**Sur1 Knockout Mice**

A MODEL FOR K\textsubscript{ATP} CHANNEL-INDEPENDENT REGULATION OF INSULIN SECRETION*

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Victor Seghers‡‡, Mitsuhiko Nakazaki‡‡, Franco DeMayo‡, Lydia Aguilar-Bryan¶, and Joseph Bryan‡‡

From the Departments of ‡‡Molecular and Cellular Biology and ¶¶Medicine, Baylor College of Medicine, Houston, Texas 77030

Sur1 knockout mouse β-cells lack K\textsubscript{ATP} channels and show spontaneous Ca\textsuperscript{2+} action potentials equivalent to those seen in patients with persistent hyperinsulinemic hypoglycemia of infancy, but the mice are normoglycemic unless stressed. Sur1–/– islets lack first phase insulin secretion and exhibit an attenuated glucose-stimulated second phase secretion. Loss of the first phase leads to mild glucose intolerance, whereas reduced insulin output is consistent with observed neonatal hyperglycemia. Loss of K\textsubscript{ATP} channels impairs the rate of return to a basal secretory level after a fall in glucose concentration. This leads to increased hypoglycemia upon fasting and contributes to a very early, transient neonatal hypoglycemia. Whereas persistent hyperinsulinemic hypoglycemia of infancy underscores the importance of the K\textsubscript{ATP}-dependent ionic pathway in control of insulin release, the Sur1–/– animals provide a novel model for study of K\textsubscript{ATP}-independent pathways that regulate insulin secretion.

ATP-sensitive potassium channels (K\textsubscript{ATP} channels)\textsuperscript{1} are a unique combination of a K\textsuperscript{+} inward rectifier (either K\textsubscript{IR}6.1 or K\textsubscript{IR}6.2) and a sulfonylurea receptor (SUR1 or SUR2) transport ATPase superfamily members (1–3). These channels respond to mic unless stressed. Hypoglycemia of infancy, but the mice are normoglycemic those seen in patients with persistent hyperinsulinemic hypoglycemia (1, 5). Surprisingly, two recent studies involving disruption of K\textsubscript{ATP}-independent pathways that regulate glucose-stimulated insulin secretion by setting the resting membrane potential below the activation threshold for voltage-gated Ca\textsuperscript{2+} channels (4).

Mutations in human Sur1 or K\textsubscript{IR}6.2 cause a recessive form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI) characterized by oversecretion of insulin despite severe hypoglycemia (1, 5). Surprisingly, two recent studies involving disruption of K\textsubscript{ATP} channels in mice produced a quite different picture. Targeted overexpression of a dominant-negative K\textsubscript{IR}6.2 subunit, K\textsubscript{IR}6.2\textsubscript{G132S}, in β-cells reduced channel activity producing animals that were hypoglycemic at birth but became increasingly hyperglycemic secondary to β-cell death (6).

K\textsubscript{IR}6.2 null mice, K\textsubscript{IR}6.2–/–, on the other hand, completely lack β-cell K\textsubscript{ATP} channels but exhibit a less severe phenotype (7). The K\textsubscript{IR}6.2–/– animals have nearly normal blood glucose levels, showing mild glucose intolerance when challenged with glucose. These animals are reported to release a small amount of insulin in response to glucose, whereas isolated, perfused islets show a small first phase of glucose-stimulated insulin secretion and no second phase. The normal blood glucose levels have been attributed to insulin hypersensitivity secondary to the loss of SUR2/K\textsubscript{IR}6.2 K\textsubscript{ATP} channels in skeletal muscle.

SUR1 null mice, Sur1–/–, unlike their K\textsubscript{IR}6.2–/– counterparts, are not insulin-hypersensitive. Isolated Sur1–/– islets exhibit a pattern of glucose-stimulated insulin release consistent with regulation by an underlying K\textsubscript{ATP}-independent pathway (or pathways), the nature of which is unknown (8–12). The Sur1–/– mice are both significantly more hyperglycemic when glucose-loaded and significantly more hypoglycemic when fasted than the control animals. Potential physiologic mechanisms of regulation of blood glucose by K\textsubscript{ATP}-independent insulin secretion are discussed.

**EXPERIMENTAL PROCEDURES**

**Animals and Genotyping**—The 5′-end of the mouse Sur1 gene was cloned from a mouse genomic library constructed in λ phage. The approach is the symbol for the Sur1 gene is Abcc1. A targeting vector was constructed that contained 5.5 kilobase pairs of DNA, including the 5′- and 3′-intronic sequence flanking a puromycin resistance cassette that replaced the second exon of Sur1. Thymidine kinase was included downstream of the 3′ region of homology for negative selection. The vector was linearized and used to electroporate RW4 embryonic stem cells (Genome Systems, Inc., Palo Alto, CA). Transfected cells were selected with G418 (3 μg/ml) and 1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil (3.5 μM). Colonies were picked after 12 days and screened for targeted disruption by Southern blotting. Correct recombination was verified by polymerase chain reaction from both the 5′ and 3′-ends of the targeted region. Embryonic stem cells were injected into C57BL/6 blastocysts using standard techniques. Chimeric males were selected and crossed with wild type C57BL/6 females to generate Sur1–/– heterozygotes, which were bred to obtain Sur1–/–, Sur1+/–, and Sur1+– animals. Animals were maintained on a 12-h light/dark cycle and fed standard rodent chow.

