Insulin Sensitivity and Negative Insulin Feedback after Pancreas Transplantation in Insulin-Dependent Diabetic Patients

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Abstract. The aims of this study were to determine the change in the rate of insulin-stimulated glucose disposal (insulin sensitivity) and the ability of insulin to inhibit its own secretion in four pancreas-kidney transplant recipients with insulin-dependent diabetes mellitus. Insulin sensitivity (glucose infusion rate, GIR) was measured by a euglycemic hyperinsulinemic clamp technique before and 2, 6 and 12 months after transplantation. The GIR values in the four recipients were normalized within 2 months and remained normal for 12 months after transplantation, despite long-term steroid therapy for immunosuppression. Physiological hyperinsulinemia (50–70 $\mu\text{U}/\text{ml}$) suppressed plasma C-peptide, but its nadirs were still higher than the basal levels in normal controls. Taking into account evidence of a minimal increase in the concentration of circulating insulin that inhibits insulin secretion in healthy subjects and evidence of increased insulin secretion in pancreas recipients, the authors speculate that defective feedback inhibition of insulin secretion could contribute, at least in part, to the disproportionate basal hyperinsulinemia in patients with a denervated, transplanted pancreas in the absence of insulin resistance.

Key words: Pancreas-kidney transplantation, Insulin sensitivity, Negative insulin feedback
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FEEDBACK inhibition of insulin secretion by insulin is thought to be operative in normal [1–5] and obese [2, 4] individuals and in patients with non-insulin-dependent diabetes mellitus [6, 7], in whom basal, but not glucose-stimulated insulin secretion is inhibited by insulin itself. However, this insulin beta cell feedback inhibition was reported to be defective in insulin-dependent diabetic patients after pancreas transplantation [8–10]. The disorder may be related to the lack of autonomic input from the central nervous system, which is considered essential in the modulation of pancreatic islet function [11, 12].

A point of interest is that in heterotopic pancre-

as transplantation, fasting plasma concentrations of not only insulin but also of C-peptide immunoreactivity (CPR) are inappropriately high, and this has been explained by both systemic venous drainage of pancreas allografts and insulin resistance resulting from prednisolone treatment for immunosuppression. To evaluate this explanation, we examined if insulin sensitivity and negative insulin feedback could change during the time course after pancreas transplantation with continuous immunosuppressive therapy in patients with insulin-dependent diabetes mellitus (IDDM).

Subjects and Methods

Patients

The patients studied were four females, aged 31–

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37 years, with 20–25-year histories of IDDM. Three had undergone simultaneous pancreas-kidney transplantation (cases 1–3) and one had undergone pancreas-after-kidney transplantation (case 4) because of end-stage nephropathy (Table 1). In all 4 recipients, the venous drainage of the pancreas allograft was into the right iliac vein, thereby providing systemic venous rather than portal drainage of pancreatic endocrine secretions. Exocrine drainage was diverted into the bladder. Within 18 h posttransplantation, all recipients achieved normoglycemia and were able to maintain plasma glucose and HbA_{1c} levels within the normal range up to 12 months after transplantation without insulin therapy. The immunosuppressive regimen included azathioprine, cyclosporin A and methylprednisolone as shown in Table 1. None of the four recipients had symptomatic hypoglycemia after transplantation, although one (case 4) had reduced fasting plasma glucose levels (55–65 mg/dl). The control group consisted of 10 normal healthy subjects matched for age (Table 1).

The nature and purpose of the study were explained to the subjects before they gave their voluntary consent to participate. The study protocol was approved by the Institutional Review Board of Tokyo Women's Medical College.

