Defective Glucagon Secretion During Sustained Hypoglycemia Following Successful Islet Allo- and Autotransplantation in Humans

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Defective glucagon secretion during hypoglycemia is characteristic of long-standing type I diabetes. To determine whether this defect can be corrected by successful intrahepatic islet transplantation, we performed studies of hypoglycemia in four nondiabetic patients with chronic pancreatitis who had undergone total pancreatectomy and successful intrahepatic islet autotransplantation, in two type I diabetic recipients of successful intrahepatic islet allotransplantation, and in matched control subjects. We examined *1*) whether intrahepatic islet autotransplantation provides glucagon secretion during prolonged periods of hypoglycemia and *2*) whether intrahepatic islet allotransplantation in type I diabetic patients and consequent long-term normoglycemia reestablishes native α-cell responses to hypoglycemia. Glucagon secretion was assessed during 3-h hypoglycemic hyperinsulinemic clamp studies. The islet autograft recipients were studied 63 ± 19 months posttransplant, and all were insulin-independent and normoglycemic (HbA_{1c}, 5.8 ± 0.2%). Neither allograft recipient required exogenous insulin and maintained HbA_{1c} levels of 5.7 and 6.4% 30 and 34 months posttransplant, respectively. All recipients were normoglycemic (fasting glucose: autograft recipients, 5.6 ± 0.1 mmol/l; allograft recipient #1, 6.3 mmol/l; allograft recipient #2, 5.8 mmol/l) at the time of study. During hypoglycemia, no increase in glucagon secretion was observed in either the auto- or allotransplant recipients, whereas healthy control subjects and recipients of kidney transplantation had significant increases in glucagon. In contrast, both allo- and autograft recipients had glucagon responses to intravenous arginine. These data uniquely demonstrate that: *1*) intrahepatic islet transplant grafts secrete glucagon in response to arginine, but fail to secrete glucagon in response to sustained hypoglycemia; and *2*) the restoration of sustained normoglycemia for over 2 years in type I diabetic patients may not reestablish glucagon responses from the native pancreas during hypoglycemia. Transplantation sites other than the liver may be required to achieve normal glucagon secretion from the transplanted islets. *Diabetes* 46:23–27, 1997

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RIA, radioimmunoassay.

Glucagon secretion and glucose counterregulation during hypoglycemia are defective in virtually all patients with long-standing type I diabetes (1–5). While it is well established that intrahepatic islet transplantation can reestablish β-cell secretory function and normoglycemia (6–8), whether this procedure can also correct defects in glucagon response to hypoglycemia is not presently known.

Studies of α-cell function after islet transplantation have been significantly limited by the lack of success of this procedure in humans. While significant information has been derived from studies of subjects undergoing pancreatic islet autotransplantation (6), studies in subjects undergoing successful islet allotransplantation are required to clearly define α-cell function in patients with type I diabetes. Consequently, we performed hypoglycemic clamp studies in four nondiabetic patients with chronic pancreatitis who had undergone total pancreatectomy and successful intrahepatic islet autotransplantation and two type I diabetic recipients of successful intrahepatic islet allotransplantation *1*) to determine whether intrahepatic islet autotransplantation will provide glucagon secretion during sustained hypoglycemia and *2*) to ascertain whether intrahepatic islet allotransplantation and consequent long-term normoglycemia will restore native α-cell responses to hypoglycemia in type I diabetic subjects.

RESEARCH DESIGN AND METHODS

Study subjects. Four patients with chronic painful pancreatitis who had undergone pancreatectomy and successful intrahepatic islet autotransplantation and two type I diabetic recipients of simultaneous kidney and intrahepatic islet allotransplant (allograft recipients #1 and #2) (Table 1) were studied. The details of the surgical procedures of both auto- (6,7) and allotransplantation (8) have been previously reported. All transplant recipients were able to be maintained off exogenous insulin with fasting plasma glucose levels <6.4 mmol/l (115 mg/dl). However, one allograft recipient (allograft recipient #2) elected to use 5–10 units of intermediate acting (NPH) insulin daily to prevent postprandial hyperglycemia. Insulin therapy in this patient was withheld for 48 h before the clamp studies. Immunosuppressive drugs in the allograft recipients included standard doses of azathioprine, cyclosporine, and prednisone (8). Both type I diabetic islet allograft recipients had a history of diabetic retinopathy and clinical evidence of autonomic neuropathy. The type I diabetic allograft recipients reported a history of severe hypoglycemia with hypoglycemic unawareness before the islet-kidney transplant. Four patients matched for age, sex, and BMI with the islet autograft recipients served as healthy control subjects. Two nondiabetic recipients of renal transplantation were also studied, and these subjects were matched with the allograft recipients for age, sex, and BMI. Both kidney transplant patients were similarly treated with cyclosporine, azathioprine, and prednisone. None of the control subjects was taking medication known to interfere with glucagon secretion. Islet allograft and kidney transplant patients were taking between 7.5 and 12.5 mg prednisone per day. All medications were withheld on the day of study.

