Increased Plasma Amylin in Type 1 Diabetic Patients After Kidney and Pancreas Transplantation

A sign of impaired β-cell function?

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OBJECTIVE — In response to hyperglycemia, β-cells release insulin and C-peptide, as well as islet amyloid pancreatic polypeptide, which is involved in glucose homeostasis. After successful pancreas-kidney transplantation (PKT), type 1 diabetic patients may revert to a nondiabetic metabolism without exogenous insulin therapy and re-secrete all β-cell hormones.

RESEARCH DESIGN AND METHODS — Using mathematical models, we investigated hormone (amylin, insulin, C-peptide) and metabolite (glucose, free fatty acids) kinetics, β-cell sensitivity to glucose, and oral glucose insulin sensitivity index (OGIS) in 11 nondiabetic type 1 diabetic patients after PKT (BMI 25 ± 1 kg/m^2, 47 ± 2 years of age, 4 women/7 men, glucocorticoid-free), 6 matching nondiabetic patients after kidney transplantation (25 ± 1 kg/m^2, 50 ± 5 years, 3 women/3 men, on glucocorticoids), and 9 matching nondiabetic control subjects (24 ± 1 kg/m^2, 47 ± 2 years, 4 women/5 men) during a 3-h 75-g oral glucose tolerance test (OGTT).

RESULTS — PKT patients had higher fasting amylin (19 ± 3 vs. control subjects: 7 ± 1 pmol/l) and insulin (20 ± 2 vs. control subjects: 10 ± 1 μU/ml; each $P < 0.01$) levels. Kidney transplant subjects showed increased OGTT plasma insulin at 90 min and C-peptide levels (each $P < 0.05$). In PKT patients, plasma glucose from 90 to 150 min was 9–31% higher ($P < 0.05$ vs. control subjects). Amylin clearance was comparable in all groups. Amylin's plasma concentrations and area under the concentration curve were up to twofold higher in PKT patients during OGTT ($P < 0.05$). OGIS was not significantly different between groups. β-Cell sensitivity to glucose was reduced in PKT patients (−64%, $P < 0.009$). Fasting plasma amylin was inversely associated with β-cell sensitivity to glucose ($r = -0.543$, $P < 0.004$).

CONCLUSIONS — After successful PKT, type 1 diabetic patients with nondiabetic glycemia exhibit increased fasting and post–glucose load plasma amylin, which appears to be linked to impaired β-cell function. Thus, higher amylin release in proportion to insulin might also reflect impaired β-cell function in type 1 diabetic patients after PKT.

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Simultaneous pancreas-kidney transplantation (PKT) is the treatment of choice in type 1 diabetic patients with end-stage renal disease. Successful pancreas transplantation in type 1 diabetic patients results in sustained normalization of fasting plasma glucose and HbA_{1c} (A1C) levels without exogenous insulin therapy (1–8), which additionally protects the transplanted kidney from exposure to hyperglycemia. However, qualitative and quantitative defects of β-cell function have been demonstrated in most, but not all, PKT patients with nondiabetic glycemic control (9–13).

Autoimmune β-cell destruction in type 1 diabetic patients does not only result in an absolute deficiency of insulin, but also a deficiency of C-peptide and islet amyloid polypeptide (amylin), both of which are cosecreted with insulin, but are not of vital importance. Although the role of C-peptide in metabolism is still debated (14), amylin is clearly involved in glucose homeostasis through the inhibition of gastric emptying and postprandial hepatic glucose production, eventually reducing postprandial glucose excursions (15). Most recently, the synthetic amylin analog pramlintide was shown to improve glycemic control in type 1 diabetic patients, leading to a reduction of daytime glucose excursion and improvement of A1C (16–19).

