K$_{\text{ATP}}$ channel-deficient pancreatic $\beta$-cells are streptozotocin resistant because of lower GLUT2 activity

Jin Xu,1 Li Zhang,1 Andrew Chou,1 Tim Allaby,1 Guy Bélanger,1 Jerry Radziuk,1,2 Bernard J. Jasmin,1,2 Takashi Miki,3 Susumo Seino,3 and Jean-Marc Renaud4

1Department of Cellular and Molecular Medicine and 2Ottawa Hospital and Ottawa Health Research Institute, University of Ottawa, Ottawa, Canada; and 3Division of Cellular and Molecular Medicine, Graduate School of Medicine, Kobe University, Kobe, Japan

Submitted 15 May 2007; accepted in final form 9 November 2007

Xu J, Zhang L, Chou A, Allaby T, Bélanger G, Radziuk J, Jasmin BJ, Miki T, Seino S, Renaud J-M. K$_{\text{ATP}}$ channel-deficient pancreatic $\beta$-cells are streptozotocin resistant because of lower GLUT2 activity. Am J Physiol Endocrinol Metab 294: E326–E335, 2008. First published November 27, 2007; doi:10.1152/ajpendo.00296.2007.—In wild-type mice, a single injection of streptozotocin (STZ, 200 mg/kg body wt) caused within 4 days severe hyperglycemia, hypoinsulinemia, significant glucose intolerance, loss of body weight, and the disappearance of pancreatic $\beta$-cells. However, in ATP-sensitive K$^+$ channel (K$_{\text{ATP}}$ channel)-deficient mice (Kir6.2$^{-/-}$ mice), STZ had none of these effects. Exposing isolated pancreatic islets to STZ caused severe damage in wild-type but not in Kir6.2$^{-/-}$ islets. Following a single injection, plasma STZ levels were slightly less in Kir6.2$^{-/-}$ mice than in wild-type mice. Despite the difference in plasma STZ, wild-type and Kir6.2$^{-/-}$ liver accumulated the same amount of STZ, whereas Kir6.2$^{-/-}$ pancreas accumulated 4.1-fold less STZ than wild-type pancreas. Kir6.2$^{-/-}$ isolated pancreatic islets also transported less glucose than wild-type ones. Quantification of glucose transporter 2 (GLUT2) protein content by Western blot using an antibody with an epitope in the extracellular loop showed no significant difference in GLUT2 content between wild-type and Kir6.2$^{-/-}$ pancreatic islets. However, visualization by immunofluorescence with the same antibody gave rise to 32% less fluorescence in pancreatic islets. The fluorescence intensity using another antibody, with an epitope in the COOH terminus, was 5.6 times less in Kir6.2$^{-/-}$ than in wild-type pancreatic islets. We conclude that Kir6.2$^{-/-}$ mice are STZ resistant because of a decrease in STZ transport by GLUT2 in pancreatic $\beta$-cells and 2) the decreased transport is due to a downregulation of GLUT2 activity involving an effect at the COOH terminus.

In an attempt to determine whether the compensatory mechanisms in Kir6.2$^{-/-}$ mice would allow for slower development of diabetic symptoms compared with wild-type mice, we administered STZ to both groups of mice. Unexpectedly, we found that, contrary to wild-type mice, Kir6.2$^{-/-}$ mice develop no diabetic symptoms following a single STZ injection (200 mg/kg body wt; see RESULTS); i.e., Kir6.2$^{-/-}$ mice appeared to be STZ resistant. The present study was thus designed to document the STZ resistance in Kir6.2$^{-/-}$ mice.

In pancreatic $\beta$-cells, the K$_{\text{ATP}}$ channel is made up of four Kir6.2 subunits, which make up the pore of the channel, and four SUR1 subunits, which are regulatory subunits (6, 21). Homozygous null mice for the Kir6.2 gene (Kir6.2$^{-/-}$ mice) have no K$_{\text{ATP}}$ channel activity in several tissues, including pancreatic $\beta$-cells (14). These mice have normal plasma glucose and insulin levels during prolonged fasting and secrete insulin following a mixed meal (21) but not during hyperglycemia induced with parenteral glucose administration (14). Despite the latter deficiency, Kir6.2$^{-/-}$ mice only have a mild glucose intolerance: they are only slightly more hyperglycemic than wild-type mice following an intraperitoneal glucose injection (14). It therefore appears that Kir6.2$^{-/-}$ mice develop compensatory mechanisms allowing them to be less dependent on insulin to prevent hyperglycemia. One of these mechanisms may involve greater basal and insulin-induced glucose uptake by adipose tissues and skeletal muscles (13).

Streptozotocin (STZ) is a glucosamine-nitrosourea compound that shows selective cytotoxicity to pancreatic $\beta$-cells and is widely used to generate diabetic animal models (2, 7, 10). STZ is believed to cause DNA alkylolation (4), leading to the activation of poly(ADP-ribose) polymerase for DNA repair (1, 3, 16). This process consumes an excessive amount of NAD$^+$ (1, 3). It has been suggested that the loss of NAD$^+$ then impairs ATP production, causing a lethal energy depletion (1).

