Endocrine Responses of Type 1 (Insulin-Dependent) Diabetic Patients Following Successful Pancreas Transplantation

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Summary. The aim of the present study was to evaluate the insulin and glucagon responses to various stimuli in patients following pancreatic transplantation. Four Type 1 (insulin-dependent) diabetic patients with end-stage renal failure who had received a cadaveric segmental, neoprene-injected, pancreas transplant, in association with kidney transplantation, were investigated. Free-insulin, pancreatic glucagon, and growth hormone concentrations were measured after both oral and intravenous glucose tolerance tests, and following tolbutamide, arginine and arginine plus somatostatin infusions. Tests were performed 1 month (three cases) and 30 months (one case) after surgery, when no insulin administration was required. Four non-diabetic kidney grafted patients, matched for duration of graft survival and immunosuppressive treatment (steroids, azathioprine and anti-lymphocyte-globulins), served as control subjects. Impaired glucose tolerance was present in all diabetic and control patients. This was possibly related to immunosuppressive treatment. In comparison with control subjects, insulin release was normal in response to arginine and tolbutamide but was reduced in response to oral and intravenous glucose, while glucagon and growth hormone release were similar in both groups. Somatostatin was less effective in diabetic patients than in control subjects in suppressing insulin and glucagon release.

Key words: Type 1 diabetes, pancreas transplantation, pancreas, insulin, glucagon, growth hormone, kidney transplantation, somatostatin (cyclic).

Pancreas transplantation is intended to restore endogenous insulin secretion and to normalize metabolism in patients suffering from Type 1 (insulin-dependent) diabetes mellitus [1]. Pancreatic transplantation in a diabetic patient who is a candidate for kidney transplantation, because of end-stage renal failure, does not constitute an undue added risk. For this reason, pancreas transplantation has usually been performed together with kidney transplantation at the E. Herriot Hospital in Lyon. Details of the surgical procedure have been presented elsewhere [2]. When the surgical procedure is successful, there is a dramatic improvement in the diabetes, so that insulin treatment is no longer needed. Restored insulin secretion is also evident [3–6]. The aim of our study was to evaluate the insulin and glucagon response to provocative stimuli in such patients. This is of importance as the grafted pancreas is heterotopic, is away from the usual neuro-humoral environment, and secretes into the general circulation instead of the portal system.

Patients and Methods

Patients

Four Type 1 diabetic patients, who had undergone kidney and pancreas transplantation apparently successfully, were investigated. Clinical data are shown in Table 1. Segmental pancreas transplantation was performed according to our technique [2]. Briefly, a segment of the pancreas (body and tail), injected intraductally with neoprene in order to suppress exocrine function, was vascularized by anastomosis to iliac artery and vein.

During the period of investigation, all the patients had been insulin-independent since transplantation. Immunosuppressive treatment was based on multiple blood transfusions or thoracic duct drainage and on administration of prednisolone (10–20 mg/day), azathioprine (0–100 mg/day) and equine anti-lymphocyte-globulins (8 mg·kg^{-1}·day^{-1}) [7]. No major renal rejection episodes occurred in three diabetic patients (Nos. 1, 2, 4), but in the fourth patient, three episodes of rejection occurred (on days 70, 130 and 230). These episodes were treated with massive doses of prednisolone (4 mg·kg^{-1}·day^{-1} for 2 days), and a condition of subclinical chronic renal rejection followed. As a control group, four non-diabetic kidney grafted patients (Nos. 5, 6, 7, 8) were subjected to the same studies. Clinical data are

Table 1. Clinical details of the four Type 1 diabetic patients transplanted with kidney and pancreas (Nos. 1–4) and four control subjects transplanted only with kidney (Nos. 5–8)