Although amylin secretion has not yet been investigated in type 1 diabetic patients after successful PKT, it may be completely restored in these patients. Based on this assumption, the association between amylin secretion and plasma concentration on the one hand and glycemia, β-cell function, and insulin sensitivity on the other, in patients after pancreas transplantation, might provide further insight

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Abbreviations: AUC, area under the concentration curve; FFA, free fatty acid; OGIS, oral glucose insulin sensitivity index; OGTT, oral glucose tolerance test; PKT, pancreas-kidney transplantation.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Characteristics as well as basal levels and total and suprabasal AUC of glucose, insulin, C-peptide, and amylin; parameters of insulin and amylin kinetics; estimates of β-cell function; and insulin sensitivity during the OGTT in type 1 diabetic patients after PKT, nondiabetic patients after kidney transplant, and nondiabetic control subjects*

	PKT patients	Kidney transplant patients	Control subjects
n	11	6	9
Age (years)	46.5 ± 2.4	49.9 ± 4.5	46.5 ± 3.2
Sex (F/M)	4/7	3/3	4/5
BMI (kg/m^2)	25.1 ± 0.8	25.3 ± 0.7	24.2 ± 0.9
A1C (%)	6.0 ± 0.1*	5.4 ± 0.2	5.5 ± 0.2
Creatinine clearance (ml/min)	68.1 ± 4.0†	58.8 ± 6.4†	103.1 ± 8.0
RRsys/diast (mmHg)	134 ± 9/78 ± 3	136 ± 5/86 ± 3	118 ± 3/79 ± 3
Fasting glucose (mg/dl)	95.6 ± 2.9	93.7 ± 3.7	91.2 ± 2.9
Glucose AUC (mol/l per min)	1.4 ± 0.1	1.4 ± 0.1	1.1 ± 0.1
Glucose ΔAUC (mol/l per min)	0.34 ± 0.05	0.38 ± 0.11	0.19 ± 0.06
Basal insulin (μU/ml)	19.5 ± 1.7‡	13.1 ± 2.5	10.3 ± 1.1
Insulin AUC (nmol/l per min)	65.9 ± 5.1	98.0 ± 27.1§	53.7 ± 8.3
Insulin ΔAUC (nmol/l per min)	44.8 ± 4.2	83.8 ± 26.2§	42.6 ± 7.3
Basal C-peptide (ng/dl)	249 ± 18	360 ± 78‖	168 ± 26
C-peptide AUC (nmol/l per min)	397 ± 16	710 ± 156¶	421 ± 37
C-peptide ΔAUC (nmol/l per min)	249 ± 16	496 ± 111¶	321 ± 29
Basal amylin (pmol/l)	18.5 ± 3.2‡	13.5 ± 3.3	6.7 ± 1.1
Amylin AUC (nmol/l per min)	7.2 ± 1.0§	8.8 ± 4.0	3.6 ± 0.5
Amylin ΔAUC (nmol/l per min)	3.8 ± 0.6	6.3 ± 3.5	2.4 ± 0.4
Basal insulin secretion (pmol/l per min)	50.7 ± 3.6§	73.3 ± 15.8¶	34.2 ± 5.2
Total insulin secretion (nmol/l)	25.3 ± 1.1	46.1 ± 10.7¶	26.6 ± 2.4
Hepatic insulin extraction (%)	56.2 ± 3.3§	66.0 ± 2.8	67.2 ± 3.1
Basal amylin secretion (pmol/l per min)	0.54 ± 0.12§	0.49 ± 0.16	0.16 ± 0.03
Total amylin secretion (nmol/l)	0.27 ± 0.06	0.30 ± 0.10	0.13 ± 0.02
Amylin-insulin cosecretion factor	1.05 ± 0.23§	0.62 ± 0.12	0.48 ± 0.07
Amylin clearance (min)	0.033 ± 0.004	0.045 ± 0.013	0.035 ± 0.003
β-Cell sensitivity to glucose	85.5 ± 8.6‡	136.7 ± 19.3	235.7 ± 54.3
Adaptation index (nmol · min^{-1} · ml^{-1})	164 ± 6	244 ± 27¶	189 ± 17
OGIS (ml/min per m^2)	412 ± 13	383 ± 43	450 ± 20

Data are means ± SE. ANOVA with Dunnet and Bonferroni post hoc: *$P < 0.05$, PKT patients vs. control subjects and kidney transplant patients; †$P < 0.001$ vs. control subjects; ‡$P < 0.01$, PKT patients vs. control subjects; §$P < 0.05$ vs. control subjects; ‖$P < 0.01$ vs. control subjects; ¶$P < 0.05$, kidney transplant patients vs. control subjects and PKT patients. ΔAUC, suprabasal area under curve; RR sys/diast, systolic and diastolic blood pressure.

