Pancreas Transplantation in Diabetic Humans Normalizes Hepatic Glucose Production During Hypoglycemia

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Although successful pancreas transplantation in humans with type I diabetes mellitus restores glucose-induced insulin secretion, provides freedom from insulin treatment, and normalizes fasting glucose levels, much less is known about its effects on counterregulation of hypoglycemia. To determine whether pancreas transplantation normalizes glucagon secretion and hepatic glucose production (HGP) during hypoglycemia, we performed hyperinsulinemic hypoglycemic clamps in successful recipients of pancreas allografts. Recipients were found to have glucagon secretory responses during hypoglycemia that were similar to those of control subjects (incremental glucagon response: recipients, 147 ± 34 ng/L; control subjects, 161 ± 43 ng/L, NS) but were significantly higher than those of matched subjects with type I diabetes (23 ± 9 ng/L, $P < 0.01$). HGP rates at the end of 120 min of hypoglycemia were also significantly higher in recipients and control subjects than in subjects with diabetes (pancreas recipients, 1.92 ± 0.33 mg · kg^{-1} · min^{-1}; control subjects, 2.05 ± 0.18 mg · kg^{-1} · min^{-1}; subjects with type I diabetes, 0.58 ± 0.12 mg · kg^{-1} · min^{-1}). A comparison with a third group of nondiabetic kidney transplant recipients demonstrated that the beneficial effects on glucose counterregulation were a result of pancreas transplantation and not the associated immunosuppressive therapy. We conclude that pancreas transplantation restores hypoglycemia-induced glucagon secretion and HGP, thereby allowing for normalization of glucose recovery from hypoglycemia. *Diabetes* 43:661–666, 1994

Successful pancreas transplantation in humans with type I diabetes provides freedom from insulin therapy and restores glucose-induced phasic insulin secretion, normal fasting plasma glucose levels, and normal to near-normal levels of glycosylated hemoglobin (1–6). This procedure also reduces the peripheral insulin resistance characteristic of type I diabetes (7) and provides postmeal carbohydrate metabolism similar to that of nondiabetic subjects using the same immunosuppressive therapy (8). However, the effects of pancreas transplantation on metabolic responses during hypoglycemia in pancreas recipients has not been nearly as well delineated. Long-standing type I diabetes is well known to be associated with defective glucagon and epinephrine responses to hypoglycemia (9–12), which in turn sets the stage for prolonged and severe episodes of hypoglycemia. Consequently, defining the effects of pancreas transplantation on counterregulation of hypoglycemia is of vital clinical importance if this therapy is to be used for patients disabled by severe and recurrent hypoglycemia.

The only previous studies in this area have demonstrated improvement in the ability of pancreas recipients to recover from short-term hypoglycemia produced by an intravenous pulse of insulin (13,14). However, such studies are seriously limited because intravenous pulses of insulin result in large interindividual differences in the depth and timing of glycemic nadirs. Furthermore, a greater dose of insulin had to be given to the pancreas recipients to overcome the peripheral insulin resistance caused by immunosuppressive therapy and to achieve similar glucose nadirs in the pancreas recipients and normal control subjects (13). Because hepatic insulin sensitivity has been reported to be normal in diabetic patients (15,16) and in pancreas recipients (7), this greater insulin dose might have resulted in more pronounced suppression of hepatic glucose production (HGP) in pancreas recipients, thereby limiting their potential to recover from insulin-induced hypoglycemia.

Consequently, we now report counterregulatory responses in pancreas recipients using the more precisely controlled conditions provided by the hyperinsulinemic hypoglycemic clamp technique to determine whether pancreas transplantation in type I diabetes patients normalizes defects in the glucagon secretory response and HGP during hypoglycemia. Control studies were performed in age-, sex-, and body mass index (BMI)-matched normal (nondiabetic) volunteers, type I diabetic patients who had not received transplants, and nondiabetic kidney transplant patients receiving the same immunosuppressive drugs as the pancreas recipients.