**Photolabeling**—Brains from control and Sur1+/– mice were used to isolate membranes as described (13). Sur1 was identified by photolabeling with 125I-azidoiodoglibenclamide as described previously (14).

**Glucose and Insulin Measurements**—Blood glucose levels were measured using Precision Q-ID glucose sensors (MediSense, Inc., Bedford, MA). Microcontainer® serum separator tubes (Becton Dickinson, Franklin Lakes, NJ) were used to extract serum. Plasma insulin was measured with a rat insulin ELISA kit using mouse insulin as a standard (Crystal Chem, Inc., Chicago, IL). Medium insulin was measured using a rat insulin RIA kit (Linco Research, Inc., St. Charles, MO). Fed and fasted tail blood samples were taken from randomly feeding animals or following a 16-h fast. Intraperitoneal glucose and insulin tolerance tests were done on 12–16-week-old male mice following a 16-h fast. Animals were injected intraperitoneally with glucose (1.2 g/kg of body weight) or insulin (0.1 units/kg of body weight) solubilized in 0.9% NaCl. Animals

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‡ These authors contributed equally to this work.

To whom correspondence should be addressed: Dept. of Molecular and Cellular Biology, Rm. 108C, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Tel.: 713-798-4007; Fax: 713-798-0545; E-mail: j Bryan@bcm.tmc.edu.

† The abbreviations used are: K\textsubscript{ATP} channels, ATP-sensitive K\textsuperscript{+} channels; SUR, sulfonylurea receptor; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; K\textsubscript{IR}6, potassium inward rectifier; KRBB, Krebs-Ringer bicarbonate buffer.

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were anesthetized with 65 mg/kg of body weight of sodium pentobarbital approximately 15 min prior to drawing blood from the retro-orbital venous sinus.

Analysis of the Isolated Islets and Islet Cells—Animals were anesthetized using sodium pentobarbital as described above. Islets were isolated by digestion of the pancreas by intraductal injection of 1 mg/ml collagenase P (Roche Molecular Biochemicals) dissolved in Krebs-Ringer bicarbonate buffer (KRB) solution as described elsewhere (15, 16). Collected islets were used directly or dispersed mechanically into single cells in a Ca2+-free KRB solution for electrophysiological measurements. For batch experiments, islets were preincubated in RPMI 1640 medium containing 2.8 mM glucose, and then the islets (10 islets/well in 24-well plates on Transwell™ permeable supports, 6.5 mm in diameter, 8.0 μm pore size; Corning Inc., Corning, NY) were incubated overnight in 0.75 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum and test concentrations of glucose equilibrated with 5% CO2/95% air, pH 7.4, at 37 °C. Perfusion was done following Komatsu et al. (17), with modifications. One hundred islets in a column of Bio-Gel P-10 (Bio-Rad) were continuously perfused at 37 °C with Hepes-NaHCO3 KRB with 1 mg/ml bovine serum albumin (equilibrated with 5% CO2/95% air, pH 7.4) at a flow rate of 0.75 ml/min. After perfusion for 40 min under 2.8 mM glucose, solutions were changed as indicated in the figure. Test substances were added to the basal medium without adjustment of the final osmolality.

Electrophysiology—Dispersed islet cells were cultured for 1–3 days in RPMI 1640 medium containing 11.1 mM glucose with 100 μg/ml streptomycin, 100 IU/ml penicillin, and 10% fetal calf serum at 37 °C in 5% CO2. Standard patch clamp techniques were used to record ion channel currents (18). The pipette solution used for cell-attached recording contained 140 mM KCl, 2 mM CaCl2, and 11 mM HEPES, pH 7.2. The standard internal solution for whole-cell recording contained 50 mM KCl, 35 mM K2SO4, 2.0 mM MgCl2, 11 mM EGTA, 1.2 mM CaCl2, and 11 mM HEPES, and with or without ATP at the indicated concentrations, pH 7.2. The pipette solution for perforated patch recording contained 40 mM K2SO4, 50 mM KCl, 10 mM HEPES, 2.0 mM MgCl2, and 0.5 mM EGTA, pH 7.2. Amphotericin B (240 μg/ml dissolved in 0.4% Me2SO) was included in the pipette to perforate the membrane. Isolated islet cells were superfused with KRBB containing 2.8 mM glucose, 129 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.2 mM MgCl2, 1.2 mM KH2PO4, and 5.0 mM NaHCO3, pH 7.4, for at least 30 min before recording. Currents were recorded using an AXOPATCH-1C (Axon Instruments Inc., Foster City, CA) amplifier and analyzed using pCLAMP (Axon Instruments Inc.). All experiments were performed at room temperature (22–25 °C). β-Cells were identified by a combination of their morphology and the presence of ATP-sensitive potassium currents.

