Pancreatic islet transplantation: studies on the technique and efficacy of islet isolation and transplantation

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8 Metabolic control after intrasplenic islet autotransplantation in dogs: ß-cell secretory capacity, insulin action, and the enteroinsular axis*

Introduction

Islet transplantation is considered a promising therapeutic approach to insulin-dependent diabetes mellitus, but its potential for long-term physiological glucose regulation has been incompletely investigated. Fasting normoglycemia and insulin independence can be achieved by islet transplantation in large animals and man. However, clinical data following successful islet transplantation are anecdotal [1], and detailed functional assessment received limited attention in experimental studies, as well [2–6]. We, therefore, investigated the insulin secretory capacity by intravenous arginine stimulation during 35 mM glucose clamps, the action of insulin during euglycemic clamps, and the efficacy of physiological meal stimulation, in 8 long-term islet autotransplanted dogs. Our preliminary data suggested that hyperglycemic potentiation of the enteroinsular axis may be the main mechanism to limit postprandial glucose excursions after islet transplantation [7]. However, no studies of the insulinotropic effects of gut hormones after islet transplantation have yet been published. We, therefore, also investigated the insulinotropic effect of exogenous administration of the major gut hormone glucagon-like peptide-1 7–36 amide (GLP-1) in the autograft recipients under hyperglycemic clamp conditions that mimicked the postprandial 8–9 mM glycemia observed after islet transplantation.

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Research design and methods

Dogs

Experiments were performed in 38 adult, female, outbred, beagles (Harlan CPB, Zeist, The Netherlands) weighing 8–18 kg. Eight dogs underwent total pancreatectomy and intrasplenic islet autotransplantation — the other dogs served as controls. The animals were maintained on a regular diet of semi-liquid dog food (50 energy% carbohydrate, 20 energy% fat and 30 energy% protein; Complete Dog Food D-B, Hope Farms, Woerden, The Netherlands) twice daily and had free access to water. After islet transplantation the diet was supplemented with 2 g/day protease-lipase-amylase pellets (Pancreas pellets, Organon, Oss, The Netherlands).

Graft preparation

After a 24-h fast, total pancreatectomy was performed for islet isolation and autotransplantation in anesthetized animals [8]. Islets were isolated from the pancreas by intraductal perfusion with 1600 U/ml collagenase type V or XI (Sigma Chemical Co., St. Louis, MO) in Hanks' balanced salt solution (Flow Laboratories, Irvine, Ayrshire, UK), digestion for 8-20 min at 37°C, and gentle trituration of the tissue at 4°C. The tissue was resuspended in the bottom layer of step density gradients (1.095, 1.085, 1.075, and 1.045 g/ml) of ~70,000-M_r dextran (Sigma) in Hank's solution, and purified islets were collected from the two uppermost layers after centrifugation at 500 *g*. The total volume of isolated islets and the purity (relative islet volume) were assessed by morphometry [9] in multiple aliquots totalling 0.05% of the purified islet suspension, which was autotransplanted by reflux via two splenic vein tributaries, while clamping the splenic pedicle and short gastric vessels.

Metabolic studies

Glucoregulation was tested at 6–9 months posttransplant, and in the controls, under the following conditions: *1*) after a meal to study physiological stimulation; *2*) following conventional, intravenous bolus glucose stimulation (intravenous glucose tolerance test, IVGTT); *3*) following an intravenous arginine bolus during a ~35 mM glucose clamp, to determine the insulin secretory capacity; and *4*) during a hyperinsulinemic euglycemic clamp to measure tissue sensitivity to insulin, and insulin clearance. In addition, in the experimental group, the insulin response to ~8.5 mM glucose clamps with or without the co-infusion of GLP-1 was compared, in order to determine the

effect of the peptide under glycemic conditions close to the posttransplant ~8–9 mM postprandial glycemia.

The tests were carried out with an interval of at least 2 days in conscious dogs after an overnight 18-h fast. The animals were resting in Pavlov slings, except during meal tests. Blood was drawn and boluses were administered through 17-gauge Teflon cannulas inserted in foreleg veins, and infusions were delivered using syringe pumps (Terufusion STC-521; Terumo, Tokyo, Japan) through 20-gauge Teflon cannulas inserted in hindleg veins. Blood samples were collected on ice in EDTA-containing tubes. Plasma was separated at 4°C within 15 min, and samples for insulin assay were kept below –20°C pending radioimmunoassay using dog insulin as standard [8]. Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Brea, CA).

