Low-Dose Ovine Corticotropin-Releasing Hormone Stimulation Test in Diabetes Mellitus With or Without Neuropathy

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The function of the hypothalamic-pituitary-adrenal (HPA) axis was evaluated in insulin-dependent diabetics without (group I, n = 10) or with (group II, n = 10) established symptomatic neuropathy and in age- and weight-matched normal controls (n = 11). Since the corticotropin (ACTH)/cortisol response to the minimal-effective dose of corticotropin-releasing hormone ([CRH] 0.03 μg/kg body weight) represents a useful tool for HPA axis examination, all subjects were tested with the low-dose ovine CRH stimulation test. Experiments started at 8:30 AM, when CRH was injected after two basal blood samples were withdrawn, and lasted 2 hours. Basal serum levels of ACTH were similar in the three groups. Administration of CRH induced a small but significant increase in ACTH levels in all subjects; however, the CRH-induced ACTH increase was significantly higher in normal controls than in diabetic groups I and II. Furthermore, a significantly lower ACTH response was observed in group II than in group I. In contrast, basal and CRH-induced cortisol levels were significantly higher in diabetics than in normal controls. Comparisons between diabetic groups showed that both basal and stimulated cortisol secretion was significantly higher in group II than in group I. When peak ACTH responses to CRH and basal cortisol levels were combined, a significant negative correlation was found ($r = .545$, $P < .02$). These data show that even uncomplicated diabetes mellitus is associated with adrenal hyperfunction. Such an alteration is more pronounced in the presence of neuropathy.
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HYPERFUNCTION of the hypothalamic-pituitary-adrenal (HPA) axis has been associated with diabetes mellitus in the absence of other pathologic conditions such as depression, obesity, or weight loss, which are known to produce HPA activation. HPA hyperactivity has been documented by the presence of elevated circulating levels of cortisol and/or corticotropin (ACTH) and resistance to dexamethasone suppression.[1-9] However, in the light of discrepant results, until now it is unclear whether HPA hyperfunction is present in uncomplicated diabetes and contributes to the development of chronic complications, or whether diabetic neuropathy is a required condition for increased HPA activity.

To clarify this issue, we measured serum levels of ACTH and cortisol in diabetic patients with or without neuropathy under basal conditions and in response to stimulation with a minimal-effective dose (0.03 μg/kg) of corticotropin-releasing hormone (CRH). This test was chosen because it has been proven to provide a more accurate and reliable evaluation of HPA regulation in normal and pathologic conditions[10] than tests where maximal-stimulatory doses of CRH (1 μg/kg body weight or 100 μg per subject) were used.[11,12]

SUBJECTS AND METHODS

We studied 11 normal subjects, 10 diabetic patients with no clinically detectable somatic or autonomic neuropathy (group I), and 10 diabetic patients with established symptomatic polyneuropathy (group II). The age of the patients (mean ± SE) was 41.2 ± 2.4 years in group I and 43.1 ± 1.9 in group II. The duration of diabetes ranged from 4 to 18 years.

Since the onset of diabetes, patients had been treated with insulin, and at the time of the study they were hospitalized for adjustment of insulin therapy.

Experiments started after optimization of insulin therapy, which was similar in both groups (dose [mean ± SE] of intermediate-duration monocomponent insulin plus short-acting monocomponent insulin administered together once or twice daily: 31.1 ± 2.4 IU/24 h for group I and 33.1 ± 1.9 for group II). Insulin therapy remained unchanged during the whole period of the study because all patients achieved good control of metabolic status (Table 1). None of them had clinical or laboratory evidence of ketosis or associated endocrine or other intercurrent disease. Patients were also screened for history of depressive illness or recent weight loss. Depression was excluded by specific interviews performed by two psychiatrists according to the Hamilton Depression Rating Scale.[13,14] In all patients, creatinine clearance (mean ± SE) was in the normal range (group I, 98.2 ± 4.9 mL/min; group II, 97.2 ± 5.3). All patients underwent ophthalmologic examination to detect the presence of diabetic retinopathy. Diagnostic criteria were as follows: After papillary dilatation, all patients underwent ophthalmoscopy performed by the same experienced ophthalmologist. The finding of at least three microaneurysms was considered a sign of retinopathy. This sign was found in two patients of group I and three patients of group II. In these patients, retinopathy was confirmed by fluoroangiography. These five patients were included in the study.

