Glucose Uptake, Utilization, and Signaling in GLUT2-Null Islets

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We previously reported that pancreatic islet β-cells from GLUT2-null mice lost the first phase but preserved the second phase of glucose-stimulated insulin secretion (GSIS). Furthermore, we showed that the remaining secretory activity required glucose uptake and metabolism because it can be blocked by inhibition of oxidative phosphorylation. Here, we extend these previous studies by analyzing, in GLUT2-null islets, glucose transporter isoforms and glucokinase expression and by measuring glucose usage, GSIS, and glucose-stimulated insulin mRNA biosynthesis. We show that in the absence of GLUT2, no compensatory expression of either GLUT1 or GLUT3 is observed and that glucokinase is expressed at normal levels. Glucose usage by isolated islets was increased between 1 and 6 mmol/l glucose but was not further increased between 6 and 20 mmol/l glucose. Parallel GSIS measurements showed that insulin secretion was not stimulated between 2.8 and 6 mmol/l glucose but was increased by >4-fold between 6 and 20 mmol/l glucose. Stimulation by glucose of total protein and insulin biosynthesis was also markedly impaired in the absence of GLUT2. Finally, we re-expressed GLUT2 in GLUT2-null β-cells using recombinant lentiviruses and demonstrated a restoration of normal GSIS. Together, these data show that in the absence of GLUT2, glucose can still be taken up by β-cells, albeit at a low rate, and that this transport activity is unlikely to be attributed to GLUT1 or GLUT3. This uptake activity, however, is limiting for normal glucose utilization and signaling to secretion and translation. These data further demonstrate the key role of GLUT2 in murine β-cells for glucose signaling to insulin secretion and biosynthesis. *Diabetes* 49:1485–1491, 2000

Glucose uptake is the initial step in glucose-stimulated insulin secretion (GSIS) by pancreatic β-cells. In rodents, the low-affinity (K_M = 17 mmol/l) glucose transporter GLUT2 is the only glucose transporter normally detected in pancreatic β-cells (1–3). Decreased expression of this transporter occurs simultaneously with the loss of GSIS in numerous animal models of type 2 diabetes (4). The decreased expression of the transporter is probably secondary to other diabetic metabolic alterations (5), in particular increases in plasma free fatty acid (6,7) or glucocorticoid levels (8,9). However, this decreased expression generally does not appear to be sufficient to limit glucose access to glucokinase and, therefore, to prevent GSIS (10,11).

Recently, we generated GLUT2-null mice by homologous recombination (12). We demonstrated, in perifusion experiments, that the absence of GLUT2 from mouse islets leads to the loss of the first phase of insulin secretion. However, a second phase of secretion was still present and was dependent on glucose metabolism because poisoning the mitochondria with antimycin A1 completely abrogated this secretory response. This result indicated that, even in the complete absence of GLUT2, glucose could still be taken up by β-cells and metabolized to generate a secretory signal. We had further shown that glutamine plus leucine, or glyceraldehyde, induced a normal secretory response. This finding indicated a normal functioning of the signaling pathway either from amino acids or from the triose step in glycolysis to insulin granule exocytosis. Together, these data suggested that the loss of first-phase insulin secretion could be the result of the absence of the low-affinity GLUT2 transporter or, because glucose could still be taken up by β-cells, that an alternative glucose uptake process was activated, although glucokinase expression would be strongly reduced. Indeed, it has been suggested that expression of glucokinase could be stimulated by the presence of GLUT2 through either an increase in glucokinase mRNA or direct interaction of GLUT2 with glucokinase (13–15).

Here, we further analyzed glucose metabolism and signaling in GLUT2$^{-/-}$ islets. We demonstrated that glucokinase was normally expressed but that glucose utilization was only slightly increased between 1 and 6 mmol/l glucose and not further increased with higher glucose concentrations, indicating a limiting uptake capacity. The remaining second phase of secretion of GLUT2$^{-/-}$ islets was, however, still increased in a glucose concentration–dependent manner with a 4- to 7-fold stimulation between 6 and 20 mmol/l glucose. Recovery of first-phase secretion was restored by re-expressing GLUT2 with the use of recombinant lentiviruses. These data demonstrate the important role of GLUT2 in murine islets for normal glucose metabolism and signaling to secretion and insulin biosynthesis.

RESEARCH DESIGN AND METHODS

Animals. GLUT2-null mice were from our colony (12). Control mice were either of the GLUT2$^{+/-}$ or the GLUT2$^{+/+}$ genotype; no difference in any of the parameters studied was observed between these 2 groups. Because the GLUT2$^{-/-}$ mice die between 2 and 3 weeks of age, these studies were performed with ~2-week-old mutant and control mice.

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BSA, bovine serum albumin; GSIS, glucose-stimulated insulin secretion; IBMX, isobutylmethyl xanthine; KRBH, HEPES-buffered Krebs Ringer; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride.