Glucose-stimulated insulin secretion in pancreatic $\beta$-cells comprises an ATP-sensitive K$^+$ channel (K$_{\text{ATP}}$ channel)-dependent and -independent pathway (14, 21). For the K$_{\text{ATP}}$ channel-dependent mechanism, glucose-stimulated insulin secretion first involves the transport of glucose into the cytosol by glucose transporter 2 (GLUT2). As glucose is metabolized, the intracellular ATP concentration increases, and that of ADP decreases. The changes in ATP and ADP cause the closing of the K$_{\text{ATP}}$ channels, which results in the depolarization of the cell membrane. Voltage-dependent calcium channels are then activated, allowing for a calcium influx. Finally, calcium triggers the exocytosis of insulin-containing granules (20, 21).

Address for reprint requests and other correspondence: J.-M. Renaud, Dept. of Cellular and Molecular Medicine, Univ. of Ottawa, 451 Smyth Rd., Ottawa, ON, Canada K1H 8M5 (e-mail: jmrenaud@uottawa.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
STZ even if they overexpress GLUT1 (4, 5, 19). Finally, blocking K<sub>ATP</sub> channel activity in wild-type pancreatic β-cells reduces GLUT2 activity (26). We therefore hypothesized that Kir6.2<sup>−/−</sup> mice are STZ resistant because STZ is not transported in the cytosol as GLUT2 activity is downregulated in Kir6.2<sup>−/−</sup> pancreatic β-cell. To test this hypothesis, we measured, with the use of wild-type and Kir6.2<sup>−/−</sup> mice, how STZ accumulates in the pancreas following a single intraperitoneal STZ injection and glucose uptake in isolated pancreatic islets. GLUT2 was also visualized and quantified by immunofluorescence and Western blots by using two antibodies, one with an epitope in the extracellular loop and the other with an epitope in the COOH terminal.

MATERIALS AND METHODS

Animals

Kir6.2<sup>−/−</sup> mice were generated as previously described by Miki et al. (14). Genetic background was mixed over four generations with C57BL6 mice, which were used as wild-type mice. Mice were bred, fed ad libitum, and housed according to the guidelines of the Canadian Council for Animal Care. Kir6.2<sup>−/−</sup> mice did not require any specialized care. Animals used in this study were all between 8 and 12 wk old. When tissue samples were collected, mice were anesthetized with a single intraperitoneal injection of 2.2 mg ketamine, 0.44 mg xylazine, and 0.22 mg acepromazine per 10 g of body mass. After tissue extraction, mice were euthanized with an overdose of anesthetics. The Animal Care Committee of the University of Ottawa approved all experimental procedures used in this study.

Effects of STZ on Plasma Glucose and Insulin

STZ (Sigma) was dissolved in ice-cold 10 mM citric sodium solution, pH 4.5, and mice were injected intraperitoneally at a dose of 200 mg/kg body wt within 10 min of dissolution. Untreated mice were injected with citric buffer. Body weights were monitored before and 4 days after the injection. For one group of mice, blood samples (from hindlimb saphenous vein) and pancreases were collected 4 days after the STZ injection from nonfasted mice. For a second group of mice, glucose tolerance test was carried out in untreated and STZ-treated mice; here mice were fasted for 16 h before being injected intraperitoneally with 1 g glucose/kg body wt. Blood samples (25 μl) were then collected every 30 min for 2 h.

Blood samples were centrifuged at 10,000 g (MB Microcapillary Centrifuge, International Equipment) to remove blood cells. Glucose was determined as described by Renaud and Moon (17). Insulin was determined with the use of wild-type and Kir6.2<sup>−/−</sup> pancreatic β-cell. To test this hypothesis, we measured, with the use of wild-type and Kir6.2<sup>−/−</sup> mice, how STZ accumulates in the pancreas following a single intraperitoneal STZ injection and glucose uptake in isolated pancreatic islets. GLUT2 was also visualized and quantified by immunofluorescence and Western blots by using two antibodies, one with an epitope in the extracellular loop and the other with an epitope in the COOH terminal.

Histology of Pancreas, Isolated Islets, and Liver

Pancreatic islets were isolated by using collagenase digestion as described by Wollheim et al. (25). Briefly, collagenase type XI (0.7 mg/ml, Sigma) was dissolved in ice-cold physiological saline solution (PSS) containing (in mM) 136 NaCl, 5.4 KCl, 1 MgCl<sub>2</sub>, 0.41 Na<sub>2</sub>HPO<sub>4</sub>, 0.41 KH<sub>2</sub>PO<sub>4</sub>, 5 glucose, and 10 HEPES, pH 7.4. Pancreas was injected, via the pancreatic duct, with 2–3 ml of the collagenase solution and was incubated for 10 min in collagenase solution at 37°C. After five washes in PSS, to which 1 mM CaCl<sub>2</sub> was added, islets were hand picked under an inverted microscope. After 24 h incubation in RPMI-1640 medium, containing 11 mM glucose (GIBCO) supplemented with 10% FBS (GIBCO) and 1% penicillin-streptomycin (GIBCO), islets were exposed for 30 min to 1 mM STZ (first dissolved in 0.9% NaCl before being added to RPMI-1640 medium). After being washed twice in ice-cold RPMI-1640 medium, islets were incubated in RPMI-1640 medium for another 15 h before being stained for insulin as described in Histology of Pancreas, Isolated Islets, and Liver.