RESEARCH DESIGN AND METHODS

Eight type I diabetic recipients of pancreas allografts with iliac vessel anastomoses and systemic venous drainage who had received transplants between 21 and 78 months previously were studied (Table 1). All recipients were normoglycemic, had normal HbA_{1C} levels, and mildly elevated serum creatinine levels attributed to cyclosporine therapy. None were receiving exogenous insulin or other medications for diabetes. Immunosuppression was achieved with the triple-drug regimen of azathioprine (mean dose of 97 ± 23 mg/day), cyclosporine (330 ± 54 mg/day), and prednisone (10 ± 1 mg/day). Ten normal volunteers and 9 patients with type I diabetes of similar duration, matched for sex, age,

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HGP, hepatic glucose production; BMI, body mass index; RIA, radioimmunoassay.

TABLE 1
Patient clinical characteristics

	Normal control subjects	Pancreas recipients	Diabetic patients	Kidney recipients
n	10	8	9	5
Sex (M/F)	4/6	3/5	4/5	3/2
Age (years)	34 ± 2	35 ± 3	33 ± 2	36 ± 3
BMI (kg/m^2)	24.8 ± 1.5	24.8 ± 2.0	24.3 ± 0.8	26 ± 2.0
HbA_{1c}	5.1 ± 0.1	5.3 ± 0.2	8.0 ± 1.1	5.2 ± 0.1
Serum creatinine level (μM)	80 ± 9	141 ± 9	88 ± 9	141 ± 18
Diabetes duration (years)	—	18 ± 2	22 ± 3	—
Time since transplant (months)	—	39 ± 7	—	54 ± 17

Data are means ± SE. Normal ranges: creatinine, 27–115 μM; HbA_{1c} 4.3–6.0%.

and BMI, were studied to obtain control data. Five kidney recipients receiving the same immunosuppressive therapy and matched for renal function were also studied to control for the effects of immunosuppressive therapy. Patients with coronary heart disease or a history of seizures were excluded from the study. All patients gave informed, written consent to participate, and the study was approved by the University of Minnesota Human Subjects Committee.

All patients consumed a diet containing 300 g carbohydrate for 3 days immediately preceding the study. They were admitted to the University of Minnesota General Clinical Research Center on the evening before the study. The last subcutaneous injection of intermediate-acting insulin in diabetic patients was administered 24 h before the study. Euglycemia was maintained in diabetic patients by continuous intravenous insulin infusions that were adjusted hourly based on the blood glucose value. Studies in diabetic patients were canceled or postponed to another day if their blood glucose levels fell below 3.9 mM overnight. All subjects underwent a 12-h overnight fast. On the morning of the study, subjects were at bed rest and remained supine for the duration of the study. One catheter was inserted into an antecubital vein and another into a forearm vein of the same arm for infusion of test substances. A third catheter was placed retrogradely into a vein in the dorsal aspect of the contralateral hand for blood sampling. This hand was placed in a heated box (55–60°C) to provide access to arterialized venous blood. A primed (9 μCi), continuous (0.15 μCi/min) infusion of [3-^{3}H]glucose (Du Pont-NEN, North Billerica, MA) was started at −120 min and continued throughout the study. Normoglycemia was maintained in diabetic patients by means of variable intravenous insulin infusions until the beginning of the study. At time 0, the maintenance infusion of insulin was discontinued in diabetic subjects and a 1 mU · kg^{-1} · min^{-1} insulin infusion (Humulin, Lilly, Indianapolis, IN) was begun in all patients and continued for 180 min. Plasma glucose was measured at 5-min intervals and allowed to decline to 3.1 mM over 60 min. To maintain the plasma glucose level at 3.1 mM, 20% dextrose labeled with [3-^{3}H]glucose to achieve a specific activity equal to the serum at time 0 was infused. After the insulin infusion was discontinued at 180 min, the [3-^{3}H]glucose infusion was maintained as long as it was needed to maintain the plasma glucose at 3.1 mM and was stopped as soon as the plasma glucose level began to rise. All substances were infused using Harvard pumps (model 22 multi, Harvard Bioscience, South Natick, MA). Serum samples were obtained every 10–20 min for determination of specific activity, insulin, C-peptide, and glucagon.

