The Effect of Pancreatic Polypeptide on Glucose Disposal After Surgical Alterations of the Pancreas

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Surgical alterations of the pancreas result in anatomic changes that can affect postoperative glucose metabolism. Pancreas transplantation results in reduction of beta-cell mass, systemic release of insulin, and denervation. The authors hypothesized that such alterations affect peripheral glucose disposal to induce an "insensitivity" to endogenously (systemically) released insulin. Additionally, they hypothesized that surgically induced deficiency of the postprandial hormone, pancreatic polypeptide, might contribute to altered glucose disposal. The authors studied two surgical models in dogs known to be devoid of pancreatic polypeptide—70% proximal pancreatectomy (PPx) and PPx plus distal pancreas autotransplantation (PAT/B). Oral glucose challenge and euglycemic hyperinsulinemic clamp studies were performed before and after a 16-day "pulsed" infusion of pancreatic polypeptide. Both surgical procedures resulted in elevations in the integrated glucose response after oral glucose, which was not affected by pancreatic polypeptide infusion. Euglycemic clamp studies showed decreased hepatic glucose output (R_a) and overall glucose disposal (R_d) in the fasted state for both surgical groups. The transplant animals demonstrated significant decreases in R_d during the hyperinsulinemic challenge (3.2 ± 0.01 *versus* 5.7 ± 0.01 mg/kg/minute at 60 to 120 minutes for PAT/B *versus* control). After 16 days of pancreatic polypeptide infusion, however, basal R_a, as well as basal and 60- to 120-minute R_d values, were returned to control values in the transplant group. The authors conclude that pancreas transplantation results in altered glucose disposal, possibly due to an altered effectiveness of systemically released insulin. They conclude that pancreatic polypeptide is an important modulator of peripheral insulin action. Therefore, the role of pancreatic polypeptide must be taken into account when evaluating postoperative glucose metabolism in canine models of pancreas transplantation.

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PANCREAS TRANSPLANTATION USING vascularized grafts has gained increasing acceptance in the treatment of type I diabetes. The surgical alterations of the pancreas necessary for transplantation, however, might be predicted to be associated with alterations in carbohydrate metabolism. These alterations include β-cell reduction (use of segmental grafts or loss through preservation techniques), systemic drainage of pancreatic venous effluent, and loss of innervation.

We have investigated several alterations in carbohydrate metabolism after pancreas transplantation using an animal model that employs the segmental pancreas autotransplant. Elevations in glucose-stimulated insulin release and a possible "insensitivity" to the endogenous insulin released have been documented in these pancreas autotransplant models.[1,2] Others have suggested a relationship between pancreas resection *per se* and alterations in peripheral sensitivity to insulin or regulation of insulin release. Bonner-Weir et al.[3] have hypothesized that decreased insulin secretion after β-cell reduction has a causal effect; Seymour et al.[4] have suggested that a deficiency in a postprandial hormone, pancreatic polypeptide, also may affect glucose metabolism adversely.

Hormone deficiencies, including pancreatic polypeptide, have received scant attention as possible adverse postoperative factors after transplantation. Pancreatic polypeptide (PP) is a 36-amino-acid peptide found in pancreatic islet F cells located primarily in the head of the pancreas.[5] Surgical resection of this area in dogs and humans essentially obliterates basal and nutrient-stimu-

lated levels of PP. Evaluation of the PP-deficient state of chronic pancreatitis in dogs and humans demonstrated that deficiency of this peptide is associated with the loss of insulin-mediated suppression of hepatic glucose production.[4,6] The relationship of PP to postoperative glucoregulation after pancreas transplantation, however, remains unclear.

The current studies were designed to evaluate the hypothesis that pancreas transplantation leads to alterations in glucoregulation due to postsurgical anatomic changes and resultant PP deficiency. We investigated this hypothesis in the canine autotransplant model, which allows examination of the effects of transplantation devoid of the effects of rejection or immunosuppression.

Methods

Surgical Procedures

Adult female mongrel dogs, each weighing 15 to 20 kg, were selected for one of two surgical procedures after completion of preoperative studies (see Protocol). All procedures were carried out under National Institutes of Health and University of Virginia (Protocol 1126-04-85) guidelines. The long-term stability of the models has been previously documented.[1,2]

Overnight fasted animals were anesthetized with sodium pentobarbital (25 to 30 mg/kg body weight), intubated, and given halothane anesthesia (0.5% to 1.0%) by volume respirator. Intravenous fluids and warming blankets maintained normovolemia and normothermia. The abdomen was entered through a midline incision.