Plasma STZ and Uptake by Different Tissues In Vivo

Mice were intraperitoneally injected with citric buffer (untreated mice, time 0) or 200 mg STZ/kg body wt. Blood, pancreas, liver, hindlimb skeletal muscles, and cardiac muscle samples were collected 5, 10, 15, 30, and 60 min after the STZ injection. After centrifugation of blood cells, a volume of 60% perchloric acid (PCA) was added to the plasma to give a final concentration of 6%. Pancreas, liver, cardiac muscle, and skeletal muscle samples were homogenized in 2 ml 6% PCA. Plasma and tissue proteins were removed by centrifugation at 10,000 g. STZ was measured in the supernatant as described by Schnedl et al. (19) to obtain the plasma and tissue total STZ content. Mice were also injected with 0.5 μCi [14C]sucrose (extracellular marker, Amersham) in 100 μl citric buffer, and the radioactivity from the STZ extracts (plasma and tissues) was counted by using a WinSpectral liquid scintillation counter (model 1414, Wallac Instruments). Quenching was corrected by counting 1 μCi of [14C]sucrose in 6% PCA. For each tissue, the extracellular volume was calculated from the [14C]sucrose counts; the extracellular STZ content was then calculated and subtracted from the total STZ content to obtain the intracellular STZ content.

Experiments showed that measurements of extracellular spaces were consistent if carried out 20–30 min after the [14C]sucrose injection (after 40 min, the remaining plasma [14C]sucrose was too small to properly measure extracellular space; data not shown). Thus for these experiments [14C]sucrose was injected 20–30 min before tissue sampling for the STZ measurements: i.e., the STZ and [14C]sucrose were injected separately.

Glucose Uptake by Isolated Pancreatic Islets

Radioactive-labeled STZ was not available, and the chemical STZ determination was not sensitive enough unless a very large number of isolated pancreatic islets were used from a large number of mice. As mentioned in the introduction, the presence of GLUT2 is critical for STZ transport in pancreatic islets. Therefore, to determine whether less STZ is transported in pancreatic β-cells because of lower GLUT2 activity, we measured glucose uptake by using the technique described by Zao et al. (26), except for the use of 1.37 μCi 2-deoxy-D-[1-14C]glucose (“H-2-DG), a glucose marker, and 2.7 μCi [3H]sucrose, an extracellular marker (Amersham). Islets were incubated at 37°C for 1, 5, 10, or 20 min. Radioactivity was counted by using the WinSpectral liquid scintillation counter. Quenching was corrected by counting 1 μCi of 3H-2-DG and [14C]sucrose in 6 M urea.

Histology of Pancreas, Isolated Islets, and Liver

Hematoxylin and eosin staining. Pancreases were embedded in optimum cutting temperature compound and were frozen in isopentane precooled in liquid nitrogen. Cross-sections, 7 μm thick, were postfixed with 4% paraformaldehyde (pH 7.4) at 4°C, stained for 2 min in hematoxylin solution, and dipped five times in eosin solution. After washes in tap water for 10 min, cross-sections were soaked twice for 10 s in 90% alcohol, twice for 10 s in 100% alcohol, and twice for 10 s in xylene. Finally, cross-sections were mounted with coverslips by using Permunt.

Insulin staining. After fixation in 4% paraformaldehyde at 4°C and overnight incubation in 10% sucrose solution at 4°C, pancreas or pancreatic islets were embedded in optimum cutting temperature compound and were frozen in isopentane precooled in liquid nitrogen. Cross-sections, 7 μm thick, were exposed for 30 min at room temperature to 10% donkey serum (fluorescence staining) or 5% skim milk [diaminobenzidine (DAB) staining] before being exposed over-
night at 4°C to mouse monoclonal anti-insulin (1:400 dilution for fluorescence staining and 1:1,000 for DAB staining, Sigma). Visualization of insulin was done either by incubating cross-sections for 1 h at 37°C with Alexa-Fluor 488 donkey anti-rabbit IgG (1:200 dilution, fluorescence staining) or in the presence of horseradish peroxidase-conjugated donkey anti-mouse IgG (1:200 dilution, Chemicon). In the latter case, cross-sections were incubated 6 min in 0.5% DAB in PBS buffer (containing 0.1% vol/vol 0.3% H₂O₂, Sigma) for color development.

**GLUT2 staining.** After fixation, pancreas and liver cross-sections were incubated overnight at 4°C with either a rabbit anti-GLUT2 (1:400 dilution, Alpha Diagnostics) with a 16-amino acid epitope in the COOH terminal [anti-GLUT2(C-term)] or a rabbit anti-GLUT2 (1:1,500 dilution, Chemicon) with a 67-amino acid epitope in the extracellular loop between transmembrane domains I and II [anti-GLUT2(ExLoop)]. Cross-sections were then incubated for 1 h at 37°C with Cy3-conjugated donkey anti-rabbit IgG (1:200, dilution, Jackson ImmunoResearch).

**Measurement of fluorescence intensity for GLUT2.** Cross-sections were observed using a Carl Zeiss Axioskop2 microscope (Germany) to which was attached a MicroPublisher camera (QImaging). Pictures were saved and analyzed by using Northern Eclipse software (version 6.0, EMPIX Imaging). GLUT2 fluorescence was normalized relative to the insulin surface area to take into account the different islet sizes as follows. First, the color pictures for both insulin and GLUT2 staining were changed to grayscale. For each individual islet, a line was traced around the insulin-stained area in one picture, and the program calculated the surface area within the line. The line was then copied to the exact same position on the GLUT2 picture, and the fluorescence was then determined over the same surface area. The fluorescence was then divided by the insulin surface area. For the liver, the fluorescence intensity was measured from the entire picture because the surface area was always the same between pictures.

