Effects of Hyperglycemia on Function of Isolated Mouse Pancreatic Islets Transplanted Under Kidney Capsule

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The insulin release from isolated pancreatic islets grafted under the kidney capsule was examined by means of a modified kidney-perfusion technique. The grafts, consisting of 150 C57BL/6 or 250 C57BL/Ks mouse islets, were implanted syngeneically under the left kidney capsule of normoglycemic or alloxan-induced diabetic recipients 4 wk before the perfusion. In both mouse strains, islets grafted to normoglycemic animals showed an immediate distinct peak of insulin release when challenged with high glucose, whereas no response was observed from islets grafted to hyperglycemic mice. In a similar way in C57BL/Ks mice, arginine stimulated insulin release from the islet grafts in normoglycemic but not in hyperglycemic recipients. Insulin treatment of the diabetic recipients, however, partially normalized the insulin response to glucose. Islet grafts were removed in toto and analyzed for contents of insulin, glucagon, somatostatin, and DNA or rates of glucose-stimulated (pro)insulin biosynthesis. In both mouse strains, islets implanted into hyperglycemic animals contained significantly less insulin, and their rates of (pro)insulin biosynthesis were markedly decreased. Insulin treatment only marginally affected these parameters. The glucagon content of the grafted islets was unaffected by the hyperglycemia in both strains of mice, whereas a significant decrease in the somatostatin content was observed in the C57BL/Ks mice. We concluded that grafted islets exposed to prolonged hyperglycemic stress become functionally impaired in mice of both strains. Our perfusion technique of islet-graft–bearing kidneys in combination with biochemical studies on the removed grafts provides a suitable model for studies of the effects of prolonged hyperglycemia on islet β-cell function
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Exposure to hyperglycemia for as short a period as 48 h seems to induce an impairment of glucose-induced insulin release from pancreatic islets (1). These findings are notable in view of the possible therapeutic potential of transplantation of isolated islets as a cure for diabetes mellitus, although glucose homeostasis probably will not be completely normalized immediately after transplantation. We were therefore interested in developing an animal model in which the function of transplanted islets can be evaluated after various periods of hyperglycemia. The aim of our investigation was to use a perfusion technique (2,3) to study the time course of insulin release from syngeneic islets grafted under the kidney capsule of diabetic mice of different strains. Furthermore, (pro)insulin biosynthesis and the hormone and DNA contents of the transplanted islets were evaluated in vitro after removal of the graft. By varying the number of implanted islets and the postimplant therapeutic program of the recipients, the grafts could be subjected to different periods and degrees of hyperglycemia after implantation.

MATERIALS AND METHODS

Animals. Male and female inbred C57BL/6 and C57BL/Ks mice aged 3–5 mo were used as islet donors. Syngeneic males of the same age and strain served as recipients. Some of the animals had been made diabetic by an intravenous injection of alloxan (ALX; 75 mg/kg body wt; Sigma, St. Louis, MO) 7 days before transplantation. All animals had free access to tap water and pelleted food (type R3; Ewos, Anticimex, Södertälje, Sweden) throughout the experiment.

Islet isolation and transplantation. Pancreatic islets were prepared by a collagenase (Boehringer Mannheim, Mannheim, FRG)-digestion method from overnight-fasted mice (4). Groups of ~150 islets were cultured free floating for

Glucagon 1 ng/L = 1 pg/ml	Somatostatin 1 pM = 1.64 pg/ml
Insulin 1 pM = 0.139 μU/ml	

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1–2 days in RPMI-1640 medium (Flow, Irvine, UK) supplemented with 10% (vol/vol) calf serum (Statens, Stockholm) as previously described (5). After culture, 150 C57BL/6 or 250 C57BL/Ks mouse islets were implanted beneath the kidney capsule of ether-anesthetized mice of the same strain as previously described (6).

Five different recipient groups were designated as follows: *1*) C57BL/Ks mice transplanted with 250 islets and otherwise untreated; *2*) ALX-induced diabetic (ALX-D) C57BL/Ks mice transplanted with 250 islets; *3*) ALX-D C57BL/Ks mice transplanted with 250 islets, and thereafter treated with insulin injections (800 mU/day ultralente; Novo, Copenhagen); *4*) C57BL/6 mice transplanted with 150 islets and otherwise untreated; *5*) ALX-D C57BL/6 mice just transplanted with 150 islets.