Partial pancreatectomy. These animals underwent 70% proximal partial pancreatectomy (PPx). Proximal dissection was continued until the entire gastroduodenal vascular arcade was skeletonized with duodenal viability preserved. The proximal pancreas (duodenal lobe) was removed, leaving only the splenic lobe intact. The weight of the resected tissue ranged from 28 to 37 gm. Splenectomy was performed and the coronary vein ligated, ensuring that the venous drainage from the pancreas was routed through the splenic vein into the portal vein. The pancreatic duct was left open and allowed to drain freely into the peritoneal cavity.

Pancreas autotransplant. Partial pancreatectomy was carried out in the fashion described above. The splenic artery was identified at its celiac axis origin and the splenic vein was identified. Hypothermic preservation by perfusion of chilled heparinized normal saline solution (500 units heparin per liter) was performed in a retrograde fashion by cannulating the distal splenic artery with an 18-gauge catheter. The distal pancreas was placed in the pelvis and the venous anastomosis was accomplished in an end-to-side fashion to the iliac vein. An end-to-side arterial anastomosis was completed between the splenic and the external iliac artery. The pancreatic duct was anastomosed to the urinary bladder, as previously described.[1]

Provocative Tests

Four animals in each group were allowed to recover from surgery for at least 1 month and regain their preoperative weight. Each animal was studied twice, with studies performed at least 2 weeks apart. These animals were maintained on daily pancreatic exocrine supplementation (Viokase, 10 gm/day, A. H. Robbins, Richmond, VA). All provocative tests were performed on animals who were studied in the awake state while standing comfortably in loosely applied harnesses. No study was initiated unless the dog was clinically healthy, eating regularly, and cleared by the veterinarian staff.

Test meals. Test meals were performed on animals after an overnight fast. Three basal samples (−20, −10, and 0 minutes) were collected on each animal before receiving 20 g/kg canned dog food meal (Alpo, Alpo Dog Food Company, Richmond, VA; constituents: 9% protein, 6% fat, 1.5% fiber, and the remainder made of water). Sequential samples at 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes were collected for glucose, insulin, and pancreatic polypeptide determinations. Each sample was quickly centrifuged and placed on ice. Serum glucose levels were immediately determined by the glucose oxidase method on a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin samples were frozen (−70°C) at the conclusion of the experiment. The insulin assay is a double antibody assay performed routinely by the University of Virginia Diabetes Core Laboratory Facilities. Serum pancreatic polypeptide was determined by measuring immunoreactive pancreatic polypeptide using standard radioimmunoassay techniques.[7]

Oral glucose tolerance tests. All animals were fasted overnight. An 18-gauge catheter then was placed in a peripheral leg vein, and two basal samples were obtained. A 20% glucose solution was administered by oral tube over a 3-minute period by delivering 2 g/kg glucose. Serial samples are obtained until 240 minutes as described for the test meals studies. All dogs tolerated this method well with no adverse reactions.

Euglycemic clamp studies. Using a modification of the technique described by Andres et al.[8] and DeFronzo et al.,[9] euglycemia was maintained at a basal level by servocontrolled intravenous infusion of a 20% glucose solution and a separate infusion of U100 regular insulin (Eli Lilly Co., Indianapolis, IN) at a rate of 0.5 mU/kg/minute. Infusion of glucose was performed through a central venous catheter introduced by the saphenous vein of the hind limb. Insulin was administered through a peripheral catheter, and blood samples were obtained from a central venous catheter placed 12 inches centrally from the glu-

cose-infusion catheter to avoid sampling error due to admixing. The infusion of insulin was started with a loading dose (1 mU/kg/minute) of insulin for 5 minutes, at which time a rate of 0.5 mU/kg/minute was maintained for the duration of the experiment. Serum glucose was then maintained at the basal value (±10%) for the duration of the study by a computer-assisted calculation of a space-of-distribution formula that determined the required rate of glucose infusion with a variable speed, high-capacity infusion pump (Harvard Apparatus Co., S. Natick, MA). After baseline blood samples were obtained, a bolus infusion of tritiated glucose (2.5 μCi/kg 3-^{3}H-glucose, New England Nuclear, Boston, MA) was followed by a continuous infusion of 31.25 nCi/kg/minute (0.555 mL/minute) through a peripheral catheter. Baseline blood samples were obtained every 10 minutes beginning 100 minutes after the start of the constant rate tritiated glucose infusion. After three or more of these samples indicated stable plasma glucose levels, a primed continuous 2-hour infusion of insulin began (t = 0). All insulin infusates were prepared in 50 mL 0.9% saline containing 2 mL of each animal's whole blood to prevent adsorption of insulin onto the infusion apparatus.