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TABLE 1
Patient characteristics

Patient	Age (years)	Sex	BMI (kg/m²)	HbA_{1c} (%)	Islets ($n \times 10^3$)	Fasting glucose (mmol/l)	Months post-transplant	Basal glucagon (ng/l)	Δ glucagon (hypoglycemic) (ng/l)	Δ glucagon (intravenous arginine) (ng/l)
Autograft recipients										
#1	47	F	25.1	3.7	412	4.9	78	35	−3	30
#2	36	F	19.4	4.8	401	5.3	23	93	13	47
#3	46	F	24.1	5.9	265	6.5	107	93	9	24
#4	32	F	21.8	6.4	325	5.3	42	87	9	24
All (± SE)	40 ± 4	4F	22.6 ± 1.3	5.2 ± 0.6	351 ± 35	5.6 ± 0.3*	63 ± 19	77 ± 14*	4 ± 4†	34 ± 5†
Control subjects (± SE)	38 ± 4	4F	22.5 ± 1.7	5.0 ± 0.2	—	5.2 ± 0.2	—	146 ± 29	114 ± 45	134 ± 22
Allograft recipients										
#1	44	M	20.4	5.7	528	6.3 ± 0.2	34	146	1	167
#2	34	F	19.0	6.4	502	5.8 ± 0.2	30	292	−42	99
Kidney transplant recipients										
#1	45	M	24.1	5.0	—	4.8	36	127	76	ND
#2	29	F	17.9	4.9	—	5.4	24	120	138	ND

Patient profiles and measurements of glycemia and basal and stimulated glucagon responses for the islet autotransplant group, islet allotransplant group, control subjects, and kidney transplant control subjects. Δ glucagon is as defined in METHODS. ND, no data. *$P < 0.05$ (autograft recipients vs. control subjects); †$P < 0.02$ (autograft recipients vs. control subjects).

Metabolic studies. All subjects were admitted to the University of Minnesota Clinical Research Center the night before the study. Hypoglycemic clamps were performed following a 12-h overnight fast, as previously described (9). Briefly, on the morning of the clamp study, a catheter was inserted into a forearm or antecubital vein for infusion of the test substances. A second catheter was placed retrograde into a dorsal hand vein on the contralateral side and utilized for blood sampling. This hand was placed in a heated box (55°C) to arterialize venous blood. At time zero, a 1 mU · kg^{-1} · min^{-1} insulin infusion (Humulin; Lilly, Indianapolis, IN) was begun and continued for 3 h. Plasma glucose was determined at 5-min intervals and allowed to decline to 3.1 mmol/l (55 mg/dl) over the first 60 min. Thereafter, plasma glucose was clamped at this level by means of a variable rate 20% dextrose infusion. All substances were infused using Harvard pumps (Harvard Bioscience, S. Natick, MA). Serum samples were obtained at 10- to 20-min intervals for the determination of insulin, C-peptide, and glucagon. The results of the clamp studies in the two kidney transplant recipients have been previously reported as a portion of another study (9).

Arginine stimulation tests were performed by the intravenous injection of 5 g arginine (given as 10% arginine HCl; KabiVitrum, Clayton, NC) over 30 s, with time 0 set halfway through the arginine injection. Samples for glucagon were collected from the contralateral arm at baseline and 2, 3, 4, 5, 7, and 10 min after the arginine injection. Acute glucagon response to arginine was defined as the mean of the peak three glucagon values between 2 and 5 min following the arginine injection with the basal value subtracted.

Analytical methods. Plasma glucose was determined at bedside with a Beckman glucose analyzer (Fullerton, CA). All samples for glucagon determination were collected in pre-chilled tubes containing 2.5 mg EDTA per 500 U Trasylol per 1 ml blood, placed on ice, and centrifuged within 30 min. Glucagon was measured by radioimmunoassay (RIA) with antibody 04A, which was obtained from Dr. R. Unger (University of Texas, Dallas, TX) (10). Insulin was measured by standard double antibody RIA, as described previously (11). C-peptide was measured by RIA (12) using guinea pig anti-human C-peptide antibody provided by Novo Biospecific (Wilton, CT). The actual concentrations of commercial 20% dextrose intravenous solutions were confirmed by direct measurement.