Table VIII.1

Metabolic control at 6–9 months after intrasplenic islet autotransplantation in dogs

Parameter	Controls	Grafts
Fasting		
Glucose (mM)	5.3±0.1	5.3±0.1
Insulin (pM)	62±3	57±2
Intravenous glucose tolerance test		
Glucose clearance, K_g (–%/min)	2.4±0.1	0.9±0.1*
Acute insulin response (pM)	305±37	37±8*
Glucose clamp at 35 mM		
Secondary insulin response (pM)	922±120	81±20*
Arginine i.v. at 35 mM glycemia		
Acute insulin response (pM)	693±77	172±44*
Postprandial		
Glucose excursion (mM)	0.2±0.1	2.9±0.7*
Insulin response (pM)	119±10	164±15†
Hyperinsulinemic euglycemic clamp		
Insulin action, S_i (10^2.L.kg^{-1}.min^{-1} per pM)	38±5	21±3†
Insulin clearance (ml.kg^{-1}.min^{-1})	42±4	34±3
First insulin level (pM)	157±9	172±13
Second insulin level (pM)	732±63	840±74

Means ± SE of data obtained in 8 grafted dogs and 30 normal control dogs. Individual fasting levels were calculated as means of baseline values of all tests. * $p < .0001$ *vs* controls., † $p < .05$ *vs* controls

A test meal consisting of 500 ml of the regular semi-liquid meal was given to the animal in seclusion, to ensure ingestion within 15 min of presentation of the meal. Blood specimens were drawn by puncturing external jugular veins at –5, 0 (immediately before presentation of the meal), 15, 30, 60, 90, and 120 min for assessment of the integrated responses above baseline of plasma glucose and insulin during two postprandial hours. IVGTTs were performed by bolus infusion over 30 s of 0.5 g/kg glucose as a 40 % solution, and measurement of plasma glucose and insulin at –5, 0, 1, 3, 5, 10, 15, 30, 45, and 60 min, to determine both glucose clearance (K_g) from 10 min as reported previously [8], and the weighed acute insulin response above baseline from 0–3 min. For the assessment of the insulin secretory capacity, a variable rate infusion of a 40% glucose solution was started from 0 min at 150 ml/h to raise and maintain plasma glucose at ~35 mM, guided by on-line plasma glucose analysis every five min to manually control the infusion rate. At 50 min, 2 g arginine hydrochloride was administered as a 10% solution over 30 s, and blood for insulin assay was drawn at –5, 0, 15, 30, 45, 50, 52, 53, 54, 55, 60, 70, and 80 min. The secondary insulin response to 35 mM glucose was expressed in the mean increment from 45 to 50 min above baseline. The acute insulin response to arginine was expressed in the mean increment from 52 to 55 min over the mean 45–50 min prestimulus level. Tissue sensitivity to insulin was measured by a 2-step sequential hyperinsulinemic, euglycemic clamp. After taking baseline samples for glucose and insulin assay at –15, –10, –5, and 0 min, insulin (Actrapid, Novo, Copenhagen, Denmark) was infused at 10 mU/min from 0 min, and at 50 mU/min from 90–180 min, during which a variable glucose infusion (20% solution) — guided by glucose analysis as before — kept plasma glucose at the mean fasting level. Samples for plasma insulin assay were drawn every 10 min from 60 to 90, and from 150 to 180 min, for calculation of the insulin sensitivity index, S_i (10^2.L.kg^{-1}.min^{-1} per pM), as previously described [10]. Insulin clearance was expressed in the high-dose insulin infusion rate per kg body weight, divided by the corresponding mean insulin level, during the final 30 min. For assessment of the insulinotropic effects of GLP-1 each animal underwent two hyperglycemic experiments on separate days, one with and another without the simultaneous infusion of synthetic human glucagon-like peptide-1 7–36 amide (code 7168, Peninsula, Merseyside, St. Helens, UK) dissolved in 0.9% saline containing 0.1% bovine serum albumin. Net peptide content rather than gross weight was used for calculation of the dose. After taking baseline samples a variable 40% glucose infusion was started at 20 ml/h to raise and maintain plasma glucose at ~8.5 mM, guided by glucose analysis as before. From 50 to 80 min 1.75 pmol.kg^{-1}.min^{-1} GLP-1 was infused in the

