

Mechanisms of Glucose Hypersensitivity in β -Cells From Normoglycemic, Partially Pancreatectomized Mice

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Increased β -cell sensitivity to glucose precedes the loss of glucose-induced insulin secretion in diabetic animals. Changes at the level of β -cell glucose sensor have been described in these situations, but it is not clear whether they fully account for the increased insulin secretion. Using a euglycemic-normolipidemic 60% pancreatectomized (60%-Px) mouse model, we have studied the ionic mechanisms responsible for increased β -cell glucose sensitivity. Two weeks after Px (Px14 group), Px mice maintained normoglycemia with a reduced β -cell mass (0.88 ± 0.18 mg) compared with control mice (1.41 ± 0.21 mg). At this stage, the dose-response curve for glucose-induced insulin release showed a significant displacement to the left ($P < 0.001$). Islets from the Px14 group showed oscillatory electrical activity and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) oscillations in response to glucose concentrations of 5.6 mmol/l compared with islets from the control group at 11.1 mmol/l. All the above changes were fully reversible both in vitro (after 48-h culture of islets from the Px14 group) and in vivo (after regeneration of β -cell mass in islets studied 60 days after Px). No significant differences in the input resistance and ATP inhibition of ATP-sensitive K^+ (K_{ATP}) channels were found between β -cells from the Px14 and control groups. The dose-response curve for glucose-induced MTT (C,N-diphenyl-N''-4,5-dimethyl thiazol 2 yl tetrazolium bromide) reduction showed a significant displacement to the left in islets from the Px14 group ($P < 0.001$). These results indicate that increased glucose sensitivity in terms of insulin secretion and Ca^{2+} signaling was not due to intrinsic modifications of K_{ATP} channel properties, and suggest that the changes are most likely to be found in the glucose metabolism. *Diabetes* 48:1954–1961, 1999

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AM, acetoxymethyl derivative; $[Ca^{2+}]_i$, cytosolic Ca^{2+} ; FFA, free fatty acid; IPGTT, intraperitoneal glucose tolerance test; K_{ATP} , ATP-sensitive K^+ ; KRBB, Krebs-Ringer bicarbonate buffer; MTT, C,N-diphenyl-N''-4,5-dimethyl thiazol 2 yl tetrazolium bromide; Px, pancreatectomy; RIA, radioimmunoassay.

Blood glucose level is maintained within a narrow range through a mechanism coupling changes in extracellular glucose concentrations to insulin secretion (1,2). With hyperglycemia, increased glucose metabolism raises the ATP/ADP ratio, which closes ATP-sensitive K^+ (K_{ATP}) channels (3); the subsequent depolarization causes bursts of electrical activity (4), cytosolic Ca^{2+} ($[Ca^{2+}]_i$) oscillations (5), and insulin release (6). Chronic hyperglycemia, however, results in impaired glucose-induced insulin secretion, a defect that has been considered to play a major role in the pathogenesis of type 2 diabetes (7). Recently, it has been shown that a raised sensitivity to glucose precedes the loss of glucose-induced insulin secretion in hyperglycemic rats (8). However, hyperglycemia did not appear to be the cause of the heightened β -cell sensitivity to glucose that has also been described in different normoglycemic conditions such as partial pancreatectomy (8), insulin resistance (9), glucose infusion (10), or normal pregnancy (11). A common feature of these normoglycemic conditions with enhanced glucose-stimulated insulin secretion is the increased workload placed on β -cells, suggesting that the rise in glucose sensitivity represents an adaptive response of the β -cells to an increased demand for insulin. Changes at the level of the β -cell glucose sensor, the glucose-phosphorylating enzymes glucokinase and hexokinase (12,13), and the β -cell glucose transporter GLUT2 (14) have been described in situations of β -cell hyperfunction. It is not clear, however, whether these changes fully account for the increased insulin secretion.

We recently reported an increased glucose sensitivity in transplanted islets when normoglycemia was restored in diabetic recipients through the transplantation of a limited β -cell mass. In this model, increased sensitivity was attributed to a resting decrease in the fraction of blockable K_{ATP} channels in transplanted β -cells (15). However, these mice were hyperglycemic for 1 week after transplantation, and therefore the influence of previous hyperglycemia on changes in glucose sensitivity could not be completely ruled out. The aim of the present study was to determine the ionic mechanisms responsible for β -cell increased glucose sensitivity. The 60% pancreatectomy (Px) model was chosen because it is known that after 60% Px, normoglycemia is maintained despite the reduced β -cell mass, thus excluding the effects of hyperglycemia (8).

RESEARCH DESIGN AND METHODS

Partial pancreatectomy model. 60% Px was performed on 8- to 12-week-old male C57B1/6 mice (B&K Universal, North Humberston, U.K.) using the method described previously in rats (16). After an overnight fast, mice were anesthetized by intraperitoneal injection of 50 mg/kg barbital sodium (Normon Laboratory, Madrid), 90 mg/kg ketamine (Parke-Davis S.L., Barcelona), 0.8 mg/kg atropine (Braun Medical S.A., Jaen, Spain), and 4 mg/kg diazepam (Roche S.A., Madrid). After midline abdominal incision, the splenic lobe of the pancreas was mobilized by partially breaking the mesenteric connections to the stomach, small bowel, and retroperitoneum. Pancreatic tissue was removed by gentle abrasion with cotton applicators, with care being taken to leave major blood vessels intact. The excised portion included the splenic lobe of the pancreas but not the small flap attached to the pylorus. To establish the 60% Px in mice, in a set of preliminary experiments the pancreatic remnant was excised and weighed immediately after the partial pancreatectomy. The weight was compared with that of normal pancreas of control (nonpancreatectomized) mice of similar age and weight. The extent of pancreatectomy was modified until the weight of the pancreatic remnant was 40% of normal pancreas. Control mice (shams) underwent laparotomy and mobilization of the pancreas. Body weight, nonfasting blood glucose concentrations, and plasma free fatty acids (FFAs) were measured at between 9 and 11 A.M. on the day of surgery and days 5, 7, 10, 14, 30, 45, and 60 after Px. An intraperitoneal glucose tolerance test (IPGTT) was performed in four control mice, four mice 14 days after 60% Px, and five mice 60 days after 60% Px. After overnight fasting, blood samples (20 μ l) were obtained by tail snip before (0 min) and 15, 30, 60, and 120 min after an intraperitoneal injection of glucose (1 g/kg). Blood glucose was obtained from the snipped tail and measured with a portable glucose meter (Accutrend GC; Boehringer Mannheim, Mannheim, Germany). FFAs were assayed using a kit from Boehringer Mannheim.

