Diminished Fraction of Blockable ATP-Sensitive K^+ Channels in Islets Transplanted Into Diabetic Mice

Bernat Soria, Franz Martín, Etelvina Andreu, Juan Vicente Sanchez-Andrés, Victor Nacher, and Eduard Montana

The reasons for the poor outcome of islet transplantation in diabetic patients are not well known; a better understanding of the pathophysiology of transplanted islets is needed. To study the mechanism coupling secretagogue stimuli with insulin release in transplanted islets, we determined the effects of glucose, tolbutamide, and carbamylcholine on the β-cell membrane potential and cytosolic calcium concentrations ($[Ca^{2+}]_i$) of islets syngeneically transplanted into normal and streptozocin-induced diabetic mice. In both groups, normoglycemia was maintained after transplantation. Islets transplanted into normal recipients showed similar changes in β-cell membrane potential and $[Ca^{2+}]_i$ oscillations to those in control islets. In contrast, when islets were transplanted into diabetic mice, bursts of electrical activity were triggered at lower glucose concentrations (5.6 mmol/l) than in control islets (11 mmol/l), and maximal electrical activity was achieved at lower glucose concentrations (11 mmol/l) than in control islets (22 mmol/l). When membrane potential was plotted as a function of glucose concentration, the dose-response curve was shifted to the left. Compared with control islets, glucose-induced $[Ca^{2+}]_i$ oscillations were broader in duration (22.3 ± 0.6 s vs. 118.1 ± 12.6 s; $P < 0.01$) and higher in amplitude (135 ± 36 nmol/l vs. 352 ± 36 nmol/l; $P < 0.01$). Glucose supersensitivity was attributed to a resting decrease in the fraction of blockable ATP-sensitive K^+ (K^+_{ATP}) channels in transplanted islets that maintained normoglycemia with a limited β-cell mass. ***Diabetes*** **45:1755–1760, 1996**

From the Department of Physiology and Institute of Neurosciences (B.S., F.M., E.A., J.V.S-A), School of Medicine, University of Alicante, Aptdo. 374, 03080 Alicante, Spain; the Laboratory of Diabetes and Experimental Endocrinology (V.N., E.M.), Endocrine Unit (13-2), CSUB-Hospital of Bellvitge, University of Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

Address correspondence and reprint requests to Prof. Bernat Soria, Department of Physiology and Institute of Neurosciences, School of Medicine, University of Alicante, Aptdo. 374, E-03080 Alicante, Spain. E-mail: Bernat.Soria@ua.es.

Received for publication 22 April 1996 and accepted in revised form 26 July 1996.

AM, acetoxymethyl; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; STZ, streptozocin.

The efficacy of islet transplantation in restoring normoglycemia in diabetic rats was first demonstrated 20 years ago (1), but the first successful transplants of islet cells in diabetic patients were only recently reported (2–4). Up to 31 December 1994, a total of 243 adult islet allotransplants had been reported in type I diabetic patients (5); however, insulin independence was achieved in only 19 recipients, and, even in these patients, it had limited duration (6). Why transplanted islets survive only a limited time in diabetic patients is not well understood. Early and late failures of islet autotransplants in animal models suggest that nonimmunological factors could play a role in these poor outcomes (7). The physiological basis of these failures is unknown; thus a better understanding of the pathophysiology of transplanted islets is needed to overcome the problems that currently hamper islet transplantation.

It is well established that stimulatory glucose concentrations (> 7 mmol/l in mouse β-cells) induce a decrease in K^+ conductance (8,9) because of the closure of K^+_{ATP} channels (10,11). The subsequent depolarization causes bursts of electrical activity (12), $[Ca^{2+}]_i$ oscillations (13,14), and insulin release (15–17). In summary, current models accept that K^+_{ATP} conductance modulates the dose-response curve to glucose in ionic events (18,19). So far it is not known to what extent features of this model can be applied to transplanted islets. Thus, in our study we investigated the mechanism coupling secretagogue stimuli with insulin release in islet cells syngeneically transplanted under the kidney capsule of normal and streptozocin-induced diabetic mice.

RESEARCH DESIGN AND METHODS

Animals and treatments. Male inbred C57Bl/6 mice (B & K Universal, U.K.), age 8–12 weeks, were used as donors and recipients of the transplantation. Animals were made diabetic by a single intraperitoneal injection of streptozocin (STZ) (Sigma, St. Louis, MO), 160 mg/kg of body weight, freshly dissolved in citrate buffer (pH 4.5). Before transplantation, diabetes was confirmed by the presence of blood glucose levels higher than 20 mmol/l, weight loss, and polyuria. Blood glucose was obtained from the snipped tail and measured between 9:00 A.M. and 11:00 A.M., in nonfasting conditions, with a portable glucose meter (Accutrend GC, Boehringer Mannheim, Mannheim, Germany).

