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Minkowski Award, 1980, Athens

Inge-Bert Täljedal was born in 1942. After medical studies in Uppsala and Stockholm, he defended his doctoral thesis at Umeå in 1967. His research has mainly dealt with physiological aspects of the pancreatic islets. Dr. Täljedal has been a member of the EASD Council, the Diabetologia Editorial Board, the Swedish Medical Research Council, and the Swedish Council for Planning and Co-ordination of Research. Since 1980 he has been Professor of Histology at the University of Umeå, Sweden.

Functional aspects of mouse islets transplanted to the kidney

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Summary To test whether the transplantation of pancreatic islets affects their basic functions, collagenase-isolated mouse islets were inserted under the left renal capsule of recipient animals. After various periods of time, grafts were removed from the kidney and examined for insulin content and secretory dynamics in a perifusion system. During syngeneic (C57BL/6, BALB/c) or subsyngeneic (NMRI) intrastrain transplantation, the graft insulin content fell drastically during the first week and stayed low for at least 6 weeks; first-phase secretion in general appeared suppressed. Immunosuppression by cyclosporin A had little effect on (sub)syngeneic grafts but markedly improved the performance of allotransplants. Daily injections of the calcium antagonist, verapamil, enhanced the insulin secretory responses of isolated grafts, whether (sub)syngeneic or allogeneic. In syngeneic and subsyngeneic grafts, the potentiating effect of acetylcholine on glucose-induced insulin release was markedly diminished, whereas that of caffeine was not. Transplanted islets also exhibited a subnormal responsiveness to the inhibiting action of noradrenaline. It is concluded that chronic denervation and transplantation of pancreatic islets may cause fundamental changes in the beta-cell responses to physiological regulators of insulin release. [Diabetologia (1994) 37 [Suppl 2]: S112–S116

Key words Acetylcholine, caffeine, cyclosporin A, insulin secretion, islets, noradrenaline, transplantation, verapamil.

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As early as in 1922, Allen [1] demonstrated that denervated pieces of pancreas can prevent glucosuria in dogs. Fifty years later, similar observations were made with transplanted islets in the diabetic rat [2].

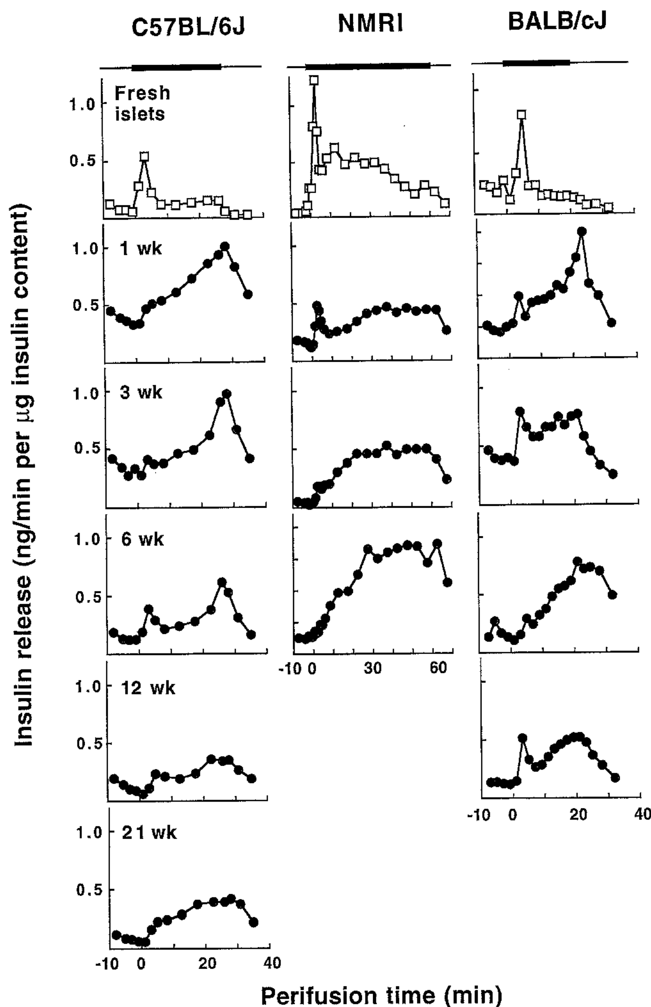

Fig. 1. Dynamics of glucose-induced insulin release from perifused fresh islets (top panels, open symbols) and grafts (solid symbols) 1–21 weeks after intra-strain transplantations among C57BL/6J, NMRI, and BALB/cJ mice. The sequence of glucose concentrations during each perifusion was basal (2.8 mmol/l), stimulatory (11 mmol/l in BALB/cJ, 16.7 mmol/l in C57BL/6J and NMRI), and basal, as indicated by the thin and thick horizontal bars at the top of each column of panels. Each curve represents the mean of 2–5 experiments

Many such experiments in vivo, as well as numerous observations on isolated islets in vitro, suggest that the glucostat function of the beta cell is largely independent of its normal innervation and anatomical surrounding. With this in mind, attempts are being made to develop islet transplantation into a valid therapeutic measure for diabetes mellitus.

