Glucose Stimulates \( \text{Ca}^{2+} \) Influx and Insulin Secretion in 2-Week-old \( \beta \)-Cells Lacking ATP-sensitive \( K^+ \) Channels*

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In adult \( \beta \)-cells glucose-induced insulin secretion involves two mechanisms (a) a \( K_{\text{ATP}} \) channel-dependent \( \text{Ca}^{2+} \) influx and rise of cytosolic \( [\text{Ca}^{2+}]_c \), and (b) a \( K_{\text{ATP}} \) channel-independent amplification of secretion without further increase of \( [\text{Ca}^{2+}]_c \). Mice lacking the high affinity sulfonylurea receptor (Sur1KO), and thus \( K_{\text{ATP}} \) channels, have been developed as a model of congenital hyperinsulinism. Here, we compared \( [\text{Ca}^{2+}]_c \), and insulin secretion in overnight cultured islets from 2-week-old normal and Sur1KO mice. Control islets proved functionally mature: the magnitude and biphasic kinetics of \( [\text{Ca}^{2+}]_c \) and insulin secretion changes induced by glucose, and operation of the amplifying pathway, were similar to adult islets. Sur1KO islets perfused with 1 mM glucose showed elevation of both basal \( [\text{Ca}^{2+}]_c \), and insulin secretion. Stimulation with 15 mM glucose produced a transient drop of \( [\text{Ca}^{2+}]_c \), followed by an overshoot and a sustained elevation, accompanied by a monophasic, 6-fold increase in insulin secretion. Glucose also increased insulin secretion when \( [\text{Ca}^{2+}]_c \), was clamped by KCl. When Sur1KO islets were cultured in 5 instead of 10 mM glucose, \( [\text{Ca}^{2+}]_c \) and insulin secretion were unexpectedly low in 1 mM glucose and increased following a biphasic time course upon stimulation by 15 mM glucose. This \( K_{\text{ATP}} \) channel-independent first phase \( [\text{Ca}^{2+}]_c \), rise was attributed to a Na\(^+\)-, Cl\(^-\), and Na\(^+\)-pump-independent depolarization of \( \beta \)-cells, leading to \( \text{Ca}^{2+} \) influx through voltage-dependent calcium channels. Glucose indeed depolarized Sur1KO islets under these conditions. It is suggested that unidentified potassium channels are sensitive to glucose and subserve the acute and long-term metabolic control of \( [\text{Ca}^{2+}]_c \), in \( \beta \)-cells without functional \( K_{\text{ATP}} \) channels.

Fine control of insulin secretion by pancreatic \( \beta \)-cells is critical for glucose homeostasis: excessive release causes hypoglycemia, whereas insufficient insulin leads to diabetes. Glucose exerts its control on \( \beta \)-cells via two major, hierarchical signaling pathways (1). The triggering pathway requires functional ATP-sensitive \( K^+ \) channels (\( K_{\text{ATP}} \) channels)\(^2\) to produce the essential rise in cytosolic \( \text{Ca}^{2+} \) concentration ([\( \text{Ca}^{2+}]_c \)) through the following sequence of events. When the glucose concentration increases, oxidative glycolysis in \( \beta \)-cells accelerates, leading to changes in cytosolic adenine nucleotides, which close \( K_{\text{ATP}} \) channels in the plasma membrane. The resulting depolarization opens voltage-dependent \( \text{Ca}^{2+} \) channels, permitting \( \text{Ca}^{2+} \) influx and a rise in \( [\text{Ca}^{2+}]_c \), that triggers exocytosis of insulin granules (2–8). Simultaneously, glucose activates an amplifying pathway, also termed the augmentation or \( K_{\text{ATP}} \) channel-independent pathway, which does not increase \( [\text{Ca}^{2+}]_c \), further but augments the secretory response to the triggering \( \text{Ca}^{2+} \) signal. The mechanisms for this metabolic amplification have not been fully identified but are distinct from neuro-hormonal amplification (1, 9–13).