Analytical methods. Plasma glucose was immediately measured with a Beckman Glucose Analyzer (Fullerton, CA). Samples for glucagon determinations were collected in prechilled tubes containing 2.5 mg EDTA and 500 U Trasylol/ml blood, put on ice, and centrifuged within 30 min. Glucagon was measured by radioimmunoassay (RIA) with antibody 04A obtained from Dr. R. H. Unger (University of Texas, Dallas) (17). Insulin was measured by double RIA as described previously (18). C-peptide was measured by RIA using a guinea pig anti-human C-peptide antibody provided by Novo-Biospecific (Wilton, CT) (19). Glucose-specific activity was determined in plasma that was deproteinized according to the method of Somogyi (20) and later dried (21). Rates of HGP and glucose utilization (rate of glucose disappearance) were determined with the equations of Steele et al. (22) as modified by DeBodo et al. (23).

Statistical analysis. Data are expressed as means ± SE. Incremental rates are the slopes of increment determined by the simple regression method (24). Statistics for intergroup comparisons were performed by analysis of variance followed by Fisher's least significant difference or Student's *t* test (24). $P < 0.05$ was considered statistically significant.

RESULTS

Glucose and insulin levels. The group of diabetic subjects began the study with higher glucose values than control subjects or recipients of pancreas or kidney transplants (Fig. 1). (Note: To maintain clarity in the figures, the data for