Statistics—One-way analysis of variance was used to evaluate the significance of measurements. Tukey’s post-test was used to compare the variability of the population.

RESULTS

Targeted Inactivation of the Sur1 Gene—The Sur1 gene was inactivated by replacing the second exon in mouse embryonic stem cells with a puromycin resistance cassette (Fig. 1A). The targeting vector was transfected into 129/SvJ embryonic stem cells by electroporation. Puromycin resistant clones were isolated and screened by Southern blotting to identify homologous recombinants. Two clones were injected into C57 BL/6 blastocysts to generate germline chimeras. The identification of Sur1+/+ and Sur1+/- mice is shown (Fig. 4A). Sur1+/- and Sur1-/– mice were also identified using PCR (Fig. 1C). Sur1-/- mice were isolated and photolabeled with 3 nM [3H]azidothymidine in the presence or absence of unlabeled 1 μM glibenclamide and then solubilized and separated by SDS-polyacrylamide gel electrophoresis.

was established using the patch clamp technique. In the whole cell recording mode, control cells show a marked increase in K+ current that peaks at approximately 2 min (3.21 ± 0.62 nS/PF) after membrane rupture and dialysis of intracellular nucleotides and then proceed to “rundown” in the absence of ATP (Fig. 2A). The Sur1-/- cells exhibit no comparable K+ currents. Comparison of the peak values of the normalized conductances of ATP-sensitive potassium currents. Quantitation of the percentage of animals with regulated or heterozygous β-cells indicates that their densities of KATP channels are the same (Fig. 2A, inset), implying a mechanism for regulation of channel density although the number of genes has been halved. Inclusion of ATP (1 mM) in the patch pipette reduces the peak currents and rate of channel rundown.

Sur1-/- β-Cells Show Spontaneous Electrical Activity—The electrical activity of Sur1+/+ and Sur1+/- β-cells in low glucose solutions is markedly different. KATP channels are active in control cells (downward deflections in Fig. 2B, top trace); the average resting membrane potential was −62.4 ± 12.3 mV, n = 48. By contrast, the membrane potential of the Sur1-/- cells fluctuated between −45 and −25 mV (82%, n = 28; average value, −32.6 ± 6.2 mV, n = 28) as a result of spontaneous action potentials (Fig. 2B, bottom traces) that were not suppressed by diazoxide but were inhibited by the Ca2+ channel blocker nifedipine.

Sur1-/- Islet Histology Is Nearly Normal—Terminal deoxynucleotidyl transfer-mediated nick end labeling assays on control and Sur1-/- adult islets showed no differences in apoptosis. Staining for insulin and somatostatin showed no differences, whereas glucagon staining revealed that the peripheral distribution of α-cells was disrupted with an increased number of glucagon positive cells within the interior of the central β-cell mass (data not shown). The cause of this redistribution is unclear, but it has been reported for the KIR6.2-/- mice (7) and

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**Fig. 1. Generation and screening of Sur1-/- animals.** A, schematic of the first 5 exons of the 39-exon Sur1 gene. Exon 2 was replaced with the puromycin resistance gene, and a negative selection marker encoding thymidine kinase (TK) was placed downstream of the 3′ region of DNA homology. B and C, Southern blotting and polymerase chain reaction (PCR) were used to identify Sur1+/+, Sur1+/-, and Sur1-/- mice. D, neuronal membranes from Sur1+/+, Sur1+/-, and Sur1-/- mice were isolated and photolabeled with 3 nM [3H]azidothymidine in the presence or absence of unlabeled 1 μM glibenclamide and then solubilized and separated by SDS-polyacrylamide gel electrophoresis.
for the β-cell specific knockout of the insulin receptor mice (19), among others.

**Sur1 Knockout Mice**

**FIG. 2.** Electrophysiology of Sur1+/+, Sur1+/−, and Sur1−/− β-cells. A, K+ currents increase in Sur1+/+ but not Sur1−/− β-cells as cytoplasmic ATP falls during whole-cell recording, as shown in a plot of normalized conductance versus time. The holding potential was −70 mV; test voltage pulses, 100 ms duration of 10 mV, were applied every 3 s. The control currents peaked at about 2 min and then proceeded to rundown. No currents were detected in the Sur1−/− β-cells. The inset shows that the normalized conductance from control and heterozygous Sur1−/− β-cells are equivalent. Inclusion of 1 mM ATP in the pipette solution reduces the peak currents and rate of rundown. The error bars are ± S.D.; n = 10 cells. B, Sur1−/− β-cells exhibit spontaneous action potentials. Recording from Sur1+/+ control cells in cell-attached mode illustrates the presence of KATP channels in the patch, whereas Sur1−/− cells exhibited spontaneous action potentials that were suppressed by the L-type Ca2+ channel blocker nifedipine but not by diazoxide. The holding potential was 0 mV; cells were incubated in 2.8 mM glucose.