Data from islet autograft recipients and control subjects are expressed as mean ± SE. Data for the islet allograft recipients and matched kidney transplant recipients are expressed as individual values. Statistical comparisons between islet autograft recipients and control subjects were performed by Student's t test. P values <0.05 were considered statistically significant.

RESULTS

Patient characteristics. Islet autograft recipients were studied a mean of 63 months (range, 23–107 months) post-transplant, and all were insulin-independent after receiving a mean of 351,000 islets each (range, 265,000–412,000 islets) (Table 1). Both recipients of islet allotransplantation had diabetes for a duration of at least 25 years before transplant. Each received >500,000 islets that had been procured from the pancreas of single brain-dead cadaver donors (8), and neither required insulin.

Serum glucose and HbA_{1c}. HbA_{1c} levels were normal in all patients, although slightly elevated in allograft recipient #2 (Table 1). Fasting glucose levels were normal in both islet auto- and allograft recipients and were <6.4 mmol/l in all patients on the day of the clamp study (Table 1). During the clamp, all patients achieved the target glucose nadir (3.1 mmol/l [55 mg/dl]) within 60 min of initiation of the insulin infusion, and this level was maintained for the duration of the clamp study (Figs. 1 and 2).

Insulin and C-peptide levels. Fasting serum insulin values were 42 ± 10 pmol/l in the autograft recipients, 60 and 120 pmol/l in allograft recipients #1 and #2, respectively, and 48 ± 12 pmol/l in the control group. During the 3-h insulin infusion, serum insulin values increased to 390 ± 102 pmol/l in the autograft recipients, 444 and 408 pmol/l in allograft recipients #1 and #2, respectively, 390 ± 42 pmol/l in the control subjects, and 360 and 403 pmol/l in kidney transplant patients #1 and #2, respectively. In response to insulin infusion, endogenous insulin secretion was suppressed in all patients, as measured by reductions in serum C-peptide. C-peptide decreased below basal levels within 60 min with similar suppression occurring in all patients (nadir C-peptide: autograft recipients, 0.07 ± 0.03 pmol/ml; allograft recipient #1, 0.19 pmol/ml; allograft recipient #2, 0.11 pmol/ml; control subjects, 0.08 ± 0.05 pmol/ml; kidney transplant patients #1 and #2, 0.06 and 0.07 pmol/ml).

Glucagon responses to hypoglycemia and intravenous arginine. Fasting glucagon was 77 ± 14 ng/l in the four autograft recipients, 146 and 292 ng/l in the two allograft recipients, 146 ± 29 ng/l in the control subjects (autograft recipients vs. control subjects, $P < 0.01$), and 127 and 120 ng/l in kidney transplant recipients #1 and #2, respectively (Table 1). Glucagon levels failed to increase above basal levels during the hypoglycemic clamp in the islet autograft recipients,

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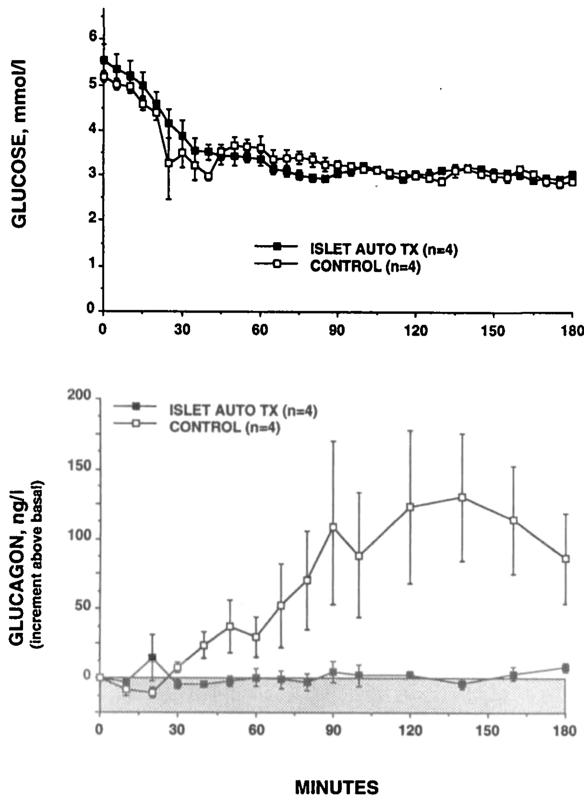

FIG. 1. Glucose and glucagon levels during hypoglycemic clamp in four recipients of islet autotransplantation after total pancreatectomy and four matched healthy control subjects. Hyperinsulinemic (1 mU · kg^{-1} · min^{-1} administered intravenously beginning at time 0) hypoglycemic clamps were performed with a target glucose nadir of 3.1 mmol/l (55 mg/dl). Despite an identical degree of hypoglycemia, islet autograft recipients failed to increase glucagon levels during the clamp study, while the control subjects demonstrated a significant increase in glucagon (control subjects vs. autograft recipients, $P < 0.01$).

