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Rapid communications

Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans

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Summary. Amylin is a 37-amino acid pancreatic polypeptide, probably involved in the pathophysiology of Type 2 (non-insulin-dependent) diabetes mellitus. We have determined amylin in human plasma by extraction-based radioimmunoassay (Sep-Pak C18). Of 23 healthy control subjects plasma amylin was determined as 11.9 ± 3.5 ng/l. Of 27 patients with Type 2 diabetes receiving insulin the amylin levels were lower, and in 16 patients with Type 2 diabetes on oral medication they were higher than in the control subjects: 8.2 ± 4.4 ng/l ($p < 0.01$) vs 18.8 ± 9.9 ng/l ($p < 0.05$). In 14 Type 1 (insulin-dependent) diabetic patients we found extremely low mean amounts of amylin: 2.9 ± 1.9 ng/l ($p < 0.002$). Thus, basal amylin appears to be associated with the capacity to release insulin. An oral glucose load stimulated the release of amylin, this was more pronounced in patients with Type 2 diabetes than in healthy subjects. An excellent correlation of mean amylin with mean insulin concentrations was obtained ($r = 0.949$). In patients with Type 2 diabetes amylin was reduced congruent to a decrease in C-peptide during a hyperinsulinaemic, euglycaemic glucose clamp experiment ($r = 0.971$ for linear correlation between C-peptide levels and amylin). We conclude, that amylin and insulin are co-secreted in humans, and that the amylin release is under feedback-control by insulin.

Key words: Amylin, diabetes associated polypeptide, islet amyloid polypeptide.

Human insulinoma, as well as pancreatic islets of humans and animals with Type 2 (non-insulin-dependent) diabetes mellitus contain amylin, a 37-amino acid peptide (relative molecular mass: 3850 Dalton), which forms destructive pancreatic hyalinosis/amyloidosis-deposits. Co-secretion of amylin with insulin has been supposed because of their co-localisation within the same secretory granules of Beta cells [1, 2].

By extraction based amylin – radioimmunoassay (RIA), we compared, amylin concentrations of healthy subjects with those of diabetic patients under basal conditions, as well as during stimulation of insulin release by oral glucose intake. In another approach we studied plasma kinetics of amylin in Type 2 diabetic patients during suppression of endogenous insulin release by the administration of exogenous insulin.

Subjects and methods

All the subjects studied gave their informed consent. Five Type 2 diabetic patients (55 ± 10 years) were enrolled in a hyperinsulinaemic, euglycaemic glucose clamp experiment: at an i. v. infusion rate of insulin 240 pmol $\cdot m^{-2} \cdot min^{-1}$, plasma levels of glucose were maintained between 4.5 and 5.6 mmol/l.

For the oral glucose tolerance test seven healthy control subjects and seven Type 2 diabetic patients ingested 75 g of glucose. Within each experiment venous blood samples were withdrawn at definite time intervals for determination of the different parameters (Figs. 1 and 2).

Analytical procedures

Serum insulin and C-peptide were measured by commercial RIA (RIA-gnost Insulin, Behringwerke AG, Marburg, FRG; RIA-mat C-Peptid II, Byk-Sangtek, Dietzenbach, FRG). Serum glucose was determined by the Parallel Analyser from American Monitor Corporation, Indianapolis, Ind., USA.

Extraction from plasma and RIA of amylin

For extraction of amylin from EDTA-plasma (at least 5 ml) we used Sep-Pak C18 cartridges (Waters/Millipore, Milford, Mass., USA), with methanol/trifluoroacetic-acid (TFA)/water as the mobile phase. The peptide was desorbed by 2×2 ml of a solution of methanol/TFA/water, 90/0.5/9.5 by volume. Vacuum-concentration ("Speed-Vac", Savant Instruments, Farmingdale, NY, USA) yielded dry plasma extracts (stored at –25 °C). For amylin RIA we used components available from Peninsula Laboratories, Belmont, Calif., USA. The second antibody-immunoprecipitating reagent was from