Patients (number)	Sex	Age (years)	Duration of diabetes (years)	Complications			HLA		Date of transplantation	Time-lapse transplantation tests (months)	Number of renal rejection episodes	Post transplantation values		Steroid administration		Final outcome
				Eye	Kidney	Nervous system	A	B				Fasting blood glucose (mmol/l)	Plasma creatinine (mmol/l)	During the days of testing (mg/day)	Total dose received between transplantation and testing (mg/day)	
1	M	41	20	+	+	+	2	12/17	15/2/81	1	0	6.30	100	15	27	Death (myocardial infarction)
2	F	27	25	+	+	+	3/9	18/35	29/3/81	1	0	6.10	70	10	17	Alive with functioning pancreas and kidney
3	F	40	36	+	+	+	9	35/14	20/9/78	30	3	5.50	200	15	18	Death (sepsis)
4	F	31	19	+	+	+	29/30	18	16/6/81	1	0	5.00	130	15	22	Death (mesenteric infarction)
5	M	24	0	/	/	/	9/32	18/22	27/2/81	1	0	4.50	140	20	36	Alive and well functioning
6	M	29	0	/	/	/	2/30	12/21	7/4/81	1	0	4.60	110	20	45	Alive, with a rejected kidney
7	F	51	0	/	/	/	1	12/8	27/4/79	22	4	4.17	109	20	18	Alive and well functioning
8	M	26	0	/	/	/	3/9	7/35	3/4/81	1	0	3.80	130	20	50	Alive and well functioning

shown in Table 1. Due to the small number of diabetic patients and to the different time-lapse from transplantation, the control subjects were selected to be matched for immunosuppressive treatment and duration of grafting. The matching of the patients is as follows: 1 versus 5, 2 versus 6, 3 versus 7, 4 versus 8.

Test Procedures

The diabetic and control patients were subjected to the following tests: an oral glucose tolerance test (75 g glucose with samples at 0, 15, 30, 45, 60, 90, 120, 180 min); an intravenous glucose tolerance test (0.5 g glucose/kg body weight with samples at 0, 5, 10, 20, 30, 40, 60, 90 min); a tolbutamide infusion (1 g IV with samples at 0, 3, 5, 10, 20, 30, 40, 60 min); an arginine infusion (25 g, IV, as a 10% solution given over 30 min with samples at 0, 5, 10, 20, 30, 45, 60, 90, 120 min) and an arginine plus cyclic somatostatin infusion (Clin-Midy, Montpellier, France) (250 μg bolus IV at time 0 min and 250 μg IV infusion over 120 min, samples as in the arginine test). These tests were performed in the recumbent position at 09.00 h after an overnight fast. Not all patients were submitted to all tests. Informed consent was obtained from all diabetic and control patients before testing.

Assays

Blood samples were obtained at the time intervals indicated in the test procedures, by means of an indwelling catheter inserted into a forearm vein or into the subclavian vein and kept patent by a slow saline infusion (0·154 mol/l). Blood was placed into tubes containing disodium EDTA and aprotinine (10,000 KIU/ml) and centrifuged at 4 °C. Blood glucose levels were evaluated by an enzymatic method. Plasma was stored at −20 °C until assayed for free-insulin (IRI) [8, 9], growth hormone (GH) [10] and immunoreactive glucagon (IRG). IRG was assayed using the antibody R-78 at a working concentration of 1:24000; the antibody was C-terminus directed and therefore reacted only with pancreatic glucagon. Incubation lasted 48 h at 4 °C; separation of free- from bound-hormone was obtained by precipitation with polyethyleneglycol 20% and centrifugation at $2000 \times g$ for 20 min. Free-IRI was assayed according to the method of Nakagawa et al. [8] and Heding [9]. Kits for IRG were supplied by Serono-Immunochemicals, Biodata, Milan, Italy, and kits for free-IRI by Novo, Copenhagen, Denmark. In our laboratory, fasting IRI, IRG and GH levels are 0–20 mU/l, 50–200 mIU/l and 0–20 mIU/l, respectively. The inter- and intra-assay variation were, respectively, 13.4% and 6.4% for GH, 15% and 9% for IRG, 11% and 10% for IRI.

