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Insulin action and insulin binding following pancreas transplantation

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Summary. Insulin action and insulin specific binding to erythrocytes were examined in ten recipients of a pancreatic segment and renal graft (Group 1), in nine non-diabetic kidney recipients (Group 2) and in ten age- and weight-matched healthy control subjects (Group 3). All transplant recipients were normoglycaemic without need of insulin, received the same immunosuppression and had good renal graft function at 11-18 months post-transplantation, when the investigation was performed. Using the insulin clamp technique, insulin action was expressed as the metabolic clearance rate of glucose at insulin infusion rates of 1.0 (MCR_{submax}) and 10.0 (MCR_{max}) $mU \cdot kg^{-1} \cdot min^{-1}$. In comparison with the healthy control subjects, fasting free insulin and C-peptide levels were significantly higher in Groups 1 and 2, but no differences between Groups 1 and 2 were found ($p>0.05$). Mean values ± SEM of MCR_{submax} in Groups 1, 2 and 3 were 6.30 ± 0.55, 6.09 ± 0.69 and 10.52 ± 1.10 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively, and of MCR_{max} 12.65 ± 0.78, 13.14 ± 0.92 and 19.28 ± 1.42 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively. Insulin action was significantly decreased in Groups 1 and 2 at the low as well as the high insulin infusion rates but there was no difference between the two groups of recipients ($p>0.05$). No differences in binding data (specific binding, number of binding sites per cell) were found. It is concluded that insulin resistance is common to all immunosuppressed organ recipients and is not related to the pancreas graft. The decreased maximal response to insulin and normal insulin binding to erythrocytes tend to suggest a post-receptor defect in insulin action.

Key words: Pancreas transplantation - Insulin resistance - Insulin action - Insulin binding

Introduction

It is generally accepted that systemic insulin delivery accounts for elevated insulin levels after successful pancreas transplantation (van Goor et al. 1986; Nason et al. 1988; Lugagne et al. 1989; van der Burg et al. 1989; Diem et al. 1990). An additional cause of hyperinsulinaemia might be insulin resistance. The present study was therefore designed to examine whether insulin action in recipients of pancreas and kidney differs from that in healthy subjects and if it does, whether the difference is related to immunosuppressive therapy or to the presence of the pancreatic graft. In order to differentiate a possible receptor or post-receptor defect in insulin action, we used the hyperinsulinaemic clamp method at two insulin infusion rates and we determined the insulin binding to its receptors on erythrocytes.

Subjects and methods

Subjects. Insulin action and receptor binding was studied in ten Type 1 (insulin-dependent) diabetic patients with end-stage diabetic nephropathy at 11-18 months post-successful pancreas and kidney transplantation (Group 1), in nine non-diabetic kidney recipients at 11-14 months post-transplantation (Group 2) and in ten age- and weight-matched healthy control subjects (Group 3). The purpose, nature and potential risks of the study were explained to all the subjects and written consent was obtained before their participation. The characteristics of each group are listed in Table 1. In Group 1, together with the kidney, a pancreatic segment was transplanted whose splenic artery had been anastomosed to iliac arteries. The splenic vein of the graft was anastomosed to iliac veins. The pancreatic duct was occluded by prolamine. All the transplant recipients were normoglycaemic and did not require exogenous insulin. The highest glycaemia on the day preceding the investigation was 7.5 mmol/l 1h postprandially. Immunosuppressive therapy was the same in Groups 1 and 2 and consisted of prednisone (10 mg per day), cyclosporine A (trough

Table 1. Characteristics of individual groups of subjects (mean ± SEM)