**Western Blot Determination of GLUT2 Content**

These measurements were carried out using only the anti-GLUT2(ExLoop) because the anti-GLUT2(C-term) did not work under Western blot conditions. Isolated pancreatic islets were transferred to a lysis buffer containing 5% SDS, 80 mM Tris-HCl, pH 6.8, 5 mM EDTA, and protease inhibitor pill (Roche Diagnostics) freeze-thawed five times, and sonicated three times for 30 s (Branson Ultrasonic). The lysate was then centrifuged for 10 min at 10,000 g and 4°C to remove cell debris. Total protein content was determined in the supernatant by using the bicinchoninic acid protein assay (Pierce Chemical) with bovine serum albumin as standard. Aliquots of 10 or 20 μg protein were then subjected to electrophoresis on 10% SDS-PAGE and were transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature with 5% skim milk and were incubated for 1 h at room temperature or overnight at 4°C with the anti-GLUT2(ExLoop) (1:500 dilution). Following four 10-min washes, membranes were incubated for 1 h at room temperature with horseradish peroxidase-labeled donkey anti-rabbit IgG (1:5,000 dilution, Chemicon). After four 10-min washes, bands were visualized by using SuperSignal West Pico Chemiluminescence (Pierce) and were quantified with a density scan (Kodak Image Station). The same procedure was then repeated for actin quantification by using monoclonal anti-actin antibody (1:500 dilution, Sigma) and horseradish peroxidase-labeled anti-mouse IgG antibody (1:500 dilution, Sigma).

After quantification, the intensities of the GLUT2 bands were first divided by the intensities of the actin band. The resulting ratios (within the same blot) from wild-type and Kir6.2−/− samples were then expressed as a percent of the mean ratio from wild type. This calculation allowed us to obtain an estimate of the variability for both wild-type and Kir6.2−/− data.

**Statistical Analysis**

Data are expressed as means ± SE. Split-plot ANOVA designs were used for body weight, plasma glucose and insulin, and glucose measurements, with the treatment mice in the whole plot and treatment time in the split-plot ANOVA because data over time were collected from the same mice. Two-way ANOVA design was used for STZ measurements because a different mouse was used for each time period. ANOVA calculations were made by using the general linear model procedures of the Statistical Analysis Software (SAS Institute). When a mean effect or an interaction was significant, the least significant difference was used to locate the significant differences (22). The t-tests were used for fluorescence intensity and Western blot data because in each case only two means were compared. The word “significant” refers only to statistical difference (P < 0.05).

**RESULTS**

**STZ Effects on Plasma Glucose and Insulin**

Nonfasting plasma glucose levels were not different between wild-type and Kir6.2−/− mice, being 10–11 mM (Fig. 1A) as previously reported (14). Four days after a single intraperitoneal injection of 200 mg STZ/kg body wt, the plasma glucose level of nonfasted wild-type mice had increased to 39 mM. However, STZ failed to cause an increase in plasma glucose level in Kir6.2−/− mice over the same time period. In another experiment, Kir6.2−/− plasma glucose remained normal for up to 15 days after the STZ injection (data not shown). STZ-treated wild-type mice had three times less plasma insulin than untreated wild-type mice, whereas insulin levels in STZ-treated Kir6.2−/− mice were significantly higher than in untreated Kir6.2−/− mice (Fig. 1B). Four days after the STZ injection, Kir6.2−/− mice had lost 5% of their body weight, which was significantly less than the 9% loss observed for wild-type mice (Fig. 1C).

Plasma glucose levels of fasted, untreated wild-type and Kir6.2−/− mice (Fig. 1D) were almost half of those observed in nonfasted mice (Fig. 1A), as previously reported (14). Untreated Kir6.2−/− mice were slightly more hyperglycemic than wild-type mice 30 min after an intraperitoneal glucose injection (1 g/kg body wt), and both groups of mice returned their plasma glucose levels back to normal within 2 h (Fig. 1B), as previously reported (14). The STZ treatment impaired the glucose tolerance to a large extent in wild-type mice but not in Kir6.2−/− mice. The initial increase in plasma glucose after the injection was much greater in STZ-treated wild-type than Kir6.2−/− mice. Furthermore, after 2 h, STZ-treated Kir6.2−/− mice had returned their plasma glucose levels back to normal, whereas in STZ-treated wild-type mice the plasma glucose was still significantly above preinjection levels.

**Effects of STZ on Pancreatic β-Cells**

Four days after the STZ injection in mice, pancreatic islets were smaller in STZ-treated wild-type mice compared with untreated wild-type mice (Fig. 2, A and C). Furthermore, in untreated wild-type mice, the insulin-containing cells comprised 77% of the total pancreatic islet surface area compared with only 4% in islets from STZ-treated mice (Fig. 2, E and G). STZ had no such effects on the pancreatic islets of Kir6.2−/− mice. First, there was no apparent cellular damage in islets of STZ-treated Kir6.2−/− mice (Fig. 2, B and D). Second, the insulin-stained area represented 85% of the pancreatic islet...
total surface area in both untreated and STZ-treated Kir6.2−/− mice (Figs. 2F and 3G).