\( K_{\text{ATP}} \) channels in \( \beta \)-cells are composed of the high affinity sulfonylurea receptor (SUR1-ABCC8) and the pore-forming \( \text{K}_{\text{IR6.2}} \) (\( \text{KCNJ11} \) subunit) (14). Mice lacking \( K_{\text{ATP}} \) channels in their \( \beta \)-cells (Sur1KO or Kir6.2KO mice) are valuable models to investigate the regulation of insulin secretion without using pharmacological agents. They were originally developed (15–17) with the aim of understanding the pathology of congenital hyperinsulinism, a disease due, in 50% of cases, to inactivating mutations of \( K_{\text{ATP}} \) channels (2, 18–20). However, unlike the infants who suffer from severe hypoglycemia during the neonatal period, Sur1KO or Kir6.2KO mice are not hypoglycemic except for the first 48–72 h (15, 16). In vitro islet studies done in these mice have yielded controversial results. Whereas, several groups reported that these islets are poorly sensitive to glucose stimulation, at least in the absence of simultaneous activation of protein kinases A or C (15–17, 21), we observed acute stimulation of insulin secretion by glucose in Sur1KO islets, which we attributed to both triggering and amplifying pathways (22). Smaller but distinct effects of glucose in the same model were also demonstrated by others (23, 24).

Previous in vitro studies of islets lacking \( K_{\text{ATP}} \) channels have been restricted to adult animals. Because \( \beta \)-cells undergo functional maturation during the postnatal period (25, 26), it is unclear whether the behavior of adult Sur1KO islets is similar to that of young Sur1KO islets or has been altered by adaptive processes, thereby invalidating all extrapolations to a human pathology of infancy. In the present study we investigated the

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‡ The abbreviations used are: \( K_{\text{ATP}} \) channel, ATP-sensitive potassium channel; SUR1, sulfonylurea receptor 1; \( [\text{Ca}^{2+}]_c \), cytosolic \( \text{Ca}^{2+} \) concentration; DibAC3, bis-(1,3-dihexylbarbituric acid)trimethine oxonol.
Glucose Effects in Sur1KO Islets

effects of glucose on islets from 2-week-old Sur1KO and normal mice. Our aim was to determine whether glucose can control insulin secretion in these young β-cells lacking K_{ATP} channels and to assess whether such control is exerted via changes in the triggering Ca^{2+} signal or via the amplifying pathway. In the course of these studies, we discovered that β-cell [Ca^{2+}]_{i} is under acute and long term control by glucose even in the absence of K_{ATP} channels. Our observations provide a plausible explanation for the lack of hypoglycemia in Sur1KO mice and indicate that K_{ATP} channels are not the only metabolic sensor involved in glucose regulation of Ca^{2+} influx in β-cells.

EXPERIMENTAL PROCEDURES

The study was approved by, and the experiments were conducted in accordance with the guidelines of the Animal Research Committees of the respective institutions.

Solutions and Reagents—The medium used for islet isolation was a bicarbonate-buffered solution containing (in mM): NaCl 120, KCl 4.8, CaCl_{2} 2.5, MgCl_{2} 1.2, and NaHCO_{3} 24. It was gassed with O_{2}:CO_{2} (94%:6%) to maintain pH 7.4 and was supplemented with 1 mg/ml bovine serum albumin and 10 mM glucose. A similar medium was used for most experiments after adjustment of the glucose concentration and addition of test substances. Increases in the KCl concentration (to 10 or 30 mM) were compensated for by decreases in NaCl. Na^{+}-free solutions were prepared by substitution of NaCl and NaHCO_{3} with choline chloride and choline-bicarbonate, respectively, and 1 mM atropine was added to prevent the muscarinic effects of choline. Cl^{-}-free solutions (in mM): sodium isethionate 125, KHCO_{3} 4.8, calcium acetate 3.5, magnesium acetate 1.2, and NaHCO_{3} 19.2. Total calcium was increased from 2.5 to 3.5 mM to maintain the same concentration of ionized Ca^{2+} as in Cl^{-}-containing solutions (27).

Unless indicated otherwise, reagents were from Sigma or Merck AG (Darmstadt, Germany). Diazoxide (a gift from Schering-Plough, Brussels) and tolbutamide were added from stock solutions in Me_{2}SO. Thapsigargin and ryanodine (Alomone, Jerusalem, Israel), and forskolin (Calbiochem), atropine was added to prevent the muscarinic effects of choline. Glucose Effects in Sur1KO Islets—Cultured islets were distributed in batches of 20–40 before being transferred into perfusion chambers (29). The islets were then perfused (flow rate, 1 ml/min) at 37 °C with test solutions the composition of which is indicated in the figure legends. Effluent fractions were collected at 2-min intervals and saved for insulin assay using rat insulin as a standard, and ethanol to precipitate bound insulin (30). At the end of the experiments, the islets were recovered and their insulin content was determined after extraction in acid–ethanol. Fractional insulin secretion rate was then calculated as the percentage of islet insulin content that was secreted per minute.