Four groups of mice were studied: 1) Px14 group (mice studied 14 days after 60% Px) ($n = 30$); 2) Px60 group (mice studied 60 days after 60% Px) ($n = 14$); 3) control14 group (sham-Px mice of similar body weight to Px14 mice) ($n = 12$); and 4) control60 group (sham-Px mice of similar body weight to Px60 mice) ($n = 8$).

Islet isolation, cell isolation, and culture. Islets of Langerhans were isolated as previously described (17). Briefly, after pancreas digestion with collagenase (Collagenase A; Boehringer Mannheim) in a stationary bath at 37°C, islets were separated by centrifugation and hand-picked under a stereomicroscope. Once isolated, islets were dissociated into single cells in a low-calcium medium as previously described (18). Cells were then centrifuged, resuspended in culture medium (RPMI 1640 supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 5.6 mmol/l glucose), and plated on glass coverslips. Cells were kept at 37°C in a humidified atmosphere of 95% O₂, 5% CO₂ and used between 6–10 h of culture, to avoid reversibility of the changes. When indicated, isolated islets were cultured during 48 h in the same medium and kept at 37°C in a humidified atmosphere of 95% O₂, 5% CO₂. For intracellular recording experiments, islets were isolated by microdissection as previously described (19).

Electrophysiology

Patch clamp experiments. Patch pipettes were pulled from Clark Electromedical glass capillaries (Reading, U.K.) using a two-stage puller (Mecanex BB-CH, Geneva), with resistances in the range of 3–12 M Ω when filled with a standard solution (values in mmol/l): 5 KCl, 135 NaCl, 10 HEPES, 2.5 CaCl₂, and 1.1 MgCl₂, pH 7.4. Bath solution contained (in mmol/l) 140 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, and 1 EGTA, pH 7.2. Solutions containing ATP (Sigma, St. Louis, MO) were applied through an RSC-100 rapid solution changer (Biologic, Claix, France). K_{ATP} channel unitary currents were registered from excised membrane patches in the inside-out configuration (20). Currents were measured using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA) and stored in a tape recorder (DAT, DTR-1202; Biologic) for subsequent analysis with a homemade program. Experiments were replayed through an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) at 1 kHz and sampled at 10 kHz. Pipette potential was held at 0 mV throughout the record. The experiments were carried out at room temperature (20–24°C). Dose-response curves were obtained by integrating the area corresponding to channel activity in the presence of different ATP concentrations (2.5, 5, 10, 20, 40, and 2,000 μ mol/l) and fitting these data to the Hill equation.

Intracellular recording. Membrane potential of the β -cell was recorded as described (19). Microdissected islets were fixed with micropipins to the bottom of a 50- μ l chamber and perfused at a rate of 0.8 ml/min with fresh modified Krebs medium and constantly gassed with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4. Different glucose concentrations (0, 5.6, 11.1, 16.7, and 22.2 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 for in the figures. Bath temperature was maintained at 36 \pm 1°C by a peltier controlled by a thermostat. The temperature of the chamber was continuously monitored with a microthermistor. Recordings were made with an Axoclamp 2B amplifier (Axon Instruments). Data acquisition was performed with Axotape version 2.0 (Axon Instruments) and data analysis with MicroCal Origin version 3.7 (MicroCal Software, Northampton, MA).

[Ca²⁺]_i measurement

Indo-1 loading. Collagenase-isolated islets were incubated for 1 h at 37°C in modified Krebs medium supplemented with 5.6 mmol/l glucose and 3% bovine serum albumin. The medium was continuously bubbled with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4. Islets were loaded as described (21). Islets were loaded with Indo-1 (Molecular Probes, Eugene, OR) by incubation for 1 h at room temperature in the described medium with 2 μ mol/l of the acetoxymethyl derivative (AM). Indo-1 AM was added as a concentration stock solution in DMSO (Sigma) (final DMSO concentration 0.3% vol/vol). To facilitate the solubilization of Indo-1/AM in physiologic media, the mixture also contained 10% (wt/wt) of the nonionic surfactant polyol pluronic F127 (Molecular Probes). After loading, islets were incubated with a modified Krebs medium supplemented with 1% bovine serum albumin. The Krebs medium was kept at 37°C and was constantly gassed with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4.

Superfusion of islets. Each islet was then transferred to an open chamber (300 μ l volume) mounted on the stage of an epifluorescence Nikon-Diaphot inverted microscope (Nikon, Tokyo). Islets of similar, medium size (100–200 μ m 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% bovine serum albumin and constantly gassed with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4. Different glucose concentrations (3, 5.6, 11.1, and 16.7 mmol/l) were added to the superfusion medium. The test agents reached the chamber with a delay of 15 s. These delays have been corrected for in the figures. Bath temperature was maintained at 36 \pm 1°C by heating a stainless steel ring controlled by a thermostat. The temperature of the chamber was continuously monitored with a microthermistor.

Measurements of [Ca²⁺]_i. [Ca²⁺]_i was monitored as described (21) by measuring the fluorescence emitted by Indo-1. Indo-1 was excited at 350 \pm 5 nm by means of a 100 W mercury lamp. The level of ultraviolet excitation light compatible with a good signal-to-noise ratio 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 at 410 \pm 5 and 480 \pm 5 nm, respectively, and detected by two photomultipliers (Thorn EMI 9924B; Middlesex, U.K.). An increase in [Ca²⁺]_i produces a rise in Indo-1 fluorescence at 410 nm; conversely, a fluorescence decrease is observed at 480 nm (22). The fluorescence ratio (F₄₁₀/F₄₈₀) was determined online and filtered at 10 kHz. [Ca²⁺]_i was estimated using the equation proposed by Grynkiewicz et al. (22). Autofluorescence levels were measured from control islets and found to be <15% of the total fluorescence.