Isolated pancreatic islets from wild-type mice retained a round shape with large number of cells surrounded by a membranous tissue when incubated for up to 2 days as shown for one islet in Fig. 3A. Fifteen hours after a 30-min exposure to 1 mM STZ, all wild-type pancreatic islets had lost their integrity. Many pancreatic islets had disappeared, and cells were found dispersed at the bottom of the culture dish (Fig. 3C). Some islets were still observed, but they had fewer cells and no membranous tissues surrounding them as shown for one islet in Fig. 3B. Contrary to wild-type islets, none of the Kir6.2−/− pancreatic islets were affected by STZ because both untreated and STZ-treated Kir6.2−/− pancreatic islets looked like the untreated wild-type pancreatic islets shown in Fig. 3A. Insulin was clearly visible in untreated wild-type pancreatic

Fig. 2. STZ caused cell damage in pancreatic islets of WT but not Kir6.2−/− mice. Pancreases were removed 4 days after STZ injection. Cross-sections of pancreas were then stained with hematoxylin and eosin (H&E) or with anti-insulin antibody. *P < 0.05 vs. untreated mice.
islets (Fig. 3D) and in untreated (Fig. 3F) and STZ-treated (Fig. 3G) Kir6.2\(^{-/-}\) islets but was significantly reduced in the few remaining STZ-treated wild-type islets (Fig. 3E).

**STZ and Glucose Uptake by Pancreas**

In wild-type mice, plasma STZ levels reached a peak value of 0.9 mM 5 min after the injection (Fig. 4A). Thereafter, plasma STZ levels progressively decreased, approaching 0 by 60 min. In Kir6.2\(^{-/-}\) mice, plasma STZ levels did not exceed 0.7 mM and were significantly less than in wild-type mice for the first 10 min. In pancreas, most of the measured STZ appeared to be intracellular because there were small differences between total and intracellular STZ contents (Fig. 4B). The intracellular pancreatic STZ peaked 5 min after the injection, reaching 55 nmol/100 mg wet wt tissue in wild-type mice compared with only 16 nmol/100 mg wet wt tissue in Kir6.2\(^{-/-}\) mice, a 4.1-fold difference. Thereafter, pancreatic STZ levels dropped dramatically in wild-type mice and were no longer significantly different from the levels in Kir6.2\(^{-/-}\) mice 10 min after the injection.

Liver accumulated by far the largest amount of STZ in both wild-type and Kir6.2\(^{-/-}\) mice, reaching maximum levels by 15 min with no decrease thereafter (Fig. 4C). Liver STZ levels were not significantly different between wild-type and Kir6.2\(^{-/-}\) mice. Cardiac (data not shown) and skeletal muscle did not accumulate STZ in the cytosol (Fig. 4D). The small amounts of STZ measured in skeletal muscle appeared to be all extracellular.

STZ transport into the cytosol depends on the presence of GLUT2, and the lower STZ uptake by the pancreas is probably because of lower GLUT2 activity in Kir6.2\(^{-/-}\) pancreatic islets. To verify this possibility, we estimated glucose uptake in isolated pancreatic islets by using its marker, \(^3\)H-2-DG [similar measurements for STZ was not possible because 1) the yield of pancreatic islets per mouse was too low for the sensitivity of the STZ chemical determination and 2) of the unavailability of radioactive-labeled STZ]. The uptake of \(^3\)H-2-DG was also reduced by 1.8- to 2.5-fold in Kir6.2\(^{-/-}\) compared with wild-type pancreatic islets (Fig. 5).

**Quantification and Visualization of GLUT2 Protein Content**

Two anti-GLUT2 antibodies were used to detect GLUT2 in pancreas. The first antibody, called anti-GLUT2(ExLoop), had its epitope located in the extracellular loop between the transmembrane domains 1 and 2 of GLUT2. With the use of this antibody under Western blot conditions, a single band was observed just above the 50-kDa line (Fig. 6A). The band intensity from 20 μg proteins was twice as much compared with 10 μg proteins, suggesting that the quantification was linear with the initial amount of proteins. On average, the band intensities were not significantly different between wild-type and Kir6.2\(^{-/-}\) samples (Fig. 6B).

Using the same antibody under immunofluorescence conditions, the signal was observed only in the pancreatic islets, which were visualized with anti-insulin; pancreatic acinar cells were not labeled with anti-GLUT2(ExLoop) (Fig. 7, A–D). Furthermore, the anti-GLUT2(ExLoop) appeared to label the cell membrane and not the cytosol. The fluorescence intensities, expressed relative to the anti-insulin area, were on average 32% less in Kir6.2\(^{-/-}\) than in wild-type pancreatic islets (Fig. 7E). When liver cells were exposed to anti-GLUT2(C-term), there was no significant difference in fluorescence intensity between wild-type and Kir6.2\(^{-/-}\) mice (Fig. 7, E–H).