	Group 1	Group 2	Group 3
No. of subjects	10	9	10
Age (years)	39.0 ± 2.3	41.5 ± 2.9	39.5 ± 1.3
Male/Female	3/7	6/3	5/5
Body mass index (kg/m^2)	22.9 ± 0.6	25.9 ± 1.7	24.3 ± 0.5
Fasting glycaemia (mmol/l)	4.47 ± 0.10	4.72 ± 0.13	5.01 ± 0.11
1h postprandial glycaemia (mmol/l)	6.54 ± 0.23	5.99 ± 0.29	5.52 ± 0.15
HbA1c (%)	6.90 ± 0.31	7.56 ± 0.75	7.01 ± 0.33
Coefficient of glucose disappearance (%/min)	1.28 ± 0.15	NP	1.93 ± 0.14
Serum creatinine (umol/l)	116 ± 10	124 ± 9	84 ± 5

NP = not performed

blood levels 300-600 ng/ml by non-specific RIA) and azathioprine (50-75 mg per day). All transplant recipients showed stable good function of the renal graft at the time of the study. None had been treated for a rejection episode during the previous 6 months.

Insulin clamp study. Insulin action was measured by the euglycaemic clamp technique (DeFronzo et al. 1979). Before the study, a catheter was inserted into an antecubital vein for glucose and insulin administration. To ensure arterialization of the blood, a second catheter for blood sampling was inserted in most transplant recipients into the arteriovenous fistula, which had been employed as an access for haemodialysis in the pre-transplant period. In four recipients and in all the healthy subjects the second catheter was placed into a forearm vein and the hand was then inserted into a heated box that was maintained at 65°C. Crystalline porcine insulin was administered over two consecutive 90-min periods. Each infusion was begun at a high rate and followed by an exponential declining, analogically to the method of DeFronzo et al. (1979), before achieving a constant rate of 1.0 and 10.0 mU·kg^{-1}·min^{-1} after 10 min. Glycaemia was maintained at 5.0 mmol/l with a coefficient of variation up to 6.0%. As a measure of insulin action the submaximal and maximal metabolic clearance rate of glucose (MCR_{submax} and MCR_{max}) calculated during the last 30 min of each 90-min period was used. The metabolic clearance rate of glucose was calculated by dividing the glucose utilization rate, determined according to DeFronzo et al. (1979), by the mean glucose concentration during the study (Gottesman et al. 1984). Serum free insulin (IRI), C-peptide and glycaemia were measured on two consecutive days in the fasting state. Postprandial glycaemia was measured 1h after ingestion of mixed meal containing 60g carbohydrate. Glucose disappearance constant (KG) was measured after administration of 0.33g glucose/kg intravenously. Glycaemia was measured from 10-60 min and IRI and C-peptide were measured at times 0,4,15,30 and 60 min following glucose administration. The area under the curve of insulin and C-peptide concentration (AUC_I and AUC_{CP}) was calculated.

Analytical methods. Blood glucose was measured by the glucose-oxidase method on a Beckman Analyzer. HbA1C was determined colorimetrically (Standefer et al. 1984). IRI was assessed by RIA after treatment with polyethylene glycol to remove insulin antibodies (Nagakawa et al. 1973) using kits manufactured by the Institute of Atomic Energy (Swierk, Poland). C-peptide was determined by RIA using kits from Serono diagnostics, S.A. (Switzerland).

Insulin binding. Insulin binding to erythrocytes was measured according to a modification of the method of Gambhir et al. (1978). Erythrocytes were incubated in quadruplicate (400 ul) with 0.2 ng of human mono-^{125} I (Tyr A14)-insulin (specific activity 2000 ci/mmol, Amersham, Amersham, UK) and with increasing concentrations of unlabelled insulin (0-10^5 ng/ml) at 15°C for 3.5 h. Specific binding was calculated as the total minus the non-specific binding, defined as the amount of ^{125}I-insulin bound in the presence of 10^5 ng/ml unlabelled insulin, and was corrected to a standard erythrocyte concentration (2.10^9/ml). Binding parameters were analysed by Scatchard analysis (Scatchard 1949) and by the average affinity profile plot method of DeMeyts and Roth (1975). Insulin specific binding (at tracer insulin concentration) and number of binding sites per cell were calculated.