Three groups of mice were studied: *1*) STZ-Tx group (n = 9): STZ-induced diabetic mice transplanted with 400 islets; *2*) Tx group (n = 4): normoglycemic non-STZ-injected mice transplanted with 150 islets; and *3*) control group (n = 12): normoglycemic non-STZ-injected and nontransplanted mice.

Membrane potential and $[Ca^{2+}]_i$ oscillations were measured in the transplanted islets of 3 animals of the STZ-Tx group and 4 animals of the Tx group; they were also measured in the native pancreatic islets of the Tx-group mice and in 6 mice of the control group. β-cell mass was determined in the grafts of 6 mice of the STZ-Tx group and in the pancreases of 6 mice of the control group not used for membrane potential and $[Ca^{2+}]_i$ oscillation measurements.

Islet isolation and transplantation. Islets were isolated as previously described (20). Briefly, after pancreas digestion with collagenase (Collagenase P, Boehringer Mannheim Biochemicals, Mannheim, Germany) in a stationary bath at 37°C, islets were separated on a density gradient (Hystopaque-1077, Sigma Immunochemicals, St. Louis, MO), and hand-picked under a stereomicroscope. The islets were placed into 200-ml pipette tips, allowed to sediment, and then gently transferred to a polyethylene tubing PE-50 (Becton Dickinson, Sparks, MD) with the help of a 1-ml Hamilton syringe (Hamilton, Reno, NE). The tubing was folded and centrifuged for 1 min at 400*g* to pellet the islets; after centrifugation, the pipette tip was again connected to the Hamilton syringe. With the mouse under light ether anesthesia, the kidney was exposed through a lumbar incision. A capsulotomy was performed in the lower pole of the kidney and the tip of the tubing advanced under the capsule to the upper pole where islets were injected using the Hamilton syringe.

Grafts and pancreas removal. Grafts were harvested 4 weeks after transplantation. With the mouse under light ether anesthesia, the kidney was exposed and the graft identified as a white patch. The kidney capsule surrounding the graft was incised and removed with the graft (20). Immediately after graft removal, either individual well-defined islets were microdissected from the graft to measure membrane potential and $[Ca^{2+}]_i$, or the whole graft was weighed and fixed in Bouin's solution to measure β-cell mass. For pancreas removal, a midlaparotomy was performed and the pancreas was exposed, the animal was killed, and the pancreas was dissected from surrounding tissues, excised, blotted, weighed, and fixed in Bouin's solution.

Electrophysiology. The β-cell membrane potential was recorded as previously described (21). Once isolated, islets were fixed with micropins to the bottom of a 50-µl chamber and perifused at a rate of 0.8 ml/min with fresh modified Krebs medium and constantly gassed with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4. Different glucose concentrations (5.6, 11, and 22 mmol/l) were added to the superfusion medium. The test agents reached the chamber with a delay of 3 s (these delays have been corrected in the figures). Bath temperature was maintained at 36 ± 1°C by heating a thermostat-controlled stainless steel ring. The temperature of the chamber was continuously monitored with a microthermistor. Recordings were made with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). Data acquisition was performed with Axotape version 2.0 (Axon Instruments) and data analysis with MicroCal Origin version 3.7 (MicroCal Software, Northampton, MA).

Cytosolic Ca^{2+} measurement

Indo-1 loading. Once microdissected from the graft or isolated from the pancreas, the islets were incubated for 1 h at 37°C in modified Krebs medium supplemented with 5 mmol/l glucose and 3% bovine serum albumin (BSA). The medium was continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4. Isolated islets were loaded as previously described (13). Islets were loaded with Indo-1 (Molecular Probes, Eugene, OR) by incubation for 1 h at room temperature in the described medium with 2 µmol/l of the acetoxymethyl derivative (AM) (Sigma Immunochemical). Indo-1 AM was added as a concentration stock solution in dimethyl sulfoxide (DMSO) (Sigma Immunochemical; final DMSO concentration 0.3% vol/vol). To facilitate the solubilization of Indo-1 AM in physiological media, the mixture also contained 10% (wt/wt) of the nonionic surfactant polyol pluronic F127 (Molecular Probes, Junction City, OR). After loading, islets were incubated with a modified Krebs medium supplemented with 1% BSA. The Krebs medium was kept at 37°C and constantly gassed with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4.

Superfusion of islets. Each islet was then transferred to an open chamber (vol = 300 µl) mounted on the stage of an epifluorescence Nikon-Diaphot inverted microscope (Nikon, Tokyo, Japan). Islets of similar, medium size (100–200 µm diameter) were used for all experiments. Individual islets were left to settle down in the chamber for 1–2 min in the absence of perfusion. Islets usually remained attached to the glass bottom of the chamber during a perfusion experiment. Islets were superfused at a rate of 0.5 ml/min with fresh modified Krebs medium supplemented with 1% BSA and constantly gassed with a mixture of O_2 (95%) and CO_2 (5%) for a final pH of 7.4. Glucose at different concentrations (3 and 11 mmol/l), 50 µmol/l tolbutamide, and 10 µmol/l carbamylcholine were added to the superfusion medium. The test agents reached the chamber with a delay of 15 s (these delays have been corrected in the figures). Bath temperature was maintained at 36 ± 1°C by heating a thermostat-controlled stainless steel ring. The temperature of the chamber was continuously monitored with a microthermistor.