Autonomic neuropathy was assessed by evaluation of typical

Table 1. Clinical and Biochemical Data of Diabetic (groups I and II) and Control Subjects

	Controls	Diabetic Patients	
		Group I	Group II
Age (yr)	42.1 ± 1.2	41.2 ± 2.4	43.0 ± 1.9
Weight (kg)	68.4 ± 1.8	67.4 ± 1.9	66.5 ± 1.5
Duration of diabetes (yr)		11.4 ± 0.9	12.2 ± 0.8
Blood glucose (mg/dL)	86.5 ± 0.0	166.6 ± 7.5	173.4 ± 8.1
HbA$_{1c}$ (%)	6.0 ± 0.3	9.2 ± 0.6	9.5 ± 0.7
Autonomic symptoms	—	0	7S, 5H, 7U, 8G
Neuropathy deficit score (0-28)	—	0	19.1 ± 4.8

NOTE. Data are the mean ± SE.

Abbreviations: S, sweating disturbance; H, postural hypotension; U, genitourinary abnormality; G, gastrointestinal symptoms.

symptoms: sweating disturbance, postural hypotension, genitourinary abnormality, ejaculatory dysfunction, impotence, and gastrointestinal symptoms. Each category of symptoms was scored as 0 if absent and, 1 if present.

Neuropathy deficit scores were obtained by a standard neurologic examination that consisted of assessment of light touch, vibration, temperature, pain, and tendon reflex.

For each sensory modality, the score related to the autonomic levels in the legs below which sensation was impaired (1, base of toes; 2, mid-foot; 3, ankle; 4, mid-calf; 5, knee). Knee and ankle tendon responses were scored in each leg (0, present; 1, present only with reinforcement; 2, absent). Nerve conduction studies and autonomic function testing were performed as previously described.[4,15]

Vibratory thresholds were calculated as the mean of three measurements on the first left metatarsal base using a Biothensiometer (Bio-Medical Instruments, Newbury, OH).

Eleven normal men (aged 42.1 ± 1.2 years, mean ± SE) with normal body weight and without any signs of endocrine disease or family history of diabetes mellitus participated as controls in this study. All subjects were informed of the purpose of the study and gave their consent to participate.

In all subjects, two consecutive 24-hour urine samples were collected before the CRH test.

CRH Test

At 8:00 AM on the day of the experiment, two indwelling intravenous catheters were inserted into antecubital veins of opposite arms in subjects fasting from the previous evening and lying in the recumbent position. Catheters were kept patent by a slow infusion of normal saline (NaCl 0.9%). One catheter was used for administration of ovine CRH, and the other served for blood sampling. After withdrawal of two basal blood samples (−15 and 0 minutes), CRH was administered as an intravenous bolus at the dose of 0.03 μg/kg, which has been found to be a reliable threshold dose for producing small but clear-cut ACTH/cortisol responses.[10] Further blood samples were taken at 15, 30, 60, 90, and 120 minutes after CRH administration.

Assays

Plasma ACTH and cortisol concentrations were measured in all samples by specific radioimmunoassay methods[16,17] using the double-antibody technique. Samples from the same subject were tested in duplicate and in the same assay. Measurements of urinary cortisol levels were performed after extraction with dichloromethane. The assay for urinary cortisol was performed as for plasma samples using the same standard curve. In our laboratory, the normal range of urinary cortisol values is 29 to 91 μg/24 h.

The intraassay coefficient of variation was 7.1% for ACTH and 4.0% for cortisol; the interassay coefficient of variation was 9.9% for ACTH and 7.0% for cortisol. Lower limits of sensitivity were 1 pg/mL for ACTH and 0.6 mg/dL for cortisol.

Samples taken at time 0 were also used for blood glucose and hemoglobin A$_{1c}$ (HbA$_{1c}$) evaluation. The blood glucose level was measured with an IL 918 autoanalyzer (Instrumentation Laboratory, Milan, Italy) using a glucose oxidase-peroxidase procedure. HbA$_{1c}$ was assayed by high-pressure liquid chromatography after hemolysis of red blood cells, using reagents obtained from Bio-Rad Laboratories, Richmond, CA (normal range, 4.15% to 9.08%).

Urinary albumin concentrations were evaluated by radioimmunoassay with reagents supplied by Sclavo (Siena, Italy).

