# The Defective Glucagon Response From Transplanted Intrahepatic Pancreatic Islets During Hypoglycemia Is Transplantation Site–Determined

**Vinendra Gupta, David C. Wahoff, Desmond P. Rooney, Vincent Poitout, David E.R. Sutherland, David M. Kendall, and R. Paul Robertson**

**The optimal site for pancreatic islet cell transplantation is presently unclear, although the liver has been the most commonly used. However, glucagon secretion from islets that have been autotransplanted in liver has been reported to be unresponsive to hypoglycemia yet responsive to arginine. To determine whether this selective glucagon secretory defect is related to the intrahepatic site of islet implantation or to the process of transplantation per se, we studied counterregulatory responses to hypoglycemia in dogs with pancreatic islet autotransplantation in the hepatic parenchyma (the intrahepatic [IH] group, $n$ = 9) or the peritoneal cavity (the intraperitoneal [IP] group, $n$ = 9), following total pancreatectomy, and compared them with the responses in normal controls ($n$ = 10). Dogs were subjected to a hypoglycemic hyperinsulinemic (5 mU · kg$^{-1}$ · min$^{-1}$) clamp for 90 min under general anesthesia. Arterial glucose concentrations were clamped at 2.7 mmol/l for the final 45 min of the clamp. Immediately following the clamp, glucagon responses to IV arginine (5 g) were also assessed. During hypoglycemia, glucagon responses in the IH group (maximal incremental glucagon = 33 ± 21 ng/l; glucagon area under curve [AUC] = 713 ± 1,022 ng · l$^{-1}$ · min$^{-1}$) were significantly lower than either the IP (maximal incremental glucagon = 92 ± 32 ng/l; glucagon AUC = 4,090 ± 1,600 ng · l$^{-1}$ · min$^{-1}$) or control (maximal incremental glucagon = 154 ± 71 ng/l; glucagon AUC = 6,943 ± 2,842 ng · l$^{-1}$ · min$^{-1}$) group (IH vs. IP group, $P$ < 0.05; control vs. IH group, $P$ < 0.01). Glucagon responses in the IP group did not differ significantly from the control group. Epinephrine responses to hypoglycemia were similar in all groups, whereas neither of the transplanted groups (IH and IP) had pancreatic polypeptide responses. There was a prompt rise in plasma glucagon after intravenous arginine in all groups. These data indicate that glucagon unresponsiveness to hypoglycemia is specific to intrahepatically transplanted islets, rendering the liver a disadvantageous site for optimal α-cell function. *Diabetes* 46:28–000, 1997**

From the Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine (V.G., D.P.R., V.P., D.M.K., R.P.R), and the Department of Surgery (D.C.W., D.E.R.S.), University of Minnesota Medical School, Minneapolis, Minnesota.

Address correspondence and reprint requests to Dr. David M. Kendall, Box 101 UMHC, Division of Diabetes, Endocrinology, and Metabolism, University of Minnesota, Minneapolis, MN 55455. E-mail: kendall@lenti.med.umn.edu.

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AUC, area under the curve; IH, intrahepatic; IP, intraperitoneal; PICT, pancreatic islet-cell transplantation.

Pancreatic islet-cell transplantation (PICT) offers potential benefits over whole organ pancreas transplantation for the treatment of type I diabetes because PICT is technically simpler and surgically less invasive. Although long-term insulin-independence following PICT in humans has been unusual (1), a growing number of successful islet-allotransplantations has recently been reported (2–6). The introduction of transplanted islets into the portal vein with subsequent engraftment in the liver has been the most common method of PICT. Whether the hepatic environment is as conducive for the long-term function of islet grafts as other sites of islet transplantation, notably the spleen and kidney, has been increasingly questioned (7–11).

While many studies have focused on the longevity and function of pancreatic-islet β-cells that have been engrafted in the liver (6,10–15), the assessment of α-cell function after intrahepatic islet transplantation is very limited. In the first human study of α-cell function, Pyzdrowski et al. (15) demonstrated that islets transplanted in the liver failed to secrete glucagon in response to hypoglycemia that was induced by a bolus of intravenous insulin, yet retained responses to intravenous arginine. These observations have now been confirmed in human recipients of both *auto-* and *allo*-PICT (16). Since glucagon responsiveness to both hypoglycemia and arginine in pancreas organ transplantation are preserved (17,18), we considered whether hypoglycemia unresponsiveness of transplanted α-cells is related specifically to the intrahepatic site of implantation. Therefore, we studied α-cell responses to hypoglycemia and arginine of islets that were autotransplanted in the liver or the peritoneal cavity in two groups of dogs and compared their response to normal control dogs.

