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Islet amyloid polypeptide plasma concentrations in individuals at increased risk of developing Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. To study whether abnormal secretion of islet amyloid polypeptide is involved in the development of insulin resistance and impaired insulin secretion in Type 2 (non-insulin-dependent) diabetes mellitus, we measured islet amyloid polypeptide concentrations in 56 first-degree relatives of Type 2 diabetic subjects and in 10 healthy control subjects. Fasting islet amyloid polypeptide concentrations were similar in control subjects, glucose-tolerant and glucose-intolerant relatives (8 ± 1, 9 ± 1 and 11 ± 2 fmol/ml; p = NS). The area under the islet amyloid polypeptide curve measured during an oral glucose load was larger in glucose-intolerant relatives (115 ± 13 fmol/ml) compared to glucose tolerant relatives and control subjects (88 ± 3 and 79 ± 12 fmol/ml; $p < 0.05$). The insulin response during the oral glucose load was inversely correlated with the rate of glucose disposal measured during a euglycaemic hyperinsulinaemic clamp ($r = -0.725$; $p < 0.01$), while no significant correlation was observed between the corresponding values for islet amyloid polypeptide and glucose disposal ($r = -0.380$; p = NS). Hypersecretion of islet amyloid polypeptide is observed in glucose-intolerant first-degree relatives of patients with Type 2 diabetes. Since these patients are characterized by insulin resistance and abnormal first-phase insulin secretion, the putative role of islet amyloid polypeptide in the development of these abnormalities remains to be established. It is however, unlikely that islet amyloid polypeptide is involved in the development of insulin resistance as insulin-resistant relatives with normal glucose-tolerance showed normal islet amyloid polypeptide concentrations.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, insulin resistance, first-degree relatives, islet amyloid polypeptide.

Type 2 (non-insulin-dependent) diabetes mellitus is characterized by both insulin resistance and a defective insulin secretion. The underlying causes of the disease are not known but genetic factors are known to be of major importance. First-degree relatives of patients with Type 2 diabetes are thus at increased risk of developing the disease. Many of these individuals are characterized by insulin resistance despite normal oral glucose tolerance [1].

Islet amyloid polypeptide (IAPP) is a 37 amino acid polypeptide that has been ascribed a possible pathogenetic role in Type 2 diabetes [2]. The "classic" disturbances underlying Type 2 diabetes, i. e. insulin resistance, increased hepatic glucose production and impaired insulin secretion, have all been induced by IAPP in experimental settings, implying a potential role for the peptide in the pathogenesis of Type 2 diabetes [3–5]. Since most of these studies have been carried out with non-physiological high concentrations of IAPP, the putative role of IAPP in the pathogenesis of Type 2 diabetes is still debatable. Currently there is not much support for a role for IAPP in the modulation of insulin secretion [6]. If inappropriate secretion of IAPP is involved in the development of insulin resistance or abnormal insulin secretion in predisposed individuals, one would expect to find hypersecretion of IAPP in first-degree relatives of patients with Type 2 diabetes. To test this hypothesis we related IAPP concentrations to measurements of insulin secretion and insulin sensitivity in first-degree relatives of Type 2 diabetic patients and in healthy control subjects with no family history of Type 2 diabetes.

Subjects and methods

Fifty-six first-degree relatives of patients with Type 2 diabetes and 10 healthy control subjects participated in the study (Table 1). Of the first-degree relatives nine had impaired glucose tolerance (IGT), while 47 were glucose-tolerant, based on World Health Organisation criteria.

Informed consent was obtained from all subjects and the study protocol was approved by the Ethical Committee of Helsinki University Central Hospital.

Table 1. Clinical characteristics, fasting concentrations and area under the curve of glucose, insulin and islet amyloid polypeptide (IAPP) concentrations in control subjects, in glucose-tolerant (NGT) and glucose-intolerant first-degree relatives of Type 2 diabetic patients (IGT)

	Control subjects	First-degree relatives	
		NGT	IGT
n	10	47	9
Age (years)	48 ± 5	40 ± 2	47 ± 4
Body mass index (kg/m^2)	23.8 ± 1.3	24.8 ± 0.6	27.6 ± 0.9
Fasting glucose (mmol/l)	4.9 ± 0.3	5.0 ± 0.1	5.5 ± 0.2
Area under glucose curve (mmol/l)	29.5 ± 2.0	30.7 ± 0.7	44.4 ± 1.7[a]
Fasting insulin (pmol/l)	45 ± 6	49 ± 3	96 ± 22[a]
Area under insulin curve (pmol/l)	917 ± 135	1225 ± 84	2973 ± 460[a]
Fasting IAPP (fmol/ml)	8 ± 1	9 ± 1	11 ± 2
Area ander IAPP curve (fmol/ml)	79 ± 12	88 ± 3	115 ± 13[b]

[a] $p < 0.01$ vs all other groups; [b] $p < 0.05$ vs all other groups

Metabolic studies

All subjects participated in an oral glucose tolerance test (75 g glucose) after 12-h overnight fast. Venous blood samples were taken at −15, 0, 30, 60, 90, and 120 min after glucose ingestion for determination of plasma glucose, serum insulin and plasma IAPP concentrations.