The reason for the difference in the number of grafted islets between the two strains of mice was that no more than 150 islets could be given to ALX-D C57BL/6 mice without reversing their hyperglycemia (unpublished observations). Blood samples for glucose determinations were collected by puncture of the retro-orbital venous plexa immediately before the islet transplantation and at the end of the observation period 4 wk later. The glucose contents of the samples were determined with an automated glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA).

Perfusion of graft-bearing kidneys. Four weeks after transplantation, animals intended for insulin-release experiments were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (130 mg/kg body wt; Inactin Byk; Byk Gulden, Konstanz, FRG). The abdominal cavity was opened, and the intestines were moved to the right side of the animal and wrapped in moist compresses. This exposed the abdominal portions of the aorta and the inferior caval vein, which were dissected free from surrounding connective tissue by gentle rubbing with dry gauze. All branches from these vessels were ligated and cut, with the exception of the left renal artery and vein. A ligature was then tied around the aorta and the caval vein above the origin of the left renal vessels. A polyethylene catheter (inner diam 0.58 mm) connected to a peristaltic pump (Minipuls II; Gilson, Villiers-le-Bel, France) was inserted into the lower part of the abdominal aorta. The left renal vein was cut to make it possible for the perfusion medium to flow freely through the kidney. Also the left ureter was cut to avoid stasis of urine within the kidney preparation. A schematic outline of the preparation at this stage is given in Fig. 1.

The structures indicated in Fig. 1 were removed and placed in a thermostated funnel (37°C). The organ preparation was then perfused 7 (1.5 ml/min) with a bicarbonate buffer (7) supplemented with 10 mM HEPES (Kebo, Stockholm), 20 mg/ml bovine serum albumin (BSA, fraction V; Miles, Slough, UK) and 20 mg/ml dextran T70 (Pharmacia, Uppsala, Sweden). The perfusion medium was kept at 37°C and continuously gassed with 5% CO_2/95% O_2. The experiments started with 15 min of perfusion with a medium containing 2.8 mM glucose, followed by 30-min perfusion with 16.7 mM glucose and, finally, 10 min with 2.8 mM glucose. Alternatively the graft-bearing kidneys of nine animals in groups 1 and 2 were perfused with a medium containing 2.8 mM glucose for 15 min, and 8.4 mM glucose and 20 mM L-arginine (Sigma) for 20 min and, finally, 10 min with 2.8 mM