## RESEARCH DESIGN AND METHODS

**Experimental groups.** Twenty-four conditioned outbred dogs of both sexes, weighing 14.5–22.5 kg, constituted the three experimental groups. The control group consisted of ten dogs studied before PICT. The remaining two groups of nine dogs each had islet autotransplantation carried out in either the intrahepatic (IH) or intraperitoneal (IP) site, using procedures that have been described previously (19). Since the purification of islets during isolation may lower the yield of islets, seven dogs in the IP group received unpurified islets (dispersed pancreatic islet tissue). Two other dogs received IP purified islets for comparison. Unlike in humans, the canine portal system is unable to accommodate unpurified islets without complications (20), so all dogs in the IH group received purified islets. After PICT, the dogs were allowed access to standard dog meal that was supplemented with viokase (5 g/day) and water ad libitum. Four dogs were studied both before and after PICT, whereas the rest were studied once.

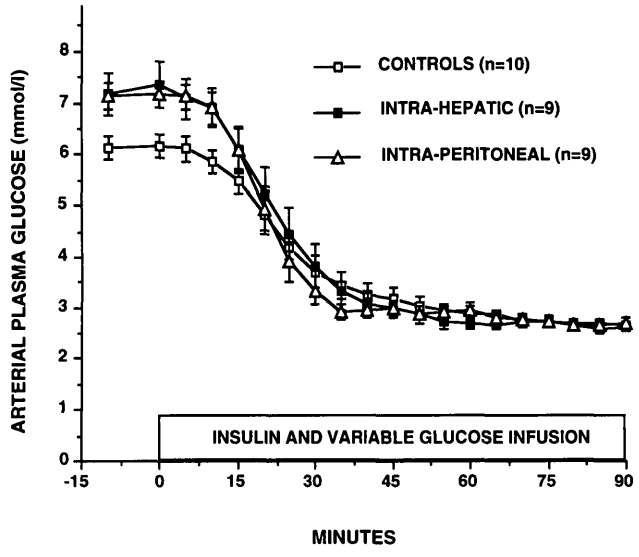


**FIG. 1. Glucose concentrations (means ± SE) during hypoglycemic hyperinsulinemic clamps in dogs that were autotransplanted with pancreatic islets in intrahepatic or intraperitoneal sites after total pancreatectomy and in nontransplanted control dogs.**

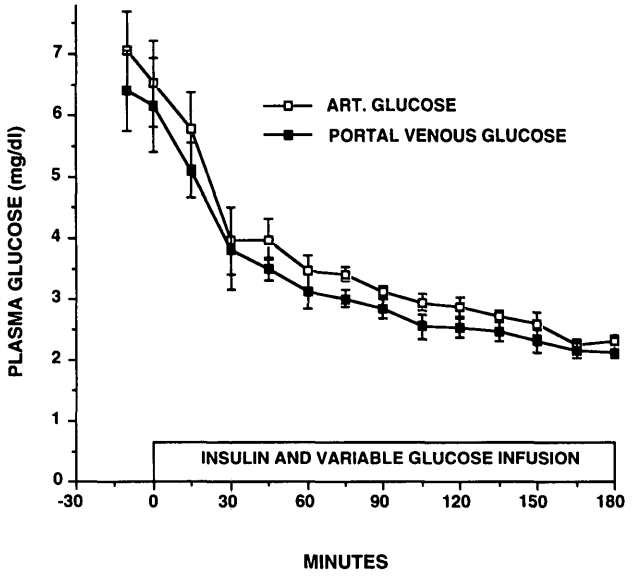


**FIG. 2. Glucose concentrations (means ± SE) in portal venous and arterial blood during a hypoglycemic hyperinsulinemic clamp in three normal dogs. In the fasting state, the glucose concentrations in portal blood were lower than those in arterial blood at all levels of glycemia.**

**Experimental design.** Transplanted dogs were studied 5.8 ± 1.8 months after successful PICT. None of the dogs studied received exogenous insulin. After an overnight fast, the dogs were anesthetized with intravenous pentothal sodium (25 mg/kg). Anesthesia was subsequently maintained with florane (1.5%), administered by mechanical ventilation in 3 l $O_2$/min. An intraarterial line was placed in the femoral artery to draw blood samples for glucose, glucagon, pancreatic polypeptide, epinephrine, and insulin. Another catheter was placed in the foreleg vein for intravenous infusions.

Two baseline samples were withdrawn –10 and 0 min before starting a hypoglycemic hyperinsulinemic clamp, during which a constant intravenous insulin infusion of 5 mU · $kg^{-1}$ · $min^{-1}$ was continued for 90 min. This study duration was selected because glucagon secretion in dogs is characteristically transient and tapers off even when hypoglycemia is sustained (21). Plasma glucose was measured at 5-min intervals and allowed to decline to 2.7 mmol/l (50 mg/dl). It was thereafter maintained at that level for the final 30–60 min of the study by a variable infusion of 20% dextrose. Harvard pumps (model 22 multi; Harvard Bioscience, S. Natick, MA) were used for both infusions. Samples were withdrawn for glucagon at 7.5- to 15-min intervals, for pancreatic polypeptide and insulin at 30-min intervals, and for epinephrine at 60, 75, and 90 min of the study.