Methods

We conducted a euglycemic hyperinsulinemic clamp study with an artificial endocrine pancreas (Nikkiso STG-22, Nikkiso Co., Tokyo, Japan), as originally described by DeFronzo *et al.* [13]. The patients' morning medications were withheld until the end of the experiment. After an overnight fast, the patients received a primed-continuous intravenous infusion of human insulin (Novolin R, Novo-Nordisk Pharmaceuticals Co., Copenhagen, Denmark) at the rate of 1.12 mU/kg/min, and venous plasma glucose was maintained at 80 mg/dl for 60–90 min. The average glucose infusion rate (GIR) during the final 30 min was used as an index of peripheral insulin sensitivity. In the

Table 1. Clinical parameters of four pancreas-kidney transplant recipients

Patients	Age (yr)	Sex	Duration of DM (yr)	BMI	FBS (mg/dl)	HbA _{1c} (%)	Transplantation (month)	Serum Cr (mg/dl)	Fasting Plasma		GIR (mg/kg/min)	MP	CsA	Az
									IRI (μ U/ml)	CPR (ng/ml)				
1	30	F	20	18.5	340	8.6	SPK 0	14.0	–	–	1.80	–	–	–
				18.1	98	5.4	2	1.5	27.5	4.8	4.03	16	200	50
				18.5	104	6.2	6	2.2	9.3	3.1	–	8	225	25
				19.9	95	5.6	12	1.9	15.6	3.3	6.85	8	275	50
2	32	F	23	17.6	162	8.7	SPK 0	8.7	–	–	3.78	–	–	–
				17.2	78	4.5	2	1.5	18.8	3.9	5.47	16	200	50
				18.0	92	5.8	6	1.9	18.2	5.0	5.23	8	250	50
				19.5	78	6.8	12	2.1	21.0	1.8	3.93	8	125	100
3	30	F	23	19.9	71	6.3	SPK 0	12.2	–	–	3.49	–	–	–
				18.7	89	4.9	2	1.3	42.7	7.2	6.05	16	–	75
4	37	F	25	15.2	197	9.5	P after K 0	2.2	–	–	4.80	6	150	25
				15.1	65	4.2	2	1.9	16.1	4.6	–	16	250	75
				15.4	57	5.5	6	2.0	8.7	5.8	–	8	250	50
				15.4	84	5.9	12	2.0	14.6	5.8	6.34*	8	225	50
Mean	32.3		22.8	17.8					19.2	4.5	5.41			
$\pm$	$\pm$		$\pm$	$\pm$					$\pm$	$\pm$	$\pm$			
SD	3.3		2.1	2.0					9.9 ^a	1.5 ^b	1.12			
Control (n=10)	31.5			21.1					7.9	1.7	5.82			
	$\pm$			$\pm$					$\pm$	$\pm$	$\pm$			
	8.7			0.2					2.8	0.5	1.7			

SPK, simultaneous pancreas-kidney transplantation; P after K, pancreas after kidney transplantation; Cr, creatinine; MP, methylprednisolone (mg/day); CsA, cyclosporin A (mg/day); Az, azathioprine (mg/day).

a: $P < 0.005$ vs. control; b: $P < 0.001$ vs. control; *determined under clamping plasma glucose at 60 mg/dl.

present study, hepatic glucose production was not determined concomitantly, because the use of a radioactive tracer ($3\text{-}^3\text{H}$ -glucose) was not approved by the Institutional Committee. Consequently, the GIR values obtained in this study could be lowered by around 0.5 mg/kg/min [14], because of steroid-induced hepatic insulin resistance, but we think that the lack of tracer infusion may not largely invalidate the present results. Before and during the course of the study, plasma samples were drawn at appropriate intervals as indicated in Fig. 1 and plasma insulin (IRI) and C-peptide (CPR) were measured with commercial radioimmunoassay kits (Phadeseph Insulin RIA, Pharmacia Diagnostics, Sweden; C-peptide Test, Shionogi Research Laboratories, Osaka, Japan).

We repeated the euglycemic clamp study at 2, 6, and 12 months after pancreas transplantation, to measure changes in negative insulin beta cell feedback regulation and insulin sensitivity.

Statistical analysis was performed by unpaired Student's *t*-test and data were expressed as the mean $\pm$ SD.