into the effects of this hormone in humans. Because amylin exerts most of its effects—such as delaying gastric emptying and suppressing postprandial hepatic glucose production—in the postprandial state, we performed a 3-h oral glucose tolerance test (OGTT) in type 1 diabetic patients who had undergone simultaneous pancreas and kidney transplantation and were on glucocorticoid-free immunosuppression and in matched nondiabetic control subjects to investigate the kinetics of amylin, insulin, C-peptide, and free fatty acids (FFAs) as well as insulin sensitivity and β-cell function and their interrelationships. To evaluate possible effects of immunosuppression and expectably reduced kidney function in the PKT patients, another group of nondiabetic patients after successful kidney transplantation matched for major anthropometric characteristics and with creatinine clearance comparable to that of PKT was studied under the described conditions.

RESEARCH DESIGN AND METHODS

— Type 1 diabetic patients ($n = 11$) after successful combined PKT were matched for age, sex, and BMI with nine healthy control subjects and six nondiabetic patients after successful kidney transplantation (Table 1). Type 1 diabetic patients (duration of type 1 diabetes: 31 ± 2 years) had received a whole pancreas with systemic venous anastomosis to the iliac vein (2.4 ± 0.5 years [range 0.8–5.2]) before the study (12). By this method of systemic drainage, pancreatic endocrine secretions bypass the physiological first-pass clearance of the liver (9,12,20). At the time of examination, the immunosuppressive regimen in PKT patients had been free of glucocorticoids for at least 5 months and included tacrolimus (2.5–8 mg/day, plasma level on study day: 13.2 ± 2.8 ng/ml) combined with either mycophenolate mofetile (1–2 g/day, $n = 9$) or azathioprine (25–50 mg/day, $n = 2$). Until the beginning of the study, none of the PKT patients had a graft rejection. Glucocorticoids were included in the initial immunosuppressive protocol but were discontinued within 3 months after successful transplantation.

The kidney transplant patients underwent successful kidney transplantation 11.2 ± 0.5 years before the study. One of the patients had had an acute rejection of the first kidney graft 9 years ago, following a successful re-transplantation of a second kidney graft 1 year later. None of the other patients suffered from any graft rejection period. At the time of examination, the immunosupressive regi-

men in kidney transplant patients included prednisolon (2.5–10 mg/day) and tacrolimus (2 mg/day, $n = 1$, plasma level on study day: 10.1 ng/ml) combined with either mycophenolate mofetile (1–2 g/day, $n = 4$) or azathioprine (50–75 mg/day, $n = 2$) and/or cyclosporine-A (75–200 mg/day, $n = 4$, plasma level on study day: 68.0 ± 2.8 ng/ml).

Normal fasting plasma glucose, A1C <6.5%, and stable creatinine were required for participation in the study. No subject was using insulin or any other hypoglycemic agent to maintain glucose control. All participants gave their written informed consent to the protocol, which had been approved by the local ethics committee.

OGTT

At Lainz hospital (Vienna, Austria), a 3-h OGTT was performed after a minimum 12-h overnight fast. A catheter (Venflon; Becton Dickinson, Stockholm, Sweden) was inserted into an antecubital vein of the forearm for blood sampling. The subjects were given the standard dose of 75 g glucose in H_2O solution (Glukodrink; Roche Diagnostics, Vienna, Austria) within 5 min and were maintained on bed rest. Venous blood samples for measurement of metabolites and hormones were drawn in the fasting state and at 10, 20, 30, 40, 60, 90, 120, 150, and 180 min after glucose load. A1C, plasma glucose, and serum creatinine were measured using routine laboratory methods (www.kimcl.at). Blood was rapidly centrifuged, and plasma and serum aliquots were stored at −70°C until further analysis.

Laboratory measurements

Plasma FFAs were determined with a commercially available enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Plasma insulin was determined with a radioimmunoassay (RIA; BioChem Immunosystems, Freiburg, Germany) and serum C-peptide by dissociation-enhanced lanthanide flouroimmunoassay (Delfia, Wallac Oy, Finland). Plasma human total amylin was measured using an enzyme-linked immunosorbent assay (Linco Research, St. Charles, MO).