To measure the glucagon response to a non–glucose-related secretagogue, arginine (in the maximally effective dose of 5 g in 50 ml water) was given as an intravenous pulse shortly after the conclusion of the clamp study. Baseline samples at –5 and 0 min, and additional samples at 2, 3, 5, and 7 min after injection, were withdrawn for the determination of glucagon.

A subset of three separate dogs underwent identical hypoglycemic clamp studies immediately after total pancreatectomy to assess whether glucagon responses might occur from an extrapancreatic site (such the gastric mucosa) in the absence of a pancreas.

To determine whether peripheral samples for glucose determination might underestimate hepatic glucose levels, another subset of four dogs underwent the hypoglycemic clamp study to compare the effect of the systemic administration of insulin on pre- and post-hepatic blood glucose levels. In these animals, simultaneous samples were obtained from a peripheral artery and the portal vein during hypoglycemia.

**Data analysis.** The maximal incremental responses of glucagon and pancreatic polypeptide and the integrated incremental glucagon areas under the curve (AUC) were determined by subtracting basal values from the peak responses. Glucagon AUC percentage was calculated as the total area expressed as a percentage of basal minus basal values of 100%. An acute glucagon response to arginine was defined as the mean of the glucagon values between 2 and 5 min, following intravenous arginine injection, with the basal value subtracted. Incremental epinephrine responses were determined by subtracting the basal from the mean of epinephrine values between 60 and 90 min of the clamp. Data are expressed as means ± SE. Statistical comparison between glucagon responses during the clamp were performed by Wilcoxon's two-sample test. Other statistical comparisons were performed by Student's *t* test.

## RESULTS

**Glucose and insulin levels.** Transplanted dogs had higher basal arterial glucose values (IH group: 7.3 ± 0.4 mmol/l [131 ± 7 mg/dl]; IP group: 7.2 ± 0.2 mmol/l [129 ± 4 mg/dl]) than the control group 6.2 ± 0.2 mmol/l (111 ± 4 mg/dl) (control vs. IH group, $P < 0.025$; control vs. IP group, $P < 0.01$) (Fig. 1). A glucose nadir of 2.7 mmol/l (50 mg/dl) was achieved in all the groups, and the rate at which the nadir was reached was similar in all groups. Basal mean serum insulin values and insulin values during the clamp were not statistically different among the groups (data not shown). Plasma glucose values in the portal vein and the femoral artery, obtained in a subset of dogs that were subjected to a stepped hypoglycemic clamp study, were similar (Fig. 2).

### Glucagon responses

***Hypoglycemic clamp.*** Glucagon data from the hypoglycemic clamp are shown in Table 1 and Figs. 3–5. Maximal incremental glucagon, glucagon AUC, and glucagon AUC percentage were significantly lower in the IH group, compared with either the IP group or controls ($P < 0.05$ for all comparisons), whereas there was no significant difference between the IP group and controls (Table 1, Fig. 4). Peak glucagon levels increased 3.4-fold over basal in controls, 1.3-fold in the IH group, and 4.5-fold in the IP group (Fig. 5). Within the IP group, the glucagon response to hypoglycemia was observed in subsets of dogs that had received either purified or unpurified islets (Δ purified glucagon = 155 ± 73; Δ unpurified glucagon = 68 ± 29). The glucagon responses were higher in the two IP dogs that received purified islets, compared with the seven animals that received unpurified islets (glucagon AUC, 9,509 ± 4,492 vs. 2,532 ± 1,262; NS), although these differences were not statistically significant. No rise in plasma glucagon in response to hypoglycemia was observed in the

TABLE 1
Data from hypoglycemic clamp study

| | Control group | IH group | IP group | Control vs. IH group | Control vs. IP group | IH vs. IP group |
|---|---|---|---|---|---|---|
| Basal glucagon (ng/l) | 65 ± 16 | 79 ± 18 | 41 ± 9 | NS | NS | NS |
| Maximal incremental glucagon (ng/l) | 154 ± 71 | 33 ± 21 | 92 ± 32 | 0.01 | NS | 0.05 |
| Glucagon area under curve (ng/l · min) | 6,943 ± 2,842 | 713 ± 1,022 | 4,090 ± 1,600 | 0.001 | NS | 0.05 |
| Glucagon area under curve (% basal · min) | 13,385 ± 3,967 | 1,213 ± 1,065 | 16,066 ± 6,002 | 0.005 | NS | 0.025 |

Data are means ± SD or *P* values.

pancreatectomized dogs that underwent a hypoglycemic clamp (Fig. 5).

