of bilateral AI patients. **(*J Clin Endocrinol Metab* 100: 3478–3485, 2015)**

Incidentally discovered adrenal masses in patients with no clinical evidence of adrenal disease are commonly encountered in everyday practice (1, 2). The estimated prevalence of these lesions is about 4% and is increasing with age, reaching a prevalence of 10% in the elderly population (2, 3). Even though they are quite common, their pathogenesis remains obscure. A substantial proportion of patients, ranging from 8.9% to 22%, presents with bilateral lesions (1, 3, 4). The formation of nodules in both adrenals supports the hypothesis that their pathogenesis might differ from that of unilateral adenomas. In fact, current studies implicate mutations in components of the

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Abbreviations: AI, adrenal incidentaloma; AUC, area under the curve; BMI, body mass index; DDAVP, desmopressin (1-desamino-8-D-arginine vasopressin); DEX-CRH, dexamethasone suppression-CRH; DHEA-S, dehydroepiandrosterone sulfate; GLM, general linear model; HPA, hypothalamic-pituitary adrenal; LDDST, low-dose dexamethasone suppression test; MRI, magnetic resonance imaging; SH, subclinical hypercortisolism; UFC, urinary free cortisol.

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cAMP pathway in a number of patients with bilateral adrenal hyperplasia associated with overt Cushing's syndrome (5–7), and recently mutations in the *ARMC5* gene were identified in 50% of such cases (8). Whether, and to what extent, these mutations are also associated with bilateral adrenal incidentalomas remains to be elucidated. Aberrant expression of various receptors has also been implicated in the pathogenesis of bilateral incidentalomas (9, 10). Recently local ACTH production by a subpopulation of steroidogenic cells has been associated with cortisol hypersecretion in macronodular adrenal hyperplasia (11). The possible role, however, of pituitary ACTH in the formation of bilateral adrenal nodules has not been investigated. In fact, the main trophic factor for the adrenals is ACTH. It is well established that chronic exposure to ACTH hypersecretion, as in longstanding Cushing's disease or, in cases of congenital adrenal hyperplasia, leads both to hyperplasia and nodular changes of the adrenal cortex (12).

The dexamethasone suppression-CRH (DEX-CRH) test has been used to discriminate between subjects with pseudo-Cushing states and patients with Cushing's disease who have falsely normal responses to a low-dose dexamethasone suppression test (13, 14). A modified version of the standard DEX-CRH test, using a considerable smaller dexamethasone dose administered once, is used as a sensitive tool to assess dysregulation of the hypothalamic-pituitary adrenal (HPA) axis in neuropsychiatric disorders (15, 16). Herein we sought to determine whether hyperactivity of the HPA system, assessed by evaluating the ACTH and cortisol responses to the DEX-CRH test, is present in patients with bilateral adrenal incidentalomas (AI), compared with normal controls and patients with unilateral AI. We opted to ensure complete ACTH suppression by giving higher doses and for a longer period of time, that is, by using the standard low-dose dexamethasone suppression test (LDDST), instead of the 1-mg overnight dexamethasone suppression test to increase the specificity of the test and eliminate possible false-positive responses.

Patients and Methods

We studied 46 patients with bilateral AI, 39 patients with unilateral AI and a similar prevalence of subclinical hypercortisolism, and 24 control subjects with normal appearing adrenal glands on computed tomography scan. Subjects were recruited from the outpatient clinics of two tertiary care centers (Evangelismos Hospital and G. Genimatas Hospital, Athens, Greece). The local ethical committee approved the study and we obtained consent from all participants.

None of the patients had signs of overt endocrine dysfunction, including stigmata of Cushing's syndrome. Pheochromocytomas and aldosteronomas were excluded by appropriate testing (normal 24 h normetanephrine and metanephrine urinary excretion, normal potassium, aldosterone, and plasma renin activity and normal aldosterone to renin ratio). We excluded congenital adrenal hyperplasia on the basis of normal 17-hydroxyprogesterone levels.

All patients were investigated as in-patients. Our practice is to discontinue, if possible, most medications for 14 days, especially those known to alter dexamethasone clearance (17). Antihypertensive medications, in particular, were discontinued 2–4 weeks prior to admission; when this was not feasible, we administered an α-adrenergic blocker and/or a calcium channel blocker for this period. Apart from one patient in the unilateral group and two patients in the bilateral group, all other patients were not on psychiatric medication.