Insulin measurements. Insulin measurements were performed as described (23). Briefly, collagenase-isolated islets were incubated for 1 h at 37°C in modified Krebs medium supplemented with 5.6 mmol/l glucose and 3% bovine serum albumin. The medium was continuously bubbled with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4. For static incubations, islets were incubated in groups of three in 1 ml fresh modified Krebs buffer with 1% bovine serum albumin plus the different glucose concentrations (2.8, 4.2, 5.6, 8.3, 11.1, 16.7, and 22.2 mmol/l) for 30 min at 37°C. For perfusion experiments, batches of 10 islets were placed in a 50- μ l chamber and perfused at a flow rate of 1 ml/min at 37°C. The islets were first perfused in 3 mmol/l glucose for 30 min to reach a state of stable insulin release. The solutions were prewarmed at 37°C and continuously gassed. Throughout the perfusion, effluent was continuously collected at 2-min intervals. The dead time was 2 min and has been corrected for in the figures. Both static incubation and perfused samples were kept at –20°C until insulin determination. For measurement of insulin content, the medium was removed and islets were washed three times in 1 ml fresh modified Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 2.8 mmol/l glucose and without BSA. Afterward, islets were sonicated 15 s \times 3 at maximum power (model W-225; Heat Systems-Ultrasonic, Farmingdale, NY) in 100 μ l of 1:10 acid-ethanol mixture (95% ethanol, 750 ml; concentrated HCl, 15 ml; H₂O, 235 ml). A 50- μ l portion of each sample was kept at –20°C until insulin determination. In addition, aliquots of 15 μ l of the same samples were stored at –80°C for protein determination. Protein content was measured by Bradford assay. Samples were done in triplicate at each point. Insulin was assayed by radioimmunoassay (RIA) using a kit from DPC (Los Angeles). The RIA included the following steps: addition of anti-porcine insulin guinea pig serum, incubation for 36 h at 4°C, addition of freshly labeled [¹²⁵I](TYR A19)-human insulin, incubation for 18 h at 4°C, and precipitation of bound insulin by ethanol. Rat insulin was used to prepare the standard curves. Standard curves and problems were run in triplicate.

β -Cell mass measurement. Each mouse was anesthetized, and the pancreas (control groups) or pancreatic remnants (Px14 and Px60 groups) were excised, blotted, weighed, fixed in Bouin's solution, and embedded in paraffin. The weight of the pancreatic tissue was determined on a Mettler A240 balance (Mettler Instrument, Hightstown, NJ), reading to 0.01 mg, as described (24,25). Sections of pancreas were stained with immunoperoxidase for the endocrine non- β -cells of the islets (15). Immunostaining used a cocktail of antibodies (Dako, Carpinteria, CA):

TABLE 1
Characteristics of experimental groups

Group	Body weight (g)	Blood glucose (mmol/l)	β-Cell mass (mg)	Non-β-cell mass (mg)
Control14 (n = 5)	33.5 ± 0.9	6.4 ± 0.3	1.41 ± 0.21	0.25 ± 0.05
Px14 (n = 6)				
Day 5		6.4 ± 0.1		
Day 7		6.2 ± 0.1		
Day 10		6.6 ± 0.2		
Day 14	33.9 ± 0.9	7.7 ± 0.2	0.88 ± 0.18*	0.12 ± 0.05*
Px60 (n = 6)	42.9 ± 1.2	7.1 ± 0.5	1.32 ± 0.15	0.15 ± 0.04*
Control60 (n = 6)	41.4 ± 0.9	6.7 ± 0.2	1.67 ± 0.33	0.28 ± 0.04

Data are means ± SE. *P < 0.001 compared with control.

rabbit anti-porcine glucagon (final dilution 1:1,000), rabbit anti-human somatostatin (final dilution 1:1,000), 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 (25). Each immunoperoxidase-stained 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 pancreas weight.

MTT assay. Collagenase-isolated islets were incubated for 1 h at 37°C in modified Krebs medium supplemented with 5.6 mmol/l glucose and 3% bovine serum albumin. The medium was continuously bubbled with a mixture of O₂ (95%) and CO₂ (5%) for a final pH of 7.4. Then, MTT (C,N-diphenyl-N',4,5-dimethyl thiazol 2 yl tetrazolium bromide) reduction was measured as previously described (26). Batches of 15 islets were incubated in 1 ml fresh modified KRBB with 1% bovine serum albumin, 0.5 mg/ml MTT (Sigma), and the different glucose concentrations (2.8, 4.2, 5.6, 8.3, 11.1, 16.7, and 22.2 mmol/l) for 30 min at 37°C. This incubation time was selected because formazan production had reached a plateau. To solubilize the formazan crystals at the end of the incubation periods, the medium was removed and islets were washed three times in 1 ml fresh modified KRB buffer supplemented with 2.8 mmol/l glucose and without BSA. Afterward, islets were sonicated 15 s × 3 at maximum power (model W-225; Heat Systems-Ultrasonic) in 100 μl isopropanol. The optical density was recorded with an automatic U-2000 spectrophotometer at 540 nm (Hitachi, Tokyo). Aliquots (15 μl) of islet extracts were stored at -80°C for protein determination. Protein content was measured by Bradford assay. Samples were done in triplicate at each glucose concentration.

Statistical analysis. Patch clamp results were expressed as means ± SD. For comparisons between two groups, the unpaired Welch's *t* test (two-tailed) was used. The rest of the results were expressed as means ± SE. Statistical differences in the dose-response curves were tested using the test of comparison of experimental curves. *P* < 0.05 was considered significant.

