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Recovery of Function and Mass of Endogenous β-Cells in Streptozotocin-Induced Diabetic Rats Treated with Islet Transplantation

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Islet transplantation corrects chronic hyperglycemia by augmentation of insulin supply from the graft tissue, but the role of endogenous β-cells after transplantation is not clear. In the present study, we examined endogenous β-cell function after glucose homeostasis had been reestablished by islet graft in streptozotocin (STZ)-induced diabetic rats. Fed plasma glucose levels in diabetic rats transplanted with a large number of islets (2500 islets) into the left kidney capsule soon became lower (139.8 ± 8.2 mg/dl) and close to the level in controls (129.7 ± 11.3 mg/dl), and IPGTT exhibited a pattern of plasma glucose response almost identical to control. The insulin and DNA contents, islet area, and the distribution of β-cells that were markedly deteriorated in islets of STZ rats were significantly restored in transplanted rats. The insulin release in response to glucose or α-ketoisocaproate was less in STZ rats, while in islets of transplanted rats the secretion recovered to levels similar to controls. On the other hand, arginine-induced insulin release was conversely hyperresponsive in STZ rats, but in transplanted rats, the response was decreased similar to controls. Thus, as the plasma glucose level normalizes, residual β-cells show a recovery of function that cannot be accounted for by the increase in mass alone.

Key Words: **islet transplantation; streptozotocin (STZ); endogenous islet; β-cell function; β-cell mass; insulin secretion; α-ketoisocaproate (KIC); arginine; KCl.**

Islet transplantation for diabetic patients has been performed clinically since the late 1980s, but the outcome has been very poor. One reason is diminishing graft mass from immune attack and ischemia, another is deteriorating function of the graft due to the continuous exposure to hyperglycemia, which is known to impair glucose-stimulated insulin secretion (1–4). The therapeutic effect of normoglycemia on the maintenance of graft has been investigated in many laboratories (1, 5, 6), but there has been little study of the direct effect of islet transplantation on the function of endogenous islets in diabetic human and animal models. Previous studies have shown that the partial improvement of hyperglycemia achieved by insulin therapy and hypoglycemic agents including phlorizin and α-glucosidase inhibitor partially restores β-cell function or has a beneficial effect on β-cell mass in type 2 diabetic animals (7–11). Accordingly, by maintaining a continuously normoglycemic condition, islet transplantation might facilitate the restoration of insulin secretory capacity in endogenous islets.

In the present study, to determine the effect of long-term normoglycemia on endogenous residual β-cells, we examined the effect of islet transplantation therapy in STZ diabetic rats. The insulin secretion in response to glucose, α-ketoisocaproate (KIC), KCl and arginine in endogenous islets of control, STZ-diabetic, and transplanted rats was investigated. In addition, the size of the islets and β-cell mass were examined histologically in order to clarify the relationship between the number of β-cells and islet function.

MATERIALS AND METHODS

Animals and animal groups. Male Wistar ST rats (250 g) were divided into two groups (Control = group 1, STZ rats = group 2, 3), one made diabetic by a single injection of 35 mg/kg streptozotocin (STZ, Nacalai Tesque, Kyoto, Japan) freshly dissolved in an equivalent volume of 0.05 M citrate buffer (pH 4.5) through a tail vein. Diabetes was confirmed by the presence of hyperglycemia, polyuria, and body weight loss. Only those rats with fed plasma glucose higher than 250 mg/dl and lower than 450 mg/dl were used for the experi-

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ments. Twelve days after STZ injection, the diabetic rats were then divided into two groups (hyperglycemic STZ rats = group 2, transplanted rats = group 3), one (group 3) receiving islet transplantation from 250 g male Wistar ST rats under the left kidney capsule. On days 7 and 12 after STZ injection, and on days 3, 7, 14, 21, 25, and 29 after transplantation, fed plasma glucose was measured between 10:00 and 13:00 with a portable glucose meter (Glutest Ace, Kyotodaiichikagaku, Kyoto, Japan). Blood was obtained from the tail snipped by a fine blade. All rats were bred under conventional conditions in an air-conditioned room with free access to tap water and standard pelleted chow.