Measurements of Islet [Ca^{2+}]_{i}—Cultured islets were loaded with the Ca^{2+} indicator fura-PE3-AM (2 μM, Teflabs, Austin, TX) for 2 h in control medium containing 5 or 10 mM glucose according to the glucose concentration during the culture. Loaded islets were then placed into the perfusion chamber of a spectrofluorimetric system, equipped with a camera, and with which [Ca^{2+}]_{i} was measured at 37 °C as described previously (31).

Measurements of Islet Cell Membrane Potential—Changes in β-cell membrane potential were evaluated using the bissoxonol fluorescent probe DiBAC_{4}(3) (Invitrogen-Molecular Probes, Eugene, OR) which, due to its anionic nature, equilibrates across the plasma membrane according to the membrane potential (32). Upon depolarization, DiBAC_{4}(3) enters the cell and binds to intracellular membranes and proteins, which results in an increase in fluorescence (33). Conversely, hyperpolarization causes extrusion of the dye and decrease in fluorescence (32, 34). These experiments were performed with albumin-free solutions. Cultured islets were washed for 30 min in albumin-free control medium containing 1 mM glucose. This medium was then replaced by a similar solution supplemented with 300 nM DiBAC_{4}(3) added from a 300 μM stock in Me_{2}SO. 30 min later the islets were transferred to the recording chamber used for [Ca^{2+}]_{i} measurements. The experiments started with a 60-min period of perfusion with a medium containing 1 mM glucose, before addition of test substances. DiBAC_{4}(3) was present at 300 nM in all solutions. Islet fluorescence (excitation at 490 nm and emission at 535 nm) was recorded beginning 10 min before addition of the first test substance. The results are expressed as a percentage of the islet fluorescence under basal conditions.

Measurements of Islet cAMP—Cultured islets were distributed in batches and incubated for 60 min in control medium without forskolin (20 islets per batch) or with 1 μM forskolin (7 islets per batch). The glucose concentration was adjusted as required. At the end of the incubation, the islets were quickly washed with ice-cold acetate buffer, containing 0.5 mM isobutylmethylxanthine, before being boiled and sonicated in the same buffer. Islet CAMP content was then determined with a commercially available kit (PerkinElmer Life Sciences), as described previously (35).

Presentation of Results—All experiments have been performed with islets from three to eight different preparations.
RESULTS

Characteristics of the Islets of 2-Week-old Control and Sur1KO Mice—Young Sur1KO mice were heavier (6.41 ± 0.06 g) than controls (5.68 ± 0.09 g, p < 0.001), and their glycemia was higher (5.8 ± 0.1 mm, range 2.6–10.3) than that of controls (5.1 ± 0.1 mm, range 2.2–9.6, p < 0.001). Islets of Sur1KO pups contained ~15% more insulin than those of controls (37.0 ± 1.0 ng/islet, n = 34 versus 30.5 ± 1.0 ng/islet, n = 32, p < 0.001 after culture in 10 mM glucose, and 32.3 ± 1.1 ng/islet, n = 28 versus 28.0 ± 1.3 ng/islet, n = 14, p < 0.05 after culture in 5 mM glucose).

We first ascertained that β-cells from young Sur1KO mice did not contain functional K\textsubscript{ATP} channels. After overnight culture in 10 mM glucose, the islets were perifused with a control medium containing 15 mM glucose. In control islets diazoxide, a K\textsubscript{ATP} channel opener, markedly lowered both [Ca\textsuperscript{2+}]\text{c} and insulin secretion. Subsequent addition of tolbutamide, a K\textsubscript{ATP} channel antagonist, reversed both effects. Neither [Ca\textsuperscript{2+}]\text{c} nor the insulin secretory rate of Sur1KO islets was affected by these drugs, indicating the lack of K\textsubscript{ATP} channels (data not shown).