The second anti-GLUT2, called anti-GLUT2(C-term), had its epitope in the COOH terminus. In wild-type pancreatic islets, fluorescence was again observed only in pancreatic islets and at the level of the cell membrane (Fig. 8, A and B). Anti-GLUT2(C-term) barely stained Kir6.2\(^{-/-}\) pancreatic β-cells (Fig. 8, C and D). On an average basis, fluorescence intensity from anti-GLUT2(C-term) was 5.6-fold less in Kir6.2\(^{-/-}\) than in wild-type pancreatic islets (Fig. 8G). Interestingly, when liver cells were exposed to anti-GLUT2(C-term), there was no difference in fluorescence intensity between wild-type and Kir6.2\(^{-/-}\) mice (Fig. 8, E–H). Western blot measurements were not done with anti-GLUT2(C-term) because it failed to work under those conditions for both wild-type and Kir6.2\(^{-/-}\) mice.

**DISCUSSION**

Injecting wild-type mice with STZ (200 mg/kg body wt) resulted within four days in severe hyperglycemia, hypoinsulinemia, body weight loss, major glucose intolerance, and disappearance of pancreatic β-cells in vivo, as has previously been reported (2, 7, 10). A major finding of this study is that STZ failed to generate these symptoms in K\textsubscript{ATP} channel-
STZ AND GLUT2 IN Kir6.2<sup>−/−</sup> PANCREATIC β-CELLS

A

B

C

D

significant difference, SE of 5 mice. *Significantly different from WT mice (ANOVA and least significant difference).

The only effects of STZ in Kir6.2<sup>−/−</sup> mice were that Kir6.2<sup>−/−</sup> mice had normal fasting and nonfasting plasma glucose and no significant decrease in plasma insulin, and pancreatic islets appeared normal and contained insulin. The only effects of STZ in Kir6.2<sup>−/−</sup> mice were 1) slightly greater glucose intolerance and 2) small weight loss, two effects that were significantly less in Kir6.2<sup>−/−</sup> than in wild-type mice. Thus the Kir6.2<sup>−/−</sup> mouse is exceptionally STZ resistant compared with wild-type mice.

When fed ad libitum, untreated Kir6.2<sup>−/−</sup> mice had lower plasma insulin levels than untreated wild-type mice, and this may be related to the fact that Kir6.2<sup>−/−</sup> mice have higher insulin sensitivity than wild-type mice (14). One noticeable difference between untreated and STZ-treated Kir6.2<sup>−/−</sup> mice was the higher plasma insulin levels in STZ-treated Kir6.2<sup>−/−</sup> mice. This is suggestive of a decrease in insulin sensitivity after STZ treatment in the Kir6.2<sup>−/−</sup> mice and would be consistent with the small decrease in glucose tolerance seen in this group.

Site of the STZ Resistance in Kir6.2<sup>−/−</sup> Mice

Two mechanisms can explain the STZ resistance of Kir6.2<sup>−/−</sup> mice: 1) plasma STZ levels are much lower, so not enough STZ is transported in the cytosol of a β-cell to reach a lethal dose or 2) pancreatic islets are themselves STZ resistant. To test the first mechanism, we determined the plasma STZ levels following an intraperitoneal injection. In wild-type mice, the plasma STZ peaked at 0.9 mM within 5 min and remained >0.6 mM for another 10 min. In Kir6.2<sup>−/−</sup> mice, the plasma STZ peaked at 0.7 mM and was significantly less than in wild-type mice for 10 min. Despite the 0.2 mM difference in plasma STZ, wild-type and Kir6.2<sup>−/−</sup> liver tissue, which had similar GLUT2 content as measured with anti-GLUT2(C-term), accumulated the same amount of STZ. This is suggestive of a decrease in insulin sensitivity after STZ treatment in the Kir6.2<sup>−/−</sup> mice and would be consistent with the small decrease in glucose tolerance seen in this group.

Fig. 4. Kir6.2<sup>−/−</sup> pancreas accumulated less STZ than WT pancreas. Mice were injected with 200 mg STZ/kg body wt. A: plasma STZ concentration in WT (●) and Kir6.2<sup>−/−</sup> (▲) mice. B–D: STZ content in pancreas (B), liver (C), and skeletal muscle (D). ●, Total STZ in WT tissue; ○, intracellular STZ in WT tissue; ■, total STZ in Kir6.2<sup>−/−</sup> tissue; ▲, intracellular STZ in Kir6.2<sup>−/−</sup> tissue. For liver, <12% of total STZ content was extracellular after 5 min, so for clarity, only intracellular STZ levels are shown. Values are means ± SE of 4–5 mice. *Value significantly different between WT and Kir6.2<sup>−/−</sup> mice (ANOVA and least significant difference, P < 0.05).

Fig. 5. Glucose uptake is less in Kir6.2<sup>−/−</sup> islets than in WT islets. Isolated pancreatic islets were incubated with 11 mM glucose, 2-deoxy-[1-3H]glucose ([3H]-2-DG), and [14C]sucrose. ●, WT; ○, Kir6.2<sup>−/−</sup>. Values are means ± SE of 5 mice. *Significantly different from WT mice (ANOVA and least significant difference, P < 0.05).
The protective effect arises from the lack of sarcolemmal $K_{ATP}$ channels. So in the following discussion we will limit ourselves to the mechanism of STZ resistance in relation to sarcolemmal $K_{ATP}$ channels because the protective effect of the mitochondrial $K_{ATP}$ channel has been discussed by Kullin et al. (9).