Statistical analysis. Statistical evaluation was performed using the Kruskal-Wallis analysis of variance on a personal computer using the program BMDP Statistical Software (University of California, Los Angeles, USA, 1979). The differences between all three groups were determined by the multiple comparison method of Nemenyi. The results are given as the mean values + SEM.

Results

The mean values of fasting and 1 h postprandial glycaemia, HbA1C , KG and serum creatinine are listed in Table 1. The three groups did not differ statistically in age, BMI, or HbA1C. Fasting glycaemia was within the normal range in all subjects. However, pancreatic graft recipients showed a value significantly lower than healthy control subjects ($p<0.05$). On the other hand, 1h postprandial glycaemia, still remaining in the normal range, was significantly higher in pancreas graft recipients than in healthy control subjects ($p<0.05$). There was no difference between Groups 1 and 2. Serum creatinine levels were significantly different only between Groups 2 and 3 ($p<0.05$). Pancreatic graft recipients had lower values of KG than healthy subjects ($p<0.01$).

Table 2. Free insulin, C-peptide and insulin binding to erythrocytes in recipients of pancreas and kidney grafts (Group 1), in non-diabetic kidney recipients (Group 2) and in healthy subjects (Group 3). Mean values ± SEM.

	Group 1	Group 2	Group 3
Fasting free insulin (pmol/l)	167 ± 19	144 ± 16	62 ± 5
Fasting C-peptide (nmol/l)	1.17 ± 0.27	1.28 ± 0.10	0.51 ± 0.05
AUC_I (pmol.min^{-1}.l^{-1})	22560 ± 2382	NP	11634 ± 1002
AUC_{CP} (nmol·min^{-1})	109.9 ± 18.0	NP	63.9 ± 6.1
Specific insulin binding (%)	4.94 ± 0.58	5.87 ± 0.99	5.89 ± 0.64
No. of binding sites per cell	25.2 ± 5.2	42.3 ± 7.86	37.8 ± 6.38

AUC_I = Area under insulin curve; AUC_{CP} = area under C-peptide curve; NP = not performed.

Mean levels of fasting IRI and C-peptide, AUC_I and AUC_{CP} during the intravenous glucose tolerance test are shown in Table 2. As compared with IRI in healthy control subjects, the parameter was elevated in Group 2 ($p<0.01$) as well as in Group 1 ($p<0.05$). Fasting C-peptide levels were also elevated in both transplant groups ($p<0.05$). However, no statistical differences were found between the two groups of transplant recipients in fasting IRI and C-peptide levels ($p>0.05$).

During the intravenous glucose tolerance test, significantly larger AUC_I ($p<0.01$) and AUC_{CP} ($p<0.05$) were demonstrated in pancreas graft recipients than in healthy control subjects.

The results of glycaemic clamp studies at both insulin infusion rates are shown in Figure 1. Compared with control subjects both groups of transplant recipients show significantly reduced MCR_{submax} ($p<0.05$), and MCR_{max} (Group 1 vs Group 3 $p<0.01$, Group 2 vs Group 3 $p<0.05$). There is no difference between both groups of transplant recipients ($p>0.05$).

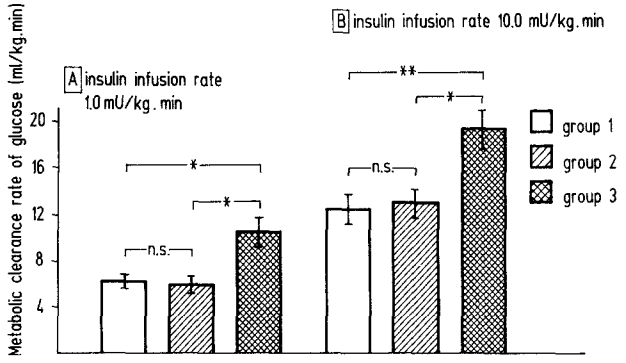

Fig. 1. Metabolic clearance rates of glucose. Mean values + SEM. * $p<0.05$, ** $p<0.01$, NS = not significant.