Protocol—Pancreatic Polypeptide Infusion

Normal control animals, PPx animals, and pancreas autotransplant (PAT/B) animals each received the test meal, oral glucose tolerance test, and tritiated glucose-euglycemic clamps as described above. Each animal then underwent a 16-day, continuous subcutaneous infusion of homogeneous bovine pancreatic polypeptide (Lilly Research Laboratories, Indianapolis, IN; lot 615-JE6-70. (Purity of this peptide is greater than 95% one peak by high-pressure liquid chromatography [HPLC].[10] Glucagon content is 0.003%; Insulin, 0.002%). Pancreatic polypeptide (PP) infusate was prepared by suspending 0.04 mg PP/kg in 2 mL 0.9% saline, and delivered by a subcutaneously placed osmotic pump (Alzet, Model 2ML2, Alza Corporation, Palo Alto, CA) at a calculated rate of 5 μL/hour (100 μg PP/kg/hour). These osmotic pumps were implanted under light pentobarbital anesthesia in a subcutaneous pocket. Additionally, each animal received a subcutaneous injection of 0.4 μg/kg pancreatic polypeptide at 8:00 A.M. each morning to recapitulate a morning postprandial "pulse" of the hormone (AM injection).

Fasting blood samples for immunoreactive pancreatic polypeptide were drawn from each dog twice daily. The first was before the subcutaneous injection, and the other was approximately 30 minutes after the morning subcutaneous injection. Thereby, "basal" and "peak" levels were drawn each day. The oral glucose tolerance test and euglycemic clamp studies were repeated on days 14 and 16 of PP infusion, respectively.

Statistical Analysis

Analysis of the data was accomplished using the SAS statistical package on a computing system (VAX-751, Digital Equipment Corporation, Maynard, MA). For the oral glucose tolerance test, the time-compensated basal-adjusted integrated values for glucose and insulin response curves were calculated.

The euglycemic clamp responses were partitioned by time into basal (−20 to 0 minutes), 30- to 60-minute, and 60- to 120-minute segments for which t = 0 at the initiation of the insulin infusion, as described above. The means for glucose and insulin values then were calculated for each period. The estimation of glucose metabolized in each period was calculated from the glucose infusion rate (M, mg/kg/minute), after adjustment for changes in serum glucose values. This value was taken as an index of overall glucose "tolerance." Means for all groups then were simultaneously compared using the general linear modeling analysis of variance, with significance determined by the least-squares means comparison method. An alpha level of 0.05 was used for significance. Coefficients of variation for the clamps also were determined on the 60- to 120-minute segment by least-squares means analysis.[11]

For the calculation of the hepatic glucose output and the overall glucose disappearance during tritiated glucose infusion, the following assumptions are made: In the basal state, when a dynamic equilibrium prevails (before insulin infusion), a glucose turnover rate (mg/kg/minute) is calculated by the isotopic dilution equation: $R_t = R_a = R_d = F/SA$, where R_t is the rate of glucose turnover, R_a is the rate of endogenous glucose production, R_d equals the rate of overall glucose utilization or disposal. F is the rate of infusion of the tracer (nCi/kg/minute), and SA is the specific activity of plasma glucose at equilibrium (nCi/mg). Because the liver is essentially the only source of glucose in the postabsorptive state, R_a can be assumed to be the rate of hepatic glucose production. In nonsteady states (during insulin infusion), R_a and R_d are calculated by Steele's equations in their algebraic form for each 10-minute interval.[12] The calculated R_a from Steele's equations minus the exogenous glucose infused during the interval is assumed to be the endogenous glucose production (hepatic glucose production, or R_a). The assay of glucose turnover by the primed-constant infusion and the pool fraction technique has been validated for both steady and nonsteady states.[12] An important recently investigated consideration of analysis of hyperglycemic clamps demonstrates that when the rate of exogenous glucose infusion equals or exceeds the calculated total glucose appearance rate, then the rate of hepatic production can be considered to be zero even though physiologically implausible negative values have been obtained. Such negative glucose

production rates are most often, but not exclusively, observed when a sudden perturbation is made that results in a large and very rapid change in plasma glucose (or insulin) levels. During the early part of the clamps (0 to 30 minutes) such incongruities may exist; for this reason no data are analyzed during the initial period of rapid insulin infusion, and peripheral levels change. Thereafter, however, and especially during the second hour, (1) relatively small changes were made in the glucose infusion and (2) the change in specific activity per unit time was small enough to minimize the error associated with the assumption made for the volume of distribution of glucose.[13] It should be noted that a systematic difference in glucose turnover rates has been observed during simultaneous infusions of different radioactive isotopes under a variety of experimental conditions in normal and abnormal states of glucose tolerance.[14,15] The differences are relatively small, however, especially in normal subjects, and differ only in the magnitude of the turnover rates. The shapes of the time course of the glucose turnover rates are identical. Furthermore, Radziuk et al.[16] have demonstrated that the tracer infusion technique can reliably estimate hepatic glucose production even when the system is not in steady state using a single-compartmental model. We report the actual hepatic glucose production values obtained from our calculations, as recently adopted by Bell et al.[15]

Results

Test Meal Studies

The response of glucose, insulin, and pancreatic polypeptide to a standard test meal challenge is shown in Figures 1 through 3. The surgical groups (PPx and PAT/B) demonstrated a similar glucose response after the test meal, which reflects the overall lack of glucose in the preparation (Fig. 1). Pancreas autotransplantation animals showed an altered insulin response when compared with PPx and control animals (Fig. 2). The pancreatic polypeptide response to a standardized test meal shows that both surgical groups had decreased basal and peak pancreatic polypeptide responses to the test meal (Fig. 3). Basal IR-PP levels in PPx and PAT/B animals were less than 50 pg/mL.