On the first day of the study protocol, blood samples were collected at 8:00 AM for measurements of cortisol, ACTH, dehydroepiandrosterone sulfate (DHEA-S), and routine biochemistry. A blood sample for cortisol was also drawn at 12:00 AM of the same day. Urine was collected during 24 hours for urinary free cortisol measurement. Subsequently patients underwent a standard 2-day LDDST (0.5 mg every 6 h for 2 d, starting at 8:00 AM). Postdexamethasone serum cortisol was measured after 48 hours, at 8:00 hours AM, 6 hours after the last dose of dexamethasone. Subsequently we administered 100 μg human CRH (Ferring Pharmaceuticals Ltd), and blood for cortisol and ACTH was withdrawn at 15, 30, 45, 60, 90, and 120 minutes after CRH. Apart from facial flushing, we did not observe other side effects during or after the procedure. Samples for ACTH measurements were kept in ice and then were spun at 4°C, and the plasma was separated and stored at −20°C until assayed.

The desmopressin (1-desamino-8-D-arginine vasopressin, DDAVP) test, when required, was performed in the morning after overnight fasting by injecting 10 μg DDAVP (DDAVP; Ferring Pharmaceuticals Ltd) as a slow iv bolus. An indwelling catheter was inserted in a forearm vein at least 1 hour earlier; blood samples were collected 15 minutes before the test, at 0 minutes and then at 15, 30, 45, 60, 90, and 120 minutes.

The diagnosis of subclinical hypercortisolism (SH) was made on the basis either of postdexamethasone cortisol levels of 5.0 μg/dL or greater or of postdexamethasone cortisol levels of 1.8 μg/dL or greater and at least one of the following criteria: 1) ACTH levels less than 10 pg/mL; 2) high urinary free cortisol (UFC) greater than 90 μg per 24 hours, corresponding to the upper normal limit of our method; and 3) midnight serum cortisol greater than 9 μg/dL (4).

For patients with bilateral adrenal tumors, we calculated the combined size of the adenomatous masses as the sum of the largest diameter(s) of each adrenal lesion.

Assays

Serum total cortisol was assayed using an automated immunochemiluminescence assay method with the ADVIA Centaur CP immunoassay system (ACS; 180 cortisol assay; Bayer; lower detection limit of the assay, 0.19 μg/dL; intraassay coefficient of variation, 8.0% for 5.43 μg/dL, 6.4% for 14.86 μg/dL, and 9.2% for 31.78 μg/dL). We used the same immunochemiluminescence assay to measure DHEA-S and UFC after dichloromethane extraction. We measured ACTH by immunoradiometric assay (Cis Bio International) with a lower detection limit of 2

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pg/mL. The reported inter- and intraassay coefficients of variation were 6.1% and 5.3%, respectively.

Imaging

Only incidentalomas with features suggestive of benign adrenocortical adenomas were included; homogeneous, smoothly marginated lesions with average, unenhanced Hounsfield units of less than 10. In patients with higher values, we considered an absolute and/or relative contrast washout value of more than 60% or a loss of signal intensity of 20% or greater on out-of-phase magnetic resonance imaging (MRI) images as indicating benignity. All but three patients with bilateral adenomas had one on each side. Two patients had double adenomas on one side and one on the other side, whereas in one patient both adrenal glands were enlarged with multiple macronodules.

Pituitary imaging was performed with MRI both before and after the injection of gadolinium.

Statistics

We used the IBM SPSS statistical package, version 20 (IBM Software Group), and GraphPad Prism, version 5.0 (GraphPad Software). Data are presented as the mean value ± SD of the mean. We checked normality with the Shapiro-Wilk test. Where necessary, logarithmic transformation was applied to normalize data before using parametric tests. The area under the curve (AUC) of cortisol and ACTH during the DEX-CRH test was calculated with GraphPad Prism. For group comparisons we used a one-way ANOVA, an unpaired *t* test, a Mann-Whitney *U* test, χ^2 analysis, or a Fisher's exact test, where appropriate. We used a repeated-measures analysis to examine changes of cortisol and ACTH during the DEX-CRH test, differences between groups, and the effect of age in the observed responses. We applied a general linear model (GLM) to control for the effect of group (where controls were assigned a score of 0, unilateral incidentalomas a score of 1, and bilateral incidentalomas a score of 2) on the cortisol response to a given ACTH level. We calculated odds ratios with a logistic regression analysis. Differences were considered significant at $P = .05$.