Calculations and statistics

Total areas under the concentration curves of glucose ($AUC_{glucose}$), insulin ($AUC_{insulin}$), C-peptide ($AUC_{C\text{-}peptide}$), and amylin (AUC_{amylin}) were calculated with the trapezoidal rule. To better compare hormone release in response to the glucose challenge, suprabasal AUCs (ΔAUC), i.e., total AUC − basal concentration × 180 min, were used. Differences between groups were assessed by performing χ^2 tests for categorical variables. Continuous variables were analyzed with ANOVA following a Dunnet or Bonferroni post hoc test (as indicated). Linear correlations are based on Spearman's correlations. Statistical analyses were performed using SPSS (SPSS, Chicago, IL), Statistica (StatSoft, Tulsa, OK), and/or MedCalc (MedCalc Software, Mariakerke, Belgium) computer software. Data are presented as means ± SE. Differences between groups at $P \leq 0.05$ were considered to be statistically significant.

Insulin secretion and clearance. Mathematical models were used to assess β-cell function, secretion, and clearance of C-peptide, insulin, and amylin from OGTT data (21,22). In PKT patients, insulin and C-peptide are delivered directly into the systemic circulation, whereas in control subjects, they undergo a first pass through the liver. Because the liver does not play any significant role in C-peptide dynamics, we analyzed the kinetics of C-peptide (23,24) instead of insulin. The secretion model described (21) and thoroughly validated elsewhere (22) reconstructs the patterns per unit volume of C-peptide release (basal secretion rate and total stimulated secretion rate) and its systemic appearance. Therefore, hepatic insulin extraction (as percentage of the secreted hormone) was simply approximated by $[1 - (AUC_{insulin}/AUC_{C\text{-}peptide})]$. Given the different drainage in PKT patients, this formula evaluates extraction in every circumstance; in addition, the calculated value refers to the whole extraction of the hormone during the entire OGTT period.

Amylin secretion and clearance. Amylin basal secretion, total secretion, clearance, and amylin/insulin cosecretion factor were estimated using a mathematical model described (21,25) and thoroughly validated with ad hoc kinetic studies (26,27) and applied in several studies (28). The amylin/insulin cosecretion factor may be regarded as a physiological parameter that relates amylin delivery to β-cell insulin secretion rate under the assumption of parallel secretion (25,27,28).

FFAs. The pattern of FFAs was assessed by calculating the slope of the curve between zero time and 60 min, thus mapping the course of FFA suppression during OGTT. The basal level and the difference between nadir and fasting FFAs were also determined.

Insulin sensitivity. Insulin sensitivity during an OGTT was assessed by the oral glucose insulin sensitivity index (OGIS), which takes into account known relationships between glucose disappearance and insulin (29). OGIS, which describes glucose clearance, has been validated against and applied instead of the corresponding value obtained from the gold standard (namely the euglycemic-hyperinsulinemic clamp test) in healthy, obese, and diabetic subjects (29–31).

β-Cell function. The adaptation index describes the capacity of the β-cell to release increasing amounts of insulin to compensate for the increasing insulin resistance (32). The adaptation index was calculated as OGIS × $\Delta AUC_{C\text{-}peptide}$. β-Cell sensitivity to glucose was calculated as total stimulated secretion rate over $\Delta AUC_{glucose}$. For comparison of β-cell dynamics between the three groups, the different ways of pancreatic venous drainage were taken into account by using C-peptide modeling (23,24) instead of insulin kinetics, since C-peptide is eliminated preliminary by the kidney. With respect to reduced renal function, thus possibly influencing C-peptide elimination kinetics, we examined a group of kidney transplant patients matched for creatinine clearance.

RESULTS

Participant characteristics

The clinical characteristics of PKT patients, kidney transplant patients, and control subjects are shown in Table 1. The groups were matched for age, sex, and BMI. When compared with the control subjects, PKT and kidney transplant patients had similar blood pressure values but a lower creatinine clearance ($P < 0.001$, PKT and kidney transplant patients vs. control subjects; NS, PKT vs. kidney transplant patients). PKT patients had higher A1C values than control subjects and kidney transplant patients ($P < 0.03$).

OGTT

Data from the OGTT for all measured compounds are shown in Fig. 1 and Table 1.