Results

Insulin sensitivity

The GIR values were restored from the subnormal pretransplantation levels ($1.80, 3.78, 3.49\text{ mg/}$

kg/min) to almost normal levels ($4.03, 5.47, 6.05\text{ mg/kg/min}$, respectively) 2 months after transplantation in cases 1–3 (Table 1). They remained within the normal range for up to 12 months after transplantation in all recipients despite continuation of immunosuppressive therapy. In case 4, a plasma glucose level of 80 mg/dl was stimulatory, so an isoglycemic level of 60 mg/dl was employed to measure the GIR, and this parameter was further improved from the pretransplantation value of 4.80 to 6.34 mg/kg/min after 12 months (Table 1). The mean GIR in the pancreas recipients did not significantly differ from that in the normal controls (5.41 ± 1.12 vs. $5.82 \pm 1.70\text{ mg/kg/min}$, respectively) (Table 1).

Suppression of plasma C-peptide

There was no anti-insulin antibody in the serum of any of the recipients. The patients' mean fasting plasma concentrations of both IRI and CPR were significantly higher than those of the normal controls (IRI: 19.2 ± 9.9 vs. $7.9 \pm 2.8\text{ }\mu\text{U/ml}$, $P < 0.005$; CPR: 4.5 ± 1.5 vs. $1.7 \pm 0.5\text{ ng/ml}$, $P < 0.001$) (Table 1). During insulin infusion, the steady-state plasma IRI reached $53.4 \pm 8.2\text{ }\mu\text{U/ml}$ in the transplant recipients and $68.0 \pm 28.1\text{ }\mu\text{U/ml}$ in the controls (NS). The patients' plasma CPR was suppressed to a lesser extent when expressed as a percentage change from the basal value ($48.6 \pm 15.2\%$ vs. $67.2 \pm 10.6\%$ in control subjects, $P < 0.01$) (Fig. 1), but the

Fig. 1. Change in plasma CPR concentrations during a euglycemic clamp study performed 2 months (●), 6 months (□) and 12 months (△) after pancreas transplantation in cases 1, 2 and 3. The shadow represents the mean $\pm$ SD in 10 normal controls.

absolute fall in plasma CPR was significantly greater in recipients than in controls (2.09 ± 1.04 vs. 1.15 ± 0.39 ng/ml, respectively, $P < 0.02$). Nevertheless, its nadirs were still higher than the basal levels in normal controls (Fig. 1). One patient (case 4) demonstrated no appreciable suppression of CPR at a glucose level of 80 mg/dl or even when the plasma IRI level reached 200 μ U/ml (Fig. 2). In this case, when the clamp was set for a lower plasma glucose level, 60 mg/dl, the plasma CPR concentration was suppressed to the same extent as in the other pancreas recipients (from 5.8 to 2.4 ng/ml) (Fig. 2).

Discussion

In this study, the insulin-stimulated glucose disposal rate was normalized in IDDM patients after pancreas transplantation and remained normal for up to 12 months after transplantation, despite long-term immunosuppressive therapy. Similarly, Luzi *et al.* [15] reported that combined kidney-pancreas transplantation reduced peripheral insulin resistance in type 1 diabetic patients. These findings are also compatible with earlier observations [16, 17] indicating that the plasma glucose disappearance rate (KG values) during intravenous glucose tolerance test was normal in pancreas recipients. Many authors [18, 19] including us [20] have shown

that short-term strict glycemic control improves insulin sensitivity in type 1 diabetic subjects. This may imply that IDDM is intrinsically an insulin-sensitive variety, standing in sharp contrast with NIDDM. Furthermore, it is suggested that normalization of insulin resistance could be an important determinant if pancreas allografts are able to maintain normal function over longer periods.