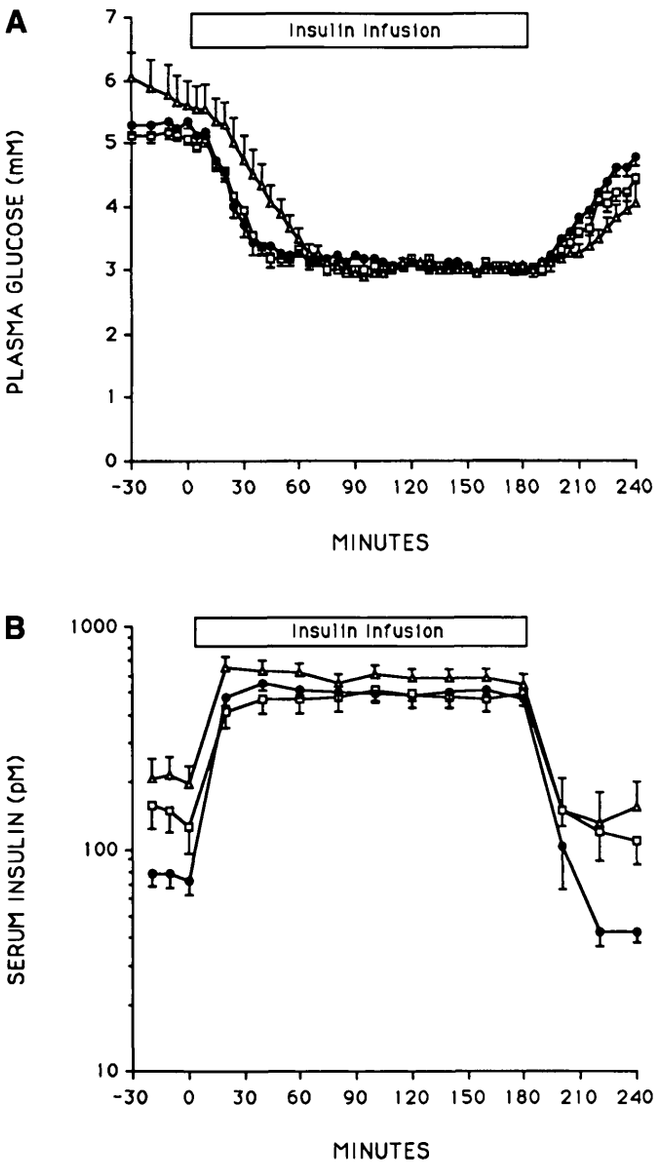

FIG. 1. Hyperinsulinemic hypoglycemic clamps were performed in pancreas transplant recipients (□, *n* = 8), subjects with type I diabetes (△, *n* = 9), and normal control subjects (●, *n* = 10). Similar glucose (*A*) and insulin levels (*B*) were achieved in all groups.

kidney transplant recipients are provided in Fig. 5 only.) The diabetic subjects also required more time to become hypoglycemic (mean plasma glucose first dropped below 3.3 mM at 65 min in the diabetic group, at 50 min in the control group, at 45 min in the pancreas transplant recipients, and at 35 min in the kidney transplant recipients). However, the rate at which the nadir was reached was similar in all groups (−0.053 ± 0.006 mM/min in control subjects, −0.039 ± 0.005 mM/min in pancreas recipients, and −0.042 ± 0.003 mM/min in kidney recipients). During 60- to 180-min intervals, identical levels of hypoglycemia were maintained in all groups (3.1 ± 0.1 mM in all groups).

The pancreas transplant group began the study with a higher mean serum insulin value than the normal control group (246 ± 54 pM in recipients vs. 89 ± 11 pM in control subjects; $P < 0.05$), but this value was not significantly higher than that in the diabetic (Fig. 1) or kidney recipient groups. Similar insulin values were achieved in all groups by 30 min. Insulin levels during the clamp study were maintained at 602 ± 36 pM in control subjects, 710 ± 67 pM in pancreas recipients, 566 ± 70 pM in diabetic subjects, and 582 ± 60 pM in kidney recipients (NS for all groups).

After discontinuation of the insulin infusion at 180 min, all subjects showed an increase in plasma glucose levels by 240 min (Fig. 1). Glucose was administered during the recovery phase to avoid serious hypoglycemia. Because the rate at which glucose was given was individualized for each subject based on the rate at which serum glucose increased, precise determination of rates of recovery for each group was not possible. However, in the diabetic group more time was required (28 ± 2 min) to reach a plasma glucose value of 3.3 mM after discontinuation of the insulin than in the control subjects (21 ± 2 min, $P < 0.05$). In contrast, the pancreas and kidney transplant recipients recovered from hypoglycemia at rates (18 ± 3 and 24 ± 4 min, respectively, to achieve a plasma glucose value of 3.3 mM after discontinuation of insulin) indistinguishable from that of the control group. At 240 min, the serum glucose values were 4.8 ± 0.1 mM in control subjects, 4.4 ± 0.1 mM in pancreas recipients, 4.1 ± 0.3 mM in diabetic subjects, and 4.2 ± 0.2 mM in kidney recipients.

Glucagon and C-peptide responses. In the basal state, glucagon levels were 176 ± 25 ng/L in control subjects, 154 ± 39 ng/L in pancreas transplant recipients, 114 ± 23 ng/L in diabetic subjects, and 192 ± 69 ng/L in kidney transplant recipients (NS, Figs. 2 and 5). During the hypoglycemic clamps both control subjects and recipients of pancreas or kidney allografts had similar increases in glucagon levels (incremental increase above basal was 161 ± 43 ng/L in control subjects, 147 ± 34 ng/L in pancreas recipients, and 146 ± 38 ng/L in kidney recipients, NS). In marked contrast, diabetic subjects had no increase in glucagon values.