Islet isolation and islet transplantation. Syngeneic Wister ST rats were used as islet donors and recipients. To obtain islets for islet transplantation and insulin secretion experiments, the rats were anesthetized with pentobarbital (50 mg/kg ip) and the pancreatic islets were isolated by collagenase digestion as described previously (12). Implantation of islets was performed 12 days after STZ injection to STZ diabetic rats (group 3) as reported previously (1). Briefly, islets for transplantation were cultured overnight in RPMI 1640 medium supplemented with 11.1 mM glucose, after which ~2500 islets were packed in a capillary tube. Recipients were anesthetized by ketamin (80 mg/kg ip), and the left kidney was exposed through a lumber incision. A capsulotomy was performed in a lower pole of the kidney, and the capillary tube was advanced under the capsule to the upper pole, where the islets were injected. Control rats and STZ diabetic rats were sham-operated.

Intraperitoneal glucose tolerance test (IPGTT). IPGTT was performed on days 25–26 after transplantation (3–4 days before sacrifice) in all rats of all three groups. After overnight fast, unanaesthetized rats were injected with a 50% glucose solution (2 g · kg^{-1} body wt) intraperitonealy. Plasma glucose values were examined 0, 15, 30, 60, 90 and 120 min after glucose injection.

Insulin secretion from endogenous islets. Twenty-nine days after islet transplantation, insulin secretion experiments and histological evaluations were performed. Before laparotomy for insulin secretion experiments, blood samples were collected from the cardiac ventriculum percutaneously by a 23-gauge fine needle under anesthesia for determination of plasma glucose and plasma insulin. Insulin secretory capacity was determined by batch incubation method. Isolated islets were preincubated for 30 min at 37°C in Krebs-Ringer-bicarbonate buffer (KRB buffer pH 7.4; 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 24 mM $NaHCO_3$) supplemented with 3.3 mM glucose and 0.2% bovine serum albumin. After preincubation, batches of islets (n = 5) were challenged at 37°C for 30 min with KRB buffer containing various concentrations of glucose or KIC with/without 200 μM diazoxide and 30 mM KCl, and 19 mM arginine. At the end of incubation, the incubation media were collected for determination of insulin release during 30 min incubation, and were assayed by RIA using rat insulin (Novo Nordisk, Bagsvaert, Denmark) as standard as has been described previously (13).

Insulin content and DNA content measurement. After drawing of an aliquot of incubation medium for insulin assay, the residual 40 μl of the medium containing 5 islets was added to 160 μl of hypoosmotic 5 mM Hepes solution (total volume 200 μl) and sonicated to lyse islet cells for assay of insulin content and DNA content. They were then frozen at −20°C until evaluation. Insulin content was determined by RIA as described above. DNA content was determined by fluometric assays as described previously (14) using bisbenzimidazol (compound Hoechst 33258, Nacalai Tesque, Kyoto, Japan) as fluorochrome and calf thymus DNA (Sigma Co., St. Louis, MO) as standard.

Histology, islet, and β-cell mass evaluation. β-cell mass was determined by the percentage of β-cell area in each islet of endogenous pancreas as described previously (11). Quantification of islet and β-cell area was performed using NIH image freeware (version 1.59, Wayne Rasband, National Institute of Health). The pancreases of the three animal groups were embedded in paraffin and sliced into three 5 μm sections. Each section was insulin-stained, and 35 mm color slides of 10 islets of each group at a magnification of ×320 were prepared. Color images were obtained as TIFF pictures by a slide scanner (Polascan Ultra35, Polaroid, Tokyo, Japan), and the contours of the islets and non-β-cells were precisely cut out by the use of Paint shop pro Ver. 4.2 (Met's corporation, Tokyo, Japan). The files were then opened on gray-scale mode of the NIH Image, and the areas of the whole islet and of non-β-cells in randomly selected islets were measured. Whole islet area was determined by measuring 25 islets in each group and represented by the percentage of the average islet area of control rat pancreas. The percentage of β-cell area of a single islet was determined by calculating the ratio between β-cell area obtained by subtracting non-β-cell area from whole islet area and whole islet area (n = 10 for each).