Measurements of cytosolic Ca^{2+}. $[Ca^{2+}]_i$ was monitored as previously described (13) by measuring the fluorescence emitted by Indo-1. Indo-1 was excited at 350 ± 5 nm by means of a 100-W mercury lamp. The level of ultraviolet excitation light compatible with a good signal-to-ratio noise was adjusted by placing neutral density filters in the light pathway to reduce photobleaching and photodamage of the preparation. The emitted fluorescence was split into two beams with a dichroic mirror (450 nm), filtered respectively at 410 ± 5 and 480 ± 5 nm, and detected by two photomultipliers (Thorn EMI 9924B, Middlesex, England). An increase in $[Ca^{2+}]_i$ produces a rise in Indo-1 fluorescence at 410 nm; conversely, a fluorescence decrease is observed at 480 nm (22). The fluorescence ratio (F_{410}/F_{480}) was determined on-line and filtered at 10 kHz. Autofluorescence levels were measured from control islets and found to be less than 15% of the total fluorescence.

β-cell mass measurement. After removal, grafts and pancreases were fixed in Bouin's solution and processed for paraffin embedding. Their weights were determined on a Mettler A240 balance (Mettler Instrument, Hightstown, NJ), reading to 0.01 mg, as previously described (20). Two-µm sections of grafts and pancreases were stained for the endocrine non-β-cells of the islets with immunoperoxidase. Immunostaining used a cocktail of antibodies (Dako, Carpinteria, CA): rabbit anti-porcine glucagon (final dilution 1:1000), rabbit anti-human somatostatin (final dilution 1:1000), and rabbit anti-human pancreatic polypeptide (final dilution 1:500). A swine anti-rabbit IgG was used as a secondary antibody. β-cell mass was measured by point counting morphometry (20). Each section was covered systematically using a 48-point grid to obtain the number of intercepts over β-cells, endocrine non-β-cells, and other tissue. The β-cell relative volume was calculated by dividing the intercepts over β-cells by intercepts over total tissue, then the β-cell mass was estimated by multiplying β-cell relative volume by graft weight. The endocrine non-β-cell mass (α, δ, and PP cells) was determined in the same way.

Statistical analysis. Electrophysiological results (Fig. 1*B* and 1*C*) were expressed as means and as standard error of the mean (X ± SE). For comparisons between two groups, the unpaired Welch's *t* test (two tailed) was used. The remaining results were expressed as means and standard error of the mean (X ± SE). For comparisons between two groups, the unpaired, two-tailed Student's *t* test) was used. $P < 0.05$ was considered significant.

RESULTS

Normoglycemia was achieved in all STZ-Tx group mice 1 week after transplantation (6.6 ± 0.9 mmol/l) and was maintained until graft harvesting (Table 1). As expected (20), no changes in blood glucose were detected after islet transplantation into normal nondiabetic recipients.

Electrophysiology and cytosolic Ca^{2+} measurement. The glucose-induced electrical activity in β-cells from control islets and from islets transplanted into STZ-Tx group mice is summarized in Fig. 1. In STZ-Tx group, β-cells (7 β-cells from 7 islets of 3 mice) showed an increased glucose sensitivity compared with β-cells from the pancreas of control group (9 β-cells from 9 islets of 6 mice) (Fig. 1*A*). Transplanted β-cells in the STZ-Tx group showed oscillatory electrical activity in response to lower glucose concentrations (5.6 mmol/l) than in the control group (11 mmol/l). Furthermore, the pattern of response elicited in β-cells from the control group with maximal glucose concentrations (22 mmol/l) was achieved in transplanted β-cells from the STZ-Tx group with 11 mmol/l glucose. When the parameters studied—membrane potential and fraction of time in active phase—were plotted as a function of glucose concentration, the dose-response curve in transplanted β-cells from the STZ-Tx group was found to be shifted left compared with β-cells from the control group (Fig. 1*B* and 1*C*). In contrast, no differences were found in the electrical activity of β-cells from the control group, transplanted β-cells from the Tx group (6 β-cells from 6 islets

TABLE 1
Characteristics of experimental groups

Group	n	Day of transplant		Day of graft or pancreas harvesting	
		Body weight (g)	Blood glucose (mmol/l)	Body weight (g)	Blood glucose (mmol/l)
STZ-Tx	9	23.3 ± 0.4	26.7 ± 0.7*	26.3 ± 0.4	6.5 ± 0.3
Tx	4	23.0 ± 0.5	7.7 ± 0.4	28.4 ± 0.8	7.6 ± 0.4
Control	12			25.0 ± 0.3	7.1 ± 0.1

Data are means ± SE. *P < 0.05 when compared with control group.

of 4 mice), and β-cells from pancreases of the Tx group (6 β-cells from 6 islets of 4 mice; data not shown).