Results

According to the criteria of the WHO Committee [11], oral glucose tolerance testing revealed an impaired glucose tolerance in all control subjects. In our diabetic patients, glucose tolerance was normal in one case, impaired in one case and diabetic in two cases, although fasting blood glucose levels were either normal or only slightly above the normal range (range 4.72–8.05 mmol/l). The IRI response to the same test was more pronounced in the control subjects than in the diabetic patients, and in particular in the two diabetic patients who had a diabetic response to the oral glucose tolerance test. IRG and GH levels during the same test were not different in the diabetic and control patients, and did not change significantly. Blood glucose levels during the IV glucose tolerance test were normal in the control subjects (Conard's K [12] being 1.75, 1.27, 2.1,
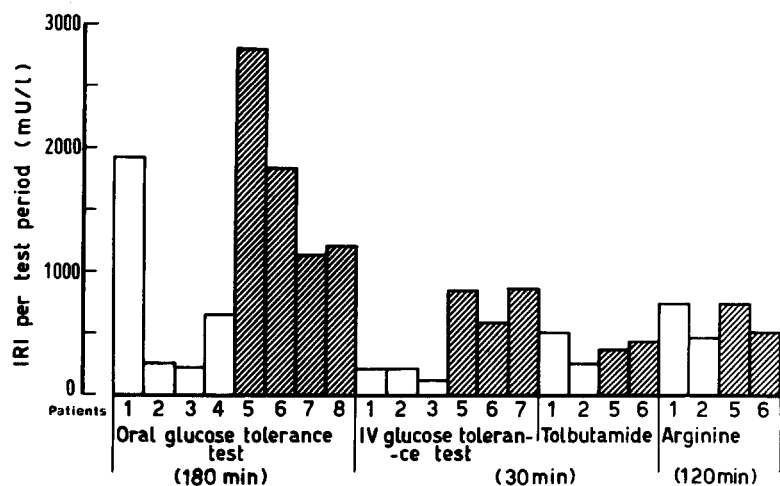
Fig. 1. Insulin secretory areas (IRI) (calculated by the trapezoidal method) after different insulinogenic stimuli in the Type 1 diabetic patients transplanted with pancreas and kidney (□; Nos. 1–4) and in non-diabetic patients transplanted only with kidney (▨; Nos. 5–8)
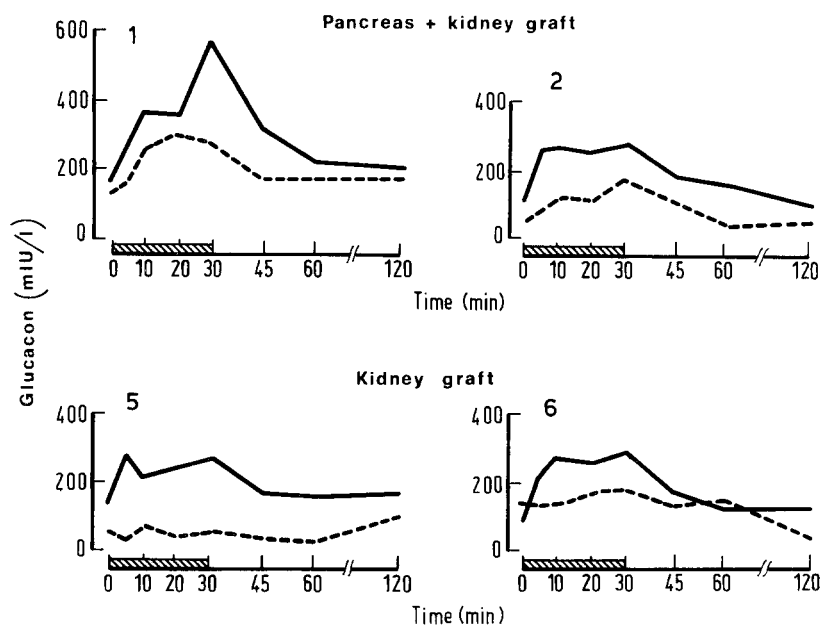