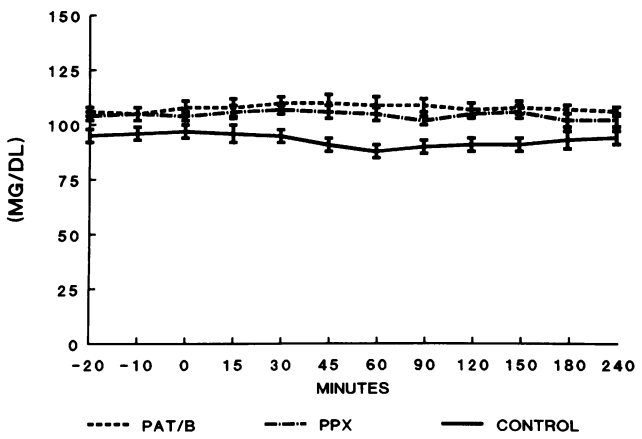

FIG. 1. Glucose response to test meal in normal controls, PPx, and PAT/B animals. Data are discussed in text (n = 8 for all groups).

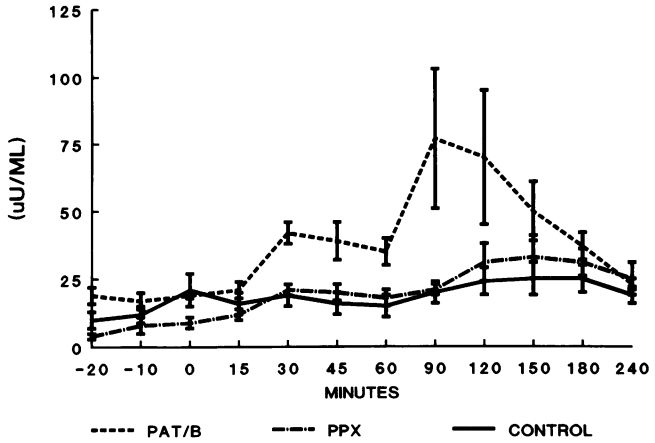

FIG. 2. Insulin response to test meal in normal control, PPx, and PAT/B animals. Data are discussed in text (n = 8 for all groups).

Pancreatic Polypeptide Response to Pancreatic Polypeptide Infusion and AM Injections

Figures 4 and 5 show the daily "basal" and "peak" (30 minutes after subcutaneous injection) pancreatic polypeptide values for the PPx and PAT/B animals for the 16 days of subcutaneous pancreatic polypeptide treatment. For the PPx animals and PAT/B animals, the average basal pancreatic polypeptide during 16 days of PP infusion was 157 ± 34 and 185 ± 21 pg/mL, respectively. The

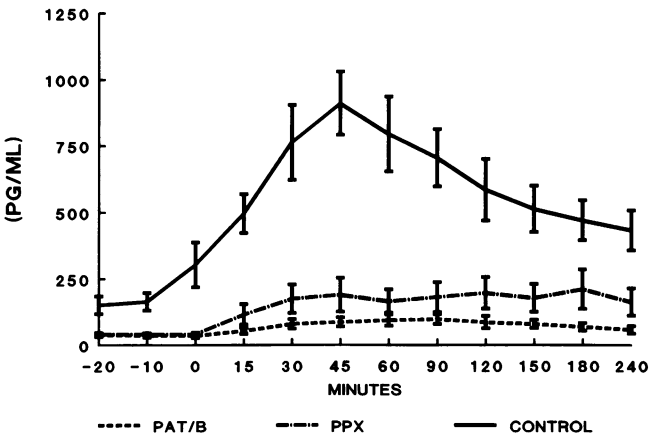

FIG. 3. Pancreatic polypeptide response to test meal in normal control, PPx, and PAT/B animals. Both surgical procedures obliterate basal and meal-stimulated levels. Data are further discussed in text (n = 8 for all groups).

