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Low Plasma Corticotropin-Releasing Hormone (CRH) Levels in Patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM)

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Abstract. Plasma CRH levels were measured in patients with non-insulin dependent diabetes mellitus (NIDDM) as the pituitary-adrenal abnormalities have been reported in NIDDM. They were also measured after oral administration of 75 g glucose to examine whether glucose increased plasma CRH along with insulin secretion. The baseline plasma CRH was significantly lower in diabetic patients than in controls. Baseline ACTH and cortisol were significantly higher in NIDDM patients than in controls. Plasma CRH, ACTH and cortisol did not change after glucose administration in either NIDDM patients or controls. Neither plasma CRH nor ACTH showed a significant correlation with plasma glucose or insulin response in NIDDM patients. These results suggest that CRH secretion is not stimulated by glucose, that plasma ACTH and cortisol are increased in NIDDM patients and that CRH is not responsible for these increases.

Key words: Corticotropin-releasing hormone, ACTH, Cortisol, Non-insulin dependent diabetes mellitus.
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CORTICOTROPIN-RELEASING hormone (CRH) is produced mainly in the hypothalamic paraventricular nucleus to control pituitary ACTH secretion. It is also produced in the extrahypothalamic brain area and in the peripheral tissues [1, 2]. It is therefore assumed that CRH in peripheral blood derives not only from the hypothalamus but from peripheral tissues.

It has already been reported that patients with diabetes mellitus had abnormalities in the pituitary-adrenal axis [3–7]. Namely, they had high urinary 17-OHCS [8] high baseline plasma ACTH and cortisol [4–7], abnormal circadian rhythm of plasma ACTH and cortisol [8, 9] and resistance to dexamethasone suppression [7, 10–12]. However, there has been no study in which plasma CRH, ACTH and cortisol concentrations were measured simultaneously. In this study, we measured plasma CRH levels before and after oral administration of 75 g glucose in normal subjects and in patients with non-insulin dependent diabetes mellitus (NIDDM) to investigate the role of CRH in the pituitary-adrenal abnormalities in NIDDM and to know whether the plasma CRH concentration changes in relation to plasma glucose or insulin.

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Subjects and Methods

Subjects

Seventeen patients (11 males and 6 females) with NIDDM were investigated. The average age was 60 ± 10.5 (mean ± SD) years. Three of them had albuminuria (10–30 mg/d*l*) but none of them had renal insufficiency. Their average serum urea nitrogen and creatinine levels were 15.6 ± 4.2

(mean ± SD) and 0.85 ± 0.22 mg/d*l*, respectively. Two of 8 patients examined had retinopathy (Scott Ia and IIa). Only one patient had neuropathy. Three patients experienced cerebral infarction (2 months, 8 months and several years prior to this examination). Five or six patients had been receiving oral hypoglycemic agents (sulfonyl urea, 1–4 Tab/day) or only diet therapy, respectively. The other patients had not received any therapy. Twelve healthy persons (8 male sand 4 females, 36 ± 8 years old) were also examined as controls.

Oral glucose tolerance test (OGTT)

In the morning after an overnight fast, 75 g glucose was administered orally to all subjects. Blood samples were collected before, and 30 and 60 min after glucose administration. Blood samples were placed into chilled glass tubes containing EDTA and centrifuged at 1500 G for 10 min at 4°C to separate plasma. Plasma samples were divided into four fractions for assay of glucose, immunoreactive insulin (IRI), CRH, ACTH and cortisol, and were stored at −20°C until extraction. CRH was extracted within 2 weeks after sampling.

Hormone assays

Two millilitters of 6M guanidine hydrochloride was added to 2 m*l* plasma and mixed for 15 sec. The mixture was applied to a SEP PAK C18 cartridge (Waters, Milford, USA) with a polystyrene syringe. The cartridge was washed with 10 m*l* 0.1 N HCl and then with 10 m*l* H_2O. CRH was eluted with 2.5 m*l* 60% CH_3CN and 40% acetic acid (0.5%). The extraction rate of [^{125}I-Try^0]-CRH (New England Nuclear Research Products, Boston, USA) added to 2 m*l* of plasma was 88.4 ± 1.6% (mean ± SD, CV=1.9%).

Immunoreactive CRH was measured by a previously reported radioimmunoassay using anti-CRH serum (Mitsubishi Petrochemical, Co. Ltd., Tokyo, Japan) and [^{125}I-Tyr^0]-CRH [13]. Each extracted plasma sample was redissolved in 1 m*l* assay buffer (0.1 M phosphate buffer, containing 0.14 M NaCl, 0.1% bovine serum albumin, 0.05% Tween 20 and 0.01% NaN_3), and duplicate 300 μl samples were used for the assay. The intra-assay variations for plasma CRH at 8.1 and 16.3 pg/m*l* were 9.4 and 14.1%, respectively. The sensitivity of CRH assay was 1 pg/m*l* plasma. Samples were measured in some assays and each assay included the samples from both controls and diabetic patients.