**Reduced GLUT2 Activity as a Mechanism of the STZ Resistance**

The resistance of Kir6.2$^{-/-}$ pancreas may be because 1) the amount of STZ transported into the cytosol is much less so that STZ no longer reach a lethal dose or 2) changes have occurred in the $\beta$-cells' cytosol, rendering STZ nonlethal. Kir6.2$^{-/-}$ pancreas accumulated much less intracellular STZ compared with wild-type pancreas. To understand the difference between the two strains of mice, it is important to first clarify in which cells STZ accumulated. We expect that in pancreas STZ was primarily in $\beta$-cells for the following reasons. Firstly, as discussed in *Site of the STZ Resistance in Kir6.2$^{-/-}$ Mice*, only GLUT2-containing cells transport STZ. Secondly, using anti-GLUT2 antibodies, we showed that GLUT2 is expressed in pancreatic $\beta$-cells but not in acinar cells.

A difference in the number of $\beta$-cells between wild-type and Kir6.2$^{-/-}$ mice may then explain the difference in pancreatic STZ uptake. In this study, mice were 8–12 wk old, and the number of pancreatic $\beta$-cells does not differ between wild-type and Kir6.2$^{-/-}$ mice until they become older than 16 wk of age (12). It is therefore unlikely that the lower STZ accumulation in Kir6.2$^{-/-}$ pancreas was because of a smaller number of $\beta$-cells in Kir6.2$^{-/-}$ pancreas. As discussed in *Site of the STZ Resistance in Kir6.2$^{-/-}$ Mice*, the lower plasma STZ content was also not a reason for the lower uptake by Kir6.2$^{-/-}$ $\beta$-cells. Using isolated pancreatic islets, we showed that the uptake of $^3$H-2-DG, a glucose marker, was significantly less in Kir6.2$^{-/-}$ than in wild-type islets. Interestingly, glipizide, a $K_{ATP}$ channel blocker, also reduces glucose uptake in pancreatic islets (26). Together these results suggest that the absence of $K_{ATP}$ channel activity reduces GLUT2 activity in pancreatic $\beta$-cells. We therefore proposed that the primary mechanism for the STZ resistance in Kir6.2$^{-/-}$ pancreatic $\beta$-cells is because of a decreased GLUT2 activity large enough to prevent lethal STZ accumulation in the $\beta$-cell cytosol.

A decrease in GLUT2 activity can either be because of lower protein content or lower intrinsic transport activity. To measure the GLUT2 protein content, we first used an antibody with an epitope in the extracellular loop between transmembrane domains 1 and 2, anti-GLUT2(ExLoop). Under Western blot conditions, the GLUT2 protein content was not significantly different between wild-type and Kir6.2$^{-/-}$ pancreatic islets. Under immunofluorescence conditions, the signal expressed relative to the islet surface area was ~30% less in Kir6.2$^{-/-}$ islets. We also used a second antibody that had an epitope in the GLUT2 COOH terminus [anti-GLUT2(C-term)], an antibody that could only work under immunofluorescence conditions. In this case, the fluorescence intensity after labeling with anti-GLUT2(C-term) was almost sixfold less in Kir6.2$^{-/-}$ islets.

Zhao et al. (26) recently reported that the fluorescence intensity, following labeling with anti-GLUT2(C-term), was dramatically reduced when $K_{ATP}$ channels were acutely

---

**Fig. 6.** Glucose transporter 2 (GLUT2) protein content measured by Western blot in WT and Kir6.2$^{-/-}$ pancreatic islets. A: example of Western blots for WT and Kir6.2$^{-/-}$ (KO) pancreatic islets using 10 and 20 µg of protein. Horizontal black line represents 50 kDa. B: GLUT2 content as a percent of WT mice (see MATERIALS AND METHODS for calculation of percent values). Open bar, WT; closed bar, Kir6.2$^{-/-}$. Values are means ± SE of 3 samples. Difference between WT and Kir6.2$^{-/-}$ mice was not significant (t-test, $P > 0.05$).

---

*compared with wild-type mice must be related to greater STZ removal by GLUT2-containing cells. Cells that express GLUT2 are pancreatic $\beta$-cells, liver, intestine, and kidney (24). The lack of difference in STZ uptake between wild-type and Kir6.2$^{-/-}$ liver and the lower uptake by Kir6.2$^{-/-}$ pancreas suggest that neither tissues could be responsible for the lower plasma STZ in Kir6.2$^{-/-}$ mice. Thus the only remaining possibility for the lower plasma STZ in Kir6.2$^{-/-}$ mice is a greater loss through the intestine and/or kidney.

To test the second mechanism of the STZ resistance, we exposed isolated pancreatic islets for 30 min to 1 mM STZ. As previously reported for rat isolated pancreatic islets (9), isolated pancreatic islets of wild-type mice examined 15 h after the STZ exposure had lost their integrity and a large portion of their insulin content. Kir6.2$^{-/-}$ isolated pancreatic islets, on the other hand, had a normal appearance and contained large amount of insulin. It is also important to note that STZ had no effects on Kir6.2$^{-/-}$ isolated pancreatic islets despite the fact that in vitro 1) the STZ concentration was greater and 2) the duration of the exposure was longer than what had been observed in vivo. We therefore suggest that Kir6.2$^{-/-}$ mice are STZ resistant solely because the pancreatic $\beta$-cells are themselves STZ resistant.