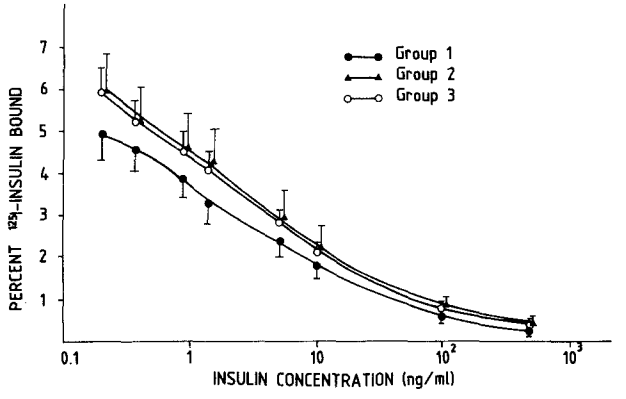

Fig. 2. Competition curves of specific ^{125}I-insulin binding to erythrocytes. Shown are mean values + SEM. The differences between individual groups are not statistically significant.

Figure 2 presents the competition curves of ^{125}I-insulin specific binding to erythrocytes. Mean values of insulin specific binding and number of binding sites per cell are given in Table 2. No statistically significant differences were found in the binding data. ($p>0.05$).

Discussion

While a technical fault is the leading cause of pancreas graft failure in the early post-transplant period, later on, rejection and other reasons, not yet sufficiently established, are more prominent. Just as in non-diabetic subjects, glucose tolerance of pancreas graft recipients depends on two major determinants: Beta-cell function and insulin action. A defect of each may contribute to the reappearance of diabetic disturbance.

In this study we have examined the insulin action in two groups of transplant recipients which were comparable as regards age, BMI, time after transplant, immunosuppression and renal graft function. In comparison with healthy subjects, we demonstrated a significant decrease in the metabolic clearance rate of glucose in both groups of transplant recipients. No differences in insulin action between the two groups of recipients were found. The mean metabolic clearance rates were reduced by approximately 40% at an insulin infusion rate of 1.0 $mU \cdot min^{-1} \cdot kg^{-1}$ and by approximately 35% at an insulin infusion rate of 10 mU/min. Fasting IRI and C-peptide levels were also not different between the two groups of recipients and were more than doubled in comparison with those in healthy subjects.

The method of blood collection was not fully standardised in our clamp studies. Because of poor venous access, arterio-venous fistulas were used in most transplant recipients, while in control subjects blood from the hand lying in a heated box was collected. However, the differences in metabolic clearance rates of glucose between the control subjects and both the transplant groups were too high to be explained by better arterialization in transplant recipients. Thus, the results indicate that insulin action is impaired in both groups of transplant recipients and that insulin resistance is roughly of the same degree. The decreased submaximal as well as maximal responses to insulin tend to suggest mainly a post-receptor defect in insulin action (Kahn 1978). In addition, no significant receptor defect was found by examination of insulin specific binding to erythrocytes. However, erythrocytes are not insulin sensitive and examination of insulin binding to other tissues would be necessary to confirm our findings.

The main cause of insulin resistance following kidney transplantation is probably corticosteroid administration, although the effect of cyclosporine has also been suggested by some (Yale et al. 1988; Öst et al. 1988; Waldstrom et al. 1990) but not proved by all investigators (Dresner et al. 1989). Rizza et al. (1982) demonstrated that 24-h infusion of cortisol in healthy subjects significantly impaired

inhibition of hepatic glucose production and stimulation of glucose utilization by insulin. The decreased insulin action at the maximal insulin infusion rate and no changes in insulin binding suggested a corticosteroid-induced post-receptor defect, as in our study. On the contrary, other authors found decreased insulin binding to erythrocytes (Yasuda et al. 1980) or monocytes (DePirro et al. 1980) induced by glucocorticoids. Ekstrand et al. (1989) demonstrated a 25% reduction in total glucose disposal in non-diabetic kidney recipients receiving an average of 8.2 mg prednisolone per day in comparison with healthy control subjects.