contralateral hindleg. Blood samples for plasma insulin were drawn at –5, 0, 10, 20, 30, and 40 min, next every 5 min until 90 min, and at 100 and 110 min. The possible insulinotropic effects of the peptide were examined by comparison of the mean insulin level, and glucose infusion rate from 65 to 80 min during the clamps.

Calculations and statistical analysis

Results are expressed as means ± SE. Integrated responses were calculated using the trapezoidal rule, and weighed by dividing by the corresponding periods. Logarithmic transformation of data was used when appropriate to normalize the distribution of the data. Differences were analysed by Student's *t*-test for paired or unpaired data and considered not significant at $p > .05$.

Results

The autografts comprised 6.5 ± 1.5 µl islets per kg body weight — which roughly corresponds to 25% of the normal native islet mass [9]. The purity (relative islet volume) of the grafts was 69 ± 10%. The recipients appeared to be in good condition, had normal stools, and steady body weights (92 ± 4% preoperative) throughout the metabolic studies.

Data from metabolic studies in the transplanted animals and the normal unmodified controls are listed in Table VIII.1. Fasting glucose and insulin levels after islet transplantation were similar as in the control animals. Intravenous glucose clearance (K_G value) after islet transplantation was 40% of the normal value. Both the acute insulin response to the glucose bolus during IVGTT, as well as the secondary insulin response to glucose during the intravenous 35 mM glucose clamp amounted to only ~10% of the control values. The insulin response curves during the IVGTT and the 35 mM glucose clamp are shown in Fig. VIII.1A and B, respectively. The insulin secretory capacity of the grafts — as estimated by arginine stimulation during the 35 mM glucose clamp — averaged 25 % of the control value (Table VIII.1). By contrast, the postprandial insulin response had increased posttransplant to ~140% of the normal response (Table VIII.1, and Fig. VIII.1C). Postprandial glycemia had also increased after transplantation and reached a mean 8–9 mM (Fig. VIII.1D). The index of insulin action during euglycemic clamps in the autografted dogs amounted to 55% of the normal value. Insulin clearance was not affected by islet transplantation (Table VIII.1).

In the transplanted animals, the postprandial insulinemia was similar as observed during the arginine test of the insulin secretory capacity (Fig. VIII.2).