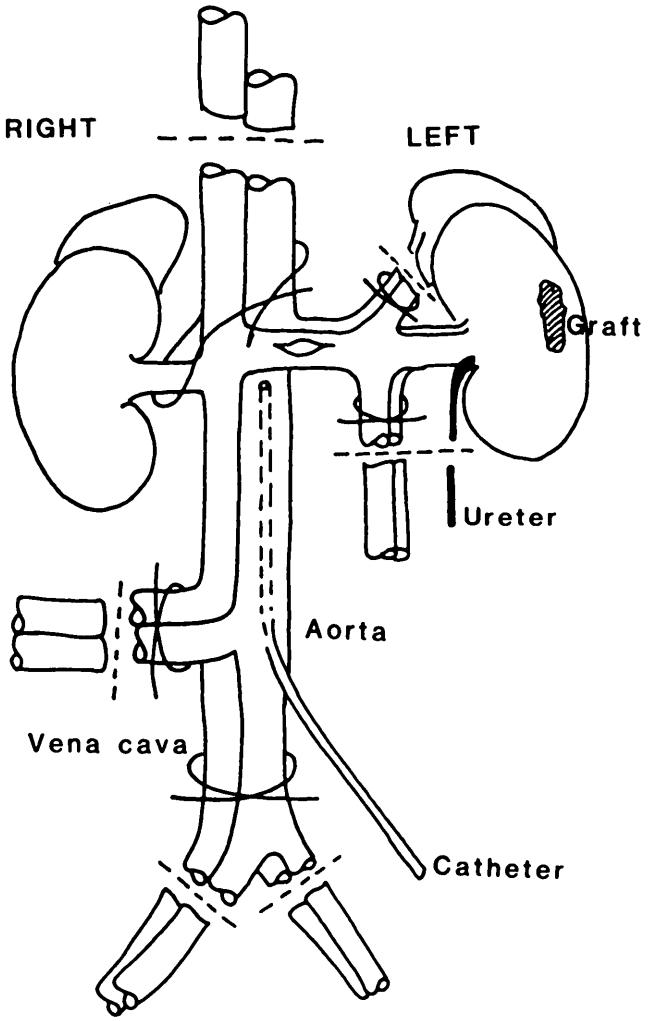

FIG. 1. Schema of preparation of graft-bearing kidney before perfusion. Polyethylene catheter was inserted into abdominal aorta. Before this, all vessels except left renal vessels had been ligated and cut. To allow perfusion medium to pass freely through kidney, an incision was made in renal vein. Throughout experiments, preparation was kept at 37°C.

glucose. A 1.5 ml sample of the effluent medium was collected every 5 min, except for the first 10 min of perfusion with the high glucose concentration or arginine, when samples were taken after 1–5, 7, and 10 min. The insulin content of these samples was measured by radioimmunoassay (8).

Light-microscopic examination. After perfusion, the kidney was dissected free from blood vessels and fat. The islet graft was clearly visible as a whitish spot under the connective-tissue capsule. This graft was excised with a margin of ~3 mm, fixed in Bouin's solution, and embedded in paraffin. Sections 7 μm thick were stained with hematoxylin and eosin and examined in the light microscope for the presence of an islet graft. Some of the sections were stained for the presence of insulin-, glucagon-, somatostatin- and pancreatic polypeptide (PP)-containing cells by the peroxidase-antiperoxidase (PAP) technique (9). The antibodies were obtained from Bio-Yeda (Rehovot, Israel; insulin), Novo (glucagon), and Dakopatts (Glostrup, Denmark; somatostatin and PP). The second antibody and the PAP complex were from Dakopatts. The specificity of the immunostaining was tested by the methods of Goldman (10) and included omis-

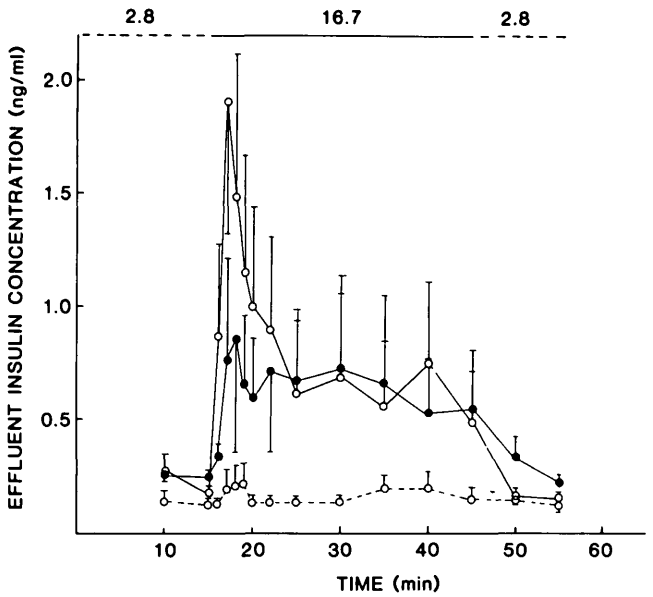

FIG. 2. Insulin concentrations in effluent medium collected from islet-graft–bearing kidneys of C57BL/Ks mice. Kidneys were perfused with medium containing 2.8 or 16.7 mM of glucose as indicated at *top*. Four weeks before perfusion experiments, 250 islets from syngeneic donors were implanted under capsule of left kidney. ○, Normoglycemic mice with intact islets in pancreas (n = 5); *broken line*, alloxan-induced diabetic mice (ALX-D), hyperglycemic mice (n = 9); ●, insulin-treated ALX-D mice (n = 5).

sion of the primary antibody and application of the primary antibody blocked with the corresponding islet hormone.

Preparation of the graft for biochemical studies. Animals not used for perfusion studies were killed 4 wk after the transplantation. The graft-bearing kidney was removed, placed in Hanks' solution, and freed from fat to expose the islet graft. The connective-tissue capsule immediately surrounding the transplant was incised and gently removed from the kidney parenchyma. Because the transplant was firmly adherent to the capsule, the graft was simultaneously removed in toto from the kidney. The preparation was placed under a stereomicroscope to facilitate the removal of the kidney capsule not covering the islet graft. The transplant and the covering kidney capsule were then manually divided into two parts of approximately the same size for further processing.