Glucose-induced [Ca\textsuperscript{2+}]\text{c} and Insulin Secretion Changes in 2-Week-old Control and Sur1KO Islets Cultured in 10 mM Glucose—When islets from 2-week-old control mice were cultured for ~18 h in 10 mM glucose and then tested in the absence of forskolin, their [Ca\textsuperscript{2+}]\text{c} and insulin responses to high glucose were similar to those previously reported for adult mouse islets (22, 29, 31). Raising the glucose concentration from 1 to 15 mM induced a transient decrease in [Ca\textsuperscript{2+}]\text{c}, followed by a large first phase increase, and a second phase characterized by oscillations that are partly masked by averaging (Fig. 1A, thick trace). Glucose-induced insulin secretion was also biphasic, with a large first phase and a flat, sustained second phase (Fig. 1C).

When the experiments were carried out in the presence of 1 μM forskolin to raise islet cAMP levels (35), basal [Ca\textsuperscript{2+}]\text{c} and the glucose-induced first phase [Ca\textsuperscript{2+}]\text{c} rise were unaffected, but steady-state [Ca\textsuperscript{2+}]\text{c} was augmented slightly (282 ± 14 nM versus 248 ± 10 nM, p < 0.05) (Fig. 1D, thick trace). In contrast, insulin secretion was augmented during both first phase (~3-fold) and second phase (~8-fold) (Fig. 1F, note the different scales in panels C and F).
Glucose Effects in Sur1KO Islets

When Sur1KO islets were cultured in 10 mM glucose and then perfused with a medium containing only 1 mM glucose, average \([Ca^{2+}]_c\) was elevated compared with control islets (Fig. 1A, thin trace). This difference was larger when the low glucose medium contained forskolin, because \([Ca^{2+}]_c\) in Sur1KO islets was further elevated (175 ± 5 versus 142 ± 7 nm, \(p < 0.001\)) (Fig. 1D). Upon stimulation with 15 mM glucose, \([Ca^{2+}]_c\) in Sur1KO islets abruptly decreased before increasing following a biphasic time course. The initial peak was similar, but the "first phase" was slightly shorter and the steady-state level was lower than in controls (Fig. 1, A and D). However, steady-state \([Ca^{2+}]_c\) in Sur1KO islets was significantly higher in 15 than 1 mM glucose, both in the absence (\(p < 0.001\)) and presence of forskolin (\(p < 0.01\)). Representative examples of these glucose-induced \([Ca^{2+}]_c\) changes in Sur1KO islets are shown in Fig. 1 (B and E). Distinct \([Ca^{2+}]_c\) oscillations were present in low glucose, and their amplitude and frequency were increased by high glucose, in the absence or presence of forskolin.

In line with the elevation of \([Ca^{2+}]_c\), Sur1KO islets cultured in 10 mM glucose-secreted more insulin (\(p < 0.001\)) than control islets in the presence of 1 mM glucose (Fig. 1C), and this difference was augmented by forskolin (Fig. 1F). Upon stimulation with 15 mM glucose, the insulin secretory rate initially decreased, as did \([Ca^{2+}]_c\), before increasing ~6-fold, albeit more slowly than in controls (Fig. 1, C and F).

Glucose-induced \([Ca^{2+}]_c\), and Insulin Secretion Changes in 2-Week-old Control and Sur1KO Islets Cultured in 5 mM Glucose—We also studied the islets after overnight culture in 5 mM glucose, a concentration closer to the average blood glucose level of the pups. Under these conditions, no insulin secretion could be measured unless the perfusion solutions were supplemented with forskolin. These experiments are presented in Fig. 2. Culturing 2-week-old control islets in the lower glucose concentration affected the acute glucose-induced \([Ca^{2+}]_c\) rise as in adult islets studied in the absence of forskolin (31): the initial dip was deeper, whereas the first and second phase increases were smaller (compare Fig. 2A and Fig. 1D, thick traces). Glucose-induced insulin secretion was biphasic, however, with 2- to 3-fold smaller responses than after culture in G10 (Fig. 2C).

Sur1KO islets were also cultured for ~18 h in 5 mM glucose and studied in the presence of forskolin. When the perfusion medium contained 1 mM glucose, \([Ca^{2+}]_c\) was low, similar to control islets (Fig. 2, A and B). Upon stimulation with 15 mM glucose, \([Ca^{2+}]_c\) dropped, then increased following a biphasic time course that was similar to the controls during the first 6 min. The duration of the first phase increase was, however, shorter, and the average steady-state elevation was smaller (Fig. 2A). This second phase was typically characterized by small rapid and large slower \([Ca^{2+}]_c\) oscillations (Fig. 2B). The insulin secretory rate of Sur1KO islets in 1 mM glucose was low, similar to controls, and glucose stimulation triggered biphasic insulin secretion, with a small, but well defined first phase and a regularly increasing second phase (Fig. 2C). Under these experimental conditions, glucose clearly induced biphasic responses in \(\beta\)-cells lacking \(K_{\text{ATP}}\) channels.