$[Ca^{2+}]_i$ responses to glucose and tolbutamide are shown in Fig. 2. Glucose-induced $[Ca^{2+}]_i$ oscillations were broader in duration and higher in amplitude (118.1 ± 12.6 s, P < 0.001; 352 ± 36 nmol/l, P < 0.001; 9 islets from 3 mice) in transplanted islets from the STZ-Tx group compared with islets from the control group (22.3 ± 0.6 s and 135 ± 36 nmol/l; 10 islets from 6 mice) and with transplanted islets from the Tx group (24.0 ± 0.9 s and 121 ± 22 nmol/l; 6 islets from 4 mice) (Fig. 2*A*). The duration and amplitude of glucose-induced oscillations were similar in islets from the control group, islets transplanted into the Tx group, and native pancreatic islets from the Tx-group (7 islets, from 4 mice; data not shown).

Tolbutamide, an oral antidiabetic agent that specifically blocks K^+_{ATP} channels (23), potentiated the glucose (11 mmol/l) effect on $[Ca^{2+}]_i$, increasing its cytosolic levels in islets from the control group (11 mmol/l glucose: 207 ± 14 nmol/l; 11 mmol/l glucose plus 50 µmol/l tolbutamide: 276 ± 21 nmol/l, P < 0.01; 6 islets from 6 mice) and in islets transplanted in the Tx group (11 mmol/l glucose: 178 ± 25 nmol/l; 11 mmol/l glucose plus 50 µmol/l tolbutamide: 285 ± 13 nmol/l, P < 0.01; 7 islets from 4 mice) (Fig. 2). Similar results were observed in islets isolated from the pancreases of the Tx group (5 islets from 4 mice; data not shown). In contrast, in islets transplanted into the STZ-Tx group, the addition of 50 µmol/l tolbutamide to 11 mmol/l glucose did not modify the already increased amplitude of $[Ca^{2+}]_i$ oscillations (11 mmol/l glucose: 293 ± 14 nmol/l; 11 mmol/l glucose plus 50 µmol/l tolbutamide: 309 ± 45 nmol/l, P = NS; 5 islets from 3 mice) (Fig. 2*B*).

In response to carbamylcholine, a muscarinic receptor agonist, changes in membrane potential (data not shown) and in $[Ca^{2+}]_i$ (Fig. 3) were similar in islets from the control group (7 islets from 6 mice), in islets transplanted into the Tx group (4 islets, from 4 mice), in native pancreatic islets from the Tx group (5 islets from 4 mice; data not shown), and in islets transplanted into the STZ-Tx group (6 islets from 3 mice).

β-cell mass. β-cell mass was determined in normal pancreases of 6 control group mice and in 6 grafts transplanted into the STZ-Tx group mice. The β-cell mass in the 400-islet grafts (0.60 ± 0.09 mg) was significantly lower than the β-cell mass in the pancreases of control mice (1.09 ± 0.14 mg; P < 0.01). The endocrine non-β cell mass (α, δ, and PP cells) was also lower in the grafts of the STZ-Tx group (0.05 ± 0.01 mg) compared with pancreases of control mice (0.26 ± 0.05 mg; P < 0.001).

DISCUSSION

We studied the mechanism coupling secretagogue stimuli with insulin release in islets transplanted into STZ-induced diabetic mice and normal mice. When islets were transplanted into diabetic recipients and normoglycemia