(Pro)insulin biosynthesis in islet grafts. The dissected grafts were incubated at 37°C in 100 μl of a bicarbonate buffer supplemented with 10 mM HEPES, 2 mg/ml BSA, 16.7 mM glucose, and 100 μCi/ml L-[4,5-^{3}H]leucine (Amersham, Amersham, UK) in humidified air containing 5% CO_2 (7). The two pieces of grafted islets were incubated for 1 or 4 h, respectively. The tissue was then washed in Hanks' solution supplemented with 10 mM nonradioactive leucine, followed by homogenization in 100 μl of redistilled water, and washing of the vial three times, each time with 100 μl redistilled water. The homogenate (400 μl) was then sonicated to disrupt any remaining intact cells. The amount of newly synthesized (pro)insulin was determined by measurements of [^{3}H]leucine incorporation into immunoprecipitated proinsulin (11). The total protein biosynthesis was measured by assay of [^{3}H]leucine incorporation into trichloroacetic acid precipitates.

Hormone and DNA content of islet grafts. A sample of 50 μl was removed from the homogenate used for the determinations of (pro)insulin biosynthesis, and transferred to tubes containing 125 μl acid-ethanol [0.18 M HCl in 95% (vol/vol) ethanol]. The samples were extracted overnight at 4°C, followed by radioimmunological assay of insulin (8), glucagon (glucagon kit no. RA-310, Milab AB, Malmö, Sweden), and somatostatin contents (somatostatin kit no. RA-306, Milab AB). Duplicate samples of 40 μl were analyzed by fluorophotometry for DNA content (12,13).

Statistical calculations. All values are means ± SE. P values were calculated by Student's two-tailed t test.

RESULTS

All ALX-D animals had serum glucose concentrations exceeding 20 mM at the time of transplantation. The mice not treated with ALX had serum glucose concentrations between 8 and 10 mM both at the time of transplantation and at the end of the transplantation period (data not shown). Transplantation of 250 islets failed to normalize the serum glucose concentration in the ALX-D C57BL/Ks mice (30.5 ± 1.4 mM; n = 21). Likewise, 150 islets transplanted to diabetic C57BL/6 mice did not reverse the diabetic state at the time of the perfusion studies (29.9 ± 3.4 mM; n = 11). The insulin-treated ALX-D C57BL/Ks mice showed an intermediate serum glucose concentration (14.8 ± 3.0 mM; n = 12) when sampled 20 h after the latest insulin injection. Microscopic examinations demonstrated islet grafts in all animals 4 wk after the transplantation. Immunocytochemical staining confirmed the presence of cells containing each of the islet hormones within the grafts of both normoglycemic and di-

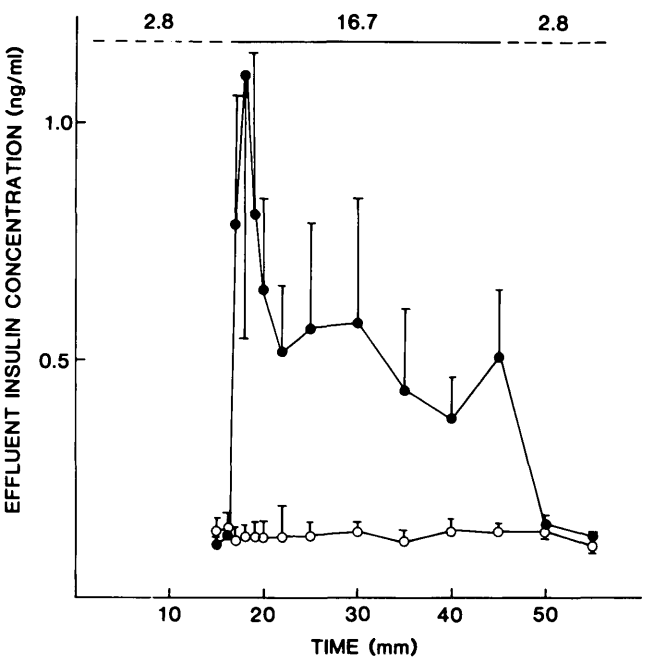

FIG. 3. Insulin concentrations in effluent medium collected from islet-graft–bearing kidneys of C57BL/6 mice. Kidneys were perfused with medium containing either 2.8 or 16.7 mM glucose as indicated at *top*. Four weeks before perfusion experiments, 150 islets from syngeneic donors were implanted under capsule of left kidney. ●, Insulin values in medium collected from grafts of normoglycemic mice (n = 5); ○, hyperglycemic animals (n = 4).