Statistical analysis. Results are presented as mean values ± SE. Statistical significance was evaluated by unpaired Student's t test. $P < 0.05$ was considered significant.

RESULTS

Characteristics of the Three Rat Groups and the Effect of Islet Transplantation

The fed plasma glucose level in STZ rats immediately before transplantation (12 days after STZ injection) was 348.2 ± 38.5 mg/dl (n = 10). As shown in Fig. 1A, plasma glucose levels in transplanted rats were clearly decreased on the third day after transplantation, were completely normalized 7 days after transplantation, and remained normal until the final day. On the other hand, the plasma glucose levels in STZ rats from 19 days to 41 days after STZ injection remained higher than in nondiabetic control rats ($P < 0.01$) and in transplanted rats ($P < 0.01$). IPGTT revealed a pattern of plasma glucose level in transplanted rats almost identical to controls, while the plasma glucose levels in STZ rats were significantly higher at all of the times examined (Fig. 1B).

The body weight of STZ rats before sacrifice was significantly lower than that of control and transplanted rats. Extremely higher plasma glucose and very lower plasma insulin levels were observed in STZ rats; on the other hand, the plasma glucose and insulin level in transplanted rats also were similar to those in control (Table 1).

Histological Evaluation of Islet and β-Cell Mass

The insulin and DNA content in STZ rats significantly and considerably decreased; however, in transplanted rats they were significantly greater than those in STZ rats (Table 2). Morphological examination revealed fewer and smaller islets, and an obviously diminished number of cells in each islet accompanied with a relative increase of the non-β-cell area in STZ rats. On the other hand, each islet in transplanted rats showed an increase in both islet and β-cell area, although the extent did not reach that of controls. Numerical assessment was used to determine the degree of alteration of islet and β-cell mass in STZ rats and the recovery in transplanted rats (Table 2).