In response to the insulin infusion, C-peptide levels were significantly suppressed in the control and transplant recipient groups (control subjects: basal = 0.319 ± 0.046 nM vs. 180-min value = 0.044 ± 0.024 nM, $P < 0.001$; pancreas recipients: basal = 0.666 ± 0.259 nM vs. 180-min value = 0.185 ± 0.063 nM, $P < 0.025$; kidney recipients: basal = 0.952 ± 0.368 nM vs. 180-min value = 0.109 ± 0.023 nM, $P = 0.001$). The group of diabetic subjects had undetectable levels of C-peptide at both time points.

Glucose utilization and HGP. Basal levels of glucose utilization were similar in all groups (Table 2 and Fig. 3).

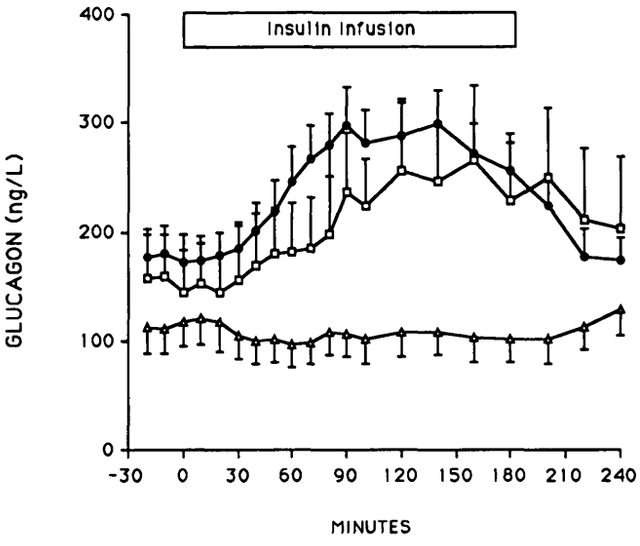

FIG. 2. Glucagon levels during hyperinsulinemic hypoglycemic clamps in pancreas transplant recipients (□, $n = 8$), subjects with type I diabetes (△, $n = 9$), and normal control subjects (●, $n = 10$). In pancreas recipients and control subjects, as opposed to diabetic subjects, increases in glucagon secretion were seen during the hypoglycemia period.

With the beginning of the insulin infusion at 0 min, the rate of glucose utilization remained stable for 30 min and then rose. The incremental rate of glucose utilization (the slope of the increment from 30 to 60 min) was similar in transplant recipients and control subjects (pancreas recipients = 0.072 ± 0.017, kidney recipients = 0.056 ± 0.022, and control subjects = 0.063 ± 0.015 mg · kg^{-1} · min^{-2}; NS) and significantly higher than the rate seen in diabetic subjects (0.015 ± 0.013 mg · kg^{-1} · min^{-2}; $P < 0.05$). However, after 90 min similar and essentially stable levels of glucose utilization were achieved in all three groups. During the glucose recovery period from 180 to 240 min, glucose utilization decreased at the same rate and to the same extent in all groups.

Basal levels of HGP were similar in all groups (Table 2, Figs. 4 and 5). Between 0 and 40 min, the infusion of insulin led to a similar rate of suppression of HGP in each group (slope of decrement in rate of HGP between min 0 and 40: control subjects = −0.011 ± 0.003 mg · kg^{-1} · min^{-2}, pancreas recipients = −0.015 ± 0.004 mg · kg^{-1} · min^{-2}, diabetic subjects = −0.011 ± 0.003 mg · kg^{-1} · min^{-2}, kidney recipients = −0.011 ± 0.003 mg · kg^{-1} · min^{-2}; NS). However, after 40 min HGP stopped falling in the pancreas transplant recipients, increased slightly and then stabilized in the normal control subjects and kidney recipients, and continued to decrease in the diabetic subjects. At 180 min, the pancreas recipients and normal control subjects had similar rates of HGP (1.92 ± 0.33 in recipients vs. 2.05 ± 0.18 mg · kg^{-1} · min^{-1} in control subjects, NS). These rates were significantly greater than that measured in the diabetic group (0.58 ± 0.12 mg · kg^{-1} · min^{-1}, $P < 0.001$). After 180 min, the rate of rise in HGP was greater in diabetic subjects than in the other groups, but despite this the absolute level of HGP remained significantly lower at 240 min in the diabetic subjects than in the control subjects and the transplant recipients (1.80 ± 0.11 in diabetic subjects vs. 2.79 ± 0.15 mg · kg^{-1} · min^{-1} in control subjects and 2.50 ± 0.22 mg · kg^{-1} · min^{-1} in pancreas recipients, both $P < 0.01$; and 2.37 ± 0.30 mg · kg^{-1} · min^{-1} in kidney recipients, $P < 0.05$). Differences