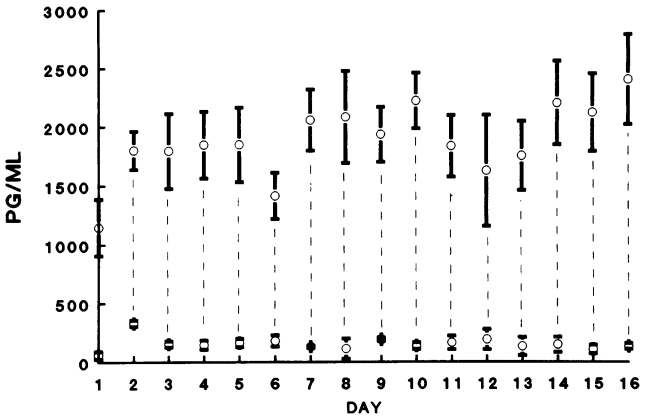

FIG. 4. Daily pancreatic polypeptide levels in PPx and PAT/B animals during chronic pancreatic polypeptide infusion with AM "pulses." The lower values represent "basal" levels, the upper values represent "peak" values approximately 30 minutes after each AM injection (n = 8 for all groups).

peak levels, averaged over these 16 days, were 1919 ± 339 and 1766 ± 175 pg/mL for the PPx and PAT/B animals, respectively.

Oral Glucose Tolerance Test

Effects of oral administration of 2 g/kg of glucose on plasma glucose and insulin in normal animals and the surgical groups before and after treatment with pancreatic polypeptide are shown in Table 1. Plasma glucoses were similar in all five groups and were not changed by the infusion of pancreatic polypeptide. Basal insulin values were elevated in the PAT/B compared with control animals, but were not affected by pancreatic polypeptide treatment. All surgical groups had elevations in basal integrated (B/INT) glucose responses. The B/INT glucose and insulin responses for the PPx and PAT/B animals were not significantly altered by pancreatic polypeptide infusion.

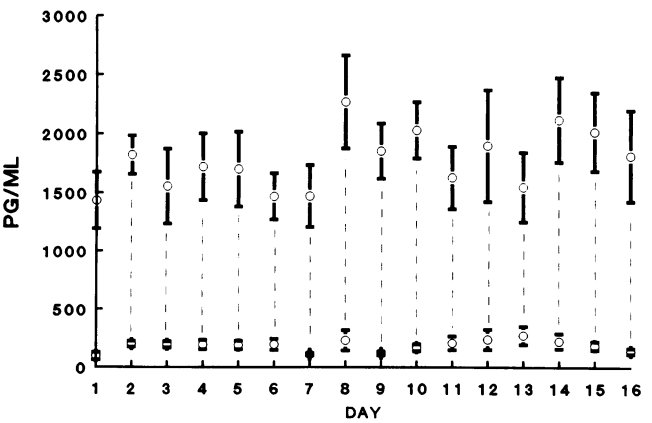

FIG. 5. Daily pancreatic polypeptide levels in PPx and PAT/B animals during chronic pancreatic polypeptide infusion with AM "pulses." The lower values represent "basal" levels, the upper values represent "peak" values approximately 30 minutes after each AM injection (n = 8 for all groups).

TABLE 1. *Oral Glucose Tests*

	Basal Glucose	Basal Insulin	B/INT Glucose	B/INT Insulin
Control	97 ± 3	9 ± 2	20 ± 6	15 ± 3
PAT/B	101 ± 2	17 ± 3*	39 ± 5	23 ± 3
PAT/B-PPT	105 ± 3	20 ± 3†	47 ± 10†	15 ± 3
PPx	100 ± 5	8 ± 3	63 ± 10‡	13 ± 5
PPx-PPT	106 ± 3	13 ± 2	68 ± 12§	14 ± 4

N = 8 for all groups.
* PAT/B *vs.* control, $p < 0.05$.
† PAT/B-PP *vs.* control, $p < 0.05$.
‡ PPx *vs.* control, $p < 0.05$.
§ PPx-PP *vs.* control, $p < 0.05$.
B/INT, basal integrated response; PAT/B, pancreatic autotransplant group; PAT/B-PPT, PAT group after 16 days of PP infusion; PPx, partial pancreatectomy group; PPx-PP, PPx group after 16 days of PP infusion.

Euglycemic Clamp Studies

The effects of 0.5 mU/kg/minute insulin infusion, with maintenance of euglycemia by the glucose clamp technique, is shown in Table 2. There were no differences in serum glucose values during the euglycemic clamp study between controls and either PAT/B or PPx animals in the basal, 30- to 60-minute segment, or 60- to 120-minute segment. Likewise, no differences occurred in the glucose values in either surgical group after 16 days of PP infusion.

Insulin values rose in the control animals from a basal value of 9 ± 3 to 31 ± 1 μU/mL during the 60- to 120-minute period of the clamp. The PAT/B animals demonstrated an increased insulin level during exogenous infusion that persisted after pancreatic polypeptide treatment (50 ± 1 and 40 ± 1 μU/mL, respectively). Likewise, the PPx animals demonstrated a slightly elevated insulin value during exogenous insulin infusion, and this was similarly unaffected by PP infusion.