The Amplifying Pathway in 2-Week-old Control and Sur1KO Islets—To test whether the amplifying pathway of glucose stimulation of insulin secretion is functional in young islets, \(K_{\text{ATP}}\)
channels were held open by diazoxide during depolarization with 30 mM KCl (1). When control islets cultured in 10 mM glucose were perfused in the absence of forskolin, depolarization in low glucose raised \([Ca^{2+}]_c\) and stimulated insulin secretion (Fig. 3A and B). Increasing the concentration of glucose to 15 mM induced a transient decrease in \([Ca^{2+}]_c\), followed by a return to steady state (Fig. 3A). Simultaneously the insulin secretory rate was doubled (Fig. 3B). Returning to 1 mM glucose in the presence of 30 mM KCl lowered insulin secretion while \([Ca^{2+}]_c\) increased slightly. This reversible increase in insulin secretion produced by glucose, without a further increase in \([Ca^{2+}]_c\), indicates that the amplifying pathway is functional in islets from 2-week-old control mice. Amplification of insulin secretion by glucose was also observed in Sur1KO islets cultured in 10 mM glucose and perfused in the absence of forskolin (Fig. 3A and B) and in both types of islets cultured in 5 or 10 mM glucose and tested in the presence of forskolin (data not shown).

**Role of cAMP in the \([Ca^{2+}]_c\) and Insulin Secretory Changes Induced by Culture of Sur1KO Islets in Low Glucose**—The concentration of glucose in the culture medium (5 versus 10 mM) had no impact on the cAMP content of Sur1KO islets whether the test incubations were performed in the absence or presence of forskolin (Fig. 4, C and D). This shows that the lower basal and stimulated \([Ca^{2+}]_c\) (Fig. 4, A and B) and insulin secretion (Fig. 4, E and F) after overnight culture in 5 mM glucose cannot be attributed to lower cAMP levels. Comparison of panels C and D in Fig. 4 (note the different scales) shows that 1 \(\mu M\) forskolin markedly augmented cAMP in young Sur1KO islets under all conditions, as previously reported for adult control islets (35), and adult Sur1KO islets (36). High glucose (G15) during the incubation increased islet cAMP levels only in the presence of forskolin (Fig. 4D), which indicates that an increase in cAMP could contribute to the responses of Sur1KO islets to 15 mM glucose in the presence of forskolin. However, all other comparisons show that the effects of glucose on \([Ca^{2+}]_c\) or insulin secretion are not attributable to changes in cAMP. This conclusion is further supported by a series of experiments using Sur1KO islets cultured in 5 mM glucose and perfused with a medium containing 1 mM glucose and no forskolin, as in Fig. 4A. Acute addition of either 1 \(\mu M\) forskolin, 0.5 mM 8-bromo-cAMP or 25 mM exendin-4 did not evoke any increase in \([Ca^{2+}]_c\), whereas the subsequent application of 15 mM glucose was consistently effective (data not shown).

**Mechanism of Glucose-induced \([Ca^{2+}]_c\) Rise in Sur1KO Islets**—In this series of experiments we investigated the possible mechanisms underlying the biphasic increase in \([Ca^{2+}]_c\) induced by 15 mM glucose in \(\beta\)-cells lacking K\(_{ATP}\) channels (Fig. 5A). Non-metabolized (37) 3-O-methylglucose did not mimic the effect of glucose (not shown). Diazoxide had no effect on basal \([Ca^{2+}]_c\), or on the glucose-induced \([Ca^{2+}]_c\), rise, and tolbutamide, even at high concentration, was similarly ineffective (Fig. 5B and C), confirming that the phenomenon is K\(_{ATP}\) channel-independent. Pretreatment of the islets with thapsigargin, to block the endoplasmic reticulum Ca\(^{2+}\) ATPase and prevent Ca\(^{2+}\) release, suppressed the initial decrease in \([Ca^{2+}]_c\) produced by high glucose but did not impair the subsequent \([Ca^{2+}]_c\) rise (Fig. 5D). Pretreatment of the islets with 100 \(\mu M\) ryanodine to prevent Ca\(^{2+}\)-induced Ca\(^{2+}\) release (38) had no effect (not shown). In contrast, partial and complete chelation of extracellular Ca\(^{2+}\) by addition of 2 or 3 mM EGTA, suppressed the glucose-induced \([Ca^{2+}]_c\) rise (Fig. 5E). Therefore, we conclude that this rise is not secondary to Ca\(^{2+}\) mobilization from intracellular stores but reflects influx of extracellular Ca\(^{2+}\).