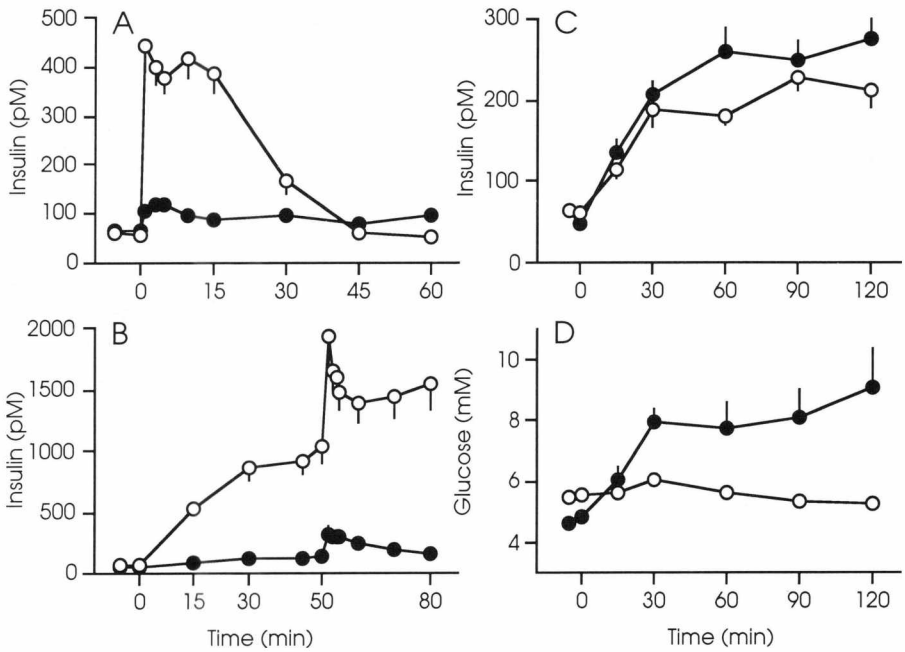

Fig. VIII.1

*Plasma insulin response to an intravenous glucose bolus (**A**), an intravenous arginine bolus during an intravenous ~35 mM glucose clamp (**B**), and plasma insulin (**C**) and glucose (**D**) responses to a mixed-meal in 30 normal control dogs (open symbols) and 8 dogs with intrasplenic islet autografts (closed symbols) at 6–9 months after transplantation. Data are means ± SE.*

The insulin secretory capacity of the individual grafts correlated significantly with both the postprandial glucose excursion ($p < .001$, Fig. VIII.3A), and — albeit marginally ($p < .05$) — with the index of insulin action (Fig. VIII.3B) in the recipients.

The insulin response curves during ~8.5 mM glucose clamps with or without the co-infusion of GLP-1 in the autografted dogs, are shown in Fig. VIII.4. Insulinemia during the final 15 min of GLP-1 infusion increased to a mean 175% of the corresponding insulin level during the glucose clamp without co-infusion of the peptide (Table VIII.2). Glucose uptake — the glucose infusion rate required to maintain the ~8.5 mM glycemia — paralleled the increment in insulin levels.

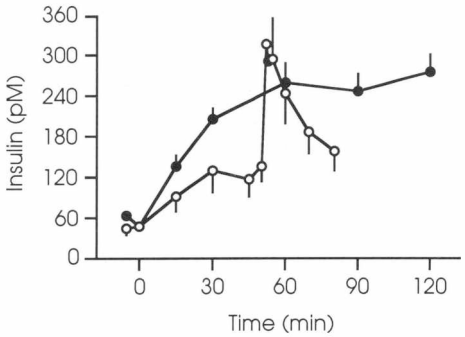

Fig. VIII.2

Comparison of the posttransplant plasma insulin responses to intravenous arginine stimulation during a ~35 mM glucose clamp (open symbols) vs mixed meal stimulation (closed symbols), indicating a quantitatively similar stimulation of the grafted ß-cells during these tests. Data are the same as the posttransplant data presented in Fig. VIII.1 B and C but at a different scale.

Discussion

We studied the long-term ß-cell secretory capacity and glycemic control in dogs with established intrasplenic islet autografts — i.e., fasting normoglycemic recipients. Both the islet dose, and site of implantation of the graft are considered important factors for successful transplantation. The long-term fasting normoglycemia after transplantation of >4 µl purified islets per kg body weight in our recipients corroborated previous reports of the critical islet mass for successful autotransplantation [4]. Of the many sites for islet grafting that have been explored, best results have been obtained with islets transplanted in the intrasplenic and intraportal sites in large animals, and the intraportal route has been favoured for technical reasons in humans [1]. In this study the spleen was chosen rather than the liver, both for genuine portal drainage of the islet hormones — because intraportal grafts may actually partially drain directly to the systemic circulation [2,3,6] — and, further, to exclude exposure of the graft

Table VIII. 2

Insulinotropic effect of GLP-1 infusion during ~8.5 mM glucose clamps in the grafted dogs

Parameter *	No GLP-1	GLP-1
Insulin level (pM)	72±19	126±17†
Glucose level (mM)	8.5±0.2	8.4±0.3
Glucose infusion ($mg.kg^{-1}.min^{-1}$)	5.8±2.0	9.4±2.8‡

* Values are means ± SE in 4 dogs, of the individual mean insulinemia, glycemia and glucose infusion rate during the final 15 min infusion of the peptide (from 65–80 min, see Fig. VIII.4). † $p < .01$, ‡ $p < .05$