TABLE 2
Glucose utilization and HGP during hypoglycemic clamps

	Normal control subjects	Pancreas recipients	Diabetic patients	Kidney recipients
n	10	8	9	5
Glucose utilization ($mg \cdot kg^{-1} \cdot min^{-1}$)				
Basal	2.47 ± 0.11	2.90 ± 0.23	2.60 ± 0.17	2.12 ± 0.25
Min 180	3.44 ± 0.27	4.04 ± 0.48	4.36 ± 0.64	4.10 ± 0.87
Min 240	2.76 ± 0.15	2.47 ± 0.23	2.06 ± 0.16	2.34 ± 0.30
Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)				
Min 180	1.39 ± 0.30	2.12 ± 0.31	3.79 ± 0.65*	2.19 ± 0.98
Min 240	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.18	0.02 ± 0.00
HGP ($mg \cdot kg^{-1} \cdot min^{-1}$)				
Basal	2.4 ± 0.11	2.89 ± 0.23	2.57 ± 0.18	2.10 ± 0.24
Min 180	2.05 ± 0.18	1.92 ± 0.33	0.58 ± 0.12†	1.34 ± 0.49
Min 240	2.79 ± 0.15	2.50 ± 0.22	1.80 ± 0.11†	2.37 ± 0.30

Data are means ± SE. * $P < 0.05$ compared with pancreas recipients or control subjects. † $P < 0.001$ compared with pancreas recipients or control subjects.

in the rates of glucose infusion between groups mirrored the differences noted for HGP in that the diabetic group required a higher rate at min 180 to maintain a serum glucose level of 3.1 nM than did the control or pancreas recipient groups (3.79 ± 0.65 $mg \cdot kg^{-1} \cdot min^{-1}$ in the diabetic group vs. 1.39 ± 0.30 $mg \cdot kg^{-1} \cdot min^{-1}$ in control subjects, $P < 0.001$; and 2.12 ± 0.31 $mg \cdot kg^{-1} \cdot min^{-1}$ in pancreas recipients, $P < 0.04$, Table 2).

DISCUSSION

These studies provide the novel information that successful pancreas transplantation normalizes defective HGP during counterregulation of hypoglycemia in patients with type I diabetes. Earlier observations by us (13) and others (14) were limited by the use of different doses of insulin for the different subject groups and the absence of glucose turnover measurements. This study offers the first examination of glucagon secretory responses and HGP during controlled hypoglycemia using the hyperinsulinemic hypoglycemic clamp in patients after pancreas transplantation. Because our data demonstrate normalization of glucose counterregulation in patients with type I diabetes after pancreas transplantation, they support the use of this therapy in individuals severely disabled by recurrent bouts of severe hypoglycemia that are refractory to intensive medical management.