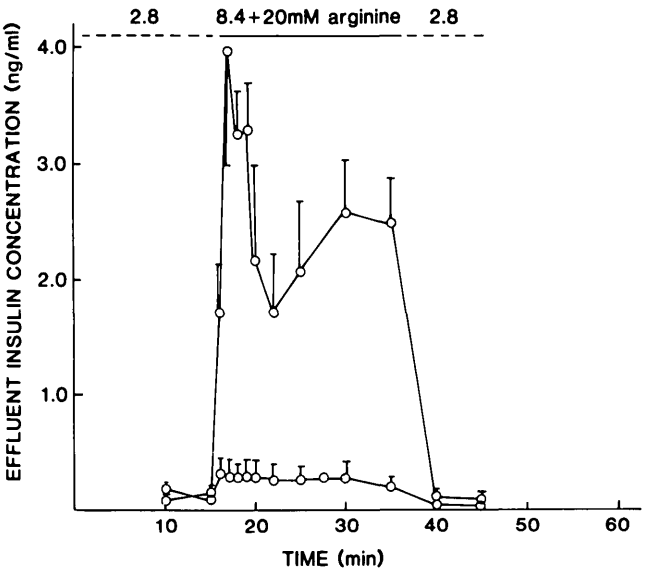

FIG. 4. Insulin concentrations in effluent medium collected from islet-graft–bearing kidneys of C57BL/Ks mice. Kidneys were perfused with medium containing 2.8 mM glucose or 8.4 mM glucose + 20 mM arginine as indicated at *top*. Four weeks before perfusion experiments, 250 syngeneic islets were implanted under capsule of left kidney. *Top curve*, normoglycemic animals (n = 4); *bottom curve*, alloxan-induced diabetic hyperglycemic animals (n = 5).

abetic animals in proportions comparable to those of native islets.

Perfusion experiments. A distinct first peak of insulin release in response to a glucose stimulus was observed during the first 5–7 min of perfusion from the graft-bearing kidneys in all normoglycemic animals (both strains), followed by an elevated and constant second phase, which promptly returned to basal levels when the glucose stimulus was removed (Figs. 2 and 3). The grafts of ALX-D animals transplanted with 150 C57BL/6) or 250 C57BL/Ks mouse islets, i.e., an insufficient amount to reverse hyperglycemia, showed no stimulation of insulin release from their grafts in response to glucose during the perfusion experiments (Figs. 2 and 3). Insulin treatment of the ALX-D C57BL/Ks mice partially normalized the insulin response to glucose (Fig. 2).

The insulin response to a stimulation with 20 mM arginine plus 8.4 mM glucose was pronounced in normoglycemic C57BL/Ks mice, with a more sustained elevation of insulin release than after glucose stimulation (Fig. 4). When grafts located in hyperglycemic mice were challenged with arginine, however, no response was observed (Fig 4).

(Pro)insulin biosynthesis. Normoglycemic animals of both strains were found to have a (pro)insulin biosynthesis of the same order of magnitude (Tables 1 and 2). After incubation for 1 h, there was a significant decrease in the (pro)insulin biosynthesis in the hyperglycemic animals, and insulin treatment of the ALX-D C57BL/Ks mice did not restore this impairment. After 4 h of incubation, however, the differences were smaller, although still significant (Tables 1 and 2).

DNA and hormone contents. No significant differences in the DNA contents of the islet grafts were observed between normoglycemic and ALX-D, non-insulin-treated recipients in any of the strains (Tables 1 and 2). The grafts removed from the insulin-treated ALX-D C57BL/Ks mice were significantly larger than the grafts removed from the normoglycemic mice. The insulin contents were markedly reduced in the diabetic mice of both strains (Tables 1 and 2). Insulin treatment of the ALX-D C57BL/Ks mice caused only a minor increase in the insulin content of the islet graft (Table 1). The islet-graft glucagon content was similar in all recipients. The somatostatin content was, however, significantly decreased in the islet grafts of the hyperglycemic C57BL/Ks mice. This was, however, not the case in the C57BL/6 mice.