Fig. 2. Plasma glucagon (mIU/l) response to arginine (——) and to arginine plus somatostatin (---) in two Type 1 diabetic patients transplanted with pancreas and kidney (Nos. 1 and 2) and in two non-diabetic patients transplanted only with kidney (Nos. 5 and 6)

respectively); whereas in the diabetic patients, one normal response (K = 1.32), one borderline response (K = 0.90) and one diabetic response (K = 0.55) were observed. The IRI response to IV glucose was normal in the control subjects, with the presence of a peak level at 5 min, but was reduced in the diabetic patients, with an absence of an early secretory peak. On the other hand, the tolbutamide test revealed no difference between the two groups; IRI release reached a peak value (range 34–70 mU/l) between 3 and 5 min in both groups. Arginine-induced IRI release was biphasic and similar in the two groups. Figure 1 shows the net IRI secretory areas (calculated by the trapezoidal method) in response to various stimuli in both groups. It is evident that striking differences were observed only in the IRI response to both oral and intravenous glucose, but not to arginine or tolbutamide. IRG (Fig. 2) and GH (Fig. 3) responses to arginine were also similar in the two

groups. When somatostatin was infused with arginine, suppression of IRI and IRG was complete in the control subjects but only partial in the diabetic patients. The effect of somatostatin on arginine-induced GH release did not vary between the groups (Fig. 3).

Discussion

Pancreatic transplantation is performed in Type 1 diabetic patients in order to provide self-regulating insulin release and to revert metabolism to normal. From the various surgical procedures which have been proposed by several authors including ourselves [1], we now choose to perform segmental transplantation [2]. In this study, we present data concerning insulin and glucagon release in four such patients in whom the diabetic state was replaced by apparently normal glucose homeosta-
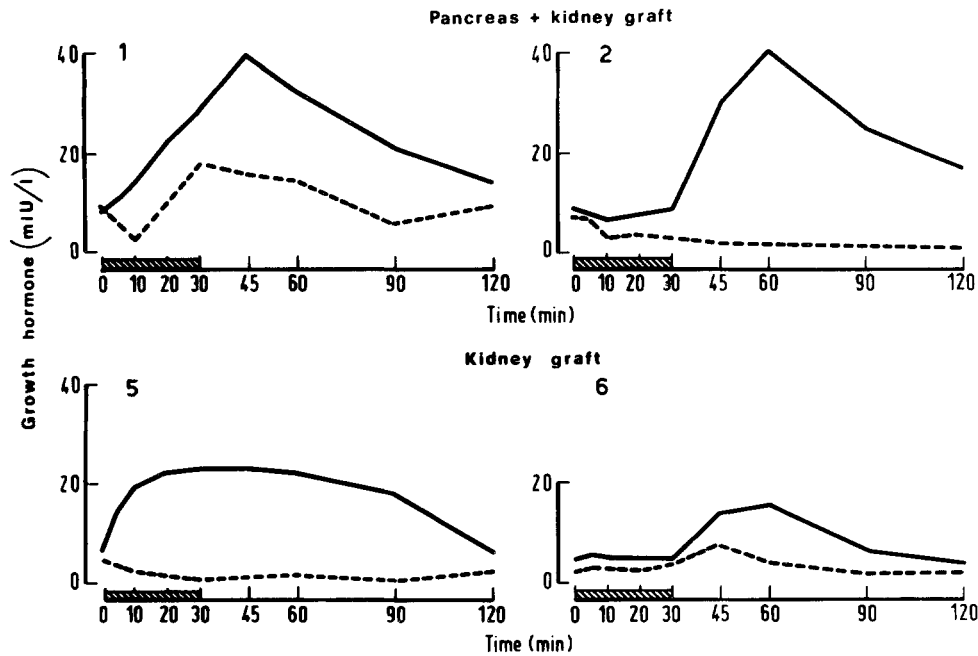
Fig. 3. Plasma growth hormone (mIU/l) response to arginine (——) and to arginine plus somatostatin (---) in two Type 1 diabetic patients transplanted with pancreas and kidney (Nos. 1 and 2) and in two non-diabetic patients transplanted only with kidney (Nos. 5 and 6)