***Arginine stimulation.*** Just before arginine injection, all the groups had similar plasma glucagon values (control group, 90 ± 9 ng/l; IH group, 67 ± 15 ng/l; IP group, 82 ± 18 ng/l). Following the intravenous pulse of arginine, there was a prompt rise in the plasma glucagon in all groups (Fig. 6). The magnitude of the acute glucagon response to arginine was, however, significantly lower in the IH (86 ± 20 ng/l) and IP (44 ± 5 ng/l) groups, compared with the controls (229 ± 40 ng/l) (control vs. IH group, $P = 0.005$; control vs. IP group, $P < 0.001$; IH vs. IP group, NS), while the IH and IP responses did not differ significantly. There were no responses to arginine in the pancreatectomized nontransplanted dogs.

***Pancreatic polypeptide responses to hypoglycemia.*** Basal pancreatic polypeptide levels (in nanograms per liter) at normoglycemia in both transplanted groups (IH group: 55 ± 11 ng/l; IP: 77 ± 8 ng/l) were lower ($P < 0.001$), compared with the controls (225 ± 11 ng/l). No significant pancreatic polypeptide increment during hypoglycemia was observed in transplant recipients, whereas the normal control dogs had intact responses (Fig. 7).

***Epinephrine responses to hypoglycemia.*** Epinephrine responses to hypoglycemia were determined in five controls, three IH dogs, and four IP dogs. Basal epinephrine values (picograms per milliliter) were similar in all the groups (control group, 339 ± 142 pg/ml; IH group, 211 ± 121 pg/ml; IP group, 301 ± 80 pg/ml). Hypoglycemia led to a significant rise in plasma epinephrine in all groups, with no significant difference in the incremental epinephrine responses among the groups (control group, 906 ± 164 pg/ml; IH group, 671 ± 284 pg/ml; IP group, 1360 ± 125 pg/ml) (Fig. 8).

## DISCUSSION

These studies were performed to determine whether defective glucagon secretion during hypoglycemia is specific to the intrahepatic site of islet transplantation or caused by the process of transplantation per se. The data reported herein establish that pancreatic islets autotransplanted intrahepatically fail to secrete glucagon during a hypoglycemic hyperinsulinemic clamp in dogs, while glucagon responsiveness during hypoglycemia is retained when the peritoneal site is used for transplantation. In contrast, intrahepatically and intraperitoneally transplanted animals retained equivalent glucagon responses to arginine (albeit quantitatively reduced in both groups). Animals in both groups had epinephrine responses during hypoglycemia, but animals in neither transplanted group had pancreatic polypeptide responses.

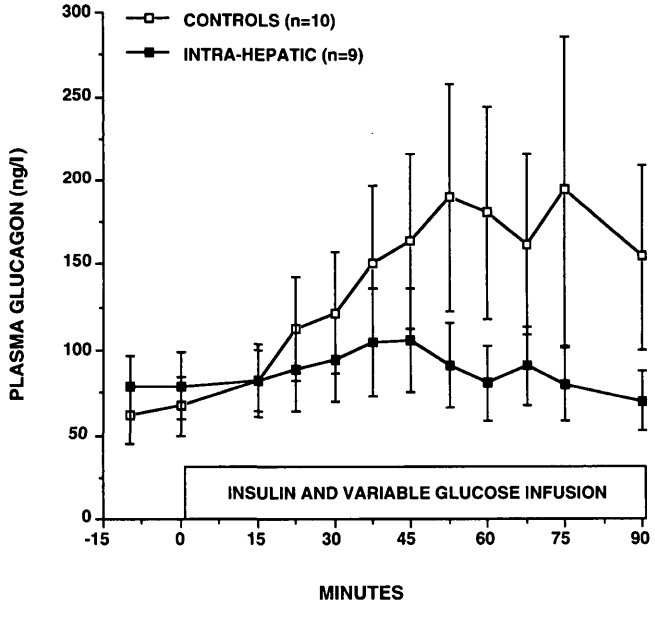


**FIG. 3. Glucagon concentrations during hypoglycemic hyperinsulinemic clamps in dogs after intrahepatic islet autotransplantation and in nontransplanted control dogs. While basal glucagon levels were similar in the two groups, the maximal incremental glucagon was significantly attenuated in the IH group, compared with controls (control vs. IH group, $P < 0.01$).**