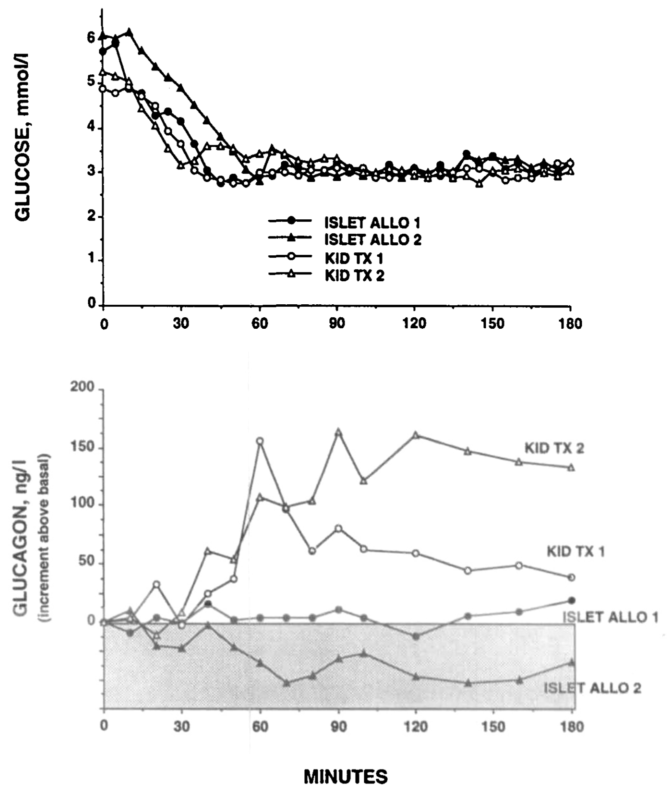

FIG. 2. Glucose and glucagon levels during hypoglycemic clamp in two diabetic recipients of islet allotransplantation and two patients following kidney transplantation matched for age, sex, BMI, and immunosuppressive treatment. During hyperinsulinemic hypoglycemic clamps, identical glucose nadirs of 3.1 mmol/l (55 mg/dl) were achieved. Islet allograft recipients demonstrated no significant increase in glucagon, while kidney transplant recipients demonstrated a significant increase in glucagon.

while the healthy control subjects showed a significant and sustained glucagon response (Fig. 1*B*; Table 1). Recipients of islet allotransplants also failed to increase glucagon levels during the hypoglycemic clamp, whereas matched kidney transplant patients had significant increases in glucagon (Fig. 2*B*; Table 1). The maximum glucagon increments during the hypoglycemic clamp (observed between 100 and 160 min of the clamp study) were as follows: autograft recipients, 4 ± 4 ng/l; allograft recipient #1, 1 ng/l; allograft recipient #2, –42 ng/l; control subjects, 114 ± 45 ng/l (autograft recipients vs. control subjects, $P < 0.01$); kidney transplant recipients #1 and #2, 76 and 138 ng/l, respectively (Table 1).

In contrast to the lack of response observed during hypoglycemia, both auto- and allograft recipients had demonstrable acute glucagon responses to arginine (Table 1). However, the mean acute glucagon responses in autograft recipients was significantly less than that observed in either allograft recipient.

DISCUSSION

To our knowledge, these are the first studies to assess glucagon secretion during prolonged hypoglycemia in humans following successful intrahepatic islet transplantation. We observed that intrahepatic islets, whether autotransplanted following pancreatectomy in nondiabetic individuals with chronic painful pancreatitis or allotransplanted in type I diabetic patients, did not secrete glucagon during a hypoglycemic clamp. In addition, the restoration of normoglycemia by islet allotransplantation in two type I diabetic patients for periods in excess of 2.5 years failed to reestablish glucagon responses to hypoglycemia despite the presence of both native and transplanted α-cells. In contrast, all islet transplant patients had demonstrable glucagon responses to intravenous arginine, confirming that functional α-cells were present.