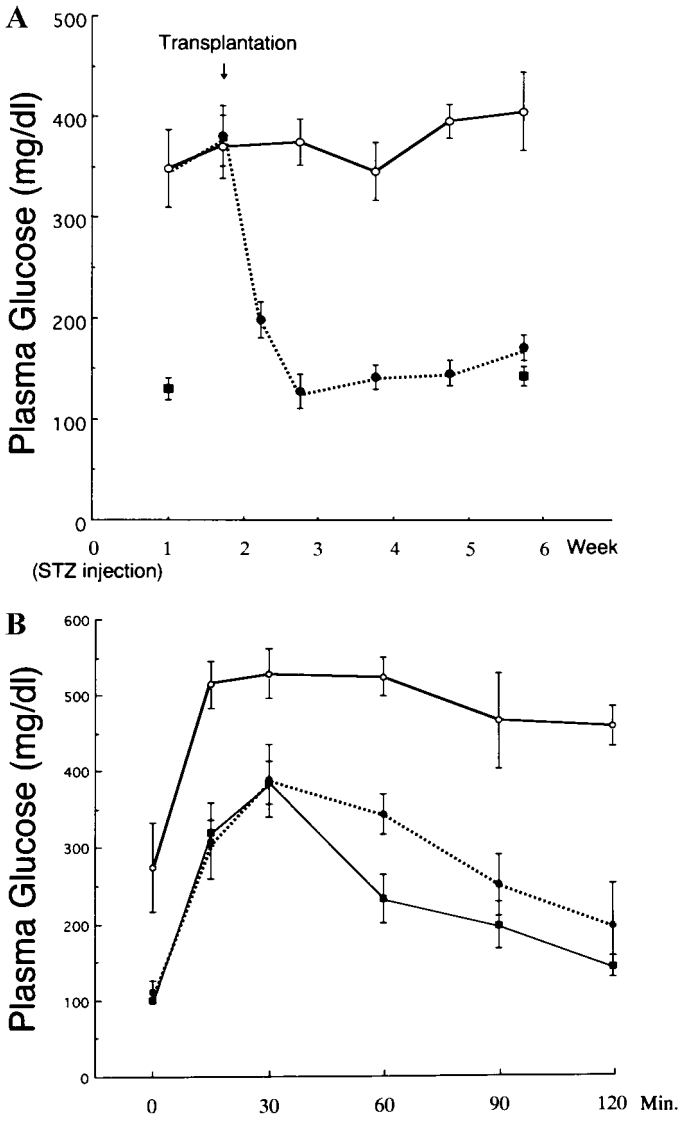

FIG. 1. Effect of islet transplantation on glucose tolerance. (A) Time course of fed plasma glucose after STZ injection (0 day) until sacrifice (41 days) in control (closed square), STZ (open circle), and transplanted rats (closed circle). The arrow indicates the day of transplantation. (B) Glucose tolerance test by IPGTT in control (closed square), STZ rats (open circle), and transplanted rats (closed circle).

Insulin Secretion in Control, STZ, and Transplanted Rats

In STZ rats, the insulin secretory response to 16.7 mM glucose or 16.7 mM KIC was significantly less than in control; however, in islets of transplanted rats, insulin secretion in response to the secretagogues improved to close to control levels (Table 3). It has been reported that arginine fails to enhance basal insulin secretion in normal rats, while abundant insulin release in response to arginine is elicited in STZ rats (15). Accordingly, to determine if the response to arginine is

TABLE 1

Body Weight, Plasma Glucose, and Plasma Insulin Level before Sacrifice in Control, STZ, and Transplanted STZ Rats

	Body weight (g)	Plasma glucose (mg/dl)	Plasma insulin (ng/ml)
Control	355.6 ± 8.5	143.46 ± 9.58	7.33 ± 1.80
STZ	281.5 ± 4.7*	406.53 ± 39.34*	1.81 ± 0.39*
Transplanted	340.2 ± 10.8	171.79 ± 12.62	8.71 ± 1.30

Note. The observation number is 10 in each group.
* $P < 0.01$ when compared with control and transplanted rats.

normalized in the islets of transplanted rats, insulin secretion stimulated by 19 mM arginine with 3.3 mM glucose was examined. Insulin release by arginine in STZ rats was as high as 768 ± 85% ($n = 5$) of that in the presence of 3.3 mM glucose, but in control rats it was similar to basal level (118 ± 11%; $n = 5$). In transplanted rats, the insulin release in response to arginine was 163 ± 22% ($n = 5$) of the 3.3 mM glucose-induced insulin secretion. The response to arginine was not significantly different in control and transplanted rats (Fig. 2A). In addition, to determine if the recovery of insulin secretion by glucose is independent of the intracellular Ca^{2+} concentration, we examined the insulin response to glucose under membrane-depolarized condition by 30 mM KCl and 200 μM diazoxide. In controls, glucose increased the insulin secretion to 133 ± 8% of that with glucose alone under such conditions. In STZ rats, the secretion was conversely reduced to 79 ± 15%, but in transplanted rats, the response recovered to the level of controls (152 ± 15%; Fig. 2B).