Analytical studies

Immunofluorescence microscopy and Western blot analysis. Pancreases were fixed with a paraformaldehyde-lysine-periodate solution, and frozen sections were prepared and processed for immunofluorescence detection of glucagon as previously described (12). Total islet lysates were prepared in a solution containing 80 mmol/l Tris-HCl, pH 6.8, 5 mmol/l EDTA, 2 mmol/l phenylmethylsulfonyl fluoride (PMSF), and 5% SDS (8). Lysates from identical numbers of control or mutant islets were separated on 10% SDS-PAGE and transferred to nitrocellulose filters, and specific proteins were detected with antibodies specific to GLUT1 (16), GLUT3 (a gift from Dr. M. Mueckler, St. Louis, MO), or glucokinase (a gift from Dr. Magnuson, Nashville, TN). Detection was performed with secondary antibodies linked to horseradish peroxidase and chemiluminescence (Amersham).

Islet isolation and in vitro culture. For islet isolation, pancreases were excised and placed in a 2-mg/ml collagenase solution (Collagenase type IV; Worthington) in Hank's balanced salt solution containing 10 mmol/l HEPES, pH 7.4. Tissue was minced and incubated for 38 min at 37°C. After washing of the digested tissue, islets were handpicked under a stereomicroscope and either used at once or cultured up to 48 h in RPMI-1640 containing 10% fetal calf serum, 10 mmol/l HEPES, 1 mmol/l sodium pyruvate, and 50 µmol/l β-mercaptoethanol. DNA content was measured as previously described (12)

Glucose utilization. Freshly isolated islets in batches of 50 were incubated for 2 h at 37°C in the presence of different glucose concentrations and tracer amounts of D-[5-^{3}H]glucose (DuPont NEN). After stopping the reaction and lysing the cells with 1 N HCl, the ^3H_2O produced was separated from the reaction supernatant by passage over Dowex 1 × 2 (Fluka, Switzerland) columns. A sample of 0.1 µCi ^3H_2O (American Radiolabelled Chemicals) was passaged over similar columns to quantify the recovery efficiency. Measurement of eluted radioactivity was performed with scintillation counting.

Insulin secretion and radioimmunoassay. Insulin secretion from freshly isolated islets or islets kept overnight in a culture medium (6) was measured in static incubations. Islets were first incubated for 2 h in the presence of 2.8 mmol/l glucose in HEPES-buffered Krebs Ringer (KRBH) solution containing 0.5% bovine serum albumin (BSA) and 0.5 mmol/l isobutylmethyl xanthine (IBMX). Addition of IBMX is required to stimulate a robust secretion of insulin from mouse islets, as previously described (12). Islets were then transferred to the same KRBH-BSA-IBMX solution containing 2.8, 6, or 20 mmol/l glucose for 2 h. Supernatants were then saved for insulin determination, and islets were lysed in ethanol-acid for measurement of total insulin content. Perifusion experiments were performed as previously described (12) using KRBH-BSA containing 2.8 or 16.7 mmol/l glucose and 0.5 mmol/l IBMX. Radioimmunoassays were performed with a kit from Linco (St. Louis, MO) using rat insulin as the standard.

Insulin biosynthesis. For insulin biosynthesis experiments, islets in batches of 30 were first incubated in KRBH containing 1.4 mmol/l glucose for 1 h at 37°C. They were then transferred to KRBH containing different glucose concentrations and kept at 37°C for 1 h. A sample of 10 µCi ^{35}S-methionine (DuPont) was added to the islets 10 min before the end of the second incubation period. Labeling was stopped by washing the islets in cold phosphate-buffered saline (PBS) solution. Islets were lysed in 300 µl PBS containing 1% Triton X-100, 5 mmol/l EDTA, 1 mmol/l PMSF, and 2 mmol/l *N*-ethylmaleimide. The supernatant was collected, three 5-µl aliquots were used for measurement of total trichloroacetic acid-precipitable radioactivity, and the rest was used for immunoprecipitation with an anti-insulin antibody (Linco). The immunoprecipitates were separated on Tris-Tricine SDS gels. Radioactive bands were detected after treatment of the gel with diphenyloxazol, drying, and exposure to X-ray films at –70°C. Quantification was performed by laser scanning densitometry.

Lentivirus preparation and use. The rat islet Glut2 cDNA from pcDNA3-Glut2 (17) was inserted as a *Bam*HI (partial)-*Xho*I fragment into the HR-CMV-lacZ vector (18). Stocks of lentiviral vectors carrying the LacZ or Glut2 genes packaged by the multiply attenuated lentivirus CMVΔR8.91 and pseudotyped with the vesicular stomatitis virus G glycoprotein envelope (plasmid pMD-G) were prepared by transient transfection of 293T-cells and titered as previously described (19,20). Islets were infected in suspension 2 h after collagenase isolation. A total of 100 islets were infected in 1 ml nonconcentrated viral stock of LacZ or Glut2 virus (5–10^5 IU/ml, multiplicity of infection 10–20) for 4 h. Islets were then diluted in 10 ml RPMI at 37°C, and perifusion experiments were carried out 36 h after infection.