**FIG. 3.** Sur1−/− islets exhibit changes in both the kinetics and extent of insulin secretion. Glucose-stimulated insulin release from control (circles) and Sur1−/− (squares) islets during continuous perifusion. A, in a short term experiment, the concentration of glucose (G) was increased from 2.8 to 16.7 mM between 10 and 50 min. Tolbutamide (Tlb), 0.3 mM, was added to the medium starting at 40 min. B, in long term perifusion experiments, the concentration of glucose was increased from 2.8 to 16.7 mM between 0 and 500 min. Note the break in the time line and scale change at 25 min. The longer time values are the mean values of seven 1-min time points flanking the given time, and the error bars are ± S.D. C, the islets in B were switched to 2.8 mM glucose to follow their rates of return to a basal secretory level. The data were normalized to an initial value of 1 by dividing by the average value immediately before switching to 2.8 mM glucose. The results from two separate experiments are plotted; the lines are the best fits of a monoexponential function to the combined data sets. The time course of insulin output after changes in glucose concentration was assayed from 100 Sur1+/+ or Sur1−/− islets during continuous perifusion.

Glucose-stimulated insulin release from control and Sur1−/− islets over an 8-h period is summarized in Fig. 3B. Note the broken time line and the change of scale on the y axis. The Sur1−/− islets showed about a 4-fold increase in their rate of insulin release within 5 min; both the control and Sur1−/− islets exhibited a slow, parallel increase in insulin output through 500 min. The rate of insulin release of the Sur1−/− islets at 400 or 500 min was approximately 30% of the controls. The results imply regulation of insulin release by a slow, KATP-independent, glucose-stimulated mechanism.
Fig. 4. Extended culture of control and Sur1−/− islets demonstrates glucose-dependent insulin secretion. Islets were cultured for 20 h at the indicated glucose concentrations before assay of released insulin. The control (circles) and Sur1−/− islets (squares) exhibited glucose-stimulated insulin release, but secretion from the Sur1−/− islets was impaired relative to control islets. At 16.7 mM glucose, the values were 494.5 ± 88.9 versus 285.1 ± 60.2 ng/ml/10 islets/20 h (n = 10; mean ± S.D.; p < 0.0005), respectively. The lines are best fits to a logistic equation; the half-maximal values are 9.0 and 9.8 mM for the control and Sur1−/− groups, respectively. For comparative purposes, the hatched bar indicates the range of blood glucose concentrations seen in randomly feeding adult animals. The inset demonstrates that secretion in response to 16.7 mM glucose is sensitive to nifedipine (1 μM). The error bars are ±S.D.

Fig. 5. Sur1+/− animals display impaired glucose tolerance but are not insulin-hypersensitive. Control and Sur1+/− male mice (12–16 weeks of age) were injected intraperitoneally with glucose (1.2 g/kg of body weight) following a 16-h fast. A, blood glucose levels of the Sur1+/− animals were significantly higher than those of the controls at 30 and 60 min (15.1 ± 3.2 mM versus 13.5 ± 2.6 mM at 15 min; n = 13 and 10; p < 0.01; and 12.8 ± 2.0 mM versus 7.4 ± 0.8 mM at 60 min; n = 14 and 9; p < 0.001). B, control animals release insulin in response to a glucose challenge, whereas the Sur1−/− animals do not. C, intraperitoneal injection of porcine insulin, 0.1 unit/kg of body weight, produced an equivalent glucose lowering effect in similarly fasted animals. The error bars are ±S.D.

To determine whether K<sub>ATP</sub> channels play a role in returning insulin secretion to a basal level after a down-shift in glucose, the control and Sur1−/− islets were switched to 2.8 mM glucose. Insulin output from the control islets dropped rapidly, t<sub>1/2</sub> = 4 min. By contrast, insulin output from Sur1−/− islets fell slowly, t<sub>1/2</sub> = 32 min, averaged for these two experiments (Fig. 3C). The results indicate K<sub>ATP</sub> channels, presumably by lowering the resting membrane potential, play a major role in wild type β-cells in "switching off" insulin release when glucose levels fall. The Sur1−/− results show that the K<sub>ATP</sub>-independent mechanism(s) has a much slower response time.