Statistical Analysis

Data were statistically analyzed with two-way ANOVA, Wilcoxon's matched-pair rank-sum test, and the Kruskall-Wallis test, as appropriate. Data are reported as the mean ± SE.

Correlation Studies

Correlation studies were attempted by combining peak ACTH responses to CRH with clinical (duration of diabetes or insulin requirement), metabolic (blood glucose or HbA$_{1c}$ levels), or other endocrine (basal cortisol levels) parameters. Data were analyzed with Spearman's linear correlation coefficient (r).

RESULTS

Clinical characteristics of controls and diabetic patients are listed in Table 1. Neurophysiologic measurements are listed in Table 2. No untoward side effect was observed in any subject after CRH injection. Blood glucose concentra-

Table 2. Sensory Threshold, Peripheral-Nerve Electrophysiology, and Cardiovascular Autonomic Function in Diabetic and Control Subjects

	Controls (n = 11)	Diabetic Patients	
		Group I (n = 10)	Group II (n = 10)
Vibratory threshold (V)	10 ± 1	16 ± 2*	46 ± 9†
Sural sensory conduction velocity (m/s)	36 ± 1	33 ± 3	23 ± 4†
Sural sensory potential amplitude (μV)	10.6 ± 3.9	5.9 ± 2.1*	0
Peroneal motor conduction velocity (m/s)	47.1 ± 1.4	42.0 ± 1.3*	32.5 ± 3.5†
Maximal − minimal heart rate (beats/min)	19 ± 2	18 ± 2	2 ± 0.4†
Valsalva ratio	1.61 ± 1.12	1.49 ± 1.14	1.18 ± 1.21†
30:15 ratio	1.23 ± 0.5	1.20 ± 0.3	1.00 ± 0.3†
Postural change in blood pressure (mm Hg)	4 ± 2	6 ± 3	20 ± 6†

NOTE. Values are the mean ± SE. Sural sensory conduction velocity, if absent, assigned a value of 20 m/s.

*P < .05 v controls.

†P < .01 v controls and diabetic group I.

Table 3. Blood Glucose Concentrations (mmol/L) During Ovine CRH Test in the Two Groups of Diabetics

	− 15 min	0 min	15 min	30 min	60 min	90 min	120 min
Group I	8.7 ± 0.5	8.6 ± 0.5	8.7 ± 0.4	8.8 ± 0.6	8.7 ± 0.7	8.6 ± 0.5	8.6 ± 0.6
Group II	8.9 ± 0.5	8.9 ± 0.6	8.8 ± 0.5	9.0 ± 0.5	9.0 ± 0.7	8.9 ± 0.5	8.8 ± 0.5

NOTE. Each point represents the mean ± SE of 10 observations.

tions in diabetics remained constant during the CRH test (Table 3). Microalbuminuria was absent in controls and diabetic groups. Basal ACTH levels were similar in all groups (Fig 1, Table 4). Administration of CRH induced a small but significant increase in all subjects (Table 4); however, the CRH-induced ACTH increase was significantly higher in normal controls than in group I (F = 5.59, $P < .05$) and group II (F = 6.73, $P < .02$). When diabetic groups were compared, a significantly higher ACTH response to CRH was observed in those without neuropathy (group I) than in those with neuropathy (group II) (F = 5.43, $P < .05$; Fig 1).

Basal levels of cortisol, the increase in cortisol (δ, peak minus baseline), and peak values during the CRH test were significantly lower in normal controls than in group I and group II diabetics (Fig 2 and Table 4). The overall cortisol response to CRH was significantly lower in normal controls than in group I (F = 6.89, $P < .02$) and group II (F = 8.10, $P < .01$) diabetics. When diabetic groups were compared, higher basal levels ($P < .02$; Table 4) and CRH-stimulated cortisol responses were found in neuropathic diabetics (group II) than in those without neuropathy (Group I) (F = 5.64, $P < .05$; Fig 2). Two patients of 10 in group I and three patients of 10 in group II had retinopathy. However, basal and CRH-stimulated ACTH and cortisol values in retinopathic subjects were similar to those observed in the other subjects of their groups.

Correlation Studies

No significant correlations between peak ACTH responses to CRH and clinical or metabolic parameters were observed. In contrast, a significant negative correlation was found between peak ACTH responses and basal cortisol levels ($r = .545, P < .002$).