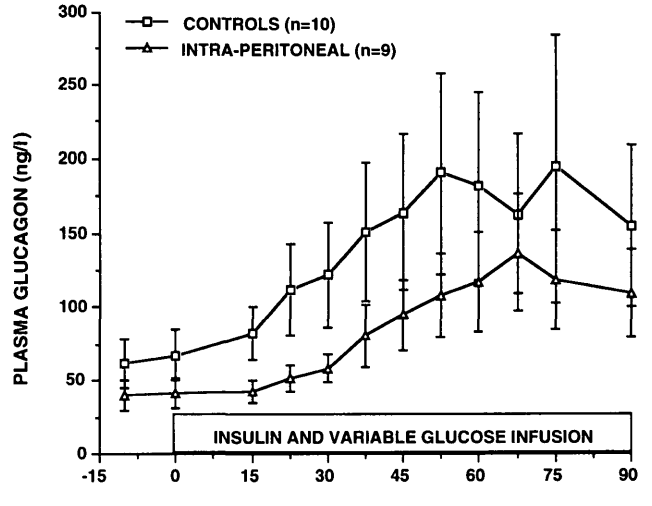


**FIG. 4. Glucagon responses during hypoglycemic hyperinsulinemic clamps in dogs after intraperitoneal islet autotransplantation and in control dogs. No significant difference in basal or incremental glucagon response was observed.**

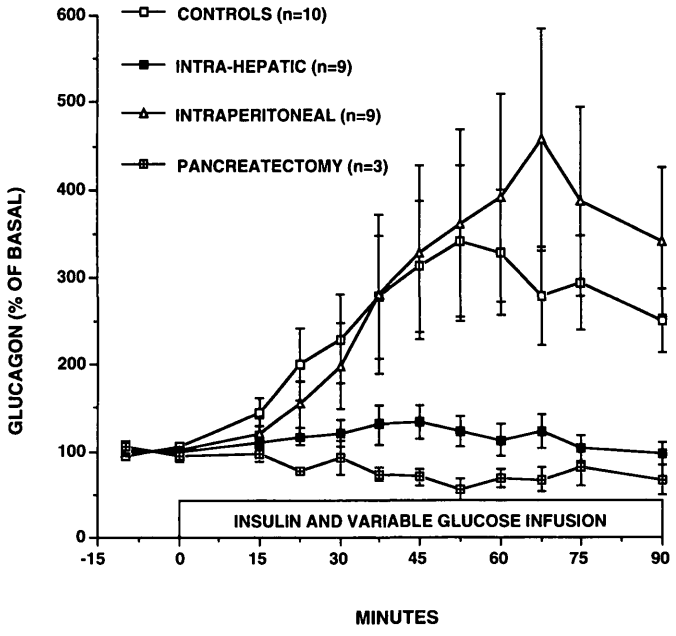


**FIG. 5. Glucagon values expressed as a percentage of basal during hypoglycemic hyperinsulinemic clamps in controls, IH, IP, and pancreatectomized dogs without islet transplantation during hypoglycemic hyperinsulinemic clamps. The IH and pancreatectomized, but not IP, dogs had significantly lower responses than those of controls (control vs. IH group, $P < 0.005$; control vs. IP group, NS).**

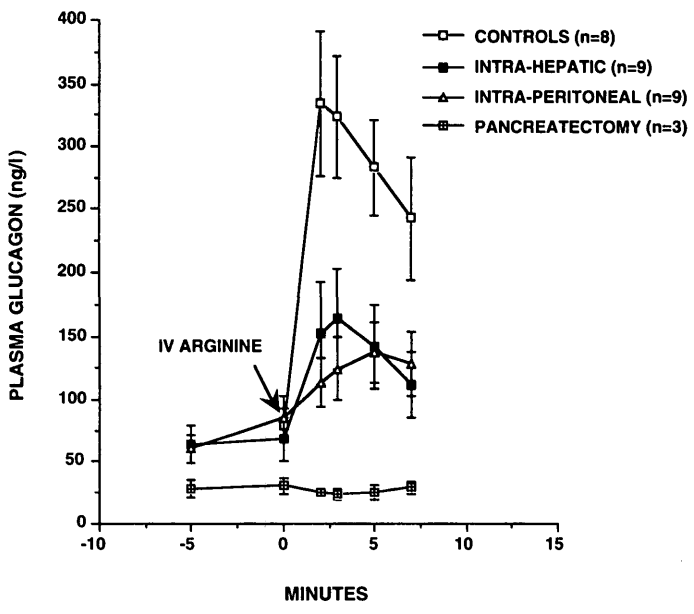


**FIG. 6. Acute glucagon responses (means ± SE) to intravenous arginine in the transplanted and control groups.**

Pyzdrowski et al. (15) previously observed that a brief intravenous insulin pulse induced hypoglycemia, but failed to elicit glucagon responses in successful human recipients of intrahepatic islet autotransplantation. The lack of glucagon response was similarly observed during sustained hypoglycemia, using the hyperinsulinemic clamp technique in human recipients of both islet autotransplantation and islet allotransplantation in two type I diabetic recipients (16). In searching for the cause of this abnormality, a generalized defect in α-cell secretory function can be excluded, because glucagon responses to intravenous arginine, although reduced in magnitude after both intrahepatic and intraperitoneal islet transplant, were present in both the human and canine studies. This is consistent with the observed presence of immunostainable glucagon in α-cells that have been obtained in liver biopsies (12,15,22) and with reports that intrahepatic islet autografts secrete glucagon after the ingestion of a mixed meal (11) and during exercise (23). The reduced magnitude of the arginine-induced glucagon response following islet transplantation is likely the result of an overall reduction in islet cell mass. However, the defect in α-cell function that we have observed after islet transplantation is specific both to the intrahepatic transplantation site and to hypoglycemia as a stimulus. This conclusion is also consistent with a recent report that islet transplantation within the splenic parenchyma prevents defective counterregulation of hypoglycemia in dogs (24).