DISCUSSION

Our technique of perfusing an islet graft is suitable for a study of the dynamics of islet hormone release after addition of different secretagogues to the perfusion medium. More detailed information was obtained than in previous functional evaluations based on estimates of the blood glucose concentrations of diabetic animals subjected to islet transplantation. This technique also offers an alternative to previous methods used for studying the long-term effects of hyper-

TABLE 1
(Pro)insulin biosynthesis and hormone contents of pancreatic islet grafts 4 wk after transplantation beneath renal capsule of C57BL/Ks mice

	Normoglycemic (n = 5)	Insulin-treated ALX-D (n = 7)	ALX-D (n = 7)
(Pro)insulin biosynthesis (10^3 dpm/μg DNA)			
1-h incubation	6.7 ± 0.9	1.6 ± 0.4†	1.3 ± 0.4†
4-h incubation	16.3 ± 3.9	6.2 ± 1.4*	6.3 ± 1.5*
(Pro)insulin biosynthesis (% of total protein synthesis)			
1-h incubation	20.0 ± 0.6	12.7 ± 2.6*	9.9 ± 3.0*
4-h incubation	13.4 ± 2.5	10.1 ± 1.7	10.6 ± 1.7
Graft content			
Insulin (ng/μg-DNA)	705 ± 65	152 ± 59†	30.2 ± 4.9†
Glucagon (ng/μg DNA)	11.5 ± 1.0	11.4 ± 1.4	14.8 ± 2.1
Somatostatin (ng/μg DNA)	1.5 ± 0.1	0.5 ± 0.1†	0.6 ± 0.1†
DNA (μg)	4.9 ± 0.4	7.3 ± 0.7*	7.4 ± 0.9

Values are means ± SE. The grafts (250 islets) were removed from normoglycemic or insulin-treated alloxan-induced diabetic (ALX-D) or nontreated ALX-D mice of the same strain. Fragments of the grafts were incubated for 1 or 4 h in a Krebs-Ringer bicarbonate buffer supplemented with 10 mM HEPES, 2 mg/ml bovine serum albumin, 16.7 mM D-glucose, and 100 μCi/ml of L-[4,5-^{3}H]leucine. The hormone contents were determined by radioimmunoassay of acid-ethanol extracts.
*P < .05, †P < .001, vs. the corresponding value for normoglycemic animals.

TABLE 2
(Pro)insulin biosynthesis and hormone contents of pancreatic islet grafts (150 islets) 4 wk after transplantation beneath renal capsule of C57BL/6 mice

	Normoglycemic (n = 7)	Alloxan-induced diabetic (n = 7)
(Pro)insulin biosynthesis (10^3 dpm/μg DNA)		
1 h	11.8 ± 2.9	2.9 ± 0.7*
4 h	30.8 ± 5.6	18.8 ± 4.4
(Pro)insulin biosynthesis (% of total protein synthesis)		
1 h	18.0 ± 1.4	6.0 ± 1.4†
4 h	15.7 ± 1.9	8.2 ± 3.0*
Graft content		
Insulin (ng/μg DNA)	1220 ± 93	78.0 ± 11†
Glucagon (ng/μg DNA)	21 ± 2	38 ± 9
Somatostatin (ng/μg DNA)	1.1 ± 0.2	2.2 ± 0.7
DNA (μg)	2.4 ± 0.3	2.3 ± 0.1

Values are means ± SE. The animals were normoglycemic or diabetic throughout the experiment. For further details, see Table 1.
*$P < .05$, †$P < .001$, vs. normoglycemic animals.

glycemia on β-cell function in that it precludes the use of cytotoxic drugs in the neonatal period (14,15), the need for extensive pancreatectomies (16), or the use of long-term glucose infusions (1,17).