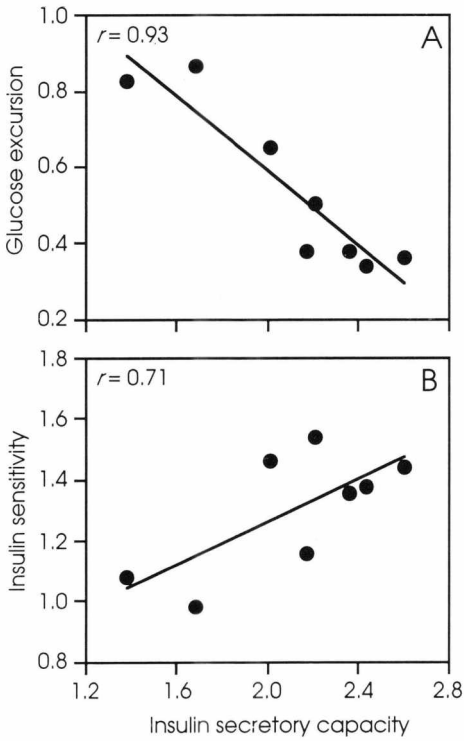

Fig. VIII.3

*Correlation in the graft recipients of the insulin secretory capacity as determined by intravenous arginine stimulation at ~35 mM glycemia, vs the postprandial glucose excursion (**A**) and insulin sensitivity during euglycemic clamps (**B**), after logarithmic transformation of the data.*

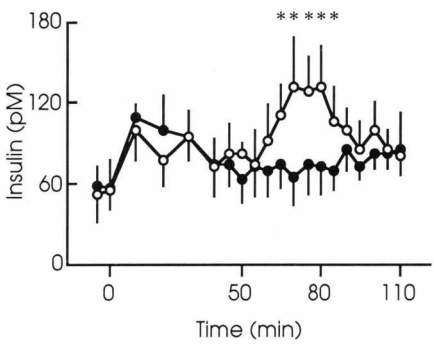

Fig. VIII.4

Plasma insulin response during intravenous ~8.5 mM glucose clamps with (open symbols) or without (closed symbols) co-infusion of GLP-1 (1.75 pmol.kg^{-1}.min^{-1}) at 6–9 months after islet autotransplantation in 4 dogs. Data are means ± SE. Significant differences between insulin levels at each time point are indicated by asterisks.

to high, intraportal, levels of endogenous gut hormones.

The poor ß-cell response and glucose intolerance during the intravenous glucose tolerance test in our graft recipients corroborate previous dog autograft reports [2,3] and studies in streptozotocin-diabetic rats — even — after isografting the average normal islet mass [6]. Because antecedent chronic hyperglycemia has been shown to impair the insulin response to intravenous

glucose [11], both the chronic postprandial hyperglycemia and a reduced ß-cell mass may have contributed to the severe reduction of the insulin response to intravenous glucose in our graft recipients. The insulin secretory capacity — as assessed by arginine stimulation under marked hyperglycemic conditions — is, in contrast, probably not affected by antecedent hyperglycemia [11,12], and, further, has been shown to be a sensitive indicator of the reduction in beta cell mass in partial pancreatectomized and diabetic subjects [13,14]. Considering the efficacy of the isolation procedure [9] the autografts comprised roughly 25% of the native islet mass, and the similar reduction of the graft's insulin secretory capacity to ~25% of the normal value would thus suggest this parameter to be a sensitive indicator of the reduction in beta cell mass in our model, as well. The insulinemia during the arginine test and the meal test was similar in the grafted animals, hence — with the assumption of near-maximal ß-cell stimulation during the arginine test [13,15] — we conclude that the grafted ß-cells were near-maximal stimulated by a meal, too. This conclusion is further substantiated by the negative correlation after islet transplantation of the insulin secretory capacity versus the postprandial glycemic increment. These findings confirm and extend our previous reports of the remarkable difference between the insulin response to intravenous glucose and a meal, both after islet transplantation in rats and dogs [6,7], as well as after the in vivo isolation of islets following obliteration of the duct of the pancreas in dogs [8].