Although we previously reported an evaluation of glucagon secretion in the recipients of intrahepatic islet autotransplantation (6), the current studies provide a more rigorous evaluation of α-cell function. Our initial series of studies (6) used an insulin bolus and were limited by the brief duration of hypoglycemia and the establishment of variable glucose nadirs. The clamp technique that was used in the studies reported herein achieved a consistent glucose nadir in all patients. In addition, studies of glucagon responses to hypoglycemia in type I diabetic recipients of successful islet transplantation have not previously been reported.

Whether the sustained correction of hyperglycemia can restore hypoglycemia-induced glucagon responses in individuals with type I diabetes is not known. Our studies provide the novel observation that glucagon secretion from the native pancreas remained defective in two patients with type

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I diabetes despite the restoration of normoglycemia for over 2.5 years. This provides very strong evidence that the simple correction of hyperglycemia (by means of islet transplantation) does not restore hypoglycemic responsiveness of the native α-cell.

As demonstrated in the accompanying manuscript by Gupta et al. (13), the absent glucagon response to hypoglycemia following islet transplantation appears to be unique to the intrahepatic implantation site. Using a canine model of autotransplantation, these studies demonstrate that, when transplanted to intraperitoneal but not intrahepatic sites, islets have intact glucagon secretory responses to hypoglycemia. This is consistent with the previous studies of Ansara et al. (14), which demonstrated that islets autotransplanted to the spleen retain glucagon responses to hypoglycemia.

Our observation that intrahepatic islet transplantation does not restore glucagon secretion in type I diabetic patients is in marked contrast to the findings we previously reported following whole-organ pancreas transplantation. Although some reports have concluded that there is no improvement in glucagon secretion after pancreas transplantation (15,16), the studies of Diem et al. (17) and Barrou et al. (9) have clearly shown that the transplantation of the pancreas restores glucagon secretion (9,17) and normalizes glucose counterregulation (9) during a hypoglycemic clamp. That intrahepatic islets successfully allotransplanted in two patients with type I diabetes did not restore glucagon secretion strongly suggests that the glucagon secretion observed following successful pancreas transplantation originates from the transplanted graft rather than the native islet.

It is not clear why intrahepatic islet transplantation fails to restore hypoglycemia-stimulated glucagon secretion. This defect is not the result of the islet isolation procedure, since islets isolated in a similar fashion yet transplanted in the peritoneum in dogs respond to hypoglycemia (13). One possibility is that changes in islet revascularization that are unique to the intrahepatic environment may account for these observations. However, studies of islet vascular growth have previously reported that normal blood flow perfusion patterns are reestablished as quickly as 14 days after transplantation (18). It should be noted in our human studies that the magnitude of the acute glucagon response to arginine was diminished following intrahepatic islet autotransplantation. This reduction could indicate a role for a generalized reduction in α-cell number in the observed absent responses during hypoglycemia. However, a generalized α-cell defect cannot entirely account for these observations, since the intrahepatic autotransplanted islets demonstrate qualitatively normal acute glucagon responses to arginine and have been previously shown to immunostain for glucagon (6). An alternative possibility is that hepatocyte glucose production may result in higher local glucose concentrations around intrahepatic α-cells than can be appreciated from measurements of peripheral blood glucose levels. If local glucose concentrations within the hepatic parenchyma fail to decrease to <60 mg/dl (3.3 mmol/l), α-cell stimulation may not occur. Under these circumstances, the α-cell itself may not be defective. Rather, the site of implantation provides an inadequate hypoglycemic stimulus, and no glucagon secretion would be expected. Experimental studies designed to abolish local hepatic glucose production were performed in dogs (V. Gupta, unpublished observations). In these studies, animals subjected to a 72-h fast and an ethanol infusion to abolish hepatic glucose production failed to demonstrate glucagon release from intrahepatic islets during hypoglycemia. Further studies using microdialysis techniques might help to clarify this phenomenon fully. It is reasonable to assert that the lack of glucagon response in patients undergoing autotransplantation may, in part, be related to the previous pancreatic islet cell damage that results directly from the pancreatic inflammation of chronic pancreatitis. However, islets allotransplanted to type I diabetic subjects were harvested from patients who did not have chronic pancreatitis. Moreover, in the canine studies of Gupta et al. (13), using healthy animals as intrahepatic islet autotransplant donors, the glucagon response to hypoglycemia was also defective.

In conclusion, we have shown that intrahepatic islet transplant grafts do not secrete glucagon in response to sustained hypoglycemia and that glucagon secretion by native and transplanted α-cells in two type I diabetic recipients during hypoglycemia was absent, despite 2.5 years of normoglycemia. If normal glucagon secretion during hypoglycemia is to be reestablished after islet transplantation, sites of implantation other than the liver may be required.

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