Levels of ACTH in plasma were measured in duplicate 100 μl samples with a commercially available immunoradiometric assay kit (Mitsubishi Petrochemical Co. Ltd.). Plasma cortisol and IRI levels were measured with radioimmunoassay kits (Daiichi Radioisotope, Tokyo, and Eiken Chemical Co., Ltd., Tokyo, Japan, respectively). The sensitivities of the assay for ACTH, cortisol and IRI were 2.5 pg/m*l*, 1.25 μg/d*l* and 5 μU/m*l*, respectively. Plasma glucose levels were measured by an electrode method.

Statistical analysis

Results are expressed as the mean ± SD unless otherwise indicated. Statistical analysis of the data was conducted with Student's *t*-test.

Results

Plasma glucose before and after glucose administration was significantly higher in NIDDM patients than in controls (Fig. 1). Baseline plasma IRI in NIDDM patients did not significantly differ from that in controls, while the plasma IRI concentration at 30 min after glucose administration was significantly lower in NIDDM patients than that in controls. Baseline plasma CRH was significantly lower in diabetic patients than in control subjects (Fig. 2). CRH in plasma did not change significantly after glucose administration. Baseline ACTH and cortisol were significantly higher in NIDDM patients than in controls. They did not change after glucose administration in either both patients or controls.

The ratio of baseline plasma ACTH to baseline CRH was significantly greater ($P<0.01$) in NIDDM patients (23.3 ± 28.8) than in controls (2.07 ± 1.05) and the ratio of plasma cortisol to ACTH was significantly lower ($P<0.05$) in NIDDM patients (0.35 ± 0.21) than in controls (0.71 ± 0.37).

No significant correlations were found among baseline plasma CRH, ACTH and cortisol in NIDDM patients (Table 1). Baseline plasma CRH did not correlate either with plasma glucose responses (the glucose concentration at 60 min

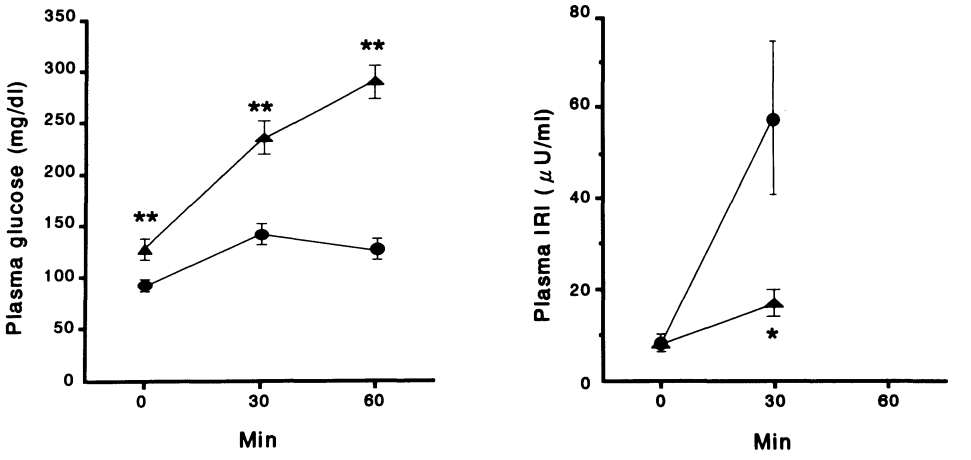

Fig. 1. Plasma glucose and immunoreactive insulin (IRI) responses in controls (●) and patients with non-insulin dependent diabetes mellitus (▲). Points and bars represent means ± SEMs. *, $P<0.05$; **, $P<0.01$.

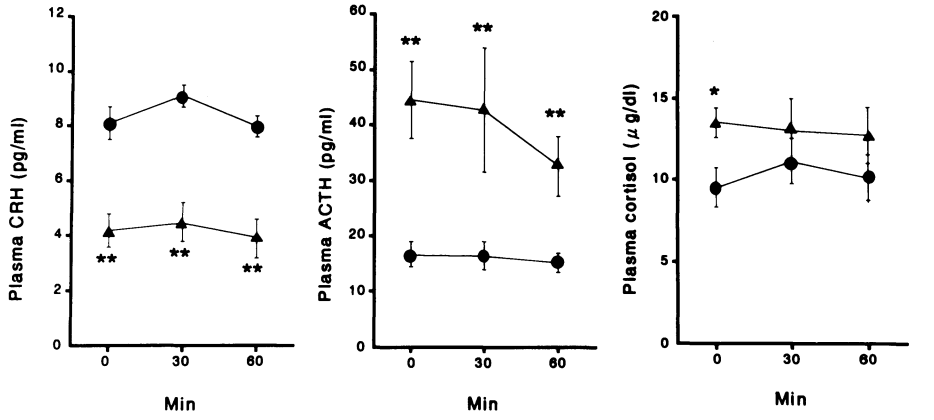

Fig. 2. Plasma CRH, ACTH and cortisol levels before and after oral administration of 75 g glucose in controls (●) and patients with non-insulin dependent diabetes mellitus (▲). Points and bars represent means ± SEMs. *, $P<0.05$; **, $P<0.01$.