In this study, there was a distinct peak of insulin release in response to a glucose challenge in all normoglycemic animals, indicating that the graft was revascularized after 4 wk. This is further supported by both morphological (unpublished observations) and functional studies of the blood flow of renal subcapsular islet grafts in rats (18) and mice (19). Moreover, the magnitude of insulin response of the transplanted islets was similar to that of native islets in perfused mouse pancreases (unpublished observations), which suggests that no derangement of the transplanted endocrine cells had taken place and that cellular interactions essential for normal islet function were unaffected by the grafting procedure (20). Also, the islet hormone contents of the grafts were comparable to those of isolated islets obtained from animals of the same strains (unpublished observations).

When islets were transplanted to ALX-D mice that remained hyperglycemic, no significant insulin secretion in response to a glucose challenge was observed. These findings of an adverse effect on the endocrine pancreas of a lasting hyperglycemia and the partial or complete reversal after insulin treatment are in line with previous observations (for review, see ref. 21). At variance with previous observations in the perfused pancreas (17,21), we observed a blunted insulin response also to stimulation with arginine. This may be explained by the longer period of a more severe hyperglycemia to which the β-cells were exposed in this study. The amount of stored insulin may be of significance in this context, because there was a marked decrease of the insulin content in the grafted islets of the hyperglycemic mice.

The islet grafts of the hyperglycemic animals also seemed to have a lower glucose-stimulated (pro)insulin biosynthesis. This observation may, however, simply reflect an increased rate of insulin turnover in these heavily degranulated β-cells. This interpretation is supported by the findings that the differences of the 4-h (pro)insulin biosynthesis values were reduced between the different treatment groups. By contrast, Halban et al. (22) demonstrated elevated (pro)insulin biosynthesis in vitro from a rat model of non-insulin-dependent diabetes mellitus. It is, however, plausible that this discrepancy is due both to inherent differences between the two models and to the degree of hyperglycemic stress to which the islets had been exposed. Thus, the islets in the grafted mice in our study were exposed to concentrations of ~30 mM glucose, whereas those of the neonatally streptozocin-injected rats were usually facing concentrations of ~10–12 mM glucose. The β-cells of the latter islets were less depleted of insulin stores, and a high glucose concentration was able to stimulate insulin release.

To support the notion that in vitro (pro)insulin biosynthesis can adapt to variations in glucose concentrations, Orland et al. (23) showed that proinsulin mRNA was elevated 3 wk after 90% pancreatectomy. However, after 14 wk, when fasting hyperglycemia was evident, both total proinsulin mRNA and the pancreatic insulin content were severely reduced. This is in agreement with previous in vitro reports indicating that high glucose concentrations do not adversely affect the (pro)insulin biosynthesis (24,25). Thus, there seems to be a considerable initial capacity for an adaptive response of (pro)insulin biosynthesis to different functional demands, but longer periods of hyperglycemia impair this response. However, whether the derangements of β-cells exposed to high glucose concentrations for a prolonged period can be reversed remains to be established.

One factor that may determine the failure of the functionally stressed β-cells is their genetic constitution. For example, similar diabetic syndromes are produced when the two single gene mutations for obesity (*ob*) and diabetes (*db*) are expressed on the same inbred background (C57BL/6), whereas on another background (C57BL/Ks), the syndrome changes from a severe-obesity moderate diabetes to a severe lethal form because of uncompensated β-cell damage (26). A difference in replicatory capacity between islet cells of these two different mouse strains when exposed to hyperglycemia of shorter duration has previously been demonstrated (27–30). Islet cells of C57BL/6 responded with an increased DNA replication, as evidenced by autoradiography when grafted into diabetic recipients, whereas C57BL/Ks islets did not (29). This could at least partially explain why 250 islets did not cure the ALX-D C57BL/Ks mice, which sometimes turned out to be the case with C57BL/6 mice (unpublished observations). Therefore, to not reverse the hyperglycemia in the latter strain, no more than 150 islets

could be given. In contradiction to a previous study in our laboratory, the islets grafted into the ALX-D C57BL/Ks mice did not decrease in size (28). If anything, they tended to grow, which might be explained by the renal subcapsular transplantation site used in this study. This implantation site has recently been shown to offer better growth conditions for grafted islets compared with the splenic site (6). Except for a decreased islet graft somatostatin content, the functional properties of the C57BL/Ks islets did not differ from those of the C57BL/6 islets. This result may indicate that the islet β-cells of these two mouse strains show a similar adaptive response to the hyperglycemic environment from both a functional and metabolic point of view.

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