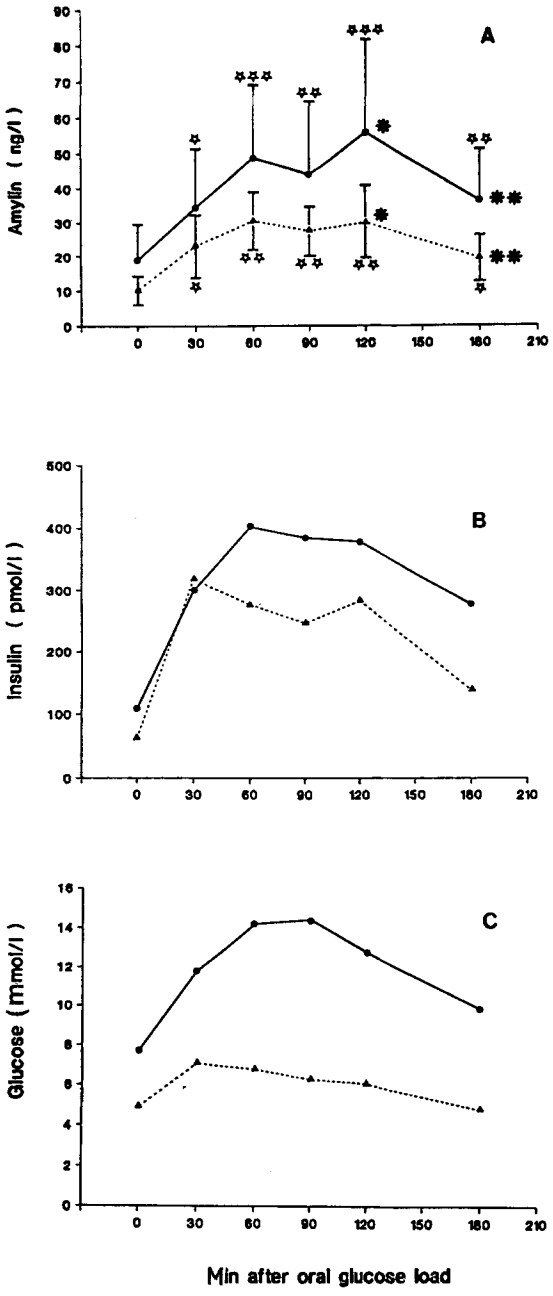

Fig. 1a–c. Time course of mean (± SD) plasma levels of amylin (**a**), insulin (**b**), and glucose (**c**) in Type 2 diabetic patients (●) or healthy control subjects (▲) after ingestion of a 75 g glucose load. Significance of differences between mean amylin levels at different times, relative to initial mean values within each group are shown thus (control subjects in brackets): *: $p < 0.1$ ($p < 0.01$); **: $p < 0.05$ ($p < 0.001$); *** $p < 0.02$. ✶ and ✶ ✶ indicate differences at the niveau of $p < 0.05$, and $p < 0.02$ for between-group comparison

Sorin/Biomedica (Saluggia, Italy). Amylin RIA was performed as a sequential assay with delayed addition of tracer (24h/24h incubation), and the second antibody separation of peptide bound from unbound. The data was processed computer-aided based on a logit/log transformation of the calibration curve.

Statistical analysis

Data is mean ± SD, significance of differences has been calculated by two tailed U-test (rank test) according to Wilcoxon, Mann, and Whitney.

Results

Characteristic data of amylin RIA

The limit of detection was 1.5 pg per RIA-tube at a linear range extending from 10 pg–70 pg per RIA-tube. Recovery of synthetic amylin from plasma was 85 ± 5%, with 75% as the lowest, and 90% as the highest extreme. Within and between run precision were 10%, and 15% respectively. All results are from duplicate samples, and have not been corrected for recoveries.

Rechromatography on Sephadex G 50 of Sep-Pak C18 extracts from two different EDTA-plasma samples (40 ml each, containing 11 and 15 pg of amylin-immunoreactive material per ml) gave only a single peak of amylin-immunoreactivity, coincident with synthetic human amylin (data not shown).