Results

The biochemical and imaging characteristics of patients and controls are shown in Table 1. Control subjects were younger compared with unilateral and bilateral AI patients, and patients with unilateral AI were younger compared with patients with bilateral AI. Patients with bilateral and unilateral AI had lower basal ACTH levels compared with controls, with no differences between patients with unilateral and those with bilateral AI. UFC levels were higher in the bilateral group compared with both the unilateral and control groups, whereas there were no difference in UFC levels between patients with unilateral AI and controls.

ACTH and cortisol levels after dexamethasone

All subjects had ACTH levels below 10 pg/mL (Figure 1A) after the LDDST. The percentage of subjects with post-LDDST ACTH levels between 5 and 10 pg/mL in the bilateral group was considerably higher (61%), compared with the control (21%) and unilateral (13%) groups ($P < .001$), irrespective of the presence of subclinical hypercortisolism. All control subjects had suppressed cortisol levels after the LDDST, whereas 12 patients with unilateral and 20 patients with bilateral AI had cortisol levels greater than 1.8 μg/dL (Figure 1B).

Table 1. Anthropometric and Hormonal Characteristics of Controls, Patients With Unilateral AIs, and Patients With Bilateral AIs

	Controls			Unilateral			Bilateral			Controls vs Unilateral	Controls vs Bilateral	Unilateral vs Bilateral
	Mean	SE	SD	Mean	SE	SD	Mean	SE	SD	*P*	*P*	*P*
n, M/F	24 (8/16)			39 (16/23)			46 (16/30)					
Age, y	40.5	2.6	12.7	49.2	2.3	14.4	61.5	1.4	9.3	<.05	<.01	<.01
BMI, kg/m^2	31.1	1.1	4.9	30.6	1.4	8.0	31.0	1.2	7.7	NS	NS	NS
Basal ACTH, pg/mL	27.7	3.7	18.0	16.9	1.8	11.3	13.6	1.2	8.3	<.05	<.01	NS
DHEA-S, μg/dL	214.3	31.5	154.1	175.4	27.3	168.4	53.4	5.2	35.2	NS	<.01	NS
UFC, μg per 24 h	38.4	2.6	12.7	39.7	5.2	31.8	77.1	7.7	52.2	NS	<.01	<.01
C after LDDST, μg/dL	0.8	0.03	0.1	2.1	0.5	2.8	2.9	0.4	3.0	<.01	<.01	NS
MNC, μg/dL				5.0	1.9	4.5	5.5	0.5	2.8			NS
Maximum size, cm				2.6	0.3	1.6	3.2	0.2	1.4			NS
Combined size, cm							5.2	0.3	2.2			
Pre-CRH ACTH, pg/mL	5.5	0.3	1.3	5.2	0.2	1.0	5.9	0.2	1.4	NS	NS	<.05
Peak ACTH 15/30, pg/mL	6.0	0.4	1.8	7.6	1.3	7.9	13.9	1.8	12.4	NS	<.01	<.01
Peak C 30/45, μg/dL	1.0	0.1	0.7	3.1	0.5	3.1	7.6	1.2	7.9	<.01	<.01	<.01
AUC C	129	89	18	380	60	375	829	828	122	<.01	<.01	<.05
AUC ACTH	838	305	62	902	101	632	1479	1138	168	NS	<.01	<.01
SH				7/39			15/30					NS

Abbreviations: C, cortisol; F, females; M, males; MNC, midnight serum cortisol; NS, nonsignificant.