Importantly, of necessity (20) the intrahepatically transplanted dogs in our study received purified islets only, whereas the intraperitoneally transplanted dogs received either purified or unpurified islets. Irrespective of purification, however, islets transplanted intraperitoneally retained glucagon responsiveness to hypoglycemia. In addition, the human studies of Kendall et al. (16) were performed in humans receiving unpurified islets that were transplanted to the hepatic parenchyma, yet no glucagon responses to hypoglycemia were found. Thus, the process of purification by itself does not explain the lack of glucagon responsivity of intrahepatically transplanted islets. We demonstrated a lack of glucagon responsivity to arginine in a subset of pancreatectomized dogs, which implies that the glucagon responses we observed in transplanted dogs were secreted from α-cells in pancreatic islets and not from cells of the gastric fundus. This is consistent with previous reports that gastric glucagon is sensitive to inhibition by insulin (25,26) and that gastric glucagon is unresponsive to hypoglycemia (27,28).

Basal pancreatic polypeptide levels were low in both of our transplanted groups of dogs, compared with the control dogs, and there were no significant increments in pancreatic polypeptide in response to insulin-induced hypoglycemia following autotransplantation in either the intrahepatic or intraperitoneal site. This suggests that, despite morphological evidence of reinnervation of islets following islet transplantation (29), vagally-mediated pancreatic polypeptide responses to hypoglycemia are not restored, a defect also observed after whole pancreas transplantation (17). Epinephrine concentrations in blood significantly increased during our clamp studies in all experimental groups. Yet, despite hypoglycemia and increased catecholamine levels, two potent stimulators of glucagon release, only pancreatic islets autotransplanted intraperitoneally secreted glucagon. Possible explanations include differences in revascularization, reinnervation, or the glycemic environment in the liver. The liver has a unique dual blood supply so that intrahepatic islets are perfused mainly by the hepatic artery and partly by the portal vein (30). To ascertain whether the glucose concentration in portal venous blood during the post-absorptive state might prevent islets from being exposed to systemic hypoglycemia, we determined glucose concentrations in blood that was sampled directly from the portal vein during the hypoglycemic clamp in a subset of dogs. Portal-vein glu-

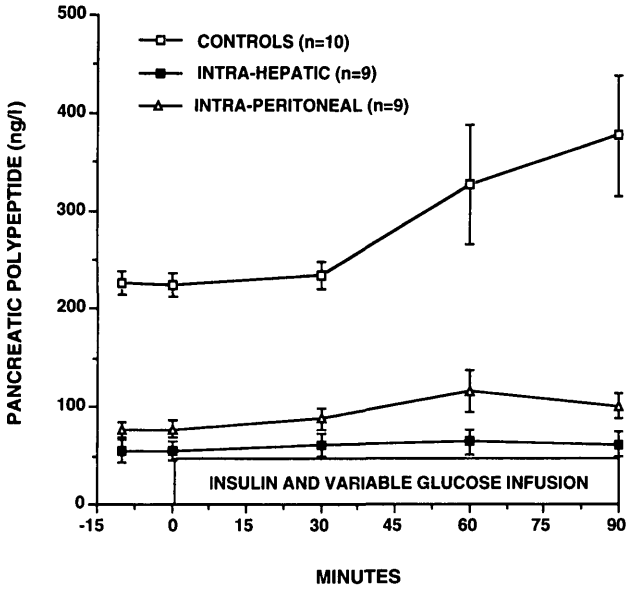


**FIG. 7. Pancreatic polypeptide responses during hypoglycemic hyperinsulinemic clamps. Pancreatic polypeptide responses were significantly lower in both the transplanted groups (IH and IP), compared with the controls.**