Glucose

Fasting plasma glucose concentration was not different in PKT patients, kidney transplant patients, and control subjects

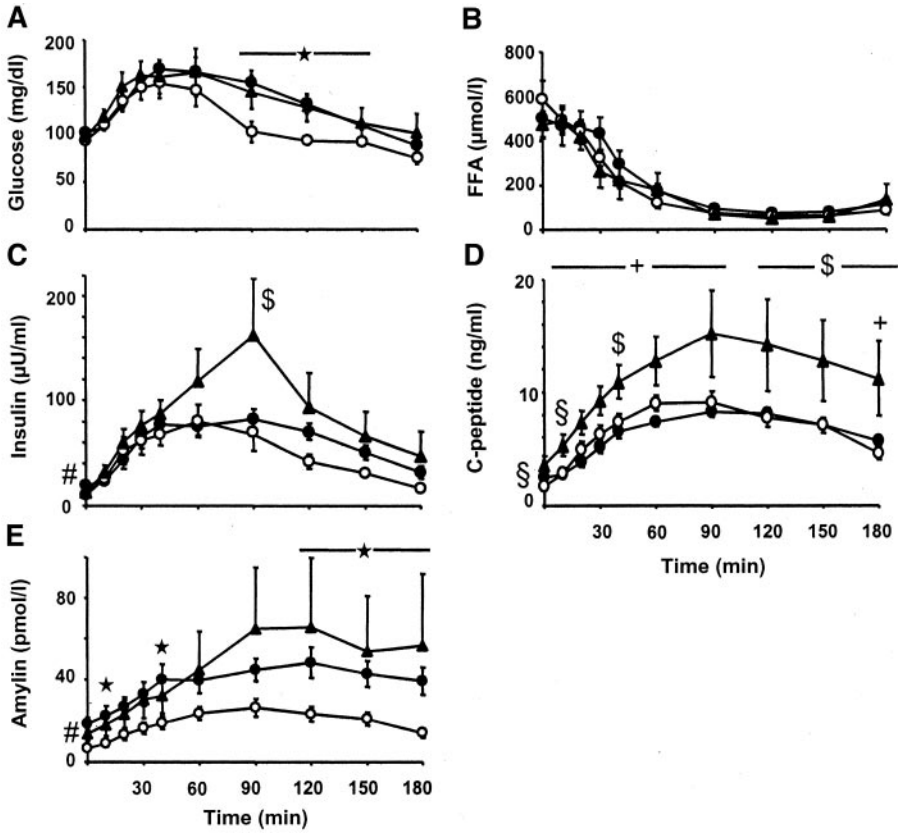

Figure 1—*Concentrations (means ± SE) of plasma glucose* (A)*, plasma FFAs* (B)*, plasma insulin* (C)*, serum C-peptide* (D)*, and plasma amylin* (E) *during the OGTT in type 1 diabetic patients after simultaneous PKT* (●)*, nondiabetic humans after kidney transplant* (▲)*, and nondiabetic control subjects* (○)*. ANOVA with Dunnet post hoc:* ★P < *0.05, PKT patients vs. control subjects;* #P < *0.01, PKT patients vs. control subjects;* $P < *0.05, kidney transplant patients vs. control subjects;* §P < *0.01, kidney transplant patients vs. control subjects;* +P < *0.05, PKT vs. kidney transplant patients.*

(Table 1 and Fig. 1*A*). While OGTT plasma glucose of kidney transplant patients did not differ from that in control subjects or PKT patients, the PKT group had higher plasma glucose values between 90 and 150 min by 9–31% (Fig. 1*A*; each $P < 0.05$).

FFAs

No parameter of FFA concentrations, kinetics, or modeling (data not shown) differed between the three groups (Fig. 1*B*).

Insulin and C-peptide

Plasma insulin concentrations were 90% higher at fasting in PKT patients ($P < 0.01$ vs. control subjects) and increased by 138% at 90 min in kidney transplant patients ($P < 0.04$ vs. control subjects). Total and suprabasal $AUC_{insulin}$ levels were higher by 82–97% in kidney transplant patients ($P < 0.05$), but similar in PKT patients when compared with control subjects (Fig. 1*C* and Table 1). C-peptide was similar between PKT patients and control subjects, but markedly increased in kidney transplant patients both at fasting (48%, $P < 0.007$ vs. control subjects) and throughout the entire course of the OGTT (Fig. 1*D* and Table 1; each $P < 0.05$ vs. control subjects and/or PKT patients). Total and suprabasal $\Delta AUC_{C\text{-peptide}}$ levels were elevated by ~50–70% in kidney transplant patients (Table 1; each $P < 0.05$ vs. control subjects and PKT patients) but were comparable between PKT patients and control subjects.