RESULTS

Metabolic evolution and β-cell mass. Normoglycemia and normolipidemia were maintained in all the mice after 60% Px (Table 1). Postprandial mild hyperglycemia was excluded, because blood glucose was always determined in fed animals. In addition, blood glucose values after IPGTT were similar in mice from control14, Px14, and Px60 groups (Fig. 1).

After 60% Px, β-cell mass increased progressively in Px14 and Px60 groups. Fourteen days after Px, β-cell mass in the pancreatic remnant was 62.5% of β-cell mass in control14 group, and by day 60 it had increased to 93.6% of the initial β-cell mass (control14 group) and 79.0% of the β-cell mass in weight-matched controls (control60 group). In contrast, the endocrine non-β-cell mass increased modestly; on day 60 after Px it was 60% of the initial non-β-cell mass (control14) and 53.4% of weight-matched control (control60 group) (Table 1).

Effect of pancreatectomy on glucose-induced insulin secretion. The glucose-induced insulin secretion from islets in control and Px14 groups is shown in Fig. 2. The relation-

ship between insulin secretion and glucose concentration was sigmoidal in both groups; however, the dose-response curve in islets from Px14 group showed a significant displacement to the left in the dose response (EC₅₀ = 7.46 ± 0.27 mmol/l) when compared with islets from control14 group (EC₅₀ = 10.24 ± 0.08 mmol/l) (*F*_(2, 56) = 11.76; *P* < 0.001). A broader glucose response range and a reduced glucose threshold for insulin secretion were seen in Px14 (Fig. 2A). Islets from Px14, when compared with the control14 group, showed increased insulin secretion in response to lower glucose concentrations (5.6 and 8.3 mmol/l glucose, respectively). Islets from Px14 group recovered a normal glucose-induced insulin secretion after 48-h culture in 5.6 mmol/l glucose (Fig. 2A). In addition, at any glucose concentration >5.6 mmol/l, islets from the Px14 group showed a higher insulin release than control14 group islets (*P* < 0.001) (Fig. 2A). Insulin content in islets from control14, Px14, and 48 h-cultured Px14 groups were similar (19.09 ± 1.56, 17.76 ± 1.25, and 20.31 ± 2.29 ng/μg protein, respectively; 15 islets from 4 mice in control14 and Px14 groups and 8 islets from 3 mice in 48 h-cultured Px14 group). Finally, protein content in islets from control14 and Px14 groups was also similar (2.72 ± 0.19 and 2.99 ± 0.34 μg protein/islet, respectively; 15 islets from 4 mice in both groups).

Perfused mouse islets responded to a stepwise increase in glucose concentration with a rapid and transient burst of insulin secretion (first phase) followed by a plateau (second

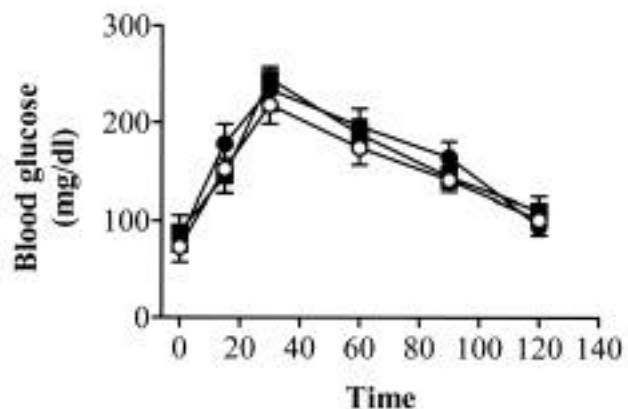


FIG. 1. Effect of pancreatectomy on glucose tolerance in control14 (○) (n = 4), Px14 (●) (n = 4), and Px60 (■) (n = 5) groups. Results are expressed as means ± SE.

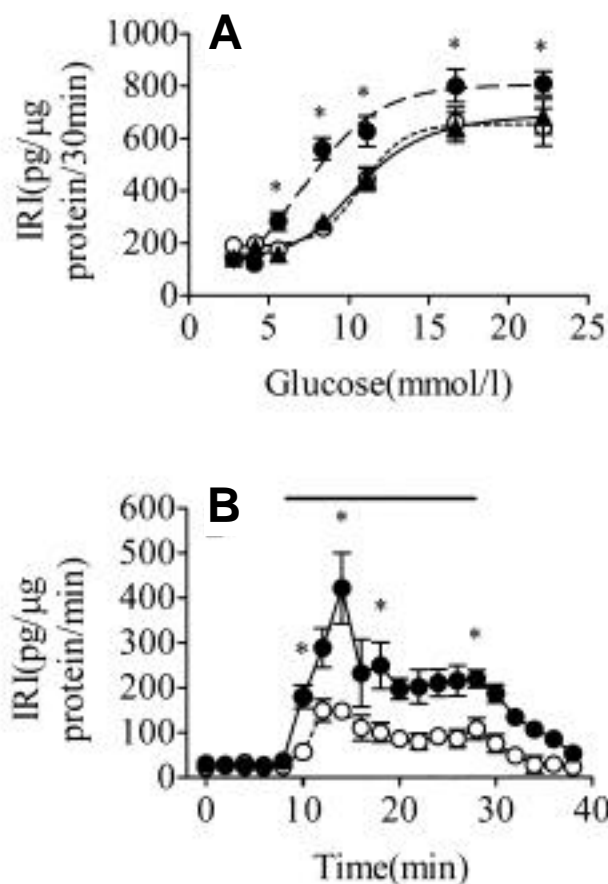


FIG. 2. Effect of pancreatectomy on glucose-induced insulin secretion. **A:** Islets from control14 (\circ), Px14 (\bullet), and Px14 cultured 48 h in 5.6 mmol/l glucose (\blacktriangle) groups were incubated in batches of three for 30 min at 37°C in 1 ml fresh modified KRBB with 1% bovine serum albumin plus different glucose concentrations. Values are expressed as means \pm SE of eight experiments. * $P < 0.05$ compared with control group. **B:** Batches of 10 islets from control14 (\circ) and Px14 (\bullet) groups were perfused at a flow rate of 1 ml/min at 37°C. After a 30-min stabilization with 3 mmol/l glucose, the islets were perfused for 10 min with 3 mmol/l glucose, then for 20 min with 22 mmol/l glucose (as indicated by the bar), and finally for 10 min with 3 mmol/l glucose. The perfusion medium was fresh modified KRBB with 1% bovine serum albumin alone. Insulin was assayed by RIA, and determinations were run in triplicate. Values are expressed as means \pm SE of six experiments. * $P < 0.001$ compared with control14 group.

phase) which lasted as long as glucose was present. Insulin release from Px14 islets increased significantly ($P < 0.001$) compared with control14 group islets (Fig. 2B).