RESULTS

Characterization of GLUT2-null islets. Islets were isolated from GLUT2-null mice or from control littermates. The islets from both genotypes were of approximately the same size as determined by microscopic examination. This was confirmed by measuring the isolated islet DNA content, which was 11.9 ± 3.4 and 9.8 ± 2.9 ng/islet (mean ± SD, n = 4 batches of 60 islets) for control and GLUT2$^{-/-}$ islets, respectively. The insulin to DNA content of the GLUT2-null islets was, however, ~40% that of control islets: 13.3 ± 3.8 and 31.8 ± 7.8 µU/ng DNA (means ± SD, n = 4 batches of 60 islets). This was due to an increased number of α-cells and a decrease in the number of β-cells. This is illustrated in Fig. 1; the volume density of α-cells was, on average, 50% higher in GLUT2$^{-/-}$ compared with control islets (12).

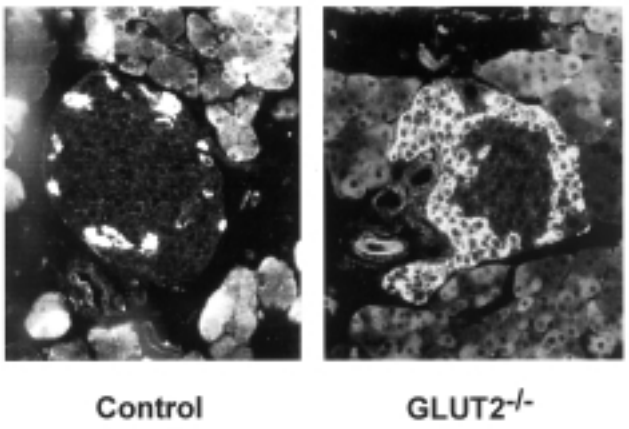

FIG. 1. Immunofluorescence detection of glucagon cells in control (left panel) and GLUT2-null islets (right panel). In the absence of GLUT2, there are more α-cells and fewer β-cells, but the average size of the islets is similar, as confirmed by measurement of total DNA content.

Glucose transporters and glucokinase expression in GLUT2-null islets. We first tested the expression of GLUT1 and GLUT3 by Western blot analysis of islet lysates obtained from batches of 40 control or GLUT2$^{-/-}$ islets. Figure 2 shows that only extremely low levels of GLUT1 could be detected in control islets after an overnight culture period and that this level was not increased in the GLUT2-negative islets. Similarly, GLUT3 could not be detected in either the control or mutant mouse islets. Glucokinase expression was evaluated by Western blot analysis using 250 islets from control and GLUT2$^{-/-}$ mice. Figure 2 shows that a similar level of expression of glucokinase was found in control and mutant islets.

Glucose utilization, insulin secretion, and insulin biosynthesis. Glucose utilization was measured in freshly isolated islet at 1, 6, and 20 mmol/l glucose. The result of these experiments is presented in Fig. 3. Whereas the measured glucose utilization in control islets increased almost linearly across these 3 glucose concentrations, there was only a small increase in glucose utilization between 1 and 6 mmol/l glucose in the absence of GLUT2 and no further increase between 6 and 20 mmol/l glucose. Similar data were obtained when islets were first kept in culture overnight and the experiment was performed in the presence of IBMX (data not shown). We then evaluated the stimulation by glucose of total protein and insulin biosynthesis. This was performed in biosynthetic labeling experiments in the presence of radioactive methionine and in a range of glucose concentrations extending from 1.4 to 20 mmol/l glucose. Figure 4*A* shows that in the absence of GLUT2 there was a marked reduction of glucose-stimulated