Amylin

In PKT patients, fasting plasma amylin levels were approximately threefold higher (Fig. 1*E* and Table 1; $P < 0.01$ vs. control subjects). In PKT patients, amylin levels were increased at 10 and 40 min as well as between 120 and 180 min during the course of OGTT, resulting in an approximately twofold elevation of total AUC_{amylin} compared with control subjects (Fig. 1*E* and Table 1; each $P < 0.05$). In kidney transplant patients, plasma amylin concentrations and suprabasal and total AUC_{amylin} levels tended to be higher than those of control subjects, but did not reach statistical significance, which was due to the extremely broad range in kidney transplant patients (AUC_{amylin} in control subjects: 1,899–6,776 nmol/l per min; in PKT patients: 3,065–14,896 nmol/l per min; and in kidney transplant patients: 2,849–28,684 nmol/l per min).

Modeling analyses

Insulin sensitivity. Insulin sensitivity, calculated using the OGIS model, was not statistically different in all groups (Table 1).

β-Cell secretion and hepatic insulin extraction. At baseline, the insulin secretion rate was 49 and 115% higher in PKT and kidney transplant patients, respectively (Table 1; each $P < 0.05$ vs. control subjects). Total insulin secretion was elevated in kidney transplant patients by 82% (Table 1; $P < 0.02$ vs. control subjects). The hepatic insulin extraction rate during the test was ~10% ($P < 0.05$) lower in PKT patients than in control subjects (Table 1).

β-Cell function. In PKT patients, β-cell sensitivity to glucose during OGTT was reduced to one-third (85.5 ± 8.6, $P < 0.009$), while that of kidney transplant patients (136.7 ± 19.3) was not different when compared with control subjects (235.7 ± 54.3). The adaptation index, a measure of β-cell adaptation to ambient insulin sensitivity, was higher by 29 and 49% in kidney transplant patients when compared with control subjects and PKT patients, respectively (Table 1; each $P < 0.05$).

Amylin kinetics. Basal amylin secretion was statistically unchanged in kidney transplant patients, but was threefold higher in PKT patients, resulting in a doubled amylin/insulin cosecretion factor in PKT patients (Table 1; $P < 0.05$ each). Total amylin secretion was not statistically different in all groups. Amylin clearance was similar in PKT patients, kidney transplant patients, and control subjects (Table 1).

Correlation analyses. In all subjects, both higher basal amylin ($r = -0.543$, $P < 0.004$) and higher AUC_{amylin} levels ($r = -0.479$, $P < 0.01$) correlated with lower β-cell sensitivity to glucose. A comparison of circulating amylin and C-peptide in PKT patients, kidney transplant patients, and control subjects at all time points during OGTT revealed a very close correlation (each $P < 10^{-6}$) (all participants: $r = 0.701$; PKT patients: $r = 0.575$; kidney transplant patients: $r = 0.737$; control subjects: $r = 0.837$). In all participants, OGIS was inversely associated with basal plasma amylin ($r = -0.437$, $P < 0.03$), as well as with suprabasal ($r = -0.557$, $P < 0.003$) and total AUC_{amylin} ($r = -0.560$, $P < 0.003$). A direct relationship was registered between basal amylin and basal insulin ($r = 0.560$, $P < 0.003$) in all subjects.

CONCLUSIONS — The main finding of this study was that there was a rise in amylin, both in the fasting state and after a glucose challenge, in type 1 diabetic patients who had undergone successful PKT. Plasma amylin concentrations in PKT patients, kidney transplant patients, and control subjects were inversely associated with β-cell function and OGIS. Despite systemic drainage, which resulted in peripheral hyperinsulinemia, after PKT, type 1 diabetic patients on a glucocorticoid-free regimen showed a degree of insulin sensitivity comparable to that in nondiabetic individuals.

Amylin

Plasma amylin is commonly elevated in insulin-resistant conditions that go along with hyperinsulinemia, such as impaired glucose tolerance, the early stage of type 2 diabetes, and obesity. However, because of β-cell destruction, amylin is completely absent in absolutely insulinopenic type 1 diabetic patients (20).

We showed that successful PKT restored and even increased plasma amylin, which, to our knowledge, has not been reported previously.