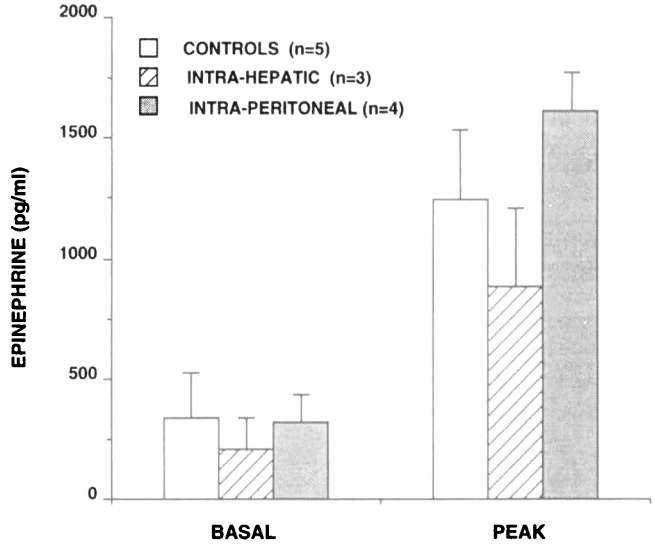


**FIG. 8. Epinephrine levels during hypoglycemic hyperinsulinemic clamps. No significant differences in incremental epinephrine responses were found among the three groups.**

cose concentrations were marginally lower than arterial glucose concentrations, providing evidence that glucose concentrations in blood perfusing intrahepatic islets decreases into the hypoglycemic range. On the other hand, hepatocytes are capable of producing glucose in response to hypoglycemia that is sensed by the central nervous system and/or portal glucoreceptors (31,32). Thus, the possibility exists that glucose diffusion from hepatocytes surrounding the islets could prevent α-cells from being exposed to adequate hypoglycemia, despite a low glucose concentration measured in the vascular supply. This possibility is enhanced by the marked glycogen deposition in the vicinity of islet transplantation that results from the high local concentrations of insulin to which the surrounding hepatic parenchyma is exposed. Currently, we favor glucose fluxes encountered locally within the liver as a potential explanation for the lack of glucagon responsiveness to hypoglycemia.

In conclusion, these studies have demonstrated that the defect in α-cell responsiveness to hypoglycemia in transplanted pancreatic islets is site-specific for the liver and does not occur if islets are transplanted into the intraperitoneal cavity. This is clinically relevant because, currently, the liver is the favored site for pancreatic islet transplantation in diabetic humans. It would appear that the liver is not an ideal location, since islets transplanted to this site will not be capable of helping patients to recover from hypoglycemia, a relevant consideration should such patients require ancillary injections of insulin to control hyperglycemia.

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## REFERENCES

1. Robertson RP: Pancreatic and islet transplantation for diabetes: cures or curiosities? *N Engl J Med* 327:1861–1868, 1992
2. Gores PF, Najarian JS, Stephanian E, Lloveras JJ, Kelly SL, Sutherland DER: Insulin independence in type I diabetes after transplantation of unpurified islets from a single donor with 15-deoxyspergualin. *Lancet* 341:19–21, 1993
3. Warnock GL, Kneteman NM, Ryan E, Seelis REA, Rabinovitch A, Rajotte RV: Normoglycemia after transplantation of freshly isolated and cryopreserved pancreatic islets in type I (insulin-dependent) diabetes mellitus. *Diabetologia* 34:55–58, 1991
4. Alejandro R, Burke G, Shapiro ET, Strasser S, Nery J, Ricordi C, Esquenazi V, Miller J, Mintz DH: Intraportal islet allografts in type I diabetes mellitus. *Transplant Proc* 24:959–960, 1992
5. Socci C, Falqui L, Davalli AM, Ricordi C, Braghi S, Bertuzzi F, Maffi P, Secchi A, Gavazzi F, Freschi M, Magistretti P, Socci S, Viguali A, DiCarlo V, Pozza G: Fresh human islet transplant to replace pancreatic endocrine function in type I IDDM patients. *Acta Diabetol* 28:151–157, 1991
6. Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Boyle PJ, Falqui L, Marchetti P, Ricordi C, Gingerich RL, Jaffe AS, Cryer PE, Hanto DW, Anderson CB, Flye WM: Results of our first nine intraportal islet allografts in type I, insulin-dependent diabetic patients. *Transplantation* 51:76–85, 1991
7. Mellgren A, Schnell Landström AH, Petersson B, Andersson A: The renal subcapsular space offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. *Diabetologia* 29:670–672, 1986
8. Warnock GL, Dabbs KD, Evans MG, Cattral MS, Kneteman NM, Rajotte RV: Critical mass of islets that function after implantation in a large mammalian. *Hor Metab Res* (Suppl.) 25:156–161, 1990
9. Hiller WFA, Klempnauer J, Lück R, Steiniger B: Progressive deterioration of endocrine function after intraportal but not kidney subcapsular rat islet transplantation. *Diabetes* 40:134–140, 1991
10. Kaufman DB, Morel P, Field MJ, Munn SR, Sutherland DER: Purified canine islet autografts: functional outcome as influenced by islet number and implantation site. *Transplantation* 50:385–391, 1990
11. Scharp DW, Marchetti P, Swanson C, Newton M, McCullough CS, Olack B: The effect of transplantation site and islet mass on long term survival and metabolic and hormonal function of canine purified islet autografts. *Cell Transplantation* 1:245–254, 1992
12. Alejandro R, Cutfield RG, Sheinvold FL, Polonsky KS, Noel J, Olson L, Dillberger J, Miller J, Mintz DH: Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 78:1339–1348, 1986
13. Sutton R, Gray DWR, McShane P, Peters M, Morris PJ: The metabolic efficiency and long-term fate of intraportal islet grafts in the cynomolgus monkey. *Transplant Proc* 19:3575–3576, 1987
14. Orloff MJ, Macedo A, Greenleaf GE, Girard B: Comparison of the metabolic control of diabetes achieved by whole pancreas transplantation and pancreatic transplantation in rats. *Transplantation* 45:307–312, 1988
15. Pyzdrowski KL, Kendall DM, Halter JB, Nakhleh RE, Sutherland DER, Robert-