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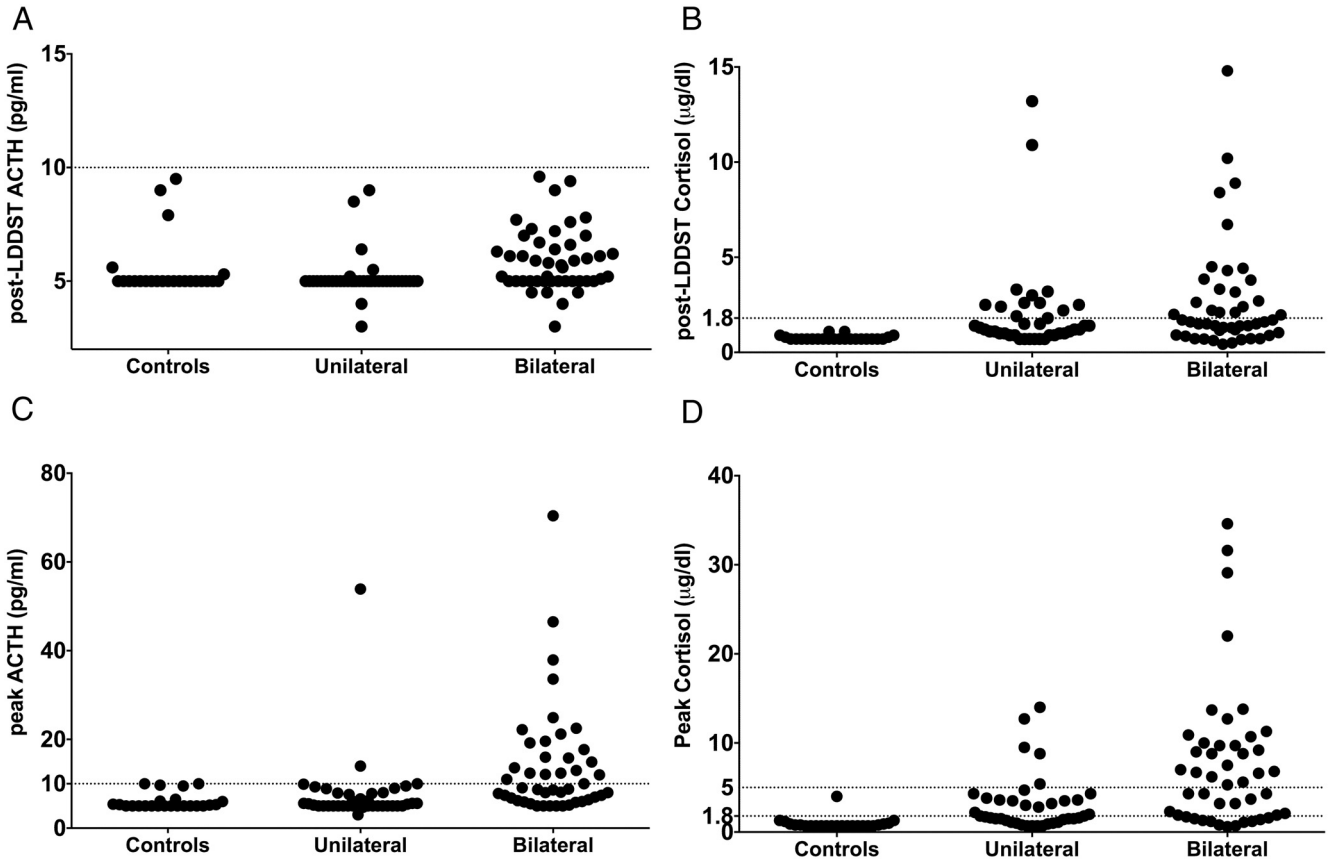

Figure 1. Post-LDDST ACTH (A) and cortisol (B) levels and post-DEX-CRH peak ACTH (C) and peak cortisol (D) levels in controls, patients with unilateral AIs, and patients with bilateral AIs. The lower detection limit of the ACTH assay is 2 pg/mL, but on many occasions the value was reported as less than 5 pg/mL. In all these cases, we present the ACTH value as 5 pg/mL in the graphs.