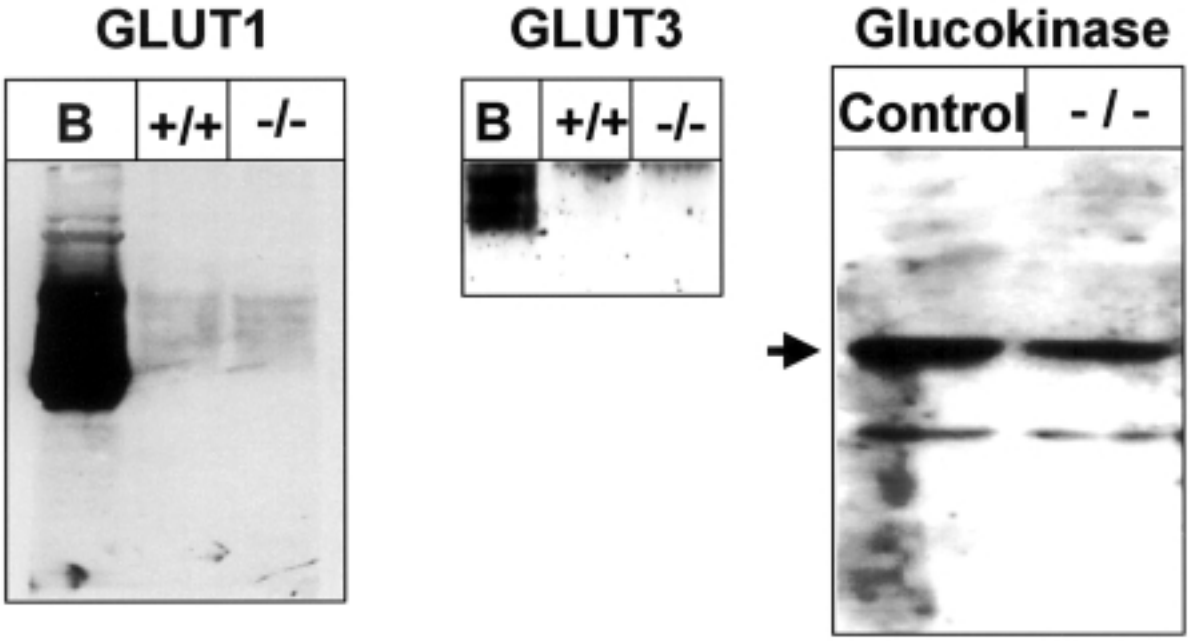

FIG. 2. Western blot analysis of GLUT1, GLUT3, and glucokinase expression in islets from control (+/+) and GLUT2$^{-/-}$ (–/–) mice. Total lysates from 40 islets were separated by gel electrophoresis on SDS-PAGE and were blotted to nitrocellulose membranes. GLUT1 or GLUT3 proteins were detected using specific antibodies. Controls (B) were from 20 µg brain membrane proteins. Only very low levels of GLUT1 could be detected in control and mutant islets. No GLUT3 could be detected. For glucokinase detection, the total lysate from 250 control or GLUT2-null islets were analyzed. Similar levels of glucokinase (as indicated by the arrow) were found in both types of islets.

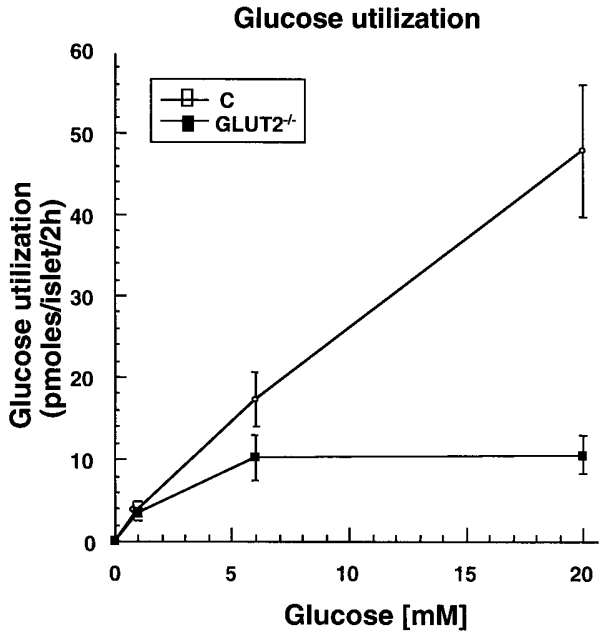

FIG. 3. Glucose utilization by islets from control and GLUT2$^{-/-}$ islets. Freshly isolated islets were incubated in the presence of the indicated concentrations of glucose and D-[5-^{3}H]glucose for 2 h, and production of ^3H_2O was then measured to determine the glucose utilization rate. Glucose utilization increased slightly between 1 and 6 mmol/l glucose but was not further increased with 20 mmol/l glucose. Data are means ± SE of 3 and 4 independent experiments for GLUT2$^{-/-}$ and control islets, respectively. C, controls.

total protein synthesis. Stimulation of insulin biosynthesis over total protein synthesis was still observed in GLUT2-null islets (Fig. 4*B*). However, this effect was markedly decreased compared with that found in the control islets.

GSIS was then measured using freshly isolated islets maintained in static incubation conditions similar to those used for glucose utilization measurements. Figure 5*A* shows that GSIS did not significantly increase between 2.8 and 6 mmol/l glucose but was increased by ~4-fold between 6 and 20 mmol/l glucose. However, the maximal amount of secreted insulin reached only 1.4% of the total insulin content. When the islets were incubated overnight in culture medium before the secretion test was performed, there was again no stimulation between the 2 lowest concentrations but a >7-fold increase between 6 and 20 mmol/l glucose (Fig. 5*B*). Additionally, the maximal amount of secreted insulin reached 4.7% of the total insulin content.