Effect of pancreatectomy on membrane potential and $[Ca^{2+}]_i$. The glucose-induced electrical activity in β -cells from control14 and Px14 islets is summarized in Fig. 3. In the Px14 group, β -cells (8 from 8 islets in 6 mice) showed an increased glucose sensitivity (Fig. 3B) compared with β -cells from control14 group islets (Fig. 3A) (7 β -cells from 7 islets in 4 mice). β -Cells from the Px14 group showed oscillatory electrical activity at glucose concentrations (5.6 mmol/l) (Fig. 3B) lower than in β -cells from the control14 group (11.1 mmol/l) (Fig. 3A). Furthermore, the pattern of response in control14 group β -cells elicited with maximal glucose concentrations (22 mmol/l) (data not shown) was achieved in β -cells from the Px14 group with 16.7 mmol/l glucose (Fig. 3B). In contrast, no significant differences were found in the electrical activity of β -cells from control and Px60 groups

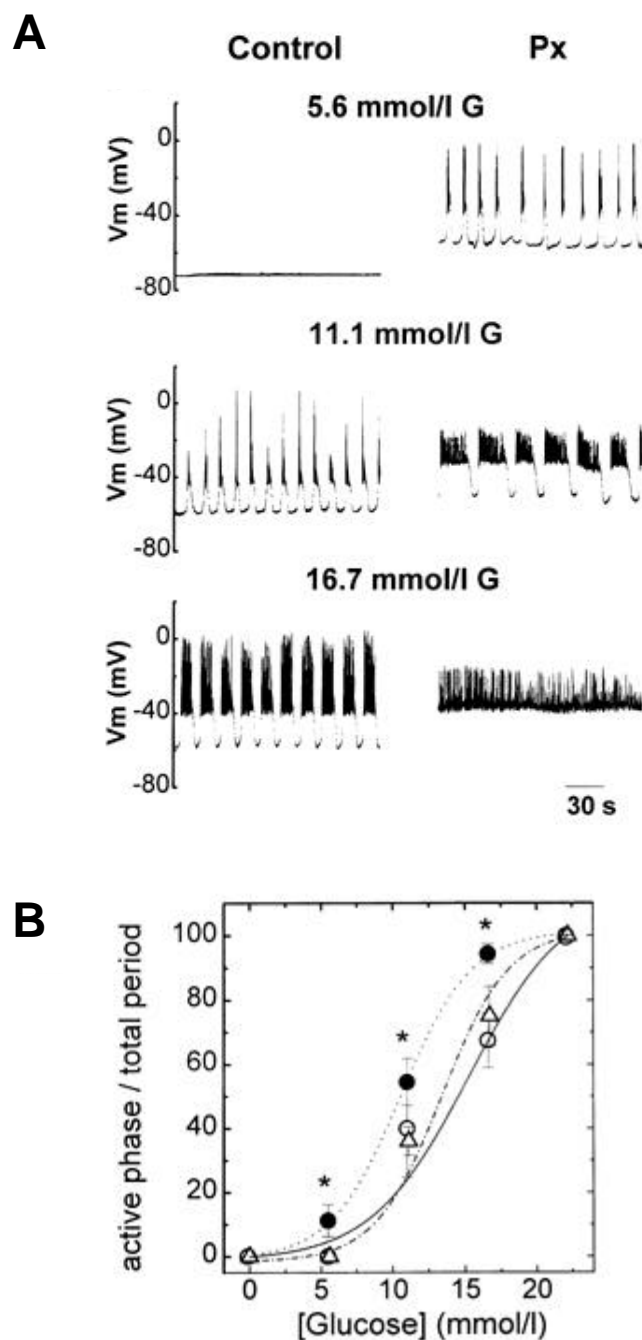


FIG. 3. Glucose-induced electrical activity in islets from pancreatectomized mice. **A:** Representative examples of the effects of different glucose concentrations (5.6, 11.1, and 16.7 mmol/l) on intracellularly recorded membrane potential of a pancreatic β -cell in the intact islet. **B:** Fraction of time in the active phase in control14 (\circ), Px14 (\bullet), and Px60 (\triangle) islet β -cells. In control14, Px14, and Px60 groups, the same islet was perfused with different glucose concentrations. G, glucose. * $P < 0.05$ compared with control14 group.

(12 β -cells from 12 islets in 4 mice) (data not shown). When the parameters studied (fraction of time in active phase) were plotted as a function of glucose concentrations, the dose-response curve in β -cells from Px14 group was found to be shifted left compared with β -cells from control14 and Px60 groups (Fig. 3B). Values for the input resistance in β -cells from control14 and Px14 groups were similar under non-