DISCUSSION

Previous studies in normal and diabetic men, in which the pituitary was challenged with high-dose CRH, did not show significant differences in the ACTH response between groups.[9] However, the use of high-dose CRH provides information on the pituitary reserve, rather than on the sensitivity of corticotropin-secreting cells to CRH. In the present study, a dose of CRH 0.03 μg/kg induced a clear-cut ACTH increase, which was small but significant in all subjects. However, the ACTH response to the minimal-effective dose of CRH was significantly lower in diabetics than in normal subjects. In addition, neuropathic diabetic patients showed significantly lower responses than patients without neuropathy. The simultaneous evaluation of cortisol levels in the three groups suggests a possible explanation for these findings. In fact, basal levels of cortisol were higher in subjects with lower ACTH responses to CRH, and the combination of these parameters showed a significant
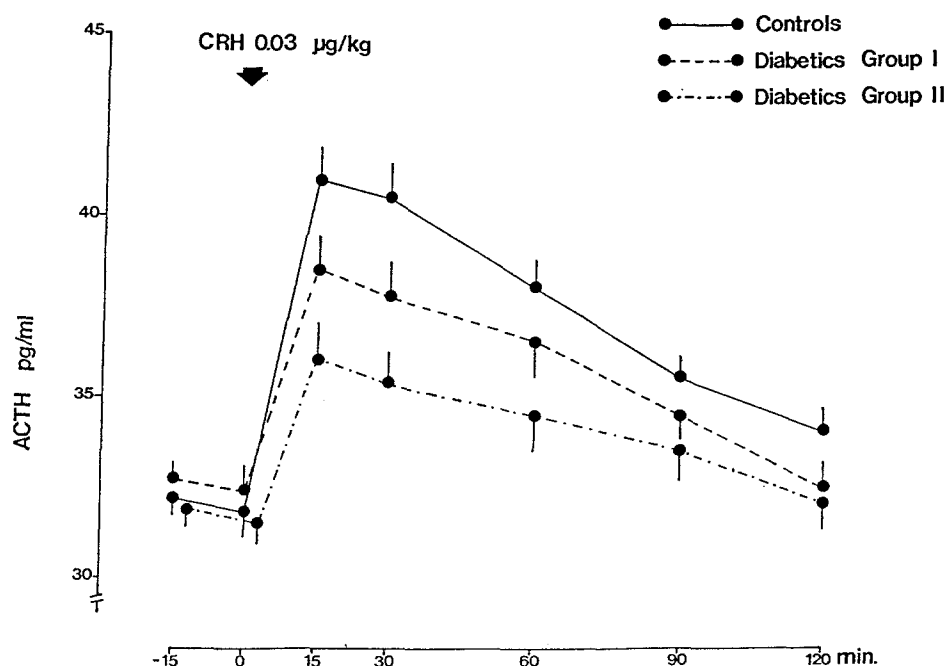

Fig 1. Plasma ACTH response to ovine CRH in diabetics (group I [n = 10] and group II [n = 10]) and normal controls (n = 11). Each point represents the mean ± SE.
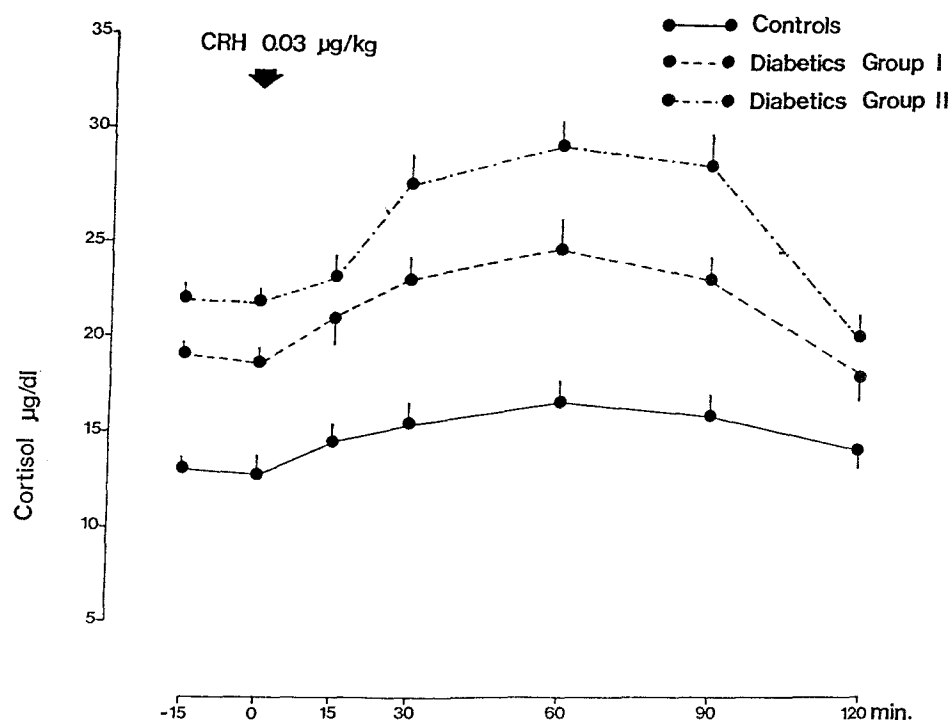
Fig 2. Plasma cortisol response to ovine CRH in diabetics (group I [n = 10] and group II [n = 10]) and normal controls (n = 11). Each point represents the mean ± SE.