In control islets, insulin was secreted at a progressively increasing rate over the experimental glucose concentration range, and the absolute value of secretion was much greater than that with mutant islets, reaching 15.3 and 18.3% of the total islet insulin content with freshly isolated or cultured islets, respectively (Fig. 5*C* and *D*). The absolute value of the islet insulin content is listed in Fig. 5.

To determine whether the glucose-stimulated insulin-secretory response of GLUT2-null islets was due to a fuel replenishment effect or a true signaling effect, we performed secretion experiments in the presence of 0.5 mmol/l leucine and 0.5 mmol/l glutamine as a source of metabolic energy and in the presence of increasing glucose concentrations. In preliminary experiments, we determined that this concentra-

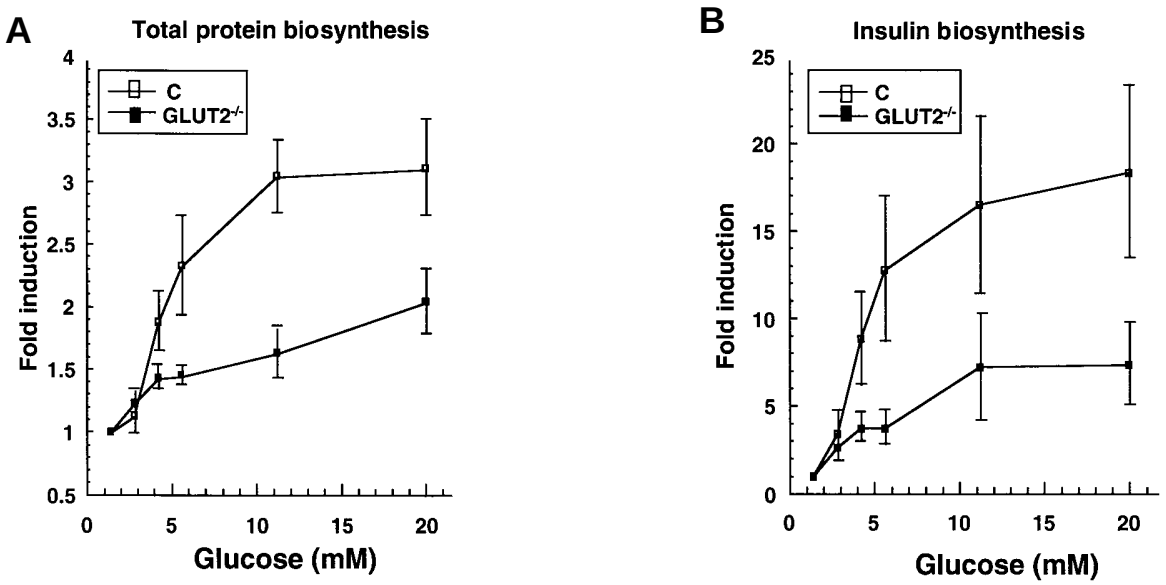

FIG. 4. Glucose-stimulated total protein (*A*) and insulin (*B*) biosynthesis. Islets were pulse-labeled for 10 min at the end of a 1-h incubation in the presence of the indicated glucose concentrations. Total protein biosynthesis was measured as trichloroacetic acid–precipitable radioactivity. Insulin biosynthesis was measured after immunoprecipitation of proinsulin and/or insulin, separation by gel electrophoresis, and laser scanning densitometry. Data are means ± SE for 4 and 5 experiments for insulin biosynthesis and total protein, respectively. C, controls.