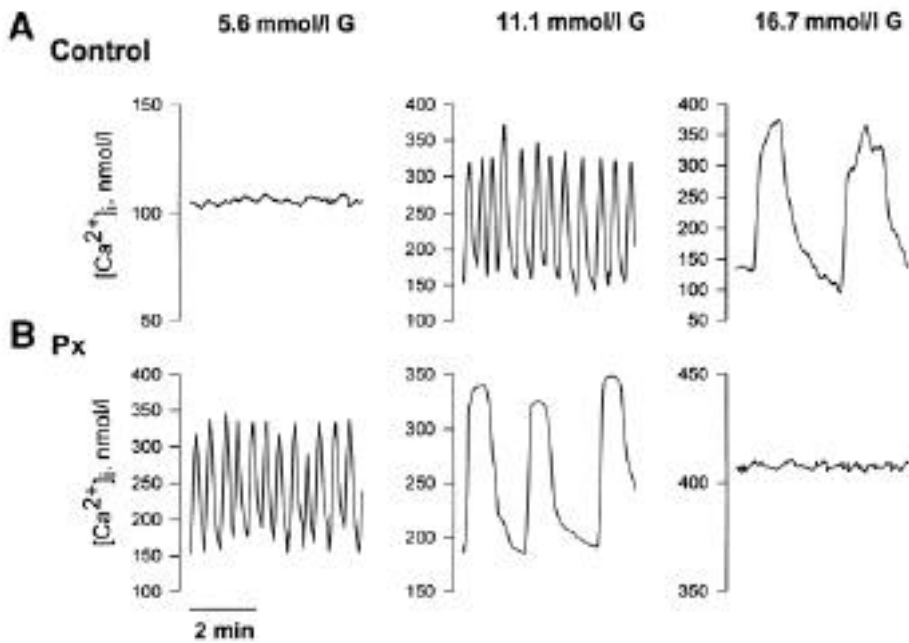


FIG. 4. Glucose-induced $[Ca^{2+}]_i$ changes in islets from pancreatectomized mice. Representative examples of the effect of different glucose concentrations (5.6, 11.1, and 16.7 mmol/l) on $[Ca^{2+}]_i$. In control14 and Px14 groups, the same islet was perfused with different glucose concentrations. G, glucose.

stimulatory (180 ± 30 and 150 ± 20 M Ω , respectively) and stimulatory (235 ± 35 and 220 ± 35 M Ω , respectively) glucose concentrations.

$[Ca^{2+}]_i$ responses to glucose are shown in Fig. 4. Islets from the Px14 group showed $[Ca^{2+}]_i$ oscillations in response to lower glucose concentrations (5.6 mmol/l) (Fig. 4B) than control14 group islets (11.1 mmol/l) (Fig. 4A). In the presence of 11.1 mmol/l glucose, $[Ca^{2+}]_i$ oscillations in Px14 group were broader in duration (121.1 ± 10.3 s, $P < 0.001$; 12 islets from 5 mice) (Fig. 4B) compared with islets from the control14 group (20.7 ± 1.7 s; 17 islets from 5 mice) (Fig. 4A). In addition, the pattern of response in islets from control14 group elicited with maximal glucose concentrations (22 mmol/l) (data not shown) was achieved in Px14 islets with 16.7 mmol/l glucose (Fig. 4B). In contrast, no significant differences were found in $[Ca^{2+}]_i$ in islets from the Px60 group (7 islets from 4 mice) compared with islets from the control14 group (data not shown).

Islets from the Px14 group recovered their normal glucose-induced $[Ca^{2+}]_i$ oscillatory pattern after 48-h culture (Fig. 5). As in islets from the control14 group, 48 h-cultured Px14 islets did not exhibit any $[Ca^{2+}]_i$ oscillatory behavior in response to 5.6 mmol/l glucose (12 islets from 3 mice) (Fig. 5B) and elicited 11.1 mmol/l glucose-induced regular $[Ca^{2+}]_i$ oscillations (Fig. 5C). Of note, 48-h culture in 5.6 mmol/l glucose did not significantly affect glucose-induced insulin release in control islets (data not shown). There were no significant differences between the $[Ca^{2+}]_i$ levels (10 min of register): 339.8 ± 31.3 nmol/l in control14 group islets perfused with 11.1 mmol/l glucose (Fig. 4A); 367.5 ± 41.3 nmol/l in Px14 group islets perfused with 5.6 mmol/l glucose (Fig. 4B); 379.4 ± 52.8 nmol/l in Px14 group islets perfused with 5.6 mmol/l glucose (Fig. 5A); and 402.7 ± 49.6 nmol/l in 48 h-cultured Px14 group islets perfused with 11.1 mmol/l glucose (Fig. 5C).

Effect of pancreatectomy on K_{ATP} channel activity. Figure 6 shows the dose-response relationship for the inhibition of K_{ATP} channel activity in the presence of different concentrations of ATP in β -cells from Px14 and control14 groups. The two representative current records (control

and Px14) shown at the top illustrate the effects of 2.5 and 40 μ mol/l ATP on K_{ATP} channel activity. Patches were initially exposed to 2 mmol/l ATP, then to 0 mmol/l ATP, then to a test ATP concentration. ATP (2 mmol/l) was applied after every test to minimize rundown. The current in 2 mmol/l ATP was

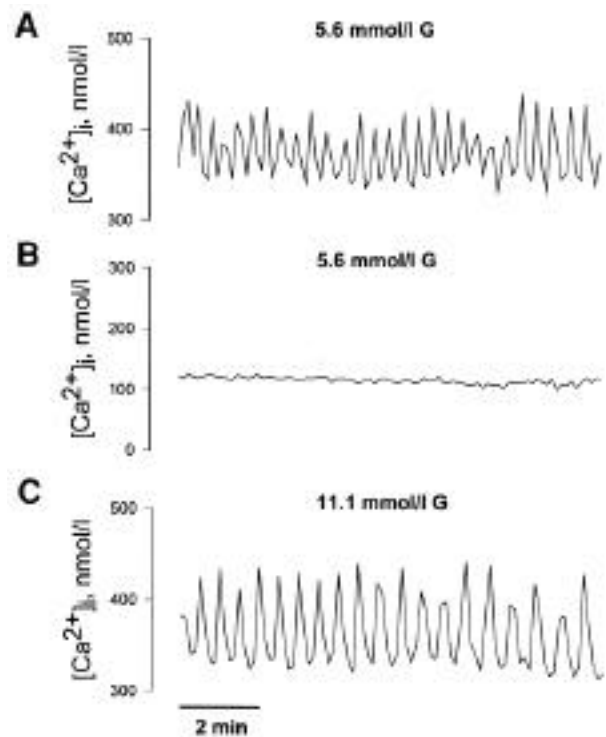


FIG. 5. Recovery of glucose-induced $[Ca^{2+}]_i$ changes in islets from pancreatectomized mice. A: Representative example of the effect of 5.6 mmol/l glucose concentration on $[Ca^{2+}]_i$ in freshly isolated islets from Px14 group. B: Representative example of the effect of 5.6 mmol/l glucose concentration on $[Ca^{2+}]_i$ in 48 h-cultured islets from Px14 group. C: Representative example of the effect of 11.1 mmol/l glucose concentration on $[Ca^{2+}]_i$ in 48 h-cultured islets from Px14 group. Groups are described in METHODS. G, glucose.