Concentration of amylin in plasma of healthy adults, and patients with Type 1 and Type 2 diabetes mellitus

The plasma-amylin of 23 healthy subjects (aged 42.6 ± 14.7 years) was 11.9 ± 3.5 ng/l (range: 6.2–17 ng/l). The 27 Type 2 diabetic patients (aged 62 ± 8.5 years) receiving insulin had lower amylin levels, and the 16 Type 2 diabetic patients (aged 56 ± 12.9 years) on oral antidiabetic therapy had higher mean amylin levels than the control subjects: 8.2 ± 4.4 ng/l (range: 1.0–15.2 ng/l; $p < 0.01$), vs 18.8 ± 9.9 ng/l (range: 5–42 ng/l; $p < 0.05$). Levels of the peptide were lowest in the plasma of 14 Type 1 diabetic patients (aged 44.6 ± 14.1 years): 2.9 ± 1.9 ng/l (range: < 1.3–6.8 ng/l; $p < 0.002$).

Data stem from independent RIA runs with three sets of plasma samples that had been collected on different occasions, each set included samples from patients out of each group.

Stimulation of amylin, release by oral glucose intake

A 75 g glucose load was given as a drink to seven healthy volunteers, (aged 37 ± 16 years), and seven Type 2 diabetic patients (aged 48 ± 12 years). As shown in Figure 1, in both groups there was a biphasic rise in plasma amylin. Peak levels at 60 and 120 min after glucose ingestion were more pronounced in the diabetic group (Fig. 1 A). Figures 1 B, C show the course of insulin, and glucose. There was an excellent linear correlation between mean levels of amylin and insulin at any time during the experiment ($r = 0.949$).

Suppression of amylin by exogenous insulin in patients with Type 2 diabetes mellitus

The hyperinsulinaemic euglycaemic clamp experiment, performed on five Type 2 diabetic patients led to a plateau of mean serum insulin levels after 60 min ranging from 320–420 pmol/l, and glucose levels ranging from 4.5–5.5 mmol/l. We observed a progressive decrease in mean concentrations of plasma amylin, excellently in par-

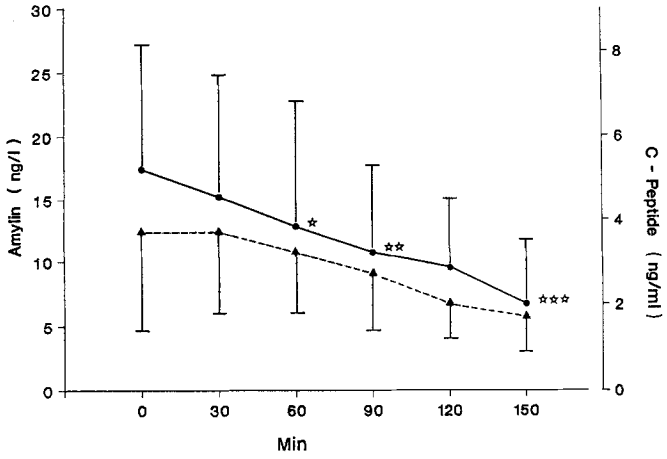

Fig. 2. Time course of mean (±SD) levels of amylin (●), and C-peptide (▲) in plasma of Type 2 diabetic patients, who underwent a hyperinsulinaemic euglycaemic clamp experiment (i.v. infusion of 240 pmol·m^{-2}·min^{-1} of insulin). Significance of differences of mean amylin values relative to pre-experimental levels: * $p < 0.1$; ** $p < 0.05$; *** $p < 0.02$ (two tailed U-test)

allel with those of C-peptide (Fig. 2). There was also a strong linear correlation ($r = 0.971$) between amylin and C-peptide.

Discussion

Data on human plasma levels of amylin have been reported by several groups [3–5, 9, 10]. Furthermore, co-secretion of amylin and insulin has been demonstrated in the isolated rat pancreas [6, 7], as well as rat Beta cells [8]. Glucose has been shown to stimulate amylin release in non-diabetic human subjects [9, 10], and i.v. insulin has abolished secretion of the peptide [9]. The correlation between serum insulin and plasma amylin has been interpreted as co-secretion of the peptides.