In our clamp studies, all subjects achieved similar serum insulin concentrations during insulin infusions and were brought to a serum glucose nadir (3.1 mM) at the same rate of glucose decline. As expected from the work of other investigators (9–12), the group with type I diabetes failed to secrete glucagon in response to hypoglycemia. In marked contrast, the glucagon secretory response of the pancreas transplant recipients was the same as that of the control subjects. Glucagon levels increased in pancreas transplant recipients as rapidly and as much as in the control subjects; this secretory response was maintained for as long as hypoglycemia was maintained. These results are consistent with the previous work of Diem et al. (13) and Bolinder et al. (14) but not that of Luzi et al. (25). The latter reported that

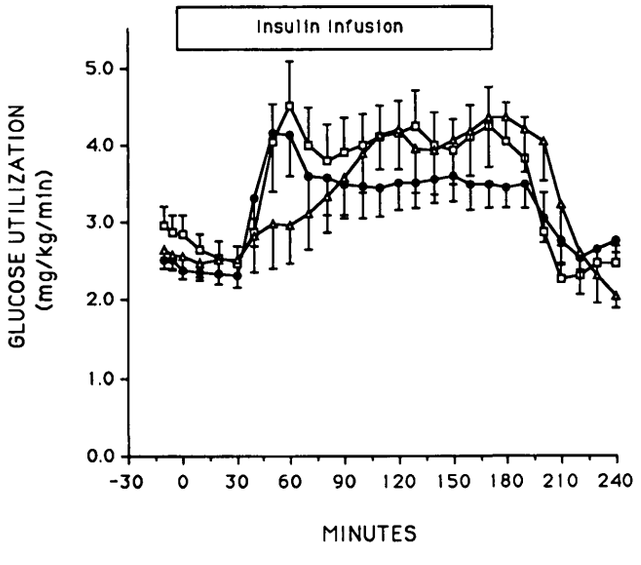

FIG. 3. Glucose utilization during hyperinsulinemic hypoglycemic clamps was measured by the isotopic dilution technique in pancreas transplant recipients (□, n = 8), subjects with type I diabetes (△, n = 9), and normal control subjects (●, n = 10). Similar rates were measured in each group from 90 to 180 min of the study.

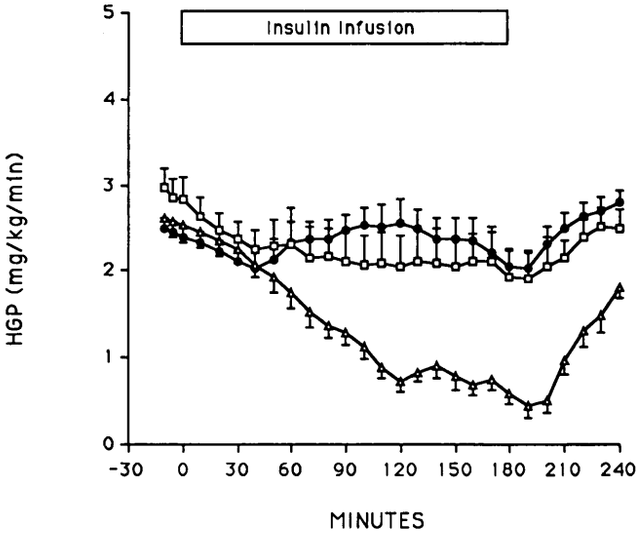

FIG. 4. HGP during hyperinsulinemic hypoglycemic clamps was measured by the isotopic dilution technique in transplant recipients (□, n = 8), subjects with type I diabetes (△, n = 9), and normal control subjects (●, n = 10). The diabetic group had significantly decreased rates of HGP during the period of hypoglycemia (60 to 180 min, $P < 0.001$).