Sur1−/− Islets Show Glucose-responsive Insulin Secretion—The glucose-responsiveness of isolated islets was tested in extended static incubations at various glucose concentrations. Insulin release from both Sur1−/− and control islets, integrated over 20 h, increased with glucose concentration (Fig. 4), with half-maximal stimulation between 9 and 10 mM. Insulin output at the lowest glucose concentrations was indistinguishable, whereas the maximum output from the Sur1−/− islets was ~60% of the control value (285.1 ± 60.2 versus 494.5 ± 88.9, p < 0.0005). This insulin secretion requires Ca<sup>2+</sup> influx and is sensitive to the Ca<sup>2+</sup> channel blocker nifedipine (Fig. 4, inset), implying that insulin output is coupled with glucose concentration via a Ca<sup>2+</sup>-sensitive mechanism. In Fig. 4, the hatched bar centered at ~6.5 mM glucose indicates the range of glucose values in randomly feeding adult control and Sur1−/− mice (see Fig. 6).

Sur1−/− Mice Are Mildly Glucose Intolerant—In response to a glucose challenge the Sur1−/− animals exhibit mild glucose intolerance (Fig. 5A); their peak glucose values are higher and their clearance times longer versus control mice (15.1 ± 3.2 versus 13.5 ± 2.6 mM at 15 min, p = 0.01, and 12.8 ± 2.0 versus 7.4 ± 0.8 mM at 60 min, p < 0.001). Consistent with the lack of first phase release from isolated islets, the Sur1−/− animals displayed mild glucose intolerance (Fig. 5B). These results suggest that mice have both insulin-induced and insulin-independent pathways for glucose disposal.

In contrast to what has been reported for the Kir6.2−/− mice (7), the Sur1−/− animals showed no increased insulin sensitivity. Following intraperitoneal injection of insulin, the glucose values of both groups dropped with no significant differences (Fig. 5C).

Sur1−/− Mice Exhibit Glucose-stimulated Insulin Secretion—The blood glucose levels of random-fed adult Sur1−/− mice were normal, and their plasma insulin/blood glucose ratios did not differ significantly from those of the controls (Fig. 6, A and B). By contrast, Sur1−/− animals fasted for 16 h were significantly more hypoglycemic than similarly fasted control animals (2.76 ± 0.65 versus 3.63 ± 0.44 mM, p < 0.05 for males, and
Comparison of the blood glucose values normalized against body weight (\(\text{ng/ml/mM}\)) show that the hypoglycemia of the \(\text{Sur1}^{--}\) mice by 5 days, the

The insulin/glucose ratios for randomly feeding animals were not significantly different (\(p > 0.05\), for \(+/+\) versus \(-/-\) males and for \(+/+\) versus \(-/-\) females), whereas the control values (\(0.88 \pm 0.15 \text{ ng/ml/mM}\)) exhibit only a weak correlation (\(r = 0.33 \pm 0.15\)) with body weight (\(p < 0.001\)). Error bars are \(\pm\)S.D. Neonatal glucose and insulin levels were measured in control and \(\text{Sur1}^{--}\) mice at 1 and 5 days of age and plotted against body weights. C, the 1- and 2-day blood glucose values show little correlation with body weight (\(r = 0.22\), \(-0.31\), and 0.04, \(n = 10\), for 1-day control animals (open circles), 1-day \(\text{Sur1}^{--}\) animals (open squares), and 2-day \(\text{Sur1}^{--}\) animals (gray squares), respectively). The mean 1-day blood glucose values for the control (3.73 ± 0.03 mM) and \(\text{Sur1}^{--}\) pups (1.73 ± 0.49 mM) are both hypoglycemic relative to the mean adult level (6.53 ± 0.09 mM), but the \(\text{Sur1}^{--}\) pups have lower glucose values, normalized against body weight, than controls (1.12 ± 0.03 versus 2.56 ± 0.44 mM/gm, \(n = 10\), \(p < 0.001\); see inset). The 5-day values for the \(\text{Sur1}^{--}\) pups (filled squares) exhibit a strong correlation with body weight (\(r = 0.91\), \(n = 12\)), whereas the control values (filled circles) exhibit only a weak correlation (\(r = 0.55\), \(n = 14\)). Comparison of the blood glucose values normalized against body weight (inset) show that the hypoglycemia of the \(\text{Sur1}^{--}\) pups resolves within 36–48 h, and by 5 days, the \(\text{Sur1}^{--}\) pups are significantly hypoglycemic relative to controls (2.11 ± 0.25 mM/gm, \(p < 0.001\) versus 2.82 ± 0.53 mM/gm, \(p < 0.001\)), \(n = 14\)). D, comparison of the 1-day plasma insulin values shows that the \(\text{Sur1}^{--}\) pups are hyperinsulinemic relative to control animals (2.82 ± 0.25 versus 1.58 ± 0.28 ng/ml, \(p < 0.05\), \(n = 26\) and 10, respectively). Within 5 days, the plasma insulin values of the \(\text{Sur1}^{--}\) animals (0.72 ± 0.15 ng/ml/mM) and 2.76 ± 0.44 ng/ml/mM) are both hypoglycemic