Thirteen subjects (four control subjects, four glucose-tolerant and five glucose-intolerant first-degree relatives of patients with Type 2 diabetes) with varying degrees of insulin resistance participated in a euglycaemic hyperinsulinaemic clamp. After obtaining three basal samples for glucose, insulin and IAPP determinations a primed constant infusion of short-acting human insulin (Actrapid, Novo Industry, Copenhagen, Denmark) was administered at a rate of 340 pmol · min^{-1} · m^{-1} for 2 h. Indirect calorimetry was employed to estimate net rates of carbohydrate and lipid oxidation.

Analytical determinations

Plasma glucose was assayed with a glucose oxidase method on a Glucose Analyser II (Beckman Instruments, Fullerton, Calif., USA). The insulin concentration in serum was measured by a double-antibody RIA (Pharmacia, Uppsala, Sweden). IAPP was extracted from 5 ml of plasma by adsorption onto a Sep-Pac C-18 cartridge which was pre-equilibrated with 0.9% NaCl. The cartridge was washed first with 0.9% NaCl and then 0.1% trifluoroacetic acid (TFA) and IAPP was eluted with 60% CH_3CN solution containing 0.1% TFA. The eluate from the oral glucose tolerance tests was lyophilized, reconstituted with RIA buffer, and subjected to RIA. The eluate from the clamp studies was lyophilized, then dissolved in 0.1 mol/l sodium phosphate buffer (pH 7.4) containing 0.05% Triton X-100 and subjected to immunoaffinity chromatography on an anti-human IAPP IgG-Affi-Gel 10 column (150 µl gel) as reported previously (7). The immunoaffinity column was washed with the phosphate buffer, IAPP eluted with a solution of 1 mol/l acetic acid containing 10% CH_3CN and 0.05% Triton X-100. The eluate was evaporated, and subjected to RIA.

Statistical analysis

All data are expressed as means ± SEM. A BMDP statistical package was used for the statistical analyses. One-way analysis of variance or the Welch test was used to test the equality of group means. Scheffe's method was used for multiple comparisons between group means. A p-value less than 0.05 was considered statistically significant.

Results

Oral glucose tolerance test

Plasma glucose, serum insulin and plasma IAPP responses to the oral glucose load in control subjects, in glucose-tolerant and in glucose-intolerant first-degree relatives are shown in Figure 1. During the oral glucose tolerance test the area under the IAPP curve was larger in glucose-intolerant first-degree relatives than in control subjects