Progress in this area so far has been slow. Apart from the technical problem of amassing islets in sufficiently large numbers, the major biological obstacle to success is generally thought to be immunological. Moreover, it remains unclear whether the secretory functions of the beta cells continue to be maintained upon their denervation and chronic dislocation.

To explore how transplantation may affect the function of the grafted beta cells we have performed a series of studies in which collagenase-isolated mouse islets were inserted under the renal capsule of normal recipient animals. After various periods of time, the grafts were recovered from the kidney and analysed for insulin content and the dynamics of insulin release in response to glucose, caffeine, acetylcholine, and noradrenaline. The influence of in vivo treatment with cyclosporin A, alone or in combination with the calcium antagonist verapamil, was also studied. A condensed survey of these investigations is given in the present report. More detailed accounts of hitherto unpublished experiments will be given elsewhere.

Materials and methods

Details of methodology have been described [3]. In brief, collagenase-isolated islets from adult donor mice were cultured at 37 °C for 24 h under air and 5 % carbon dioxide in RPMI medium containing 11 mmol/l D-glucose, 10 % heat-inactivated fetal calf serum, and antibiotics. Intra-strain transplantations of 150–600 (typically 200–300) islets to the subcapsular space of the left kidney were performed between C57BL/6J, BALB/cJ, or NMRI mice. The NMRI strain is not strictly inbred but sufficiently homogenous to permit subsyngeneic graft survival for weeks without immunosuppression. Allogeneic transplantations were from non-inbred Umeå-*ob/ob* to NMRI mice.

Grafts remained in the kidney for less than 1–21 weeks, after which time they were removed. About 80 % of each graft was recovered by peeling off the kidney capsule, to which the graft adhered. The remaining 20 % on the bare renal surface had to be microsurgically loosened together with a minute slice of the cortex. The two portions of the transplant were placed together in a perifusion device that was housed in an infant incubator kept at 37 °C. They were perifused at a rate of 1 ml/min with Krebs-Ringer bicarbonate buffer (pH 7.4) continuously gassed with 95 % oxygen and 5 % carbon dioxide and supplemented with 20 mmol/l hepes (N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid) and 1 mg/ml bovine serum albumin. The perifusate also contained D-glucose and other modifiers of insulin release as required.

The perifused grafts were extracted by ultrasonication in acid ethanol and assayed for their insulin content. Control experiments with insulin added to kidney homogenates showed that the contamination with renal tissue constituted no significant source of error. Insulin was assayed radioimmunologically with crystalline mouse insulin as standard.

Statistical analysis

Degrees of significance stated in the text (p values) were derived from two-tailed t-tests of paired data obtained in parallel experiments.

Results

Effects of in vivo treatments. When syngeneic (C57BL/6J, BALB/cJ) or subsyngeneic (NMRI) grafts were removed from the kidney after 1–3

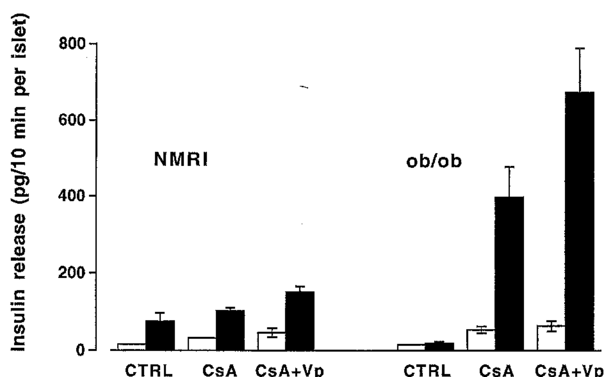

Fig. 2. Glucose-induced insulin release from 3-week-old islet grafts recovered from hosts treated with cyclosporin A (CsA) or cyclosporin A in combination with verapamil (CsA + Vp); controls (CTRL) received the vehicle for cyclosporin. The graft-bearing hosts were NMRI-mice transplanted with subsyngeneic NMRI islets (left side) or allogeneic islets from obese-hyperglycaemic Umeå-*ob/ob*-mice (right side). Columns indicate insulin output during 10 min at 2.8 mmol/l glucose (open columns) or during the subsequent 10 min of stimulation with 16.7 mmol/l glucose (solid columns). Thin bars indicate SEM for 5–8 experiments