The up to twofold higher plasma amylin levels in PKT patients could be due to its increased release and/or reduced clearance. Kidney function in both groups of transplanted patients was comparable, but worse than that in control subjects. However, amylin clearance was similar in all groups, which could be expected since amylin is predominantly cleared by the kidney. Indeed, the tight correlation of the time curves for plasma amylin and C-peptide levels (the latter is exclusively eliminated by the kidneys) revealed that the two hormones were closely associated with each other. This also substantiates the fact that amylin is primarily eliminated by the kidney, in line with previous reports (26). Finally, since hepatic amylin extraction was shown to be negligible during OGTT (27), the systemic versus portal drainage in PKT patients and control subjects, respectively, cannot be held responsible for the higher amylin concentrations in PKT patients. Therefore, the similar amylin clearance in PKT patients and control subjects indicates that elevated concentrations of plasma amylin are most likely because of enhanced secretion. To further investigate this hypothesis, we also studied another group of matched nondiabetic patients after successful transplantation of a kidney, only with normal pancreatic situs and drainage. However, because of the current guidelines on treatment of kidney transplant patients (33), regular glucocorticoid therapy had not been tapered off in our kidney transplant patients. Amylin's plasma concentration and secretion in kidney transplant patients was not significantly different from that in control subjects, but tended to be increased. In this context, it is of relevance that 5-day dexamethasone intake in healthy humans did not only increase amylin, but also insulin release (34). Our kidney transplant patients were also clearly hyperinsulinemic, most likely because of the glucocorticoid treatment. In a healthy individual, amylin is cosecreted with insulin in quite a stable proportion (35). When compared with control subjects, our kinetic analysis of amylin revealed an approximately twofold higher amylin/insulin cosecretion factor in PKT patients, but not kidney transplant patients, indicating that the β-cell releases markedly larger amounts of amylin compared with C-peptide or insulin in PKT patients only. From this it can be suggested that the trend of higher plasma amylin in kidney transplant patients after glucose challenge is rather because of increased stimulation of the β-cell that cosecretes more of both insulin and amylin. In contrast, in PKT patients, post–glucose load insulin secretion was similar to that in control subjects, whereas plasma amylin was significantly higher, which might be an early sign of β-cell damage. The lower β-cell sensitivity to glucose in these patients seems to confirm this hypothesis.

Of note, smaller amounts of amylin are also secreted from δ-cells in the pancreas and the antral and fundic mucosa (36,37). In the stomach, amylin stimulates somatostatin secretion and thereby inhibits gastrointestinal motility (36), which could result in gastroparesis and/or delayed absorption due to reduced intestinal circulation. Plasma glucose concentrations during the first 60 min of the OGTT (corresponding to the major glucose absorption period) were similar in all groups, and the diabetic patients did not show any clinical signs of gastroparesis. On the other hand, somatostatin is known to inhibit β-cell secretion. Thus, it cannot be ruled out that amylin release from the stomach stimulated somatostatin secretion, which also reduced insulin secretion and thereby worsened β-cell function in PKT patients.

β-Cell function

We studied insulin secretion through that of C-peptide instead of insulin because of the different pancreatic drainage in the examined groups. Since C-peptide is not cleared by the liver (38), we used a mathematical model for C-peptide, which allows estimation of β-cell function on the basis of peripheral hormone levels. Thus, in the PKT patients, β-cell sensitivity to glucose is markedly reduced when compared with control subjects. These findings are in line with several studies that also found defects of β-cell function in type 1 diabetic patients after PKT (10,11,13). In contrast, in kidney transplant patients, β-cell sensitivity to glucose was comparable to that in control subjects, and the adaptation index, a measure of releasing adequate increasing quantities of insulin and C-peptide to compensate for the ambient insulin resistance, was even higher than that in control subjects or PKT patients, suggesting an improved β-cell function.

It has been known for a long time that augmented plasma amylin levels precede β-cell failure and that its amyloid is deposited around the pancreatic islets of type 2 diabetic patients (39,40). In the

present study, plasma amylin was inversely correlated not only with β-cell sensitivity to glucose, but also with OGIS. Thus, elevated plasma amylin levels are a sign of both impaired β-cell function and insulin resistance and might well serve as a simple peripheral measure of β-cell function.