We have found plasma amylin to be drastically reduced in Type 1 diabetes, a situation known to be more or less devoid of release of endogenous insulin. This is a strong argument for co-secretion. Our Type 2 diabetic patients receiving insulin had lower and those on oral medication had higher mean levels of plasma amylin than the control subjects. This is evidence for co-secretion of amylin with insulin, because of the known suppression of endogenous insulin by exogenous hormone in the first group, and enhancement of insulin release by oral medication in the second group.

Other authors [4, 10] did not observe this differential behaviour. This can be explained by insensitive assay conditions used in one case [4]. The authors of the second paper [10] have, however, found slightly supranormal plasma amylin in patients with Type 2 diabetes; they did not look at patients taking insulin medication. Because of a considerable overlap of amylin levels, in our study too, significant differences have been uncovered with high numbers of patients only, fairly exceeding those of the second paper [10]. Differences between groups cannot be due to variation in RIA-performance: each of the three sets of samples used for a run of the assay contained plasma samples of patients from each group, as well as control subjects.

Stimulation of insulin release by oral glucose load is closely paralleled by release of amylin. Plasma levels of the peptides have a very strong correlation.

Release of amylin and insulin are co-suppressed by exogenous insulin, as shown in the clamp experiment. Again, a strong linkage is indicated by a high correlation between their mean plasma levels.

Serum glucose was maintained constant, and within normal levels in our clamp-experiment. Furthermore, amylin levels were low in patients on therapy with insulin, none of which had subnormal serum-glucose. Therefore, suppression of amylin-release by low glucose, as proposed previously [9] is very unlikely.

We conclude, that amylin and insulin are co-secreted, and that exogenous insulin suppresses amylin at a yet unidentified stage of its biosynthesis, storage, or release.

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References

1. Johnson KH, O'Brien TD, Betsholtz C, Westermark P (1989) Islet amyloid, islet-amyloid polypeptide, and diabetes mellitus. N Engl J Med 321: 513–518
2. Nishi M, Sanke T, Nagamatsu S, Bell GI, Steiner DF (1990) Islet Amyloid Polypeptide. J Biol Chem 265: 4173–4176
3. Nakazato M, Asai J, Kangawa K, Matsukura S, Matsuo H (1989) Establishment of radioimmunoassay for human islet amyloid polypeptide and its tissue content and plasma concentration. Biochem Biophys Res Commun 164: 394–399
4. Van Jaarsveld BC, Hackeng WHL, Nieuwenhuis MG, Erkelens DW, Geerding RA, Lips CJM (1990) Islet amyloid polypeptide in human plasma. Lancet I: 60 (Letter)
5. Hartter E, Svoboda T, Lell B, Schuller M, Ludvik B, Woloszczuk W, Prager R (1990) Reduced islet-amyloid polypeptide in insulin-dependent diabetes mellitus. Lancet I: 854 (Letter)
6. Ogawa A, Harris V, McCorkie SK, Unger RH, Luskey KL (1990) Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. J Clin Invest 85: 973–976
7. Fehmann HC, Weber V, Göke R, Göke B, Arnold R (1990) Cosecretion of amylin and insulin from isolated rat pancreas. FEBS Lett 262: 279–281
8. Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinck JW, Taborsky Jr GJ, Porte Jr D (1990) Evidence of cosecretion of islet amyloid polypeptide and insulin by β-cells. Diabetes 39: 634–638
9. Mitsukawa T, Takemura J, Asai J, Nakazato M, Kangawa K, Matsuo H, Matsukura S (1990) Islet amyloid polypeptide response to glucose, insulin, and somatostatin analogue administration. Diabetes 39: 639–642
10. Butler PC, Chou J, Carter WB, Wang Y-N, Bu B-H, Chang D, Chang J-K, Rizza RA (1990) Effect of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. Diabetes 39: 752–756

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