ACTH and cortisol levels after DEX-CRH

The GLM, repeated-measures analysis showed that ACTH and cortisol levels rose significantly ($P < .01$ for ACTH and $P < .001$ for cortisol) after the administration of CRH. The timegroup interaction was significant ($P = .002$), indicating that the increase in ACTH and cortisol levels was significant only for the bilateral group (Figure 2). The levels of ACTH and cortisol were significantly higher in the bilateral group compared with both the control group ($P < .01$ for ACTH and $P < .001$ for cortisol) and the unilateral group ($P < .01$ for ACTH and $P < .01$ for cortisol). Multiple comparisons showed no significant difference in ACTH and cortisol levels between the unilateral group and controls (Figure 2). The observed differences remained significant after adjusting for age and gender (between bilateral AI and controls, $P = .03$ for ACTH and $P < .001$ for cortisol, and between bilateral and unilateral AI, $P = .01$ for ACTH and $P = .001$ for cortisol).

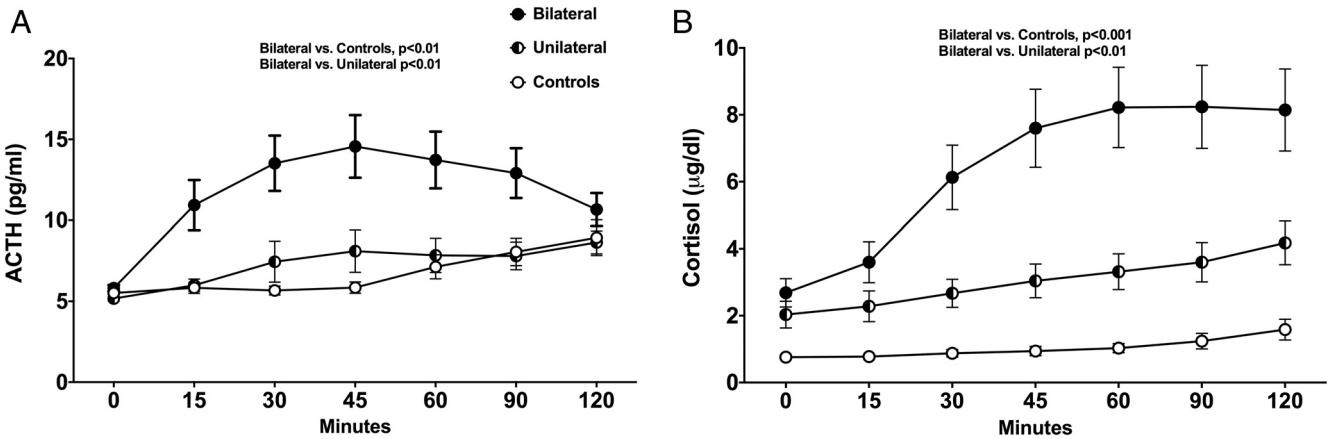

Figure 2. ACTH (A) and cortisol (B) levels during the DEX-CRH test in controls, patients with unilateral AIs, and patients with bilateral AIs.

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Peak ACTH levels at 15 and/or 30 minutes after DEX-CRH and the AUC of ACTH during the test were higher in the bilateral group compared with both the unilateral and controls, whereas there was no difference between unilateral and controls. Peak cortisol levels at 30 and/or 45 minutes after the DEX-CRH and the AUC of cortisol during the test were higher in the bilateral group compared with both the control and the unilateral groups, and they were higher in the unilateral compared with the control group (Table 1).

GLM analysis showed that for a given post-DEX-CRH peak ACTH, patients with bilateral adrenal incidentalomas had higher peak cortisol levels with a mean difference of 3.6 (95% CI 1.5–5.8, $P = .001$) compared with controls and a mean difference of 2.1 (95% CI 0.2–4, $P = .03$) compared with patients with unilateral AI. We observed no significant difference between patients with unilateral AI and controls.

Definition of response

The peak ACTH levels at 15 or 30 minutes and peak cortisol levels at 30 or 45 minutes after the administration of CRH is shown in Figure 1, C and D. All control subjects had peak ACTH levels of 10 pg/mL or less at 15 and/or 30 minutes. We defined as responders those who had peak ACTH levels of 10 pg/mL or more at 15 and/or 30 minutes followed by a significant rise in cortisol levels at 30 and/or 45 minutes.

Cortisol levels remained unchanged (maximum cortisol 1.3 μg/dL) in all but one control subject. This subject had an increase in cortisol from 1.1 μg/dL to 4 μg/dL (279%).