Basal hepatic glucose output (R_a) was 4.2 ± 0.4 mg/kg/minute in the control animals and was significantly decreased in both the PAT/B (2.9 ± 0.1 mg/kg/minute) and PPx (2.8 ± 0.1) animals. Insulin infusion rapidly suppressed these values by the 30- to 60-minute period, and this suppression persisted into the second hour. The ΔR_a (difference between basal and 60- to 120-minute values) was significantly decreased for both surgical groups when compared with controls (control = −3.4 ± 0.3; PAT/B = −2.2 ± 0.2; PPx = −2.2 ± 0.3). Pancreatic polypeptide infusion did not significantly alter ΔR_a in either surgical group.

Control animals had an overall glucose disposal (R_d) value of 4.1 ± 0.4 mg/kg/minute, which increased to 5.7 ± 0.4 mg/kg/minute during the 60- to 120-minute period.

TABLE 2. *Euglycemic Clamp Studies*

	Control	PAT/B	PAT/B-PP	PPx	PPx-PP
Glucose (mg/dL)					
Basal	95 ± 2	102 ± 2	103 ± 2	95 ± 1	101 ± 2
30–60	95 ± 1	104 ± 1	101 ± 1	95 ± 1	99 ± 1
60–120	93 ± 1	101 ± 1	102 ± 1	93 ± 3	100 ± 1
Insulin (μU/mL)					
Basal	9 ± 3	13 ± 2	15 ± 2	8 ± 1	9 ± 2
30–60	23 ± 1	32 ± 2	37 ± 1	28 ± 2	41 ± 1
60–120	31 ± 1	50 ± 2*	40 ± 1	44 ± 1	49 ± 1‡
R_a (mg/kg/min)					
Basal	4.2 ± 0.4	2.9 ± 0.1*	3.5 ± 0.5	2.8 ± 0.1†	3.1 ± 0.2
30–60	1.1 ± 0.2	0.9 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.8 ± 0.2‖
60–120	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
ΔR_a	−3.4 ± 0.3	−2.2 ± 0.2*	−2.8 ± 0.3	−2.2 ± 0.3†	−2.3 ± 0.3‡
R_d (mg/kg/min)					
Basal	4.1 ± 0.4	2.9 ± 0.1*	3.7 ± 0.1	2.9 ± 0.1†	3.1 ± 0.2
30–60	3.9 ± 0.2	2.9 ± 0.1	4.2 ± 0.1	3.4 ± 0.1	4.1 ± 0.4
60–120	5.7 ± 0.4	3.2 ± 0.1*	5.2 ± 0.1	5.0 ± 0.1	5.9 ± 0.4
ΔR_d	1.8 ± 0.5	0.3 ± 0.4*	1.5 ± 0.5§	2.3 ± 0.5¶	2.8 ± 0.5
M					
Basal	—	—	—	—	—
30–60	1.7 ± 0.2	1.2 ± 0.2	2.3 ± 0.2	2.5 ± 0.3	2.5 ± 0.3
60–120	5.5 ± 0.5	2.7 ± 0.2	3.9 ± 0.2	5.2 ± 0.2	4.6 ± 0.2

N = 8 for all groups.
* PAT/B *vs.* control, $p < 0.05$.
† PPx *vs.* control, $p < 0.05$.
‡ PPx-PP *vs.* control, $p < 0.05$.
§ PAT-B *vs.* PAT/B-PP, $p < 0.5$.
‖ PAT/B-PP *vs.* PPx-PP, $p < 0.5$.
¶ PAT-B *vs.* PPx, $p < 0.05$.

Both surgical groups had statistically decreased basal R_d values. After PP infusion, basal R_d values were comparable with controls. The PAT/B had decreased R_d values for basal, 30- to 60-minute, and 60- to 120-minute periods as well as for ΔR_d when compared with controls. Pancreatic polypeptide significantly increased basal, 30- to 60-, and 60- to 120-minute values as well as the ΔR_d to values comparable to those of the normal controls.

For M values, no real differences were demonstrated in PPx either before or after PP infusion. The PAT/B animals had a decreased M value as compared with controls (2.7 ± 0.2 *versus* 5.5 ± 0.5 mg/kg/minute, respectively), which was elevated by PP treatment to 3.9 ± 0.2 mg/kg/minute. These values did not achieve statistical significance between groups.