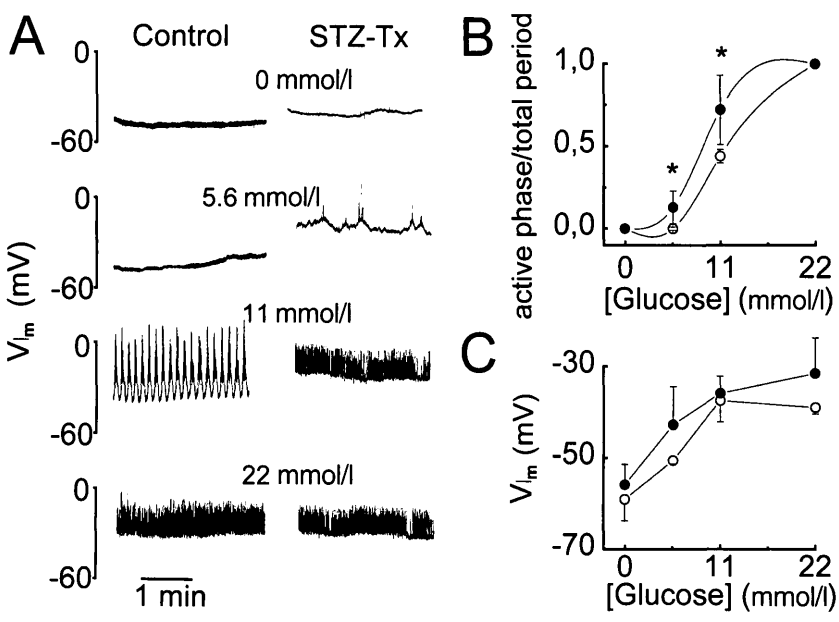

FIG. 1. Glucose-induced electrical activity in transplanted islets. *A*: representative examples of the effects of different glucose concentrations (0, 5.6, 11, and 22 mmol/l) on intracellularly recorded membrane potential of a pancreatic β-cell in the intact islet. Groups are described in Table 1. In the STZ-Tx and control groups, the same islet was perifused with the different glucose concentrations. *B*: fraction of time in the active phase in control (○) and STZ-Tx islet β-cells (●). *C*: membrane potential as function of glucose concentration, control group (○), STZ-Tx group (●). Values are expressed as means and standard error of the mean (X ± SE). *P < 0.05 when compared with control group.

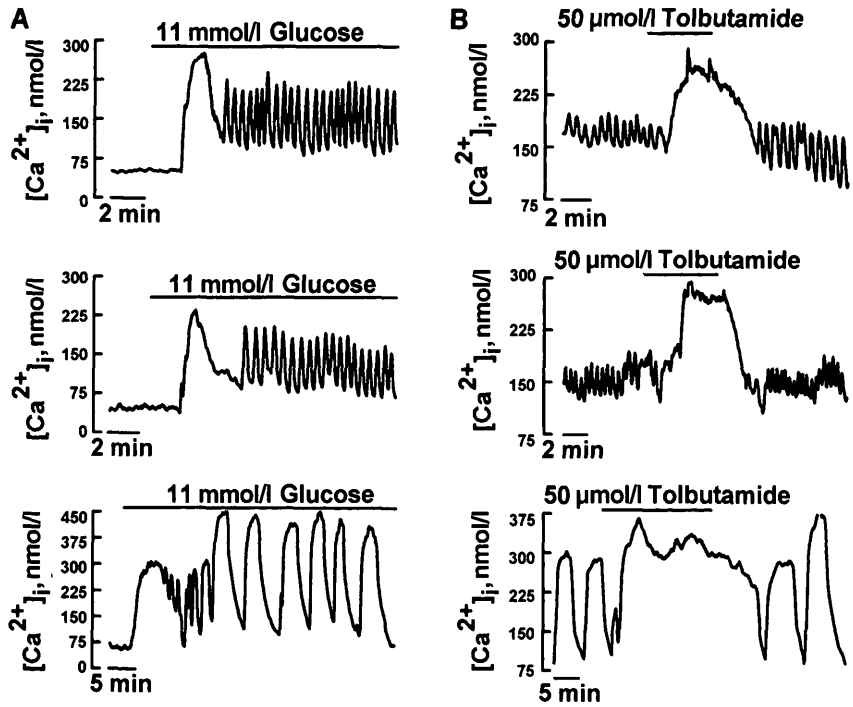

FIG. 2. Glucose- and tolbutamide-induced $[Ca^{2+}]_i$ changes in transplanted islets. *A*: representative examples of the effect of 11 mmol/l glucose on $[Ca^{2+}]_i$. Islets were perifused with 3 mmol/l glucose before the addition of 11 mmol/l glucose. 11 mmol/l glucose was added during the time indicated by the bars. *B*: representative examples of the effect of 50 μmol/l tolbutamide in the presence of 11 mM glucose on $[Ca^{2+}]_i$. 50 μmol/l tolbutamide was added during the time indicated by the bars. Upper records show the control group, middle records the Tx-group, and lower records the STZ-Tx group.

was restored after transplantation, electrical activity was elicited in transplanted β-cells at lower glucose concentrations than in control islets from the pancreases of normal mice. Furthermore, in islets transplanted into STZ-Tx diabetic mice $[Ca^{2+}]_i$, oscillations in response to glucose had broader duration and higher amplitude than in control islets. Therefore, islets that restored normoglycemia after transplantation into diabetic mice showed increased sensitivity to glucose. In contrast, islets transplanted into normal nondiabetic mice had glucose sensitivity similar to that of control islets.

Glucose-induced electrical activity in islets transplanted into STZ-induced diabetic mice was increased, and the dose-response curve was left-shifted. When islets transplanted into STZ-Tx mice were perifused with 5.6 mmol/l glucose, a concentration too low to elicit activity in control islets, they showed oscillatory electrical activity that was similar to that of control islets perifused with 11.1 mmol/l glucose. Furthermore, maximal electrical activity was achieved in STZ-Tx islets perifused with 11.1 mmol/l glucose, whereas in control islets 22.2 mmol/l glucose was required for maximal electrical activity. $[Ca^{2+}]_i$ oscillations in response to glucose in islets transplanted into STZ-Tx diabetic recipients also showed an increased glucose sensitivity with broader duration and higher amplitude of oscillations than control islets.