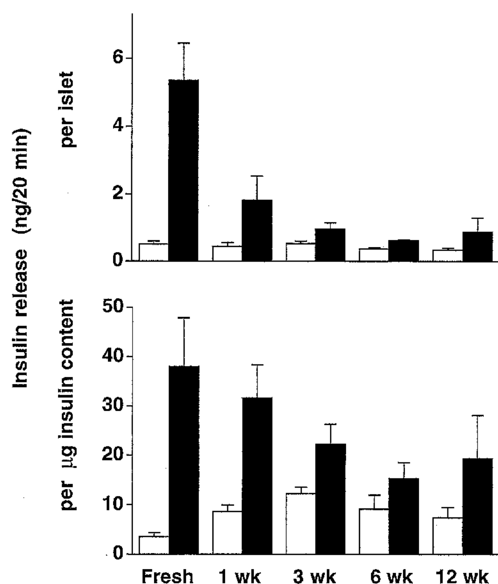

Fig. 3. Cholinergic potentiation of glucose-induced insulin release from fresh BALB/cJ-mouse islets or syngeneic islet grafts 1–12 weeks after transplantation. Results are expressed per number of islets transplanted (top panel), or per µg of insulin extracted from the grafts (bottom panel). Columns indicate insulin output during the first stimulatory 20-min pulse at 11 mmol/l glucose alone (open columns) or during the second 20-min pulse at 11 mmol/l glucose in combination with 10 µmol/l acetylcholine and 10 µmol/l eserine. Thin bars denote SEM for four to six experiments (fresh, 1 and 3 weeks) or the range of two experiments (6 and 12 weeks)

weeks, they contained about 20–30 % of the amount of insulin in fresh transplants. In allografts, the recovery of insulin after 3 weeks was only about 2 %. Immunosuppression of the graft-bearing mice by daily injections of cyclosporin A (15–25 mg/kg body weight) for 3 weeks markedly improved the insulin recovery in allografts (23 %) but not in subsyngeneic NMRI grafts.

Figure 1 illustrates the dynamics of glucose-induced insulin release from syngeneic and subsyngeneic grafts 1–21 weeks after transplantation. Regardless of the mouse strain, the first phase of secretion generally appeared diminished in comparison with the second phase.

The secretory dynamics in 3-week-old subsyngeneic NMRI grafts were about the same whether or not the transplanted mice had been treated with cyclosporin A. However, when graft-bearing NMRI-mice received daily injections of the calcium antagonist, verapamil (0.4 mg/kg mouse), in addition to cyclosporin A, the secretory response of both subsyngeneic ($p < 0.025$) and allogeneic ($p < 0.001$) grafts was significantly enhanced in comparison to cyclosporin alone (Fig. 2).

Responses to caffeine. When 1 or 5 mmol/l caffeine was included in the perifusion medium, there was a marked potentiation of the glucose-induced insulin release from 3-week-old subsyngeneic NMRI grafts (not shown). Both the early (initial 10 min) and late (subsequent 50 min) phases of the glucose-induced secretion were potentiated in the grafts, which appeared at least as responsive to caffeine as fresh islets.

Responses to acetylcholine. To perform experiments in which each transplant served as its own control, we exposed perifused grafts or fresh islets to repeated 20-min pulses of the same stimulatory concentration of glucose. The two stimulatory glucose pulses were separated by 20 min of basal secretion at 2 mmol/l glucose. During the second stimulatory pulse, the perifusion medium also contained 10 µmol/l acetylcholine and 10 µmol/l eserine.

The integrated insulin output during the two stimulatory pulses with 11 mmol/l glucose is shown in Figure 3. In fresh BALB/cJ islets, the presence of acetylcholine during the second pulse markedly potentiated the secretory response. In syngeneic grafts, the potentiating action of acetylcholine was gradually lost with transplantation time. The responsiveness to acetylcholine was clearly diminished even in 1-week-old grafts, was even less after 3 weeks, and did not reappear in 12 weeks.

Similar results were obtained in 3-week-old grafts when the glucose concentration during the stimulatory pulses was 17 mmol/l. The diminished responsiveness to acetylcholine could not be reversed by including caffeine in the perifusion medium (not shown).