**Omission of extracellular Na\(^{+}\)** transiently increased basal \([Ca^{2+}]_c\), in low glucose and augmented the increase produced by 15 mM glucose (Fig. 5F). We attribute both changes to inhibition/reversal of the Na\(^{+}/Ca^{2+}\) exchanger (39, 40). When the islets were incubated (2 h of loading) and perfused in a Cl\(^{-}\)-free medium to lower intracellular Cl\(^{-}\) (41), occasional transient increases in \([Ca^{2+}]_c\) occurred in 1 mM glucose, but the effect of 15 mM glucose was not affected (Fig. 5G). Blockade of the electronegative sodium pump with ouabain is known to depolarize...
normal β-cells (42). Ouabain (200 μM) markedly increased 
Ca$^{2+}$ in Sur1KO islets perfused with 1 mM glucose but did not
prevent high glucose from increasing it further, after an
initial drop (Fig. 5H). From these three series of experiments we
conclude that the rapid increase in [Ca$^{2+}$]$_{c}$ produced by glucose
does not result from a depolarization mediated by Na$^{+}$ influx,
Cl$^{-}$ efflux, or sodium pump inhibition.

At the high concentration of 20 mM, but not at 10 mM, tetra-
ethylammonium, a blocker of voltage-dependent and other
potassium channels (43), occasionally induced minor [Ca$^{2+}$] peaks in
1 mM glucose, marginally increased the initial peak, and augmented the
steady-state [Ca$^{2+}$]$_{c}$ rise produced by 15 mM glucose (Fig. 5I). Apanin
(100 nm), and compound UCL 1684 (100 and 500 nm), two blockers of
small conductance Ca$^{2+}$-activated potassium channels (43, 44), were
without effect on basal or the glucose-induced [Ca$^{2+}$]$_{c}$ peak (not shown). In contrast, BaCl$_{2}$, a non-
selective potassium channel blocker (43), rapidly increased [Ca$^{2+}$]$_{c}$ in
1 mM glucose, marginally increased the initial peak, and augmented the
steady-state [Ca$^{2+}$]$_{c}$ rise produced by 15 mM glucose (Fig. 5I).

Altogether these results indicate that the biphasic [Ca$^{2+}$]$_{c}$ rise produced by 15 mM glucose in islets
lacking KATP channels results from membrane depolarization. Because
nimodipine partially (at 0.1 mM) or completely (at 1 mM) prevented the
response to glucose (Fig. 5L), we conclude that this Ca$^{2+}$ influx occurs through voltage-dependent
Ca$^{2+}$ channels.

Glucose-induced Changes in Membrane Potential in Control and
Sur1KO Islets—Islets from the same
preparations were used for measurement of [Ca$^{2+}$]$_{c}$ and mem-
brane potential changes after overnight culture in 5 mM glucose.
As shown in Fig. 6A (thick trace), stimulation with 15 mM
glucose induced a biphasic [Ca$^{2+}$]$_{c}$ rise in control islets. This
rise was abrogated by diazoxide, but subsequent application of
KCl markedly increased [Ca$^{2+}$]$_{c}$. The effect of 15 mM glucose was also biphasic in Sur1KO islets, but its amplitude was
smaller and it was not counteracted by diazoxide. The changes
in membrane potential were measured in parallel experiments
using the fluorescent probe DiBAC$_4$(3) and are shown in Fig. 6B. The increase in fluorescence produced by 15 mM glucose in control islets reflects depolarization, whereas the decrease and eventual increase produced by the successive addition of diazoxide and KCl indicate repolarization and strong depolarization. This fluorescent technique, therefore, reliably detects the changes in membrane potential known to occur in normal β-cells (5–7). In Sur1KO islets, high glucose also caused depolarization (Fig. 6B). The amplitude was smaller than in control islets, but diazoxide did not reverse it. These results, therefore,
Glucose Effects in Sur1KO Islets