\(2.76 \pm 0.44\) versus 3.82 ± 0.52 mM, \(p < 0.05\), for females). This result is consistent with the inability of \(\text{Sur1}^{--}\) islets to restrict their insulin output rapidly when glucose levels fall. The data in Fig. 3C suggest the plasma insulin levels of the \(\text{Sur1}^{--}\) mice will be higher than those of the controls for nearly 3 h. Although the insulin levels are expected to equalize before the end of the fast, the \(\text{Sur1}^{--}\) mice appear to be unable to compensate for the increased insulin and bring their glucose levels back to the fasted control level. The fasted plasma insulin/blood glucose ratios support this interpretation; both male and female \(\text{Sur1}^{--}\) mice have higher mean ratios than controls (0.33 ± 0.13 versus 0.15 ± 0.07 for males and 0.24 ± 0.11 versus 0.15 ± 0.05 ng/ml/mM for females), although only the males are significantly different (\(p < 0.001\)).

\(\text{Sur1}^{--}\) Animals Exhibit Transient Neonatal Hypoglycemia—Loss of \(\beta\)-cell \(K_{ATP}\) channel activity and appearance of spontaneous \(Ca^{2+}\) action potentials in PHHI patients is associated with unregulated insulin release (20) and severe neonatal hypoglycemia (21). The clinical phenotype of PHHI patients strongly suggested that the \(\text{Sur1}^{--}\) mice would show severe,

\(\text{Sur1}^{--}\) knockout animals drop in response to fasting, but the \(\text{Sur1}^{--}\) animals have lower values (males: 2.76 ± 0.65 versus 3.63 ± 0.44 mM, \(p < 0.05\), \(n = 20\) or 19; females: 2.76 ± 0.94 versus 3.82 ± 0.52 mM, \(p < 0.05\), \(n = 16\) or 19). There were no significant differences by one-way analysis of variance in the glucose values of the control versus \(\text{Sur1}^{--}\) animals in the fed state (\(p > 0.05\) for \(+/+\) versus \(-/-\) males and for \(+/+\) versus \(-/-\) females, \(n = 21\)), whereas the control values (\(0.172 \pm 0.085\) ng/ml/mM, \(n = 69\)) were lower than the controls (1.29 ± 0.28, \(n = 52\)), 1-day control animals (filled circles) and the 5-day values for the \(\text{Sur1}^{--}\) pups (open circles). The average value was 6.53 ± 0.99 mM, \(n = 72\). B, the insulin/glucose ratios of the \(\text{Sur1}^{--}\) animals were higher than the controls, although only the difference for males was significant (0.33 ± 0.13 versus 0.146 ± 0.07 ng/ml/mM, \(p < 0.001\), \(n = 20\) or 19). The insulin/glucose ratios for randomly feeding animals were not significantly different (\(p > 0.05\), for \(+/+\) versus \(-/-\) males and for \(+/+\) versus \(-/-\) females, \(n = 21\)). The mean 1-day blood glucose values for the control (3.73 ± 0.03 mM) and \(\text{Sur1}^{--}\) pups (1.73 ± 0.49 mM) are both hypoglycemic relative to the mean adult level (6.53 ± 0.09 mM), but the \(\text{Sur1}^{--}\) pups have lower glucose values, normalized against body weight, than controls (1.12 ± 0.03 versus 2.56 ± 0.44 mM/gm, \(n = 10\), \(p < 0.001\); see inset). The 5-day values for the \(\text{Sur1}^{--}\) pups (filled squares) exhibit a strong correlation with body weight (\(r = 0.91\), \(n = 12\)), whereas the control values (filled circles) exhibit only a weak correlation (\(r = 0.55\), \(n = 14\)). Comparison of the blood glucose values normalized against body weight (inset) show that the hypoglycemia of the \(\text{Sur1}^{--}\) pups resolves within 36–48 h, and by 5 days, the \(\text{Sur1}^{--}\) pups are significantly hypoglycemic relative to controls (2.11 ± 0.25 mM/gm, \(p < 0.001\) versus 2.82 ± 0.53 mM/gm, \(p < 0.001\)), \(n = 14\)). D, comparison of the 1-day plasma insulin values shows that the \(\text{Sur1}^{--}\) pups are hyperinsulinemic relative to control animals (2.82 ± 0.25 versus 1.58 ± 0.28 ng/ml, \(p < 0.05\), \(n = 26\) and 10, respectively). Within 5 days, the plasma insulin values of the \(\text{Sur1}^{--}\) animals (0.72 ± 0.28 ng/ml/mM) were lower than the controls (1.29 ± 0.88 ng/ml), but the difference is not significant at the \(p = 0.05\) level (\(n = 12\) and 14, respectively). The inset compares the plasma insulin/blood glucose ratios (see text). The error bars in the insets are \(\pm\)S.D.
persistent hypoglycemia. This was not the case. The Sur1−/− pups were hypoglycemic 1 day after birth (1.73 ± 0.49 mg/ml), and their insulin/blood glucose ratios were inappropriately high versus control values (2.31 ± 1.56 versus 0.42 ± 0.33 mg/ml/mM, \( p < 0.001 \)), but this hypoglycemia reversed during the second day, and the insulin/glucose ratio reached a normal value (Fig. 6, C and D).