Discussion

Pancreas transplantation offers an attractive possibility for the treatment of type I diabetes. There are many reports that document the use of vascularized grafts of a segment of the whole pancreas to restore peripheral levels of glucose and insulin to "normal" values. There is increasing evidence, however, that factors other than insulin *per se* impact on glucose homeostasis in the diabetic and nondiabetic organism. Specifically, surgical alterations of the pancreas that attend transplantation result in reduced β-cell mass, systemic release of insulin, and denervation, each of which may be associated with alterations in carbohydrate metabolism. Additionally, the complex hormonal interplay between insulin and other postprandial hormones, such as glucose-dependent insulinotropic polypeptide and pancreatic polypeptide (PP), is receiving increasing attention in the understanding of overall glucoregulation. The extent to which transplantation-induced alterations in the release of other pancreatic hormones such as PP affects glucose metabolism is also unknown. We therefore sought to address the alterations in insulin's effect on glucose appearance and disposal in a model of surgical alteration such as pancreas transplantation. We hypothesized that transplantation results in a degree of peripheral insensitivity to endogenous insulin that may be explained by a deficiency in pancreatic polypeptide.

Surgical alterations of the pancreas. We and others have described changes in insulin secretory patterns and alteration in glucose handling after various surgical procedures on the pancreas.[1–4,17,18] Relevant to all surgical models is the appreciation of reduced β-cell mass that is particularly germane in transplantation due either to preservation techniques or to the use of segmental grafts. Other investigators have found differences in the response to glucose or "nonglucose" (*i.e.*, test meal or arginine) challenges in models of reduced β-cell mass.[3,18,19] It appears that in a reduced β-cell mass preparation in rats, a decreased peripheral insulin response and hyperglycemia, can occur that can result in impaired glucoregulation in the periphery. Studies in pancreas-transplanted dogs using intravenous glucose challenge corroborates this concept.[17,20]

Our data differ somewhat from those of Bonner-Weir

et al.,[3] who observed that different challenges show different degrees of impairment in the insulin response.[3] Our test meal and oral glucose tolerance test (OGTT) data show little difference in insulin response between controls and PPx animals (Table 1, Fig. 2). Pancreas transplant animals have a hyperinsulinemic response to both test meal (Fig. 2) and OGTT (Table 1). We have reported this OGTT effect previously, and this is most likely explained by direct systemic release of insulin, which thereby bypasses a "first-pass" hepatic extraction.[21] Interestingly, despite an elevated peak insulin and integrated insulin response compared with controls, the integrated glucose levels in PAT/B animals remain elevated (PAT/B, 39 ± 5; Control, 20 ± 6 mg/dL), suggesting a persistent insensitivity to the concomitant increased levels of peripheral insulin after transplantation.

We employed the euglycemic glucose clamp with measurement of tritiated glucose specific activity to evaluate hepatic glucose output (R_a) and overall glucose disposal (R_d) at a physiologically elevated level of insulin. We previously observed no significant changes in PAT/B animals during studies that produced supraphysiologic levels of insulin.[22] The current study evaluated these parameters within an equivalent glucose or test-meal–stimulated postprandial plasma insulin range of 40 to 50 μU/mL (Table 2). We determined that partial pancreatectomy or pancreas autotransplantation did not result in significant changes in hepatic glucose output (R_a) during exogenous insulin infusion. After pancreas transplantation, however, overall glucose disposal (R_d) is significantly depressed in the basal state, as well as during the 30- to 60-minute and 60- to 120-minute periods of the insulin infusion. The PPx animals did not show this effect. These data might partially explain the apparent "insensitivity" seen in the OGTT data whereby the postchallenge hyperglycemia is not corrected by apparently increased levels of insulin.

Pancreatic polypeptide deficiency and effects on glucoregulation. The reasons why alterations in surgical anatomy of the pancreas might result in physiologic alterations in insulin secretion and glucoregulation are complex. One factor may be the change in other islet hormones caused by transplantation. The deficiency of a postprandial hormone, pancreatic polypeptide, has received increasing attention in studies of animals and humans with pancreatitis and decreased insulin secretion. Pancreatic polypeptide is a 36-amino-acid peptide that is elaborated from non-β islet cells in the head and uncinate portion of the gland.[5] It is released in response to ingested nutrients and is controlled by cholinergic and adrenergic pathways.[23,24] Resection of the head of the pancreas as well as chronic pancreatitis are associated with marked deficiencies of fasting and postprandial levels of pancreatic polypeptide.

Pancreatitis with decreased pancreatic polypeptide appears to be associated with the loss of insulin's ability to suppress R_a.[4,6] Infusion of pancreatic polypeptide reverses this alteration and, additionally, is associated with an amelioration of alterations in OGTT.[4] It therefore seems likely that alterations in levels of pancreatic polypeptide may have particular relevance to pancreatic transplantation. Such alterations could occur because of loss of islet mass increased by the procedure itself or because of consequences of transplantation due to the disease process such as denervation of the residual pancreas.