Insulin resistance following pancreas transplantation should have a similar cause as in non-diabetic kidney recipients, since most transplanted subjects are on a similar immunosuppressive regimen. However, this has not yet been clearly documented. Luzi et al. (1990) recently investigated insulin sensitivity after successful pancreatic grafting at insulin infusion rates corresponding approximately to the low one in our study. Although they found insulin resistance when compared with healthy control subjects, the control group of non-diabetic recipients was too small to draw convincing conclusions. Moreover, insulin resistance could also consist in other reasons. In fact, it is a feature characteristic of Type 1 diabetes (DeFronzo et al. 1982) and may be related to the degree of glucose tolerance. Although all recipients in Group 1 fulfilled the criteria of "full function of the pancreatic graft", their KG values were significantly lower than in the healthy control subjects. Nevertheless, the three subjects of Group 1 with KG values between 0.7 and 0.98%/min had the values of MCR_{submax} 5.45, 7.46, and 9.08 and of MCR_{max} 12.45, 14.44, and 15.65 $ml \cdot kg^{-1} \cdot min^{-1}$, which all are lying only slightly below, or even above the mean values of the whole Group 1. Since peripheral hyperinsulinaemia per se is able to induce insulin resistance (Marangou et al. 1986), it could be a consequence of the heterotopic placement of the pancreatic graft. Recently, Diem et al. (1990) demonstrated basal and stimulated hyperinsulinaemia but normal C-peptide production in recipients of pancreatic graft with systemic venous drainage as compared with non-diabetic kidney recipients and healthy subjects. They conclude that hyperinsulinaemia following pancreas transplantation is mainly due to systemic insulin delivery.

While in non-diabetic kidney recipients insulin resistance is a frequent cause of secondary diabetes mellitus, in pancreas graft recipients, because of increased demands, the surviving Beta cells may not be able to secrete sufficient insulin to maintain normal glucose tolerance. A relative defect of Beta-cell function is probable in most recipients with a functioning pancreatic graft, since the Beta-cell mass may be reduced by many factors, such as conservation, ischaemia, rejection, fibrosis and size of the graft.

In addition, insulin secretion may be suppressed by cyclosporine A (Dresner et al.1989; Gillison et al. 1989; Alejandro et al.1989). In spite of that, most recipients with functioning grafts have basal and meal- or glucose-stimulated hyperinsulinaemia which may be preserved even in the case of impaired glucose tolerance or recurrence of the diabetic state (Smith et al. 1989).

We conclude that the immunosuppressive therapy currently used in pancreas transplantation induces insulin resistance, which may be at least partially responsible for the elevated insulin levels. It is probable that substitution of glucocorticoids by another immunosuppressive drug with a less pronounced diabetogenic effect could improve overall results of pancreas transplantation.

References

Alejandro R, Feldman EC, Bloom AD, Kenyon NS (1989) Effect of cyclosporin on insulin and C-peptide secretion in healthy beagles. Diabetes 38:698-703

DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237:E 214-227

DeFronzo RA, Hendler R, Simonson D (1982) Insulin resistance is a prominent feature of insulin-dependent diabetes.Diabetes 31:795-801

DeMeyts P, Roth J (1975) Cooperativity in ligand binding. A new graphic analysis. Biochem Biophys Res Commun 66:1118-1126

DePirro R, Bertoli A, Fusco A, Testa I,Greco AV, Lauro R (1980) Effect of dexamethasone and cortisone on insulin receptors in normal human male. J Clin Endocrinol Metab 51:503-507

Diem P, Abid M, Redmon JB, Sutherland DER, Robertson RP (1990) Systemic venous drainage of pancreas allografts as independent cause of hyperinsulinaemia in type I diabetic recipients.Diabetes39:534-540

Dresner LS, Andersen DK, Kahng KU, Minshi IA, Wait RB (1989) Effect of cyclosporine on glucose metabolism. Surgery 106:163-170