We hypothesized that the graft's hyperinsulinemic response to a meal, as opposed to the hypo-insulinemic response to intravenous substrates, may largely be attributed to a hyperglycemic potentiation of the enteroinsular axis — rather than the hyperglycemia per se — after a meal. GLP-1 is now considered the major insulinotropic gut factor in the enteroinsular axis, and both GLP-1 and other gut hormones such as gastric inhibitory polypeptide (GIP) are known to have a progressively greater potentiating effect on glucose-stimulated insulin release at increasing ambient glucose levels [16,17]. We recently demonstrated glucose-dependent potentiation of insulin secretion from freshly isolated canine islets in vitro, in perifusion experiments, by physiological levels of GIP and especially GLP-1 [2,18]. However, no studies of the insulinotropic effects of gut hormones after islet transplantation have yet been published. We therefore investigated the insulinotropic effect of low-dose infusion of GLP-1 during ~8.5 mM glucose clamps to mimic the postprandial glycemic conditions after islet transplantation. In man, infusion of a similar dose of GLP-1 has recently been shown to lead to near-physiological plasma GLP-1 levels [19]. The insulinemia nearly doubled during GLP-1 infusion in our grafted dogs, and the same dose of GLP-1 has been shown previously to be

less insulinotropic under milder hyperglycemic conditions in normal dogs [20]. Thus, a hyperglycemia-enhanced insulinotropic effect of GLP-1 — and probably other insulinotropic hormones like GIP, as well — may largely account for the hyperinsulinemic response to a meal after islet transplantation. The fact that the glucose infusion rate had to be increased in order to maintain the glucose level at ~8.5 mM during GLP-1 administration, is well compatible with its glucose lowering effect in both diabetic and normal subjects [21] — and GLP-1 may furthermore enhance insulin sensitivity [22], to fully explain this effect.

Although postprandial hyperglycemia as outlined above, may account for the postprandial insulinemia, the reason why the insulin secretion was inadequate to induce normal glucose levels remains to be addressed. Clearly, a reduced insulin sensitivity contributed to the postprandial hyperglycemia. Further, correlation of the graft's insulin secretory capacity with both the postprandial glucose excursion and insulin sensitivity in the grafted animals, indicated the graft's ß-cell mass to be the main determinant of the glucose intolerance. These findings corroborate studies in partially pancreatectomized animals, and newly diagnosed patients with insulin-dependent diabetes mellitus, that suggest that insulin resistance accompanies a reduced insulin secretory capacity, and furthermore may increase as the residual insulin secretory capacity decreases [14,23,24]. Aside from a reduced insulin secretory capacity, an altered pattern of insulin secretion due to the absence of neural fine regulation of insulin release from the isolated islets may also have led to the impaired glucose tolerance and insulin resistance in the autografted dogs. Firstly, the largely or exclusively neurally mediated preabsorptive insulin response is important for postprandial glucose tolerance [25], and has been shown to be either absent or diminished after transplantation of isolated islets [6,25]. Secondly, intrapancreatic neuronal co-ordination of the insulin release from native islets is required for organized pulsatile insulin secretion [26,27]. Isolated islets have been shown to secrete insulin in a pulsatile fashion both in vitro and in vivo [28–30], but the pulse intervals were abnormal in these studies, and small changes in the periodicity are known to adversely affect insulin action, and glucose handling [31]. Thus, both the deminished ß-cell mass and a deranged fine regulation of insulin release from isolated islets probably contributed to the glucose intolerance and insulin resistance in the grafted animals. A normal insulin sensitivity after intrasplenic islet autotransplantation in dogs was reported by Finegood and coworkers [5]. However, both the different methods for assessment of insulin action, and — considering the correlation of the insulin secretory capacity and insulin action

— possible differences in the insulin secretory capacity of the grafts might also account for this discrepancy.

We conclude that both a diminished ß-cell mass and a qualitatively defective insulin secretion may contribute to insulin resistance and mild postprandial hyperglycemia after islet grafting. The diminished ß-cell mass appeared to be the main determinant of these defects. The insulinotropic effects of GLP-1 and probably other gut hormones, as well, may largely account for the marked difference in the insulin response to the intravenous and oral challenges. Thus, after transplantation of a suboptimal islet mass, postprandial glucose excursions are probably largely restrained by hyperglycemic potentiation of the enteroinsular axis, which can lead to close to maximum stimulation of insulin secretion — and, eventually, may lead to functional failure of the graft. Transplantation of a larger islet mass should allow prolonged near-normal glycemic control.

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