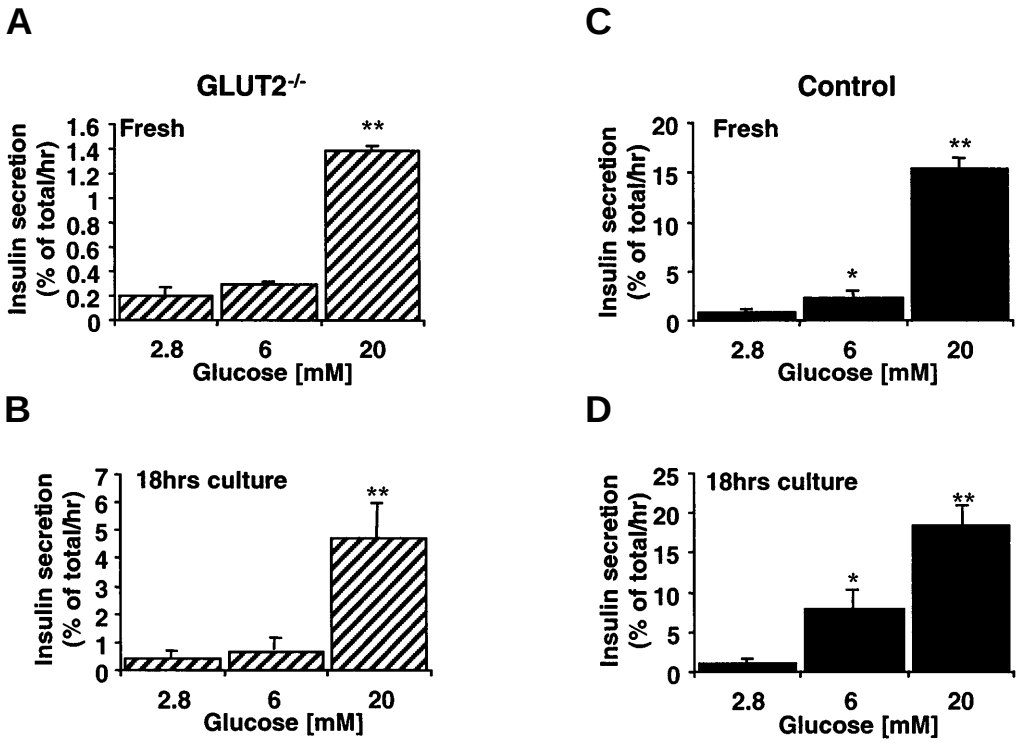

FIG. 5. GSIS by islets from control or GLUT2$^{-/-}$ mice. *A* and *C*: Freshly isolated islets from GLUT2$^{-/-}$ and control mice were preincubated for 1 h in the presence of 2.8 mmol/l glucose and then transferred for 2 h in the presence of the indicated glucose concentrations. Insulin secretion was assessed by radioimmunoassay and expressed as percent of total insulin content released per hour. Data are means ± SE of 5 experiments, each of which was performed in duplicate for each glucose concentration. The total islet insulin content was 2,310 ± 119 µU per 10 islets for the GLUT2$^{-/-}$ islets (range 2,104–2,688 µU) and 5,551 ± 509 µU per 10 islets (range 2,984–8,334 µU) for the control islets. *B* and *D*: Islets isolated from GLUT2$^{-/-}$ and control mice were cultured for 18 h before GSIS was assessed as previously described in *A* and *C*. Data are means ± SE of 3 experiments, each of which was performed in duplicate for each glucose concentration. The total islet insulin content was 3,077 ± 150 µU per 10 islets (range 3,052–3,116) for the GLUT2$^{-/-}$ islets, and 8,587 ± 614 µU per 10 islets (range 5,685–11,980) for the control islets.

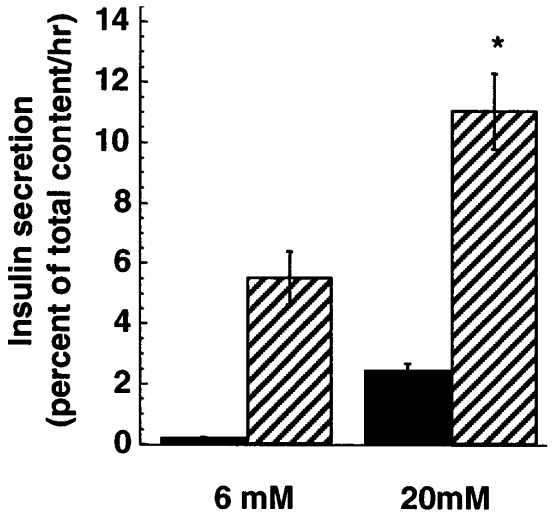

FIG. 6. GSIS by GLUT2-null islets is due to a true glucose signal. Islets were incubated for 2 h in the presence of the indicated glucose concentration and in the absence or presence of 0.5 mmol/l leucine and 0.5 mmol/l glutamine, and secreted insulin was measured. The combination of amino acids induced a strong secretory response, but addition of increasing glucose concentrations still induced an additive secretory response. ■, Glucose; ▨, glucose plus amino acids. *$P < 0.05$ vs. 6 mmol/l glucose plus amino acids.

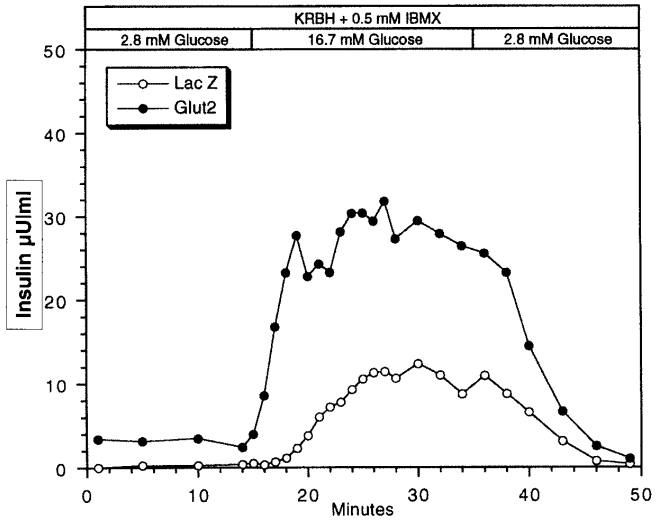

FIG. 7. Normal GSIS is restored in GLUT2$^{-/-}$ islets by GLUT2 re-expression using recombinant lentiviruses. Islets isolated from GLUT2$^{-/-}$ mice were infected over a 4-h period with recombinant lentiviruses carrying either the LacZ gene or the GLUT2 cDNA. Perifusions were performed 36 h after infection. Infection with the GLUT2 but not the LacZ lentiviruses restored normal kinetics of insulin secretion. The experiment has been repeated twice with similar results.