Ekstrand A, Ahonen J, Grönhagen-Riska C, Groop L (1989) Mechanisms of insulin resistance after kidney transplantation.Transplantion 48:563-568

Gambhir KK, Archer JA, Bradley CJ (1978) Characteristics of human erythrocyte insulin receptors. Diabetes 27:701-708

Gillison SL, Bartlett ST, Curry DL (1989) Synthesis-secretion coupling of insulin. Effect of cyclosporin. Diabetes 38:465-470

Gottesman I, Mandarino L, Gerich J (1984) Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo. Diabetes 33:184-191

Kahn CR (1978) Insulin resistance, insulin sensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27:1893-1902

Lugagne PM, Reach G, Houry S, Peuchemaure M, Rouchette J, Huggier M (1989) Portal versus peripheral venous drainage in segmental pancreatic transplantation in diabetic rats. Transplantation 47:420-423

Luzi L, Secchi A, Facchini A, Battezzati A, Staudacher C, Spotti D, Castoldi R, Ferrari G, DiCarlo V, Pozza G (1990) Reduction of insulin resistance by combined kidney-pancreas transplantation in Type 1 (insulin-dependent) diabetic patients. Diabetologia 33:549-556

Marangou AG, Weber KM, Boston RC, Aitken PM, Heggie JCP, Kirsner RLG, Best JD, Alford FP (1986) Metabolic consequences of prolonged hyperinsulinaemia in humans: evidence of induction of insulin insensitivity. Diabetes 35:1383-1389

Nagakawa S, Nakayama H, Sasaki T, Yoshino K, Yu YY, Shinozaki K, Aoki S, Mashimo K (1973) A simple method for the determination of serum free insulin levels in insulin-treated patients. Diabetes 22:590-600

Nason RW, Warnock GL, Rajotte RV (1988) Portal versus systemic insulin delivery in canine pancreatic islet cell transplantation. Transplant Proc 20:1276-1278

Öst L, Tydén G, Fehrman I (1988) Impaired glucose tolerance in cyclosporine-prednisolone-treated renal graft recipients. Transplantation 46:370-372

Rizza RA, Mandarino LJ, Gerich JE (1982) Cortisol-induced insulin-resistance in man: Impaired suppression of glucose production due to a postreceptor defect of insulin action. J Clin Endocrinol Metab 54:121-138

Smith JL, Hunsicker LG, Yuh WTC, Wright FH, van Voorhils L, Corry R (1989) Appearance of type II diabetes mellitus in type I diabetic recipients of pancreas allografts. Transplantation 47:304-311

Scatchard G (1949) The attraction proteins for small molecules and ions. Ann NY Acad Sci 51:660-672

Standefer JC, Easton RP (1983) Evaluation of a colorimetric method for determination of glycosylated hemoglobin. Clin Chem 29:135-140

van der Burg MPM, Gooszen HG, Guicherit OR, Jansen JBMJ, Frölich M,van Haastert FA, Lamers CBHW (1989) Contribution of partial pancreatectomy, systemic hormone delivery, and duct obliteration to glucose regulation in canine pancreas. Diabetes 38:1082-1089

van Goor H, Sloof MJH, Wijfels RTM, Sluiter WJ (1986) Beta cell response and insulin sensitivity after segmental pancreatic autotransplantation with systemic venous drainage. Transplant Proc 18:1152-1154

Waldstrom HE, Lavelle-Jones M, Endres D, Akimoto R, Kolterman O, Moossa AR (1990) Inhibition of insulin release by cyclosporine and production of peripheral resistance in the dog. Transplantation 49:600-604

Yale JF, Chamelian M, Courchesne S, Vigeant C (1988) Peripheral insulin resistance and decreased insulin secretion after cyclosporine A treatment. Transplant Proc 20:985-988

Yasuda K, Kitabchi AE (1980) Decreased insulin binding of human erythrocytes after dexamethasone or prednisone ingestion. Diabetes 29:811-814

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