DISCUSSION

In the present study, we investigated the effects of islet transplantation on the functional and histological characteristics of endogenous pancreatic islets in STZ diabetic rats. Whole islet area and β-cell area were increased in transplanted-STZ rats, and these morpho-

TABLE 2

Insulin and DNA Content, Islet Area, and β-Cell Area in Control, STZ, and Transplanted STZ Rats

	Control	STZ	Transplanted
Insulin content (ng/islet)	40.0 ± 1.7	4.1 ± 0.4*	21.5 ± 0.6*¶
DNA content (ng/islet)	32.9 ± 0.7	21.1 ± 1.0*	24.2 ± 0.5*†
Islet area (% of control)	100	59.2 ± 9.0*	82.7 ± 11.6¶
β-cell area (% of whole islet area)	78.4 ± 1.1	45.4 ± 2.5*	60.1 ± 2.3*¶

Note. The observation number is 10 in each group.
* $P < 0.01$ when compared with control rats; ¶ $P < 0.01$ and † $P < 0.05$ when compared with STZ rats.

TABLE 3

Insulin Secretion in Response to Glucose or KIC in Control, STZ, and Transplanted STZ Rats

	Control	STZ	Transplanted
Glucose (16.7 mM/3.3 mM)	6.58 ± 0.45	2.79 ± 0.22*	7.64 ± 0.60¶
KIC (16.7 mM/3.3 mM)	2.51 ± 0.39	1.69 ± 0.40*	3.61 ± 0.18*¶

Note. The observation number is 10 in each group.
* $P < 0.01$ when compared with control rats and ¶ $P < 0.01$ when compared with STZ rats.

logical findings may underlie the recovery of glucose-induced insulin release. We ascertained that the recovery of glucose-stimulated insulin secretion from endogenous pancreatic islets after normoglycemia was attained and maintained by islet transplantation, most probably due to the improved intracellular glucose metabolism. Normalized insulin release in response to arginine also was observed in the residual islets of transplanted rats. These findings clearly show that normoglycemia sustained by islets transplanted in sufficient abundance can functionally and histologically restore residual endogenous β-cells in STZ-treated rats.

To restore a normal level of blood glucose, we used 2500 islets for transplantation, a number that should be sufficient to restore the normoglycemic condition in diabetic rats. Although it is difficult to ascertain normoglycemia, the plasma glucose level and the glucose tolerance test have been widely used as indicators. Several investigators have found both fed and fasting plasma glucose levels to be normalized by insulin and transplantation therapy (7, 16), but few have shown completely recovered glucose tolerance. Previous reports suggested that incomplete insulin injection therapy or the transplantation of an insufficient amount of islets does not normalize either of these indices (10, 16). In the present study, the body weight of STZ rats began to increase after transplantation similarly to controls, and the plasma glucose, plasma insulin level, and glucose tolerance also were similar to controls, indicating that the normal glucose metabolism was restored and maintained by the sufficient amount of transplanted islets.

We made rats diabetic by administration of 35 mg/kg STZ to 250 g adult rats. The STZ injection could be dose-dependently controlled to the severity of the diabetic state (17). An STZ injection in the neonatal period is known to be useful in preparing mildly diabetic rats for research. Such rats are reported to be reversibly characterized by slightly lean bodies, impaired glucose-induced insulin secretion, and excessive insulin release in response to stimulation by arginine (10, 15). On the other hand, relatively large doses (50 mg/kg) of STZ injection to adult rats causes irreversible and complete destruction of the β-cells, resulting in no increase of body weight and in severe hyperglycemia due to the lack of insulin. This irreversible phenomenon in STZ rats is appropriate for islet transplantation studies because insulin is supplied only from the graft islet cells. In the present study, the rats were made mildly diabetic in a short period using the smaller dose STZ injection (35 mg/kg) to adult rats in order to retain some residual pancreatic islets, as Junod *et al.* previously described (17). The characteristics of the STZ rats used in this study resemble those of neonatally injected STZ rats in such aspects as slowly increasing body weight, impaired glucose-induced insulin release, and hyperresponsive insulin secretion stimulated by arginine, and so are more suited to study the effect of strict blood glucose level control on impaired endogenous islets.