tion of leucine and glutamine induced ~50% of the maximal secretory response obtained with 10 mmol/l of each amino acid. Figure 6 shows that even in the presence of 0.5 mmol/l leucine and glutamine, glucose still stimulated insulin secretion in a concentration-dependent manner. To evaluate whether glucose induced a secretory response through an osmolarity effect, we performed secretion experiments in the presence of 6, 20, and 60 mmol/l glucose and in the presence of 6 mmol/l glucose plus 54 mmol/l sucrose. Insulin was secreted by GLUT2$^{-/-}$ islets at the respective rates of 0.75 ± 0.4, 65 ± 24, 86 ± 27, and 4.0 ± 0.8 µU/h (5 islets per secretion test; each test was performed in triplicate). This result indicates that osmolarity could not account for the secretory effect of glucose. Lastly, to evaluate whether glucose could act without being metabolized, i.e., by a receptor dependent–like effect, we evaluated the capacity of 3-*O*-methyl-glucose to stimulate insulin secretion. Islets were exposed to 6 or 20 mmol/l glucose or to 6 mmol/l glucose plus 14 mmol/l 3-*O*-methyl-glucose. No stimulation of insulin secretion over that obtained with 6 mmol/l glucose was induced by further addition of 3-*O*-methyl-glucose (data not shown).

Rescue of the secretory response by lentivirus-mediated GLUT2 re-expression. To determine whether the lack of first-phase insulin secretion could be corrected by re-expressing GLUT2 in the islets, we constructed recombinant lentiviruses to transfer the GLUT2 cDNA in isolated islets. Lentiviruses expressing the lacZ gene were used as controls. We prepared islets from GLUT2-null mice and infected them in vitro with stocks of GLUT2- or LacZ-recombinant lentiviruses, which had been previously shown to allow expression of the respective proteins in insulinoma or islet cells. The infected islets were analyzed by perifusion experiments 48 h after isolation. Figure 7 shows that infection of GLUT2-null islets with the GLUT2 but not the LacZ-recombinant lentiviruses restored normal secretory activity.

DISCUSSION

In this study, we demonstrated that in the absence of GLUT2 from pancreatic islets, no re-expression of high-affinity glucose transporters (GLUT1 or GLUT3) could be detected. Furthermore, we showed that glucose utilization was limited when glucose concentrations were elevated and that no increase could be detected between 6 and 20 mmol/l glucose. This result was accompanied by a reduction in levels of glucose-stimulated proteins, insulin biosynthetic activity, and insulin secretion. However, even though there was no increase in glucose utilization between 6 and 20 mmol/l glucose, a >4-fold increase in secretion could be observed across this glucose concentration range. Finally, the limitation in glucose signaling was not due to the absence of glucokinase but to an insufficient rate of glucose transport because re-expression of GLUT2 leads to restoration of normal GSIS.

The facts that glucose can stimulate insulin secretion by GLUT2-null β-cells and that this secretory activity requires mitochondrial metabolism (12) suggest that glucose can still be taken up by these cells. However, the mechanism of glucose uptake in the absence of GLUT2 is not known. Our data show a very small expression of GLUT1 in control and mutant islets. It is not clear whether this transporter expression can account for the remaining uptake activity. Furthermore, we have not been able to localize this transporter to a specific cell type in mouse islets by immunofluorescence microscopy, probably because of its very low level of expression. Experiments carried out with rat islet cells have shown that culturing islets in low glucose concentrations can induce GLUT1 expression (21). This has also been demonstrated to occur in cell sorter–purified rat β-cells (22). Under these conditions, however, no high-affinity transport activity could be measured. Therefore, it is not clear whether GLUT1 re-expression can lead to changes in glucose sensing by rat β-cells. In mice, the situation seems to be different. Our

GLUT1 Western blot measurements were performed with islets kept overnight in tissue culture, and no major expression of this transporter could be detected. Furthermore, performing secretion experiments with freshly isolated islets or islets kept in vitro for up to 4 days did not lead to restoration of GSIS. GLUT1 may therefore not play any significant role in glucose uptake by GLUT2$^{-/-}$ β-cells in the living animal or in isolated islets. Because GLUT3 was not detectable at all in control or mutant islets, these data suggest that glucose uptake did not occur through either of these 2 transporters. We have not tested the presence of GLUT4 or GLUT5 because GLUT4 is expressed only in differentiated insulin-sensitive tissues and GLUT5 is mostly a fructose transporter with a very limited tissue distribution (mostly intestine and kidney in the mouse).