Denervation has been shown to cause supersensitivity in other excitable cells (24). Islets are denervated when transplanted, and initial islet reinnervation is detected 2 months after transplantation under the kidney capsule, although it is not clearly established until 3–4 months later (25). To determine whether glucose supersensitivity was caused by denervation of transplanted islets, we studied the effect of carbamylcholine on islet membrane potential and cytosolic calcium. The response to carbamylcholine was similar in transplanted and in normal pancreatic islets, indicating that muscarinic receptors were present and operative 1 month after transplantation, and suggesting that they were not involved in glucose increased sensitivity in transplanted islets.

To ascertain whether increased glucose-sensitivity was common in all transplanted islets or related to the function of transplanted islets into diabetic recipients, we compared the electrical activity and the $[Ca^{2+}]_i$ oscillations in response to glucose in islets transplanted into diabetic mice of the STZ-Tx group with the same parameters in islets transplanted into normal mice of the Tx-group. As expected (21), transplantation did not modify blood glucose values in the Tx-group. The electrical activity and $[Ca^{2+}]_i$ oscillations in the islets transplanted into normal recipients were similar to those in control islets, indicating that the mechanism coupling secretagogue stimuli with insulin release and the glucose sensitivity were normal in the Tx-group transplanted islets. Therefore, transplantation by itself was not the cause of the increased glucose sensitivity in the STZ-Tx group islets, which was probably related to the function of transplanted islets in the maintenance of normoglycemia in the diabetic recipients.

The $[Ca^{2+}]_i$ oscillations elicited by glucose in islets transplanted into the STZ-Tx group strongly resembled the slow oscillations induced by amino acids in mouse islets (26). In this model, it was suggested that the amino acid–induced closure of K^+_{ATP} channels that remained opened after glucose stimulation accounted for the appearance of these slow oscillations. It is known that tolbutamide mimics the changes in electrical activity induced by an increase in glucose concentration (19), suggesting that closure of K^+_{ATP} channels that remain open after glucose stimulation underlies the effects of tolbutamide on electrical activity and $[Ca^{2+}]_i$. To determine whether increased glucose sensitivity in STZ-Tx group islets was mediated by a reduction in the fraction of closable K^+_{ATP} channels, islets were perifused with

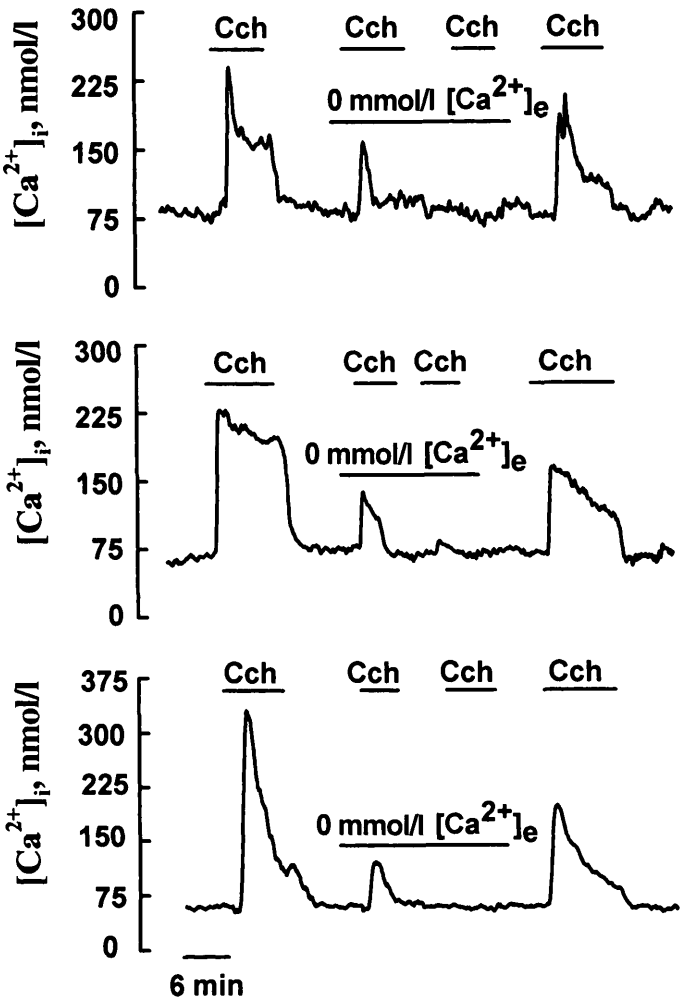

FIG. 3. Carbamylcholine-induced $[Ca^{2+}]_i$ changes in transplanted islets. In all the experiments, the islets were perifused with 3 mmol/l glucose. 10 µmol/l carbamylcholine was added during the time indicated by the bars. Upper record shows the control group, middle record the Tx-group, and lower record the STZ-Tx group.

tolbutamide. In islets transplanted in the Tx group and in control islets, the addition of tolbutamide (50 µmol/l) to glucose induced the closure of additional K^+_{ATP} channels and increased the $[Ca^{2+}]_i$ to a sustained plateau that was similar to the peak of the glucose-induced slow $[Ca^{2+}]_i$ oscillation in the STZ-Tx group. In contrast, addition of tolbutamide did not modify $[Ca^{2+}]_i$ oscillations in the STZ-Tx group, suggesting that the number of closable K^+_{ATP} channels was reduced in this group. The results indicated that the fraction of blockable K^+_{ATP} channels was diminished in islets that restored and maintained normoglycemia after transplantation into diabetic mice and accounted for the increased sensitivity to glucose.