Table 1. Correlations (r) among plasma levels of CRH, ACTH and cortisol, and responses of plasma glucose and IRI in NIDDM patients

	Baseline CRH	Baseline ACTH
Baseline ACTH	−0.066	
Baseline cortisol	0.381	−0.307
Glucose at 60 min	0.280	−0.110
Glucose response area	0.167	−0.009
IRI (30 min)	0.149	−0.374
Insulinogenic index	0.076	−0.268

and glucose response area by 60 min) or with IRI responses (IRI increment by 30 min and insulinogenic index). Plasma ACTH did not show any significant correlation either with glucose or with the IRI response. In normal subjects, neither baseline plasma CRH nor ACTH correlated with plasma glucose or the IRI response, although plasma ACTH and cortisol showed a positive correlation (r=0.341, $P<0.05$). (data are not shown).

Discussion

Present results show that the amounts of plasma ACTH and cortisol are greater in NIDDM patients than in controls. Some investigators have reported similar results [5–9, 14, 15], whereas others reported that the amounts of plasma ACTH in diabetics was similar to that in controls [16–18].

Although the discrepancies in these studies are difficult to explain, they might be explained by the difference between sampling methods. Although there was a significant difference between the age of NIDDM patients and controls in the present study, age difference is not responsible for an increase in ACTH and cortisol in NIDDM, as the aging has no significant influence on the ACTH-cortisol concentration [19]. Some investigators have reported that the increase in plasma ACTH and cortisol in NIDDM correlated positively with the duration of diabetes [7], the poor control state [15] and the degree of retinopathy [5, 8]. Very recently, Tsigos *et al.* [9] reported that diabetic neuropathy is associated with increased activity of the hypothalamic-pituitary-adrenal axis. These results were not confirmed in this study, as only a few patients had severe complications.

In the present investigation, baseline plasma CRH was lower in NIDDM patients than in controls. The sources of plasma CRH are the hypothalamic paraventricular nucleus and the peripheral tissues, i.e., pancreas, adrenal gland and intestine [1, 2]. The amount of plasma CRH showed no change after glucose administration either in normal controls or in NIDDM patients. The results suggest that CRH in the pancreas is not secreted by glucose.

We reported that the plasma CRH concentration in patients with long-term glucocorticoid administration was relatively low [13] and that plasma CRH increased in response to acute stress or more sustained and severe stress in rat [20, 21]. These results suggest that at least a part of plasma CRH is of hypothalamic origin. It has been reported that ACTH response to insulin-induced hypoglycemia was impaired in diabetics [16]. We recently observed that plasma CRH response to water immersion-restraint stress is smaller in NIDDM model rats (WBN/Kob) than in control Wistar rats (unpublished data). These observations and the present results suggest that it is unlikely that hypothalamic CRH secretion is increased in NIDDM patients and also suggest that the increased baseline plasma ACTH in NIDDM patients may not be ascribable to increased secretion of hypothalamic CRH. Some other factors may be responsible for the increase in plasma ACTH. Other ACTH releasing factors (vasopressin, angiotensin II, interleukins etc.) might be involved in the ACTH increase in NIDDM. Renal failure due to diabetic nephropathy may affect the metabolic clearance of ACTH and cortisol as it has been reported that plasma ACTH and cortisol levels were increased in renal insufficiency [22]. However, this is unlikely in the present investigation as none of the patients showed signs of renal insufficiency.

Several investigators [7–12] reported that diabetic patients (IDDM) were resistant to dexamethasone suppression. The present results show that the ratio of plasma ACTH to cortisol is greater in NIDDM patients than in controls in spite of an increase in baseline cortisol in NIDDM. These results suggest that negative feedback regulation by cortisol at the pituitary level may be impaired in NIDDM patients by some kinds of metabolic disorders in NIDDM.

Low plasma CRH in NIDDM could not be ascribed to the feedback suppression by ACTH and cortisol, as there was no significant correlation among plasma CRH, ACTH and cortisol in NIDDM patients. Diabetic patients under poor control have high plasma vasopressin levels due to increased plasma osmotic pressure. Therefore, increased synthesis of vasopressin in the hypothalamus might be involved in decreased plasma CRH in NIDDM. However, the effect of vasopressin on CRH secretion is still controversial. Plotsky *et al.* [23] reported that vasopressin acted as a tonic inhibitory influence at the central level to attenuate the secretion of CRH into the hypophysial portal circulation. On the other hand, Chrousos [24] reported that vasopressin was a potent stimulus of hypothalamic CRH secretion *in vitro*. Therefore, the cause of low plasma CRH in NIDDM remains to be clarified.

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