In the unilateral group, two patients increased their ACTH levels greater than 10 pg/mL. Only one patient had a concomitant rise in cortisol levels and was classified as responder, whereas the other had unchanged cortisol levels throughout the test (all < 1.8 μg/dL) and was considered nonresponder.

In the bilateral group, 19 patients fulfilled both criteria and were considered responders. Two additional patients increased their ACTH levels above10 pg/mL after the DEX-CRH, but their cortisol levels remained suppressed and were considered nonresponders. Within the bilateral group, we observed no difference in the percentage of patients with post-LDDST ACTH levels between 5 and 10 pg/mL between responders to DEX-CRH and nonresponders (63% vs 59%, respectively).

All responders underwent pituitary MRI and DDAVP testing that were found to be negative. None of these patients presented biochemical deterioration or progression to clinical Cushing's syndrome during a mean follow-up time of 26 ± 20 months (range 12–95 mo).

Comparison of responders vs nonresponders

The percentage of responders within the group of bilateral AI was markedly higher compared with the unilateral group (41.3% vs 2.6%, $P < .001$). Accordingly, the odds ratio for a positive response in patients with bilateral compared with patients with unilateral incidentalomas was 26.7 (97% CI 3.4–212.1, $P < .001$).

In the bilateral group, there were no differences between nonresponders and responders with regard to their age, body mass index (BMI), hormonal profile, and the prevalence of HPA axis abnormalities or SH (Table 2). Bilateral responders had larger combined adrenal size compared with bilateral nonresponders (Table 2). When only patients with bilateral AI and no evidence of SH were considered, responders were significantly younger compared with nonresponders.

The hormonal characteristics of patients with and those without SH and data on testing for aberrant receptors are given in Supplemental Table 1 and Supplemental Results.

Discussion

In the present study, we observed responses to the combined DEX-CRH test in an unexpectedly high proportion of patients with bilateral AI. As a group, these patients had higher ACTH and cortisol responses to the combined DEX-CRH test compared with both controls and patients with unilateral AI. In fact, using cutoffs derived from a control group with normal appearing adrenals on computed tomography scan, responders were almost invariably confined within the bilateral group.

Our hypothesis was that alterations in the glucocorticoid feedback sensitivity of the HPA axis might contribute to the development of bilateral lesions. So far, this likelihood has not been investigated. For this purpose we used the combined DEX-CRH test, and indeed, almost all patients with abnormal responses to the combined DEX-CRH test had bilateral AI. The test we used is to some extent different from the DEX-CRH test usually used to evaluate the integrity of glucocorticoid receptor feedback and the hypothalamic activation of the HPA axis (18, 19) in which, a 1-mg dose of dexamethasone is used the night before and the CRH test is performed in the evening, when peak ACTH and cortisol responses are higher (16, 19). In our study we performed the test in the morning and chose a higher dexamethasone dose to avoid false-positive results from inadequate dexamethasone suppression with lower doses (20). The test we used is more similar to that of Yanovski et al (13, 14), who applied this test to separate normal subjects from those with mild Cushing's disease. Successive studies evaluated the its utility mainly in pa-

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Table 2. Comparison of Anthropometric and Hormonal Characteristics Between Responders and Nonresponders to the DEX-CRH Among Patients With Bilateral AIs

	Nonresponders			Responders			
	Mean	SE	SD	Mean	SE	SD	*P*
n, M/F	25 (10/15)			21 (6/15)			
Age, y	62.8	2.0	9.9	60.0	1.9	8.6	NS
BMI, kg/m^2	30.6	1.5	7.1	31.4	1.9	8.5	NS
Basal ACTH, pg/mL	14.8	2.0	10.2	12.1	1.1	5.2	NS
DHEA-S, μg/dL	55.7	8.4	42.2	50.6	5.5	25.3	NS
UFC, μg per 24 h	64.8	6.6	33.2	91.7	14.4	66.2	NS
C after LDDST, μg/dL	3.0	0.6	2.9	2.8	0.7	3.1	NS
MNF, μg/dL	5.6	0.6	2.7	5.4	0.7	3.0	NS
Maximum size, cm	2.9	0.3	1.6	3.5	0.2	1.0	$<$.05
Combined size, cm	4.7	0.5	2.5	5.8	0.4	1.7	$<$.05
Pre-CRH ACTH, pg/mL	5.9	0.3	1.5	5.9	0.3	1.2	NS
Peak ACTH 15/30 minutes, pg/mL	7.3	0.4	2.2	21.8	3.3	14.9	$<$.01
Peak C 30/45 minutes, μg/dL	3.5	0.6	3.1	12.6	2.0	9.1	$<$.01
AUC C	419	74	372	1318	209	956	$<$.01
AUC ACTH	881	44	220	2192	299	1370	$<$.01
High UFC	5/25			7/21			NS
MNC $>$ 9	1/18			3/20			NS
Low basal ACTH, $<$ 10 pg/mL	8/25			10/21			NS
LDDST $>$ 1.8	12/25			8/21			NS
SH	8/25			7/21			NS