It is interesting to note that Kullin et al. (9) reported that the activation of $K_{ATP}$ channels with diazoxide protects rat pancreatic islets against the damaging effects of STZ. However, the protective effect in rat pancreatic islets involves the activation of mitochondrial $K_{ATP}$ channels, whereas in this study
blocked with glipizide and increased when the channels were activated with diazoxide. These changes in fluorescence intensity were observed within 30 min, and the authors argued that this time period was too short to affect the cell membrane GLUT2 protein content. Thus this study and the one by Zhao et al. (26) demonstrate that modulating KATP channel activity in /H9252-cells affects the capacity of anti-GLUT2 antibodies to bind to their epitope under immunofluorescence conditions, especially in the COOH terminus. This characteristic appeared to be specific to pancreatic islets because labeling liver GLUT2 with anti-GLUT2(C-term) gave rise to similar fluorescence intensities in wild-type and Kir6.2–/– mice.

We therefore suggest from the Western blot results with anti-GLUT2(ExLoop) that Kir6.2–/– pancreatic β-cells do not have a lower GLUT2 protein content compared with wild-type islets. Even if the 30% decrease in fluorescence from anti-GLUT2(ExLoop) represents a decrease in protein content, the decrease is not large enough to explain that glucose and STZ uptakes were, respectively, two- and fourfold less in Kir6.2–/– than in wild-type islets. In other words, the lower
STZ and glucose uptake must involve a decrease in GLUT2 activity per se.

The mechanism by which a change in $K_{\text{ATP}}$ channel activity affects GLUT2 activity cannot be determined from the results of this study. However, Zhao et al. (26) have shown that a prolonged $K^+$-induced depolarization of the cell membrane, which normally occurs when $K_{\text{ATP}}$ channels close, also results in a decrease GLUT2 activity. This suggests that the down-regulation of GLUT2 activity appears downstream of the $K_{\text{ATP}}$ channel. The last question now becomes why is the binding of anti-GLUT2(C-term) antibody reduced under immunofluorescence conditions? The glucose relative binding affinity to GLUT1 and GLUT2 depends on the amino acid sequence of the COOH terminus (8). More importantly, removal of the last 42 amino acids gives rise to a mutant GLUT2 that can no longer transport glucose across the cell membrane (15); i.e., the COOH terminus is crucial for the glucose transport by GLUT2. We thus propose that the decreased GLUT2 activity involves an effect on the COOH terminus, rendering it invisible to the antibody and blocking its contribution to the glucose transport.

**Physiological Significance of Lowered GLUT2 Activity**

Repeated or chronic exposures to high glucose concentration lead to a desensitization of the $\beta$-cells; this desensitization is a reversible physiological state of cellular refactoriness in which $\beta$-cells no longer respond to high glucose concentrations (18). Chronic exposures to high glucose levels are then expected to keep $K_{\text{ATP}}$ channels in a closed state, which eventually leads to decreases in GLUT2 activity (this study and Ref. 26). Perhaps the feedback from the closure of $K_{\text{ATP}}$ channels on the intrinsic activity of GLUT2 is a mechanism leading to the desensitization of $\beta$-cells. It is also known that chronic exposure to hyperglycemia can lead to irreversible cellular dysfunction, a process that has been termed glucose toxicity (18) because it forces pancreatic $\beta$-cells to constantly release insulin, resulting in a heavy energy demand. So the downregulation of GLUT2 activity may be a protective mechanism, limiting the exposure of the $\beta$-cell to hyperglycemia when this becomes chronic and thus limiting the damage from glucose toxicity. Such mechanisms are clearly not of acute importance in the Kir6.2$^{-/-}$ mice because the $K_{\text{ATP}}$ channels are nonfunctional.

Another interesting aspect of our findings is a comparison between Kir6.2$^{-/-}$ and GLUT2$^{-/-}$ mice. Both have no or reduced GLUT2 activity in pancreatic $\beta$-cells, but only the GLUT2$^{-/-}$ is lethal, with a life expectancy of $\approx$2–3 wk (23). Although it can be suggested that the Kir6.2$^{-/-}$ is not lethal because GLUT2 is expressed in other tissues (e.g., in the liver, as shown in Fig. 8F), restoring GLUT2 expression only in pancreatic $\beta$-cells is sufficient to rescue the GLUT2$^{-/-}$ mice to normal life expectancy (23). It thus appears that although GLUT2 deficiency in pancreatic $\beta$-cells is lethal, a concomitant deficiency in GLUT2 and $K_{\text{ATP}}$ channel is not.

In summary, $K_{\text{ATP}}$ channel-deficient mice, generated by disrupting the Kir6.2 gene that encodes for the protein subunit making the pore of the $K_{\text{ATP}}$ channel, are STZ resistant because diabetic symptoms do not develop following a single STZ injection (200 mg/kg body wt). The resistance occurs because STZ is no longer transported in the cytosol as GLUT2 activity is downregulated and not because of a decrease in protein content. The downregulation of GLUT2 activity appears to depend on a conformational change involving the COOH terminus, which is known to be critical for glucose transport by GLUT2.

**GRANTS**

This study was supported by a grant from the National Science and Engineering Research Council (NSERC) to J.-M. Renaud, a NSERC summer scholarship to A. Chou, and a Canadian Institute for Health Research grant to B. J. Jasmin.

**REFERENCES**