sis. No comparison was attempted between pre- and post-surgery condition in these pancreas-grafted patients because of absolute insulin-dependence and because of the concomitant uraemic state.

The number of patients studied is not large enough to draw general conclusions. However glucose tolerance was impaired, although fasting blood glucose levels were normal or only slightly above normal levels. In all the diabetic patients (except No. 4, who had a normal glucose tolerance test), the peak IRI level was reached between 90 and 120 min, that is delayed if compared with normal subjects but similar to that usually seen in patients with impaired glucose tolerance. Several factors might be responsible for the reduced insulin secretion observed in the diabetic patients. Firstly, only a part of the pancreas was transplanted, secondly, undetected pancreatic rejection might be present and finally a period of warm ischaemia is inevitable during surgical procedures, possibly leading to necrosis and permanent damage of the endocrine tissue. In addition, occlusion of the pancreatic duct and suppression of exocrine function may be involved, since in the experimental animal duct ligature leads to impaired glucose tolerance and reduced IRI release [13]. Furthermore, long-term fibrosis of the exocrine pancreas leads to a reduced insulin content in the experimental animal. The transplanted pancreas is also far from its original location and gastrointestinal hormones are known to stimulate IRI and IRG release in the experimental animal [14, 15]. The reduced IRI response to oral glucose might be explained on this basis only if evidence is obtained that the transplanted pancreas is less sensitive to gastrointestinal hormones. The transplanted pancreas is also denervated, and lack of the known stimulatory role played by the vagus nerve [16] and by acetylcholine [17] might

also account for the decreased insulin response to glucose. In man, however, the effect of surgical procedures, such as vagotomy, on insulin release is not completely understood [18]. At present the different IRI responses to glucose and to other stimuli, such as tolbutamide and arginine, could be because these stimuli have different mechanisms of action in eliciting IRI release [19].

Glucagon response to glucose and arginine was similar in the two groups of subjects: fasting IRG levels were within the normal range and IRG release was inhibited or unaffected by glucose, and stimulated by arginine. These data indicate that the transplanted A cell responds normally to classical stimuli. In addition, plasma IRG levels were not abnormally elevated, as is usually seen in poorly controlled diabetes [20]. Also GH release, which is known to be exaggerated in poorly controlled diabetes [20], was normal in our patients.

Somatostatin seemed to be more effective in inhibiting IRI and IRG release in the control subjects than in the diabetic patients. No explanation is available for this difference. It is known that somatostatin is able to inhibit IRI and IRG release from the perfused pancreas in situ, but not from isolated perfused islets [21]. Hypersomatostatinaemia has been described repeatedly in experimentally-induced diabetes in animals and is reversed by pancreatic transplantation in rats [22]. Although plasma somatostatin levels were not evaluated in our patients, somatostatin was equally active in both groups in suppressing GH release.

Currently uraemia represents the major cause of death in diabetic patients [23]. Chronic haemodialysis is unsatisfactory because mortality is almost double that of non-diabetic patients undergoing haemodialysis [23]. Therefore kidney transplantation represents the treatment of choice for such patients. In experienced hands

the addition of segmental pancreatic transplantation to kidney transplantation does not seem to increase the overall surgical risk, and can be considered a further step in the treatment of these patients, aiming at a complete cure not only of uraemia, but also of Type 1 diabetes itself.

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