Abbreviations: C, cortisol; F, females; M, males; MNC, midnight serum cortisol; NS, nonsignificant.

tients with Cushing's disease (17, 21–24). Our protocol, however, differs slightly from that of Yanovski et al in that we used the standard LDDST (0.5 mg oral dexamethasone given every 6 h), starting at 8:00 AM instead of 12:00 PM. Subsequently the postdexamethasone plasma cortisol sample was properly taken at 48 hours to ensure that the diagnosis of SH was not compromised. Immediately after this, ie, 6 hours instead of 2 hours after the last dose of dexamethasone, we performed the CRH test. We did not opt for a cortisol cutoff level, given that a proportion of patients with AI have high cortisol levels after dexamethasone. Instead we used an ACTH cutoff. This is because we did not use the combined DEX-CRH test to diagnose hypercortisolism but to investigate whether the dexamethasone induced ACTH suppression can be overridden by CRH, indicating dysregulation of the HPA activity. Additionally, we did not regard as responders patients who exhibited a rise only in cortisol, without a preceding rise in ACTH because autonomous cortisol secretion in some patients with adrenal incidentalomas may be intermittent.

The abnormal response to DEX-CRH was not associated with the presence or absence of SH, as shown by the same prevalence of patients with SH in both the responders and the nonresponders groups. Of note, we observed positive responses also in patients with SH, although the presence of autonomous cortisol secretion would be expected to additively further suppress HPA axis and ACTH levels. It is well recognized that these patients have low ACTH levels already and the response to CRH is usually blunted (25). Overall, our data show an escape of dexamethasone suppression after CRH and strongly support an underlying dysregulation in the direction of HPA axis hyperactivity. Such a dysregulated ACTH secretion during lifetime may lead to subtle but chronic trophic stimulation of the adrenals by repeatedly inappropriately higher ACTH levels, particularly in response to stress, favoring nodular adrenal hyperplasia. In fact, the only difference between responders and nonresponders among patients with bilateral incidentalomas was that responders had significantly larger combined size of the lesions, probably reflecting the trophic effect of dysregulated ACTH upon the adrenals.

Alternative explanations for the observed ACTH and cortisol responses to the DEX-CRH test may involve the presence of an underlying pituitary corticotroph tumor or some concomitant neuropsychiatric disorder. The possibility, however, that the observed ACTH responses after the DEX-CRH test may result from an ACTH-secreting pituitary corticotroph adenoma is rather remote, given the extremely low incidence of Cushing's disease compared with that of bilateral AI. Nevertheless, we submitted all responder patients to pituitary MRI and a DDAVP test (26, 27) to exclude the possibility of Cushing's disease and none tested positive. Moreover, most of these patients were followed up, and no evolution to overt Cushing's syndrome was noted. The possibility that the observed responses may be due to an underlying psychiatric disorder was also excluded, given that only a few patients with

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known depression or bipolar disorder were involved and were equally distributed between responders (one patient) and nonresponders (one patient). Of some relevance is also a recent study (11) that showed intraadrenal ACTH production in patients with macronodular adrenal hyperplasia and postulated that ACTH secretion by the hyperplastic adrenal glands may account for unsuppressed plasma ACTH levels in some of these patients. Both dexamethasone and CRH, however, did not have any substantial effect on this locally produced ACTH (11), and thus, possible adrenal-derived ACTH does not account for our results. In fact, we considered as responders only those with an elevated ACTH shortly after the administration of CRH to ensure that the observed ACTH rise was of pituitary origin.