Although we could not perform direct transport measurement because of the very limited number of islets that can be isolated from young GLUT2-null mice (~30–50 islets per mouse), the mechanism of glucose uptake appears to be of relatively high affinity. Indeed, as glucokinase is expressed in the GLUT2-null islets, the fact that glucose utilization is not increased beyond 6 mmol/l glucose suggests that transport activity limits the rate of glucose access to glucokinase at these concentrations. This can be the case only in the presence of a low-K_M high-affinity transport mechanism of relatively small capacity. The identity of this transport system is currently unknown.

The limiting capacity of the glucose uptake step also impacts the stimulation by glucose of total protein and insulin biosynthesis. As for secretion, stimulation of insulin biosynthesis was still detectable, but it was of a lower amplitude compared with the response of control islets. This decreased response was expected because stimulation of insulin biosynthesis also requires glucose metabolism (23) even though half-maximal stimulation is reached at a glucose concentration well below that needed to stimulate insulin secretion (24). The fact that glucose uptake was limiting for GSIS was finally demonstrated by the restoration of normal kinetics of insulin secretion by re-expressing GLUT2 with recombinant lentiviruses. In contrast, our present work does not support the previously suggested role for GLUT2 in glucokinase expression (13–15). Indeed, in these studies, forced re-expression of this transporter in insulinoma cell lines was found to be associated with an increase in glucokinase mRNA, protein, and enzymatic activity. These data were interpreted as an indication of a possible role for GLUT2 in the control of glucokinase expression. Our data clearly do not support such a role.

Finally, it was intriguing to observe that even though there was no increase in glucose utilization when the glucose concentration was raised beyond 6 mmol/l, there was still a >4-fold increase in GSIS between 6 and 20 mmol/l glucose with freshly isolated islets and a >7-fold increase with overnight cultured islets. The fact that the secretory response was increased after an overnight culture period may reflect the fact that islets, from control or GLUT2$^{-/-}$ mice, have recovered from some stress induced by the isolation procedure. The apparent discrepancy between glucose utilization and GSIS at different glucose concentrations could not be explained by differences in incubation conditions: such conditions were identical for the secretion and the glucose utilization experiments, and we could confirm that the presence or absence of IBMX did not lead to differences in glucose utilization between 6 and 20 mmol/l glucose. These findings are in agreement with a previous report demonstrating that increased intracellular cAMP levels did not modify glucose metabolism in isolated rat islets (25). We could also confirm that insulin secretion by high glucose was the result of true signaling mechanisms and not a "fuel replenishment" effect, because the glucose effect was also observed when basic energy was provided by amino acids. Increased osmolarity was also not the cause of the secretory response because high sucrose concentrations did not lead to insulin release. Finally, 3-*O*-methyl-glucose, when added in the presence of 6 mmol/l glucose, did not increase insulin secretion, which indicates that glucose metabolism was required and that if a gluco-receptor was involved in this response, it could not be activated by 3-*O*-methyl-glucose. Therefore, the lack of correlation between the increase in glucose utilization and insulin secretion is difficult to explain. However, we must remember that this secretory activity is due only to a second phase of secretion and that it requires glucose metabolism because antimycin A1, diazoxide, and nimodipine could suppress it (12). Together, these experiments indicate that the secretory response observed in the absence of GLUT2 was probably proceeding through the normal ATP-sensitive potassium channel–dependent signaling pathway. A possible explanation for the apparent dissociation between glucose utilization and GSIS is that in GLUT2-null islets, the β-cell mass is decreased and the α-cell mass is increased by ~50%. Consequently, the glucose utilization measurements cannot accurately reflect the variation of glucose metabolism in β-cells. This is especially likely because β-cells have a reduced glucose metabolism in the absence of GLUT2. Thus, glucose metabolism in β-cells may still be increased over the glucose concentration range, thereby inducing insulin secretion, but this increase may be too small to be detected in the experiments performed with intact islets.

Together, our data demonstrate that the absence of GLUT2 from pancreatic β-cells strongly impairs glucose signaling to secretion and insulin biosynthesis and that this effect correlates with a strong impairment of glucose utilization. The secretory defect results only from the absence of the transporter because re-expression of GLUT2 by recombinant lentiviruses restores a normal secretory response to glucose. Finally, even though GLUT2 was absent, glucokinase protein expression was normal, indicating no requirement for GLUT2 in this enzyme expression and/or stability. These data demonstrate the important role of GLUT2 in glucose metabolism and signaling in murine islets.

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