Hyperglycemia is known to shift the glucose concentration-insulin secretion curve to the left (27,28). In our study, nonfasting normoglycemia was restored 1 week after transplantation into STZ-Tx mice and maintained until graft harvesting 3 weeks later, excluding hyperglycemia as the cause of glucose supersensitivity in transplanted islets. Recently, increased β-cell sensitivity to glucose has been described in the presence of normoglycemia in spontaneously hypertensive rats (29) and after partial pancreatectomy (30). A common characteristic of these two models is the increased workload placed on β-cells that maintained normoglycemia despite absolute or relative reductions in β-cell mass. After a 60% reduction in β-cell mass, the islets in the pancreas remainder had to meet an increased metabolic demand to maintain normoglycemia (30). Spontaneously hypertensive rats are insulin resistant, but do not experience the compensatory increase in β-cell mass found in other insulin-resistant conditions (31,32), and therefore maintain normoglycemia with an inappropriately normal β-cell mass. To determine whether a similar situation was present in our transplantation model, we measured the β-cell mass in the grafts of the STZ-Tx group and in the pancreases of control mice. The transplanted β-cell mass that maintained normoglycemia in the STZ-Tx group was 45% lower than the β-cell mass in the pancreases of age-matched control mice, indicating that an increased metabolic demand was placed on transplanted β-cells. Because there is a positive correlation between the relative length of the depolarized phase and the amount of insulin secreted by β-cells (33), we suggest that the left-shifted glucose-induced electrical activity curve in transplanted islets was an adaptive response that increased insulin secretion and maintained normoglycemia in the presence of a reduced β-cell mass.

In summary, the mechanism coupling secretagogue stimuli with insulin secretion was preserved in transplanted islets. When islet transplantation restored normoglycemia in the diabetic recipients, we found an increased sensitivity to glucose in transplanted islets. The increased sensitivity was caused by a reduction in the fraction of closable K^+_{ATP} channels that we suggest was an adaptive response of transplanted β-cells that maintained normoglycemia with a limited β-cell mass.

ACKNOWLEDGMENTS

This work was partially supported by grants FIS 93/0329 (E.M.) and FIS 94-0014-01 (B.S.) from the Ministry of Health of Spain and Contract ERBSC1-CT 92083 from the Commission of the European Union. V. Nacher and E. Andreu were the recipients of fellowships from Fundació August Pi i Sunyer and DGICYT, respectively. We are indebted to S. Moya, A. Perez, R. García Velasco, and O. Aranda for their technical help.

REFERENCES

1. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE: Effect of transplantation site on the results of the pancreatic islet isografts in diabetic rats. *Diabetologia* 9:486–491, 1973
2. Scharp DW, Lacy PE, Ricordi C, Boyle P, Santiago J, Cryer P, Gingerick R, Jaffe A, Anderson C, Flye W: Human islet transplantation in patients with type I diabetes. *Transplant Proc* 21:2744–2745, 1989
3. Warnock GL, Kneteman NM, Ryan EAS, Evans MG, Seelis REA, Halloran PF, Rabinovitch A, Rajotte RV: Continued function of pancreatic islets after transplantation in type I diabetes. *Lancet* 2:570–572, 1989
4. Ricordi C, Tzakis A, Carrol PB, Zeng Y, Rilo HIR, Alejandro R, Shapiro R, Funq J, Demetris AJ, Mintz DH, Starzl TE: Human islet isolation and allotransplantation in 22 consecutive cases. *Transplantation* 53:107–114, 1992
5. Hering BJ, Geier C, Schultz AO, Bretzel RG, Federlin K: International Islet Transplant Registry. Justus-Liebig-University, Giessen University, Giessen, Germany, 1995
6. London NMJ: Report on the 13th Workshop of the EASD Study Group of