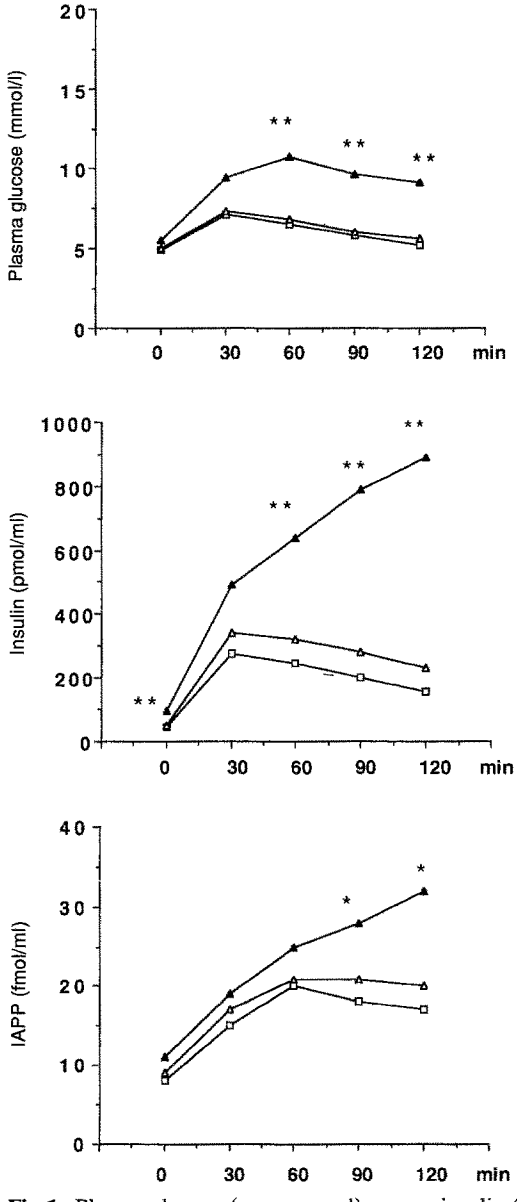

Fig. 1. Plasma glucose (upper panel), serum insulin (middle panel) and plasma islet amyloid polypeptide (IAPP) responses (lower panel) to 75 g oral glucose load in control subjects (□), in glucose-tolerant (△), and in glucose-intolerant first-degree relatives (▲). * $p < 0.05$ vs all other groups; ** $p < 0.01$ vs all other groups

and glucose-tolerant relatives ($p < 0.05$) (Table 1). Fasting insulin and IAPP concentrations ($r = 0.582$; $p < 0.001$) and the areas under the insulin and IAPP curves ($r = 0.709$; $p < 0.001$) showed a strong positive correlation.

Euglycaemic hyperinsulinaemic clamp

The glucose disposal rate in the 13 subjects studied varied between 2.3 and 9.1 $mg \cdot kg^{-1} \cdot min^{-1}$ during the euglycaemic hyperinsulinaemic clamp. Both C-peptide and IAPP concentrations were suppressed from basal by about 34% during the euglycaemic hyperinsulinaemic clamps in insulin-sensitive and insulin-resistant subjects indicating that there is a normal feedback mechanism by insulin on IAPP secretion. No correlation was observed between basal or insulin-suppressed IAPP concentrations and total glucose disposal or non-oxidative glucose disposal. The area under the insulin ($r = -0.725$; $p < 0.01$) but not IAPP ($r = -0.380$; p = NS) curve during the oral glucose tolerance test correlated inversely with the rate of insulin-stimulated glucose disposal during the clamp.

Discussion

Recent studies suggest that IAPP may be involved in the pathogenesis of Type 2 diabetes [2, 4, 5]. An overproduction of IAPP is proposed to explain the peripheral insulin resistance found in Type 2 diabetes. Insulin resistance is known to predict future development of Type 2 diabetes in first-degree relatives of Type 2 diabetic patients [8]. The observation by Butler and co-workers of similar basal and post-meal IAPP concentrations in Type 2 diabetic patients and control subjects could be taken as evidence against a role for this peptide in the pathophysiology of Type 2 diabetes [9]. However, the possibility that IAPP concentrations may be increased in the pre-diabetic stage of Type 2 diabetes cannot be excluded. If an overproduction of IAPP would contribute to insulin resistance in Type 2 diabetes, increased IAPP concentrations should be expected in this risk group. The strong correlation between both basal and stimulated insulin and IAPP concentrations support the concept that these two peptides are co-secreted from the Beta cell.

Previous studies suggesting a relationship between IAPP and peripheral glucose utilization have been performed in experimental animals employing IAPP concentrations 100–1000-fold greater than measured circulating levels. Therefore, it is questionable whether IAPP in physiological concentrations can be responsible for the insulin resistance observed in Type 2 diabetes. To address this question in man we performed euglycaemic hyperinsulinaemic clamps in a subset of the subjects. Although the first-degree relatives were insulin resistant no significant abnormalities in IAPP secretion could be observed challenging the view that hypersecretion of IAPP contributes to insulin resistance in these subjects. Furthermore, no correlation between IAPP concentrations and glucose disposal was observed.

The high IAPP responses observed in first-degree relatives with IGT is interesting. Despite having similar degrees of insulin resistance as the glucose-tolerant first-degree relatives, the IGT group is known to show impaired insulin secretion [1]. Of interest, cats with IGT show amyloid deposits in their Beta cells [10]. If this also applies to humans with IGT, increased IAPP secretion could hypothetically cause amyloid formations which would interfere with insulin secretion in these subjects.

It should be emphasized that the present study has examined associations, therefore no conclusions can be drawn on causality. Insulin-resistant first-degree relatives with normal glucose tolerance show normal IAPP concentrations challenging the view that hypersecretion of IAPP is associated with insulin resistance in these subjects. Interestingly, IGT patients showed enhanced IAPP responses to the oral glucose load. Whether this hypersecretion of IAPP is of importance in the pathogenesis of impaired (first-phase) insulin secretion observed in these subjects remains to be studied.

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