Another finding of the present study was that for a given ACTH level, patients with bilateral incidentalomas demonstrated a higher cortisol response to DEX-CRH compared with patients with unilateral incidentaloma. The most likely explanation is the significantly larger overall adrenal mass in the cases of bilateral incidentalomas found in our cohort. After all, when more ACTH-responsive cells are exposed to similar levels of ACTH, more cortisol is produced. Moreover, our group, along with other investigators (4, 28), previously reported a relationship between the size of the adrenal tumor and the degree of autonomous cortisol secretion in patients with AI. Another possibility is that the difference in the secretory behavior of bilateral compared with unilateral incidentalomas may reflect different pathogenic mechanisms (4), as has been hypothesized by several studies (29, 30).

A limitation of our study is the age difference between patients and controls. In fact, patients with bilateral masses were the oldest, whereas the controls were the youngest. It has been suggested that aging may result in HPA axis hyperactivity, probably related to a loss of hippocampal neurons and activation of vasopressinergic neurons during aging, resulting in progressive dysregulation and hyperactivity of the HPA system (20). Heuser et al (31), using the overnight DEX-CRH test in normal volunteers, demonstrated that the older group released significantly more cortisol than their young counterparts, concluding that there is an age-related increase in HPA system activity. The age effect, however, was subtle and there was actually no statistically significant effect of age on the peak ACTH or AUC of ACTH levels after the DEX-CRH test (31). Moreover, the overnight dexamethasone suppression test was used and the test was performed in the evening, when there is a stronger increase in ACTH and cortisol after CRH. Therefore, these observations cannot be extrapolated to our study in which we used a less sensitive test. Our data are also against a significant impact of age on the observed responses. Not only was the age of responders similar compared with the nonresponders, but, additionally, within the subgroup of those without SH, responders were younger than nonresponders.

Another limitation is that we did not measure dexamethasone levels. We specifically discontinued, however, medications known to affect the dexamethasone metabolism, and also, if rapid dexamethasone clearance was a significant confounder in our study, it is highly unlikely that it occurred only in patients with bilateral incidentalomas.

The etiology of our observations is elusive. Early-life events impact on HPA programming; ie, low birth weight, has been linked to an activated HPA axis and an adverse metabolic phenotype (32). There are no studies, however, on possible anatomical changes of the adrenals. Theoretically, increased peripheral cortisol metabolism or impaired adrenal steroidogenesis would also be expected to result in an overactive HPA axis to overcome the increased cortisol clearance or the initially reduced cortisol producing capacity, at least until a compensatory expansion of the overall adrenal mass occurs. Interestingly, the recently identified *ARMC5* mutations in patients with macronodular adrenal hyperplasia are associated with impaired steroidogenesis (8). Whether these mutations are present in patients with bilateral AI is unknown. Altered glucocorticoid receptor sensitivity or arginine-vasopressin release may also impact on HPA activity (20, 30). It is also possible that the observed HPA dysregulation is an epiphenomenon. Glucocorticoid excess may cause hippocampal damage resulting in HPA hyperactivity (20). Many of the DEX-CRH responders, however, did not have biochemical evidence of hypercortisolism.

In conclusion, a significant proportion of patients with bilateral AI demonstrate increased ACTH and cortisol responses to the DEX-CRH test compared with patients with unilateral adenomas. This finding provides some ground for the potential involvement of HPA dysregulation in the pathogenesis, in at least a subgroup of patients with bilateral AI. Also, in the context of incidentally detected bilateral adrenal nodules, the presence of a hyperactive HPA axis, in a proportion of patients, might hamper the diagnostic approach. The use of the DEX-CRH test might lead to the erroneous diagnosis of ACTH-dependent Cushing's syndrome, especially because, in contrast with patients with clinically overt adrenal Cushing's syndrome, many of these patients do not have fully suppressed baseline ACTH levels. Diagnostic strategies and studies to distinguish between patients with autonomous cortisol-secreting adrenal incidentalomas and those with a hyperactive HPA axis are still necessary and of utmost importance.

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