The blood glucose values of the 5-day Sur1−/− pups display a correlation with body weight that was not apparent in the controls, and the 5-day Sur1−/− pups were significantly hyperglycemic versus controls (8.29 ± 1.71 versus 6.23 ± 0.47 mg/ml, \( p < 0.001 \)). The Sur1−/− insulin/blood glucose ratio was half that of the controls, although the difference is not significant (0.09 ± 0.04 versus 0.20 ± 0.13 mg/ml/mM, \( p > 0.05 \)). This hyperglycemia in the 5-day Sur1−/− pups is consistent with the reduced insulin output of their islets at high glucose concentrations (Fig. 4).

**DISCUSSION**

The interaction of SUR and KIR6.2 subunits is tightly integrated with both required to form \( K_{\text{ATP}} \) channels (1, 2). On the other hand, \( K^+ \) currents have been reported when KIR6.2 subunits areoverexpressed using strong promoters (22, 23) or when the endogenous ER retention signals described by Zerranget al. (24) are removed (25–27). Although homomeric KIR6.2 channels have the same conductance as wild type \( K_{\text{ATP}} \) channels and exhibit low sensitivity to inhibitory ATP, they differ in all other respects. We saw no indication that KIR6.2 subunits reach the cell surface without SUR1, and we expected that their presence in the plasma membrane, unregulated by SUR1, would result in hyperpolarization, reduced insulin secretion, and profound hyperglycemia. There was no indication that other K+ channels are responsible for the loss of SUR1/KIR6.2 channels.

The loss of \( K_{\text{ATP}} \) channel activity leads to a specific \( \beta \)-cell “electrophysiological” phenotype now described in three systems: human PHHI (21, 28), KIR6.2−/− (7), and Sur1−/− \( \beta \)-cells. This phenotype is characterized 1) by the loss of \( K^+ \) currents activated by reduced intracellular nucleotides (shown for all three systems), 2) by an elevated membrane potential (also shown for all three systems), and 3) by spontaneous Ca++ action potentials (shown for PHHI and Sur1−/− \( \beta \)-cells) that increase [Ca2+], (shown for PHHI and KIR6.2−/− \( \beta \)-cells). This \( K_{\text{ATP}} \)-β-cell phenotype agrees with the “classical ionic mechanism,” or \( K_{\text{ATP}} \)-dependent pathway, proposed to rapidly control glucose-stimulated insulin secretion (4, 29).

The loss of \( K_{\text{ATP}} \) channel activity produces remarkably different alterations in glucose homeostasis in PHHI neonates versus \( K_{\text{ATP}} \)−/− mice. Although PHHI is a heterogeneous disorder (1, 30), the complexity of which is increased further by genetic imprinting (31, 32), loss of function mutations in both Sur1 and KIR6.2 have been identified in homozygous individuals whose hyperinsulinemia requires near total pancreatectomy to control the resulting hypoglycemia (1). Although insulin secretory data from isolated PHHI \( \beta \)-cells is scanty, the available results imply continuous secretion at a high rate regardless of glucose concentration (20).

\( K_{\text{ATP}} \)−/− mice, both KIR6.2−/− (7) and Sur1−/−, exhibit a far less dramatic phenotype. Neither KIR6.2−/− mice nor Sur1−/− mice are persistently hypoglycemic neonates, and as adults, both groups are normoglycemic under fed conditions. The two phenotypes differ subtly. The KIR6.2−/− mice are not significantly hyperglycemic versus controls following a 16-h fast, whereas the Sur1−/− mice are (Fig. 6). The two groups exhibit a similar mild glucose intolerance. Fasting plasma insulin values in the KIR6.2−/− and Sur1−/− animals are not significantly different from those of fasting control animals. The plasma insulin values increase to control levels when Sur1−/− animals are fed, indicating some mechanism for regulating insulin release in the absence of \( K_{\text{ATP}} \) channels; plasma insulin data have not been reported for fed KIR6.2−/− mice. In addition to being unable to properly regulate their insulin output when blood glucose falls during a fast, the Sur1−/− animals cannot properly regulate when blood glucose levels rise. This results in a peculiar plasma glucose profile, with the Sur1−/− animals being significantly more hyperglycemic when glucose-loaded, e.g. the 5-day neonatal Sur1−/− pups, and significantly more hypoglycemic when fasted, relative to control animals. The two \( K_{\text{ATP}} \)−/− animals differ markedly in their sensitivity to insulin. Although we observed no difference in insulin sensitivity (Fig. 5C), Miki et al. (7) report that the KIR6.2−/− animals are insulin-hypersensitive, suggest that this is secondary to loss of SUR2A/KIR6.2 ATP-sensitive K+ channels in skeletal muscle, and propose that the hypersensitivity accounts for the normoglycemia. This proposal suggests that the plasma insulin values of the KIR6.2−/− animals should be significantly lower than controls; otherwise, they should be hypoglycemic. The availability of SUR2A−/− animals should allow a test of this hypothesis.