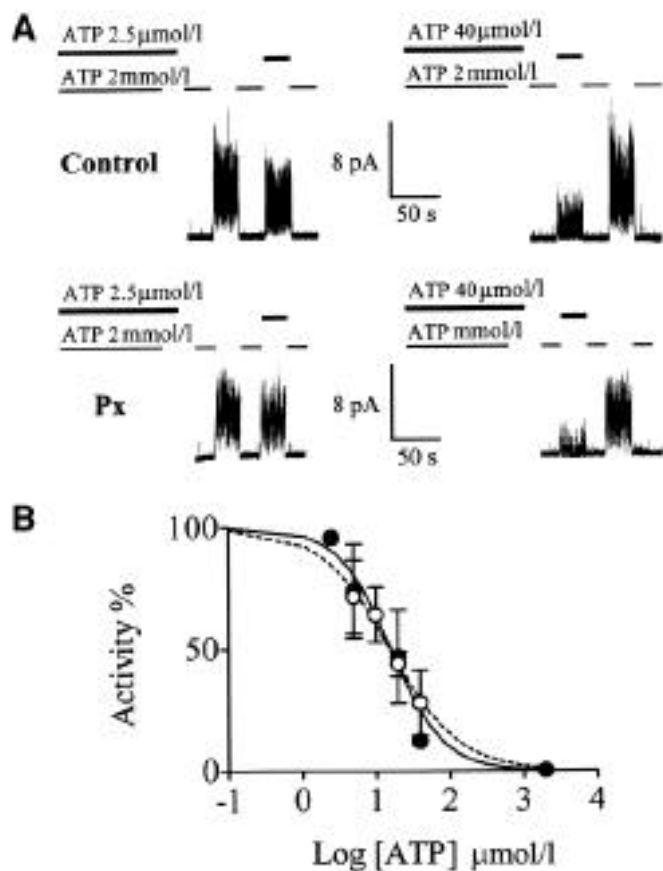


FIG. 6. Effects of ATP on K^+_{ATP} channel activity in inside-out membrane patches from pancreatic β -cells. **A:** Representative recordings of channel activity in a patch exposed to 2.5 and 40 $\mu\text{mol/l}$ ATP, as indicated above the record, in control14 and Px14 groups. **B:** Dose-response relationship for ATP inhibition of K^+_{ATP} channel activity in pancreatic β -cells from control14 (\circ) and Px14 (\bullet) groups. In this figure, the ordinate represents patch current relative to that in 0 mmol/l ATP (I_{max}). The points show means \pm SD, $n = 3$. Groups are described in METHODS.

taken as \emptyset current and subtracted from the current measured in each test solution. Current in the absence of ATP was taken as I_{max} . The average current in each ATP exposure was expressed as a fraction of I_{max} . No significant differences for ATP inhibition were found between β -cells from the Px14 and control14 groups. The data could be fitted to a Hill equation with an IC_{50} of 15.9 $\mu\text{mol/l}$ and a Hill coefficient of 1.2 for the Px14 group ($n = 3$) and an IC_{50} of 15.5 $\mu\text{mol/l}$ and a Hill coefficient of 0.9 for the control14 group ($n = 3$) (Fig. 6B). No significant difference was observed in the unitary current between β -cells from Px14 and control14 groups (data not shown).

MTT reduction. Both control14 and Px14 islets incubated in the presence of MTT showed a sigmoidal increase in formazan production as a function of increasing extracellular glucose concentration, reaching a maximal value at 11.1 mmol/l glucose (Fig. 7). However, a significant displacement to the left in the dose-response curve was observed in Px14 islets ($\text{EC}_{50} = 7.14 \pm 0.12$ mmol/l) compared with control14 group islets ($\text{EC}_{50} = 9.76 \pm 0.25$ mmol/l) ($F_{(2, 50)} = 13.41$; $P < 0.001$) (Fig. 7). Additionally, there was a broadening in the glucose response range through a reduction of the threshold to glucose metabolism. Compared with basal values, MTT reduc-

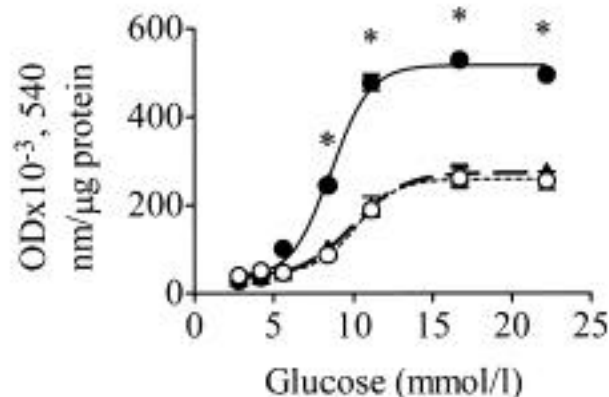


FIG. 7. Effect of pancreatectomy on glucose-induced MTT reduction. Batches of 15 islets from control14 (\circ), Px14 (\bullet), and Px14 cultured for 48 h in 5.6 mmol/l glucose (\blacktriangle) groups were incubated for 30 min at 37°C in 1 ml fresh modified KRB buffer with 1% bovine serum albumin, 0.5 mg/ml MTT, and different glucose concentrations. The formation of intracellular MTT-formazan crystals was measured by spectrophotometry at 540 nm. All determinations were run in triplicate. Values are expressed as means \pm SE of four experiments. * $P < 0.001$ compared with control14 group.

tion in Px14 islets was increased at 5.6 mmol/l glucose ($P < 0.01$) (Fig. 7); in control14 group islets, the increase appeared at 8.3 mmol/l glucose ($P < 0.05$) (Fig. 7). Moreover, Px14 islets showed a significant increase in MTT glucose reduction at glucose concentrations higher than 5.6 mmol/l ($P < 0.01$), and the maximal values were twice those observed in islets from control14 group. Islets from Px14 group recovered a normal MTT glucose reduction after 48-h culture in 5.6 mmol/l glucose (Fig. 7).