We show in the present study that islet transplantation revives endogenous residual β-cells. This is also suggested by the restoration of glucose-induced insulin secretion, insulin content, and DNA content in the islets of transplanted-STZ rats, and confirmed by the histological findings. A previous report showed that continuous hyperglycemia induces apoptosis and reduces the proliferative capacity of β-cells, which results in β-cell mass reduction and induces development of diabetes in a type 2 diabetes model animal, *Psammomys obesus* (18). Insulin therapy by sufficient islet transplantation, therefore, may protect β-cells from apoptosis. Furthermore, insulin itself may stimulate β-cell proliferation and increase β-cell mass. Pancreatic β-cells are known to be equipped with insulin receptors. Tissue-specific knockout of the insulin receptor in mice has revealed both a defect in insulin secretory response and a decrease in the number of β-cells, suggesting that insulin plays a pivotal role not only in

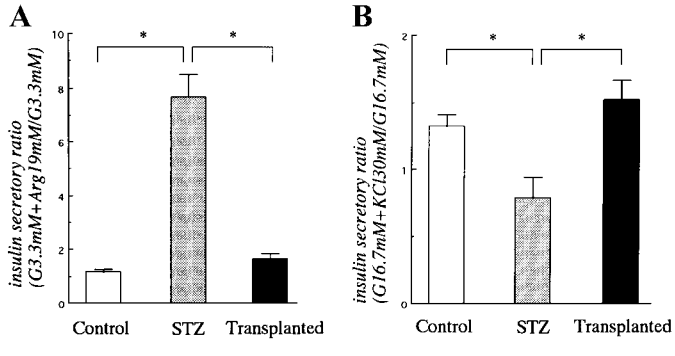

FIG. 2. Insulin secretory capacity in control (open bars), STZ (dotted bars), and transplanted rats (closed bars). (A) The ratio of insulin secretion in response to 19 mM arginine (Arg) with 3.3 mM glucose to that in response to 3.3 mM glucose in the three groups. The panel shows a representative of three separate experiments. (B) The ratio of insulin secretion in response to 16.7 mM glucose stimulation with membrane depolarization by 30 mM KCl and 200 μM diazoxide to that without membrane depolarization. *$P < 0.01$ between the groups. The observation number is five in each bar.

the regulation of insulin secretion but also in the continual replication of β-cells, via the insulin receptor (19). Abundant plasma insulin in transplanted rats, therefore, might well promote revival of the β-cells.

Our results show glucose- and KIC-induced insulin secretion to almost completely recover in transplanted rats, while the 30 mM KCl-induced insulin release with diazoxide also improves similarly. The mechanism could involve quantitative recovery of the pancreatic β-cells of transplanted-STZ rats. However, glucose metabolism is the most important factor in glucose-induced insulin release (20) and glucose metabolism in β-cells of STZ rats is known to be impaired (21). Because 30 mM KCl-induced insulin secretion with diazoxide is subject to glucose metabolism (22), transplantation therapy might ameliorate intracellular glucose metabolism and improve glucose-induced insulin secretion. Another recovery of function, normalization of arginine-induced insulin secretion, also was observed in transplanted rats. Arginine-induced insulin secretion is known to be hyperresponsive in diabetic STZ rats. Serradas *et al.* reported that normalization of the plasma glucose level by insulin treatment, but not by phlorizin or vanadate treatment, considerably decreased the hyperresponsiveness to arginine (10). These findings suggest that a supply of insulin sufficient to improve glucose tolerance is required to normalize arginine-induced insulin secretory systems in pancreatic β-cells. It has been reported that chronic hyperglycemia may lead β-cells to be insensitive to glucose stimulation (1, 23, 24). Phlorizin, independent of insulin, improves hyperglycemia and results in recovery of glucose-induced but not arginine-induced insulin secretion, further suggesting that transplantation therapy that supplies sufficiently abundant insulin can also restore β-cell function.

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