Our studies sought to document a clinically relevant state of pancreatic polypeptide deficiency, evaluate what changes in insulin glucoregulation accompany this, and attempt to correct any such changes by restoring pancreatic polypeptide in an approximately physiologic fashion. Pancreatic resection or transplantation is associated with decreased basal and meal-stimulated pancreatic polypeptide levels.[7,25,26] We performed test meals in all groups of animals to confirm the existence of pancreatic polypeptide deficiency after each surgical procedure.

Several groups have evaluated the effect of exogenous pancreatic polypeptide on glucoregulation. Hyperglycemic, obese mice have been thought to have a deficient release of pancreatic polypeptide; when treated with PP, food intake and body weight decreased.[27] Type I diabetics appear to have elevated levels of pancreatic polypeptide, as do normal people in in older age groups.[28] These observations suggest a correlation between changes in levels of pancreatic polypeptide with alterations in insulin sensitivity. This was further confirmed in both patients and animals with chronic pancreatitis in which 8-hour PP infusions (in humans) or 14-day subcutaneous PP infusions (in animals) were able to restore to normal insulin's ability to suppress hepatic glucose output.[4,6] Chronic infusions of PP at supraphysiologic levels in obese mice have been shown to "increase sensitivity" to endogenous insulin.[29] This effect was not seen in normal mice, however. In addition, acute infusions of PP appear to have little reproducible effect on insulin release or sensitivity in mice, dogs, or humans.[4,6,29,30]

Our infusion protocol sought to recapitulate physiologic "release" of PP each day in a pattern roughly equivalent to a daily meal stimulation. Additionally, we sought to maintain and document physiologic basal and "meal-stimulated" values (Figs. 4 and 5). These peripheral levels are approximately twice the values of controls; otherwise they closely parallel those seen as a result of exogenous infusion in other studies.[4,6] Our findings confirm previous evidence that postsurgical alterations in glucose metabolism might be corrected by replacement of a noninsulin factor, in this case, pancreatic polypeptide. The results of the OGTT are largely unrevealing, which agrees with other reports of PP infusions in lean mice and dogs.[6,29] There is a decrease in basal-integrated insulin response in the transplant animals after PP infusion (PAT/B *versus* PAT/

B-PP; 23 ± 3 *versus* 15 ± 3 mg/dL), which is not significant and suggests a possible insulin "suppressive" effect that has been reported by others.[31] The clamp data are more revealing. We have documented that both surgical procedures deplete the postoperative organism of endogenous pancreatic polypeptide and are associated with decreased basal R_a and R_d as described by us in preliminary discussions of these data.[26] Both surgical groups had decreases in basal R_d values after surgery. Interestingly, the transplant animals had a significant depression of R_d during exogenous insulin infusion at physiologic levels. Overall glucose disposal then was returned to normal values for basal and insulin infusion periods after chronic PP administration. Additionally, the ΔR_d is returned to normal values after PP. These findings are unique and have not been previously observed in the transplant model. The PPx animals show a decrease in R_d during insulin infusion that approximates the effect seen in PAT/B animals (but is not statistically decreased from control values). Pancreatic polypeptide infusion then results in elevations of R_d values before and during insulin infusion. These findings support the conclusion that an "insensitivity" may exist to the endogenous (systemically released) basal and postprandial insulin levels in pancreas transplants. Pancreatic polypeptide may well be necessary to "correct" or modulate insulin sensitivity. A parallel effect may also be happening in the PPx model of pancreas resection (with portal vein drainage); however, the data do not show changes that are statistically significant.

The relevance of these studies to the clinical state of transplantation in type I diabetes requires understanding of several differences and assumptions. First, clinical transplantation more recently has made use of whole gland grafts, and the deficiency of pancreatic polypeptide may not be so apparent. In fact, as alluded to earlier, the type I diabetic may have an elevated pancreatic polypeptide level; however, there are no clear data as to the secretory capability of the transplanted pancreas under these circumstances. There is evidence, however, that pancreas transplant recipients may well have decreased PP response compared with normals challenged by insulin-induced hypoglycemia.[25] Additionally, the coexistence of the diabetic state and immunosuppression may further add to variables in glucoregulation after transplantation that warrant further study. It is becoming increasingly clear, however, that pancreas transplantation, in both animal models and clinical applications, is associated with basal and stimulated hyperinsulinemic responses that nonetheless are accompanied by altered rates of glucose disposal. Elevated peripheral insulin values after transplantation are most likely explained by lack of "first-pass" hepatic extraction by systemic drainage. A resultant alteration in peripheral glucoregulation occurs, and this may be due in part to alterations in noninsulin postprandial hormone release. Our studies confirm the possible role of pancreatic polypeptide in this state, and support further investigation of alterations induced by PP deficiency.

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