negative correlation. Therefore, the reduced ACTH response to CRH in diabetics might be attributed to enhanced feedback control over corticotrope peptide release.

There may be a different and more comprehensive explanation for the reduced ACTH responsiveness to CRH in diabetics. In these patients, ACTH-secreting cells might be less sensitive to exogenous CRH because of a chronic overstimulation by elevated hypothalamic CRH release. In fact, hypothalamic hypersecretion of CRH in diabetic animals has been found to downregulate the number of CRH receptors in the anterior pituitary, and such a

mechanism has been supposed to reduce the ACTH response to exogenous CRH.[18] On the other hand, elevated peak cortisol levels cannot be explained by the same mechanism; it is possible that chronic hypersecretion of CRH is responsible for cortical adrenal hypertrophy[19-21] and cortisol hypersecretion. In fact, CRH has been shown to exert a secretagogue effect on the adrenal gland independently of pituitary ACTH release.[22-26]

A possible pathogenetic role for HPA hypersecretion in diabetes mellitus has been attributed to poor glycemic control[9] or hypothalamic effects of exogenously administered insulin.[9] The data presented here distinguish neuropathic diabetics from those without neuropathy which suggests that alterations of the HPA axis, already present in uncomplicated diabetes mellitus, worsen in the presence of neuropathy. It is possible that neuropathy and deeper HPA alterations represent two independent phenomena, both due to worsened metabolic control. However, no significant correlation was found in our patients when endocrine data were combined with previous (duration of diabetes or insulin therapy) or actual (metabolic control at the time of study) clinical or laboratory parameters. Therefore, it is possible that neuropathy itself played a role to increase adrenal activity. In this regard, neuropathic alterations of the catecholaminergic innervation of hypothalamic CRH-immunoreactive neurons have been proposed as a cause of adrenal activation.[4,27-29]

In conclusion, this study shows that uncomplicated diabetes mellitus is associated with hypercortisolism and reduced ACTH responses to the minimal-effective dose of CRH. In addition, the data show a higher adrenal activation in the presence of neuropathy.

Table 4. Urinary Cortisol Levels and Plasma ACTH and Cortisol Responses to CRH in Diabetic Groups and Controls

	Controls (n = 11)	Diabetic Patients Group I (n = 10)	Group II (n = 10)
ACTH (pg/mL)			
Basal	31.8 ± 0.8	32.8 ± 0.7	31.5 ± 0.6
Peak	41.0 ± 0.9	38.5 ± 1.1*	36.0 ± 1.0†§
Change	9.2 ± 0.3	5.7 ± 0.4*	4.5 ± 0.4†§
Cortisol (μg/dL)			
Basal	12.8 ± 0.8	18.5 ± 0.8§	21.8 ± 0.9‡§
Peak	16.6 ± 1.1	24.5 ± 1.4§	29.0 ± 1.5†§
Change	3.8 ± 0.3	6.0 ± 0.4§	7.2 ± 0.4†§
Cortisol urinary (μg/24 h)‖	62.2 ± 4.2	90.6 ± 3.7§	104.4 ± 4.6‡§

NOTE. Each point represents the mean ± SE.
*P < .05 v controls.
†P < .05 v diabetic group I.
‡P < .02 v diabetic group I.
§P < .01 v controls.
‖Mean of two measurements.

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