Artificial Insulin Delivery Systems, Pancreas and Islet Transplantation. *Diabetologia* 37:23–26, 1994
7. Alejandro R, Cutfield RG, Shienvold FL, Polonsky KS, Noel J, Olson L, Dillberger J, Miller J, Mintz DH: Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 78:1339–1348, 1986
8. Sehlin J, Taljedal IB: Glucose-induced decrease in Rb^+ permeability in pancreatic β-cells. *Nature* 253:635–636, 1975
9. J.C. Henquin: D-glucose inhibits potassium efflux from pancreatic islet cells. *Nature* 271:271–273, 1978
10. Cook DL, Hales CN: Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* 311:271–273, 1984
11. Ashcroft FM, Harrison D, Ashcroft SJH: Glucose induced closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* 312:446–448, 1984
12. Henquin JC, Meissner HP: Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic β-cells. *Experientia* 40:1043–1052, 1984
13. Valdeolmillos M, Santos RM, Contreras D, Soria B, Rosario LM: Glucose-induced oscillations of intracellular Ca^{2+} concentration resembling bursting electrical activity in single mouse islets of Langerhans. *FEBS Lett* 259:19–23, 1989
14. Santos RM, Rosario LM, Nadal A, García-Sancho J, Soria B, Valdeolmillos M: Widespread synchronous $[Ca^{2+}]_i$ oscillations due to bursting electrical activity in single pancreatic islets. *Pflügers Archiv* 418:417–422, 1991
15. Gilon P, Sheperd RM, Henquin JC: Oscillations of secretion driven by oscillations of cytoplasmic Ca^{2+} as evidenced in single pancreatic islets. *J Biol Chem* 268:22265–22268, 1993
16. Martin F, Sanchez-Andrés JV, Soria B: Slow $[Ca^{2+}]_i$ oscillations induced by ketoisocaproate in single mouse pancreatic islets. *Diabetes* 44:300–305, 1995
17. Soria B, Martin F, Sanchez-Andrés JV: $[Ca^{2+}]_i$ code for glucose-induced insulin release in mouse pancreatic islets of Langerhans. In *Insulin Secretion and Pancreatic β-cell Research*. Flatt PR, Lenzen S, Eds. London, Smith-Gordon, 1994, p. 229–235
18. Cook DL, Satin LS, Ashford MLJ, Hales CN: ATP-sensitive K^+ channels in pancreatic β-cells: spare-channel hypothesis. *Diabetes* 37:495–498, 1988
19. Henquin JC: ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic β-cells. *Biochem Biophys Res Commun* 156:769–775, 1988
20. Montana E, Bonner-Weir S, Weir GC: Beta cell mass and growth after syngeneic islet cell transplantation in normal and streptozocin diabetic C57BL/6 mice. *J Clin Invest* 91:780–787, 1993
21. Sanchez-Andres JV, Soria B: Muscarinic inhibition of the pancreatic β-cell. *Eur J Pharmacol* 205:89–91, 1991
22. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
23. Ashford MLJ, Sturgess NC, Cook DL, Hales CN: K^+-channels in an insulin-secreting cell line: effects of ATP and sulphonylureas. In *Biophysics of the Pancreatic β-cell*. Atwater I, Rojas E, Soria B, Eds. New York, Plenum Press, 1985, p. 69–76
24. Cannon WB, Rosenblueth A: *The Supersensitivity of Denervated Structures: A Law of Denervation*. New York, MacMillan, 1949
25. Korsgren O, Andersson A, Jansson L, Sundler F: Reinervation of syngeneic mouse pancreatic islets transplanted into renal subcapsular space. *Diabetes* 41:130–135, 1992
26. Martin F, Soria B: Amino acid-induced $[Ca^{2+}]_i$ oscillations in single mouse pancreatic islets of Langerhans. *J Physiol* 486:361–371, 1995
27. Brelje TC, Sorenson RL: Nutrient and hormonal regulation of the threshold of glucose-stimulated insulin secretion in isolated rat pancreases. *Endocrinology* 123:1582–1590, 1988
28. Thibault CC, Guettet C, Loury MC, Guyen JMN, Tormo MA, Baube D, Portha B, Penicaud L, Ktorza A: In vivo and in vitro increased pancreatic β-cell sensitivity to glucose in normal rats submitted to 48h hyperglycemic period. *Diabetologia* 36:589–595, 1993
29. Chen C, Hosokawa H, Bumbalo LM, Leahy JL: Mechanism of compensatory hyperinsulinemia in normoglycemic insulin-resistant spontaneously hypertensive rats: augmented enzymatic activity of glucokinase in beta-cells. *J Clin Invest* 94:399–404, 1994
30. Leahy JL, Bumbalo LM, Chen C: β-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 36:1238–1244, 1993
31. Leahy JL: Natural history of β-cell dysfunction in NIDDM. *Diabetes Care* 13:992–1010, 1990
32. Iwase M, Nunoi K, Kikuchi M, Maki Y, Kodama T, Sadoshima S, Fujishima M: Morphometrical and biochemical differences of endocrine pancreata between spontaneously hypertensive and normotensive rats with or without streptozotocin-induced diabetes. *Lab Invest* 60:102–105, 1989
33. Atwater Y, Carroll P, Xu Li M: Electrophysiology of the pancreatic β-cell. In *Molecular and Cellular Biology of Diabetes Mellitus*. Draznin B, Melmed S, LeRoith D, Eds. New York, Alan R. Liss, 1988, p. 69–78