Responses to noradrenaline. The technique of repeated glucose stimulations was also used to test the inhibitory effect of noradrenaline (Fig. 4). In fresh

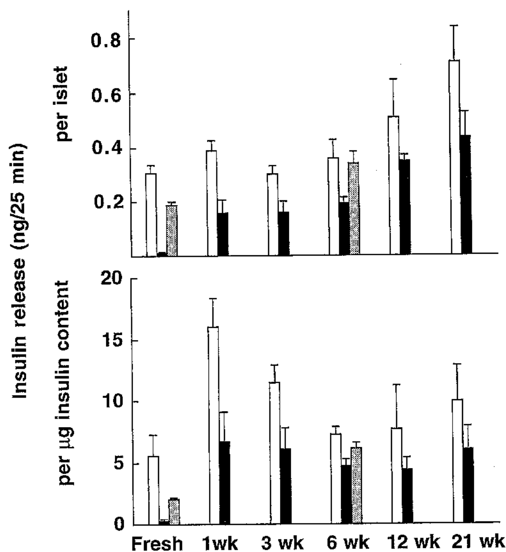

Fig. 4. Noradrenaline-induced inhibition of glucose-induced insulin release from fresh C57BL/6J mouse islets or syngeneic grafts 1–21 weeks after transplantation. Results are expressed per number of islets (top panel) or per µg of insulin extracted from the grafts (bottom panel). Columns indicate insulin output during the first 25-min stimulatory pulse at 17 mmol/l glucose alone (open columns) or during the second pulse at 17 mmol/l glucose in combination with noradrenaline (solid columns: 2.5 µmol/l; stippled columns: 0.25 µmol/l). Thin bars denote the range of two experiments (3 weeks; fresh islets with 0.25 µmol/l noradrenaline) or SEM of three to four experiments (all others)

C57BL/6J islets, the stimulatory action of 17 mmol/l glucose was inhibited almost completely by 2.5 µmol/l noradrenaline, and partially by 0.25 µmol/l. In contrast, syngeneic grafts, 1–21 weeks old, showed only modest inibitory responses to 2.5 µmol/l noradrenaline. When tested in 6-week-old grafts, 0.25 µmol/l noradrenaline caused virtually no inhibition of the glucose-induced insulin release.

Discussion

Korsgren et al. [4] perfused the kidney to study insulin release from islets transplanted under the renal capsule. Despite the technical elegance of that method, we have chosen to perifuse the islet grafts after removing them from the transplantation site. With this approach we hoped to avoid the risk of confusing any pharmacological effects on the kidney with those on the beta cells per se.

The results indicate that transplantion of the islets from pancreas to kidney induced several alterations in beta-cell function. The disappearance of insulin from allogeneic transplants is, of course, understandable in terms of immunological attack. The reason why syngeneic and subsyngeneic grafts also lost insulin in comparison with fresh islets is less clear. Quantitative morphometric measurements are needed to decide the extent to which the syngeneic beta cells disappeared or were merely degranulated.

With regard to the need for immunosuppression in the clinical context, it is noteworthy that verapamil improved the secretory capacity of both subsyngeneic and allogeneic grafts exposed to cyclosporin A. This effect of verapamil may be an indirect consequence of its action on the kidney, providing a healthier transplantation site than treatment with cyclosporin alone. Verapamil is known to prevent inhibitory effects of cyclosporin A on cortical blood flow [5] and on the ingrowth of vessels into islets transplanted to the subcapsular space [6]. In contrast, verapamil failed to interact noticeably with cyclosporin A during culture of isolated islets [7].

Syngeneic and subsyngeneic grafts from hosts without immunosuppression exhibited alterations of the glucose-induced insulin release pattern, with a comparatively modest first peak of secretion. The mechanism underlying this change is so far unknown. The fact that caffeine markedly potentiated both the early and late phases of glucose-induced secretion indicates that the grafted islets maintained a well-functioning machinery for the distal events of insulin discharge.

In contrast to caffeine, acetylcholine was a less effective potentiator in grafted as compared to fresh islets. This consequence of transplantation was evident when each graft was used as its own control, and regardless of whether the secretory rates were expressed per number of islets transplanted or per unit of insulin remaining in the grafts. This finding was unexpected, as we had anticipated the beta-cell acetylcholine receptors to be upregulated following severing of the vagus nerve. However, it is too early to decide at which level of the stimulus-secretion signal chain the decrease of cholinergic responsiveness occurred.

That a diminished responsiveness to physiological regulators may need to be taken into account as a more general phenomenon in transplanted islets is suggested by the results obtained with noradrenaline. The denervated islet grafts showed no hyperreaction to this transmittor either, but were clearly less responsive to catecholamine-induced inhibition than fresh islets.

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