In our study, physiological hyperinsulinemia (50–70 μ U/ml) inhibited basal secretion of insulin, but its nadirs were still higher than those of healthy controls. In one case, however, insulin failed to suppress plasma CPR, not only at the usual dose (1.12 mU/kg/min), but also at a five-fold greater dose (5.0 mU/kg/min) where the steady-state plasma level of insulin was approximately 200 μ U/ml. When the plasma glucose was clamped at an isoglycemic level of 60 mg/dl, however, physiological hyperinsulinemia lowered the plasma CPR to 41.0% of the basal level. This latter observation confirmed previous findings which indicated that insulin exerts feedback control over beta cell secretion only under basal conditions and has no effect at stimulatory glucose concentrations [1, 4]. The apparent "euglycemia" of venous blood in this case may accompany a mild increase in arterial blood glucose concentrations, thus preventing the insulin-induced inhibition of plasma CPR concentrations.

Fig. 2. Change in plasma CPR concentrations during glucose clamp study performed under modified glucose and insulin levels at 12 months posttransplantation in case 4.

It is of particular interest that the normal plasma glucose concentrations in pancreas transplant recipients occurred in the face of greatly increased basal plasma IRI and CPR concentrations. This is in agreement with other published data [8–10, 15–17, 21]. The increase in plasma CPR has been explained in terms of a combination of insulin resistance caused by prednisolone and alteration of C-peptide clearance secondary to impaired kidney function because CPR is cleared almost exclusively by the kidneys [22, 23]. In this study, peripheral insulin resistance, as estimated with the euglycemic clamp, was persistently normal for at least 12 months after transplantation, even in the context of long-term steroid therapy. Therefore, our results do not indicate that insulin resistance causes an increase in fasting plasma insulin in pancreas recipients. Furthermore, because steady-state plasma insulin levels achieved under an identical insulin infusion rate were comparable in subjects with pancreas transplantation and controls, the insulin clearance rate appears to be normal.

The clearance of C-peptide is known to be profoundly affected by even subtle renal tubular or glomerular dysfunction that results from either the transplant itself or the effect of cyclosporin A or other factors, thereby complicating the interpretation of peripheral C-peptide concentrations as a measure of beta cell secretion. To circumvent this difficulty, Blackman *et al.* recently utilized an approach (“deconvolution method”) that allows alterations in insulin secretion and clearance to be evaluated separately [21]. They demonstrated that when the effects of changes in C-peptide clearance were factored out, the calculated basal insulin secretion rates in 10 IDDM recipients of a combined pancreas-kidney allograft were approximately double the corresponding rates in 10 matched nondiabetic control subjects. Their finding also indicated that the increase in fasting plasma C-peptide in the recipients results from a combination of increased secretion and reduced clearance. Consequently, the increased basal plasma insulin is

attributable not only to the absence of first-pass hepatic extraction of insulin, but to the enhancement of insulin secretion. Given these considerations, we are tempted to speculate that the increased basal plasma levels of insulin might originate, in part, from the impairment of negative feedback inhibition by insulin itself. It is not clear whether insulin beta cell feedback normally plays a role in the establishment of basal insulin secretion rates. DeFronzo *et al.* [1] reported that basal insulin secretion was effectively inhibited by a small ($24 \pm 3 \mu\text{U/ml}$) physiological increase in the plasma insulin concentration in healthy subjects; that is in the range observed in pancreas-kidney transplant patients in the basal state [9, 15, 16, 19]. We therefore suppose that the feedback inhibition of insulin secretion is defective in patients with a denervated, transplanted pancreas.

In this context, it is intriguing to note that spontaneous hypoglycemia occurs after successful pancreas transplantation either during fasting or in the postprandial period [24–26]. As possible mechanisms of hypoglycemia, reduced gluconeogenic flux to the liver and defective counterregulatory responses in patients with long-standing diabetic complications seem unlikely because it has been shown that hypoglycemia-induced glucagon response and hepatic glucose production were restored after successful pancreas transplantation [27]. Instead, we propose that impaired feedback suppression of insulin secretion by insulin may play a role in the unexpected occurrence of hypoglycemia in this particular situation.

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