Impaired β-cell function in type 1 diabetes PKT might be due to *1*) previous minor lesions during transplantation, *2*) the use of specific immunosuppressive agents, and/or *3*) the effects of increased circulating amylin itself. Major graft damage seems unlikely because all PKT patients had a nondiabetic glucose tolerance test and no longer require insulin treatment. However, the PKT patients showed slightly higher A1C levels, which is also reflected by the elevated OGTT plasma glucose concentrations.

Immunosuppressive agents are needed to prevent graft rejection, but may exert a toxic effect on β-cells and impair their function. All of the PKT and kidney transplant patients regularly ingested immunosuppressive agents. However, kidney transplant patients showed increased adaptation index when compared with PKT patients and control subjects. Apart from corticoids, the immunosuppressive treatment consisted of both an antimetabolite and a calcineurin inhibitor. The majority of both patient groups was treated with mycophenolate mofetil as an antimetabolite drug. Regarding calcineurin inhibitors, all PKT patients were under tacrolimus therapy, whereas all but one kidney transplant patient ingested cyclosporine-A. Previous in vitro and in vivo comparisons of both of these drugs on β-cell toxicity yielded conflicting results. While in cell culture, tacrolimus was described to be less (41) or as toxic to β-cells (42) as cyclosporine-A, a recent meta-analysis on diabetogenity in humans (43) and a study on biopsies of transplanted pancreata in PKT patients (44) reported a more harmful effect of tacrolimus than cyclosporine-A for β-cell function. Thus, it cannot be ruled out that tacrolimus therapy at least in part contributed to β-cell impairment in our PKT patients.

Increased plasma amylin levels might exert contrasting effects on glucose and insulin metabolism in different tissues. While a high plasma amylin level is suspected to facilitate the development of type 2 diabetes by β-cell failure (40) and indicates β-cell impairment in our patients, amylin analogs such as pramlintide have been developed for the treatment of type 1 diabetes (16–19). Pramlintide improved glycemic control in type 1 diabetic patients, leading to a reduction of daytime glucose excursions as well as an improvement of A1C levels (16–19). Thus, it appears that amylin might exert beneficial metabolic effects on tissues other than the β-cell. Regardless of the mechanism, plasma amylin appears to be a marker of overt β-cell impairment in humans and might also be harmful for β-cells.

Insulin sensitivity

Based on OGIS, insulin sensitivity in PKT and kidney transplant patients was not significantly lower than that in control subjects, although kidney transplant patients tended to show the lowest OGIS values due to a combination of corticoid and immunosuppressive treatment. The similar course of plasma FFAs during OGTT also indicates comparable insulin sensitivity in the adipose tissue of all groups. This indicates that both glucotoxicity and lipotoxicity, which had certainly been present in type 1 diabetic patients with former end-stage renal failure (45,46), can be reversed after successful PKT resulting in nondiabetic glycemia.

In contrast to our findings, several previous studies showed peripheral insulin resistance and elevated FFAs in patients after PKT (47,48). However, these studies were performed in patients taking glucocorticoids, which are known to deteriorate insulin sensitivity and to be diabetogenic (49,50). Nowadays, in most cases, pancreatic transplantation is followed by initial quadruple combination of immunosuppression, including antibody induction therapy and subsequent triple therapy for maintenance of immunosuppression with calcineurin inhibitors, antimetabolites, and glucocorticoids (4,49). If possible, prednisolone is tapered off during the first year of transplantation, thereby improving glucose tolerance (49). All of our PKT patients were on glucocorticoid-free immunosuppression. Considering the impaired β-cell function in PKT patients, our data also underline the necessity of a glucocorticoid-free regimen in these patients, because steroids would not only potentiate the β-cell toxicity of immunosuppressive agents, but also accelerate and favor the re-manifestation of diabetes (44).

In conclusion, type 1 diabetic patients after successful PKT on a glucocorticoid-free regimen exhibit nondiabetic glucose levels but impaired β-cell function. The normal FFA dynamics and insulin sensitivity in PKT patients confirm the benefits and necessity of glucocorticoid-free immunosuppressive treatment. Type 1 diabetic patients after PKT show increased fasting and post–glucose load amylin and increased amylin release in proportion to insulin, which appears to be associated with impaired β-cell function. Thus, amylin might serve as a marker of β-cell impairment in type 1 diabetic patients after combined PKT.

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