son RP: Preserved insulin secretion and insulin independence in recipients of islet autografts. *N Engl J Med* 327:220–226, 1992
16. Kendall DM, Teuscher AU, Robertson RP: Defective glucagon secretion during sustained hypoglycemia following successful islet allo- and autotransplantation in humans. *Diabetes* 46:23–27, 1997
17. Diem P, Redmon JB, Abid M, Moran A, Sutherland DER, Robertson RP: Glucagon, catecholamine and pancreatic polypeptide secretion in type I diabetic recipients of pancreas allografts. *J Clin Invest* 86:2008–2013, 1990
18. Barrou Z, Seaquist ER, Robertson RP: Pancreatic transplantation in diabetic humans normalizes hepatic glucose production during hypoglycemia. *Diabetes* 43:661–666, 1994
19. Wahoff DC, Sutherland DER, Hower CD, Lloveras JK, Gores PF: Free intraperitoneal islet autografts in pancreatectomized dogs: impact of islet purity and posttransplantation exogenous insulin. *Surgery* 116:742–750, 1994
20. Kretschmer GJ, Sutherland DER, Matas AJ, Payne WD, Najarian JS: Auto-transplantation of pancreatic fragments to the portal vein and spleen of totally pancreatectomized dogs. *Ann Surg* 187:79–86, 1978
21. Davis SN, Cherrington AD: The hormonal and metabolic responses to prolonged hypoglycemia. *J Lab Clin Med* 121:21–31, 1993
22. Pipeleers-Marichal MA, Pipeleers DG, Cutler J, Lacy PE, Kipnis DM: Metabolic and morphologic studies in intraportal-islet-transplanted rats. *Diabetes* 25:1041–1051, 1976
23. Portis AJ, Warnock GL, Finegood DT, Belcastro AN, Rajotte RV: Glucoregulatory response to moderate exercise in long-term islet cell autografted dogs. *Can J Physiol Pharmacol* 68:1308–1312, 1990
24. Ansara MF, Saudek F, Newton M, Raynor AC, Cryer PE, Scharp DW: Pancreatic islet transplantation prevents defective glucose counter-regulation in diabetic dogs. *Transplant Proc* 26:664–665, 1994
25. Blazquez E, Munoz-Barragan L, Patton GS, Orci L, Dobbs RE, Unger RH: Gastric A-cell function in insulin-deprived depancreatized dogs. *Endocrinology* 99:1182–1188, 1976
26. Lefebvre PJ, Luyckx AS: Extrapancreatic glucagon and its regulation. In *Handbook of Experimental Pharmacology: Glucagon*. Lefebvre PJ, Ed. Berlin, Springer-Verlag, 1983, p. 205–226
27. Matsuyama T, Tanaka R, Shima K, Nonaka K, Tarui S: Lack of gastrointestinal glucagon response to hypoglycemia in depancreatized dogs. *Diabetologia* 15:471–474, 1978
28. Gotoh M, Monden M, Okamura J, Mori T, Shima K: Insulin and glucagon secretion after pancreatectomies: correlation of secretion and hormonal contents of remaining pancreas. *Diabetes* 38:861–867, 1989
29. Gardemann A, Jungermann K, Große V, Cossel L, Wohlrab F, Hahn HJ, Blech W, Hildebrandt W: Intraportal transplantation of pancreatic islets into livers of diabetic rats: reinnervation of islets and regulation of insulin secretion by the hepatic sympathetic nerves. *Diabetes* 43:1345–1352, 1994
30. Andersson A, Korsgren O, Jansson L: Revascularization of islets: intraportally transplanted pancreatic islets revascularized from the hepatic arterial system. *Diabetes* 38 (Suppl. 1):192–195, 1989
31. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
32. Hamilton-Wessler M, Bergman RN, Halter JB, Watanabe RM, Donovan CM: The role of liver glucosensors in the integrated sympathetic response induced by deep hypoglycemia in dogs. *Diabetes* 43:1052–1060, 1994