DISCUSSION

In this study, we investigated the stimulus-secretion coupling mechanisms in 60% Px mice, a model in which normoglycemia and normolipidemia are maintained despite reduced β -cell mass.

The metabolic characteristics and growth of the pancreatic remnant in 60% Px rats have been well characterized (16), and we anticipated that the model would have similar characteristics in mice. The 60% Px mice remained normoglycemic despite the significant β -cell mass reduction, excluding the possibility of any transitory hyperglycemia interfering with changes in glucose sensitivity. In addition, 60% Px mice were normolipidemic, excluding the possibility that altered plasma FFA levels led to the observed changes. Furthermore, progressive β -cell regeneration in the pancreatic remnant provided an excellent model to investigate the reversibility of changes in β -cell glucose sensitivity and their relationship with β -cell mass. Although β -cell mass increased markedly after partial Px, it was still reduced 14 days after Px, at which time β -cell glucose sensitivity (insulin secretion, electrical activity, and $[\text{Ca}^{2+}]_i$) was increased. In contrast, 60 days after Px, β -cell mass increased to 79% of control values and glucose sensitivity was similar in control and Px60 mice. We suggest that the increased glucose sensitivity 14 days after Px was an adaptive response of β -cells to meet the increased metabolic demand placed on a reduced β -cell mass. The reversibility of this adaptive mechanism, shown by the normal glucose sensitivity of the Px 60 group, was confirmed by the normal glucose sensitivity observed in Px 14 groups islets after a 48-h culture period.

Increased β -cell sensitivity to glucose has been shown to precede the loss of glucose-induced insulin secretion in diabetic rats and could be the event initiating impaired glucose potentiation elicited by chronic hyperglycemia (8). However, glucose supersensitivity has also been found in several normoglycemic conditions characterized by the increased workload placed on β -cells. This suggests that hyperglycemia is not the cause of increased β -cell sensitivity. Changes have been found in glucokinase, hexokinase, and GLUT2 as islets adapt to increased metabolic demand, but may not fully account for the increased glucose sensitivity (27). Recently, we described an increased β -cell glucose sensitivity in islet cells transplanted into diabetic mice that maintained normoglycemia with a reduced cell β -mass (15). The glucose-induced electrical activity and $[Ca^{2+}]_i$ response in transplanted β -cells was found to be similar to that found in Px14 β -cells. In that study, glucose supersensitivity was attributed to a resting decrease in the fraction of blockable K_{ATP} channels, which could be caused by either an increase in the glucose metabolic modulators of K_{ATP} channels or a change in the properties of the channel. To address the second possibility, we studied the input resistance and K_{ATP} channel sensitivity to ATP. No significant differences were observed in the input resistances between β -cells from Px14 and control groups, indicating that the numbers of K_{ATP} channels were similar for β -cells from both groups. Finally, patch-clamp studies did not show any modification of ATP effect on K_{ATP} channels in β -cells from Px14 mice compared with control mice. Furthermore, Px did not affect the magnitude of the unitary current. These data, together with no significant modification in channel activity, imply that there is no significant change in the open probability.

Glucose metabolism was assessed by the MTT reduction test. The intracellular reduction of the tetrazolium dye MTT allows determination of β -cell metabolic activity (26). A very good correlation has been shown between MTT reduction, glycolytic flux, glucose oxidation, and insulin secretion in INS cells and rat islets (26). The MTT reduction experiments showed a significant displacement to the left of the β -cell oxidative metabolism, with an increased glucose metabolism at all glucose concentrations tested (except 2.8 mmol/l glucose) in islets from the Px14 group. A similar pattern was observed for insulin release in islets from the Px14 group. These data suggest that metabolic steps of the stimulus-secretion coupling mechanism are probably responsible for the increased glucose sensitivity. An additional role of glucokinase in this phenomenon cannot be ruled out (12). The enhanced insulin secretion observed in Px14 islets from 5.6 mmol/l glucose could also be indicative of augmentation of the Ca^{2+} -dependent mechanism of insulin secretion by K_{ATP} channel-independent glucose signaling. In addition, GTP-dependent mechanisms (28) could be involved in the increased glucose-induced insulin secretion elicited by Px islets.

The results described here show that in β -cells from the Px14 group, 1) there is a displacement to the left of the glucose dose-response curve and β -cell oxidative metabolism, with a decrease in the glucose threshold; 2) there is an increase in the dynamic range of the system in terms of oscillatory electrical activity and $[Ca^{2+}]_i$ oscillations; and 3) there are no modifications in the channel properties of the K_{ATP} channel. In summary, we have found an increased sensitivity to glucose for insulin secretion 14 days after 60% Px, during which time normoglycemia was maintained with a reduced

β -cell mass. β -Cell glucose sensitivity returned to normal when β -cell mass increased to control mice values, indicating that the change in sensibility was a reversible adaptive mechanism in response to the increased workload placed on β -cells. The lowered glucose set-point was not due to modification of the K_{ATP} channel's intrinsic properties. Thus, the results suggest that increased glucose sensitivity in terms of insulin secretion, electrical activity, and Ca^{2+} signaling is probably due to changes in glucose metabolism.

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