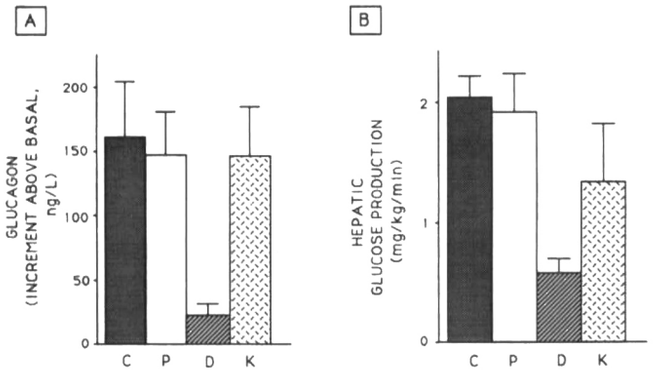

FIG. 5. Peak incremental glucagon responses (*A*) and HGP rates (*B*) at the end of the insulin infusion (min 180) are compared. Control subjects (■, $n = 10$), pancreas recipients (□, $n = 8$), and kidney recipients (▨, $n = 5$) had similar peak incremental glucagon responses and similar rates of HGP at min 180. In diabetic subjects (▨, $n = 9$) glucagon levels did not increase and a significant decrease in HGP was seen at min 180.

glucagon levels in pancreas transplant recipients during a hyperinsulinemic hypoglycemic clamp did not increase. However, Luzi et al. reported glucagon levels measured at only two serum glucose concentrations and did not measure HGP.

After the onset of the insulin infusion, all groups initially responded with a decrease in HGP rates. After 40 min, continued suppression was observed in the diabetic subjects, whereas stabilized rates were seen in the pancreas transplant recipients and the normal control subjects. Once all groups were clamped at 3.1 mM glucose, the pancreas transplant recipients and the normal control subjects continued to have HGP rates significantly greater than those in the group with diabetes. One possible explanation for the continued suppression of HGP during hypoglycemia in the diabetic group could be the level of antecedent glucose control, because this variable may be important in the counterregulatory response (26,27). However, although excellent glycemic control has been reported to diminish glucose counterregulation, overall glycemic control was only fair in our group of diabetic subjects as indicated by their mean glycosylated hemoglobin level.

The time required to recover from hypoglycemia can only be estimated because of the different rates of glucose infusion administered to individual subjects to avoid severe hypoglycemia after discontinuation of the insulin. Nonetheless, note that a serum glucose level of 3.3 mM was achieved in pancreas transplant recipients more quickly than in the diabetic subjects. Hence, the data allow the conclusion that the normalization of the HGP in response to hypoglycemia is in large part a result of restoration of hypoglycemia-induced glucagon responses in the pancreas recipients. Enhanced catecholamine secretion in response to hypoglycemia may also play a role in this normalization of HGP, but epinephrine values were not determined in this study. Our observations also are relevant to recent reports of periods of spontaneous hypoglycemia in some pancreas transplant recipients (28,29). Because pancreas recipients respond to hypoglycemia with normal rates of glucagon secretion and HGP, it is difficult to understand why they might be subject to spontaneous episodes of hypoglycemia. More intensive investigation of patients complaining of low blood glucose levels after successful pancreas transplantation will be essential to understand the pathophysiological factors underlying their symptoms.

To determine whether the improvements in counterregulation of hypoglycemia we have observed in pancreas transplant recipients resulted from the transplantation itself and not the associated immunosuppressive therapy required to prevent graft rejection or altered renal function, we studied a control group of matched nondiabetic kidney transplant recipients receiving the same immunosuppressive drugs. The metabolic responses were similar in the two transplant groups, allowing the conclusion that the restoration of the glucagon response and HGP during hypoglycemia in the pancreas recipients were a result of the transplantation itself and not related to immunosuppressive therapy or mildly diminished glomerular filtration rates.

In conclusion, we have demonstrated that pancreas transplantation restores the defective glucagon secretory response and enhances HGP during hypoglycemia in subjects with type I diabetes. Normalization of these counterregulatory mechanisms allows for normal recovery from hypoglycemia. Because severe and recurrent hypoglycemia increase both the morbidity and mortality of type I diabetes, pancreas transplantation may be considered as a therapeutic option available for patients incapacitated by hypoglycemic episodes and whose diabetes is refractory to intensive medical management.

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