The secretion profiles of isolated KIR6.2−/− and Sur1−/− islets also differ. The Sur1−/− islets show no first phase insulin secretion in response to a shift from 2.8 to 16.7 mM glucose, whereas the KIR6.2−/− animals do show some first phase release, although it is small (Fig. 3B in Miki et al. (7)). By contrast, the KIR6.2−/− islets exhibit no second phase release, whereas the Sur1−/− islets show a second phase response, although it is attenuated, measuring only 35% of the control value (Fig. 3A).

In extended perfusion experiments, the Sur1−/− and control islets exhibit a parallel increase in insulin output. Extended static incubation experiments demonstrate that insulin output from both control and Sur1−/− islets is glucose-dependent and suppressible by the Ca++ channel blocker nifedipine (Fig. 4). Furthermore, the insulin output of the Sur1−/− islets is quite similar to control islets in the range of glucose blood glucose values determined in randomly fed animals (hatched bar in Fig. 4). The reason(s) for the attenuated insulin output from the Sur1−/− islets is unclear, but it could reflect a role for SUR1 apart from \( K_{\text{ATP}} \) channels (33).

In the case of Sur1−/− mice, the loss of \( K_{\text{ATP}} \) channel activity reveals an underlying control of what would commonly be considered the 2nd phase of insulin secretion by a \( K_{\text{ATP}} \)-independent mechanism or pathway. Several \( K_{\text{ATP}} \)-independent pathways have been identified previously by pharmacologic means. One method used the potassium channel opener diazoxide to hold \( K_{\text{ATP}} \) channels in an open state, while increasing the external K+ concentration to depolarize the \( \beta \)-cell membrane, activate voltage-gated Ca++ channels, and increase [Ca2+]i (10, 34). A second method, analogous to the \( K_{\text{ATP}} \)-mouse models, used sulfonylureas to block \( K_{\text{ATP}} \) channels (8, 35). Again, [Ca2+]i, was elevated secondary to blocking \( K_{\text{ATP}} \) channels. With these strategies, it was possible to show that glucose metabolism augmented Ca++-stimulated insulin release. A third route required simultaneous activation of protein kinases A and C and did not require an increase in [Ca2+]i, but was GTP-dependent (36–39). The available data are not sufficient to determine which pathway, or perhaps a novel pathway, is responsible for the regulation of insulin release in Sur1−/− mice. Suppression of insulin release from Sur1−/− islets by nifedipine is consistent with a requirement for Ca++ influx, suggesting that a Ca++-dependent pathway is involved. On the other hand, as pointed out during the review of this paper, insulin release via the Ca++-dependent, \( K_{\text{ATP}} \)-independent pathway may be more rapid and of greater magnitude than observed from Sur1−/− islets. We have been unable to find data...
this impaired ability to restrict insulin output, a consequence of being unable to repolarize their β-cells, accounts for the increased hypoglycemia that we observed in fasting adult animals and may play a similar role in the 1-day-neonatal pups.

A key question is why the glucose levels in PHHI patients are persistently low, whereas the KATP−/− mice are normoglycemic. There is a significant body of literature on differences in insulin secretory responses between mouse and rat (and human) β-cells. When rat islets are shifted to high glucose, they show a first phase of insulin secretion following a larger, rising second phase. Mouse islets exhibit an equivalent first phase peak followed by an elevated plateau, but no rising second phase. This differential behavior has been described by a number of authors, including Lenzen (41), Berglund (42), Ma et al. (43), and Zawalich et al. (44). Our working hypothesis to explain the species differences is that KATP−/− β-cells are continuously in the second phase of insulin secretion. Insulin output is greater for PHHI β-cells than KATP−/− mouse β-cells, consistent with normal human β-cells, which, like rat β-cells, have a large rising second phase of insulin secretion. This hypothesis requires that basal insulin output from PHHI β-cells at low concentrations of glucose should be elevated, in contrast to the Sur1−/− β-cells, the basal output of which is comparable to controls. The Sur1−/− mice offer a potential background for testing this hypothesis by engineering transgenic animals to search for components (that contribute to the large rising second phase of insulin secretion in rats and humans and for compounds that modulate second phase insulin release.

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Sur1 Knockout Mice

**Sur1 Knockout Mice: A MODEL FOR KATP CHANNEL-INDEPENDENT REGULATION OF INSULIN SECRETION**

Victor Seghers, Mitsuhiro Nakazaki, Franco DeMayo, Lydia Aguilar-Bryan and Joseph Bryan

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