A

![Graph A](image)

B

![Graph B](image)

FIGURE 6. Effects of glucose, diazoxide, and KCl on [Ca^{2+}]_c, and membrane potential in control and Sur1KO islets after culture in 5 mM glucose. A, [Ca^{2+}]_c, was measured in islets loaded with fura-PE3-AM. B, the islets were loaded and perfused with the membrane potential probe DiBAC(3) as described under "Experimental Procedures." An increase in fluorescence reflects depolarization; a decrease reflects hyperpolarization. Results are expressed as a percentage of the fluorescence of each islet in 1 mM glucose. Results are means ± S.E. for 24 islets from 4 different preparations.

support the conclusion that glucose causes a small depolarization in Sur1KO islets that have been cultured overnight in 5 mM glucose. Ouabain and BaCl_2 also depolarized Sur1KO islets and induced insulin secretion in the presence of 1 mM glucose (data not shown).

DISCUSSION

We show that islets from 2-week-old normal mice are functionally mature in terms of glucose-induced [Ca^{2+}]_c rise and insulin secretion. When compared with the adult control islets used in our previous studies (22, 29, 31), no obvious differences in the magnitude or kinetics (triphasic changes and oscillations in the steady state) of [Ca^{2+}]_c changes were detected. Glucose-induced insulin secretion was typically biphasic. Although, these young control islets were smaller than adult islets (~4 times less insulin than in islets from 1-year-old mice) (22), their fractional rate of insulin secretion in response to 15 mM glucose was ~2-fold higher during either first or second phase, in the absence or presence of forskolin. Using a high concentration of KCl in the presence of diazoxide, a classic technique for clamping [Ca^{2+}]_c at elevated levels (1), we also show that glucose can increase insulin secretion without further raising [Ca^{2+}]_c. This establishes that the amplifying pathway, originally identified in adult rodent islets (9, 10), is operative in normal 2-week-old β-cells.

After overnight culture in 10 mM glucose, the behavior of 2-week-old Sur1KO islets was similar, but not identical, to adult Sur1KO islets. As in adult islets (17, 21, 22), [Ca^{2+}]_c in 1 mM glucose was higher than in controls. This is attributed to continuous stimulation of Ca^{2+} influx in depolarized β-cells without K_{ATP} channels and can explain the high basal rate of insulin secretion. Stimulation of 2-week-old Sur1KO islets with 15 mM glucose produced a transient drop of [Ca^{2+}]_c, followed by an overshoot and a sustained elevation beyond initial levels. The stimulation by high glucose was accompanied by a ~6-fold increase in insulin secretion showing young islets without K_{ATP} channels are highly responsive to glucose. The insulin content of 2-week-old Sur1KO islets was ~3-fold less than adult islets (22), and their fractional rate of insulin secretion was lower in the presence of 1 mM glucose, but similar to adult islets during stimulation with 15 mM glucose. The present results establish that our previous observations on islets from 1 year-old Sur1KO mice (22), where glucose increased insulin secretion, were not due to a progressive adaptation during aging. It is unclear why some (16, 17), but not all (23, 24), other studies using younger adult mice fail to observe a similar stimulation.

Because glucose increased [Ca^{2+}]_c in young Sur1KO islets, the increase in insulin secretion can be attributed partially to a larger triggering signal. However, when 2-week-old Sur1KO islets were depolarized with KCl, glucose increased insulin secretion without raising [Ca^{2+}]_c, further, demonstrating that the amplifying pathway is operative in these young β-cells without K_{ATP} channels, as it is in adult Sur1KO islets (22) and in young normal islets (this study). Nutrients and cAMP can interact to increase insulin secretion through an amplifying pathway (9, 11, 45). However, the increase in insulin secretion produced by glucose in Sur1KO islets was neither dependent on the presence of forskolin nor consistently associated with a rise in cAMP. On the basis of studies with incubated rat islets, it has been suggested that the combination of high glucose and cAMP could trigger insulin secretion by a mechanism that is independent of K_{ATP} channels (resistant to diazoxide) and of changes in membrane potential and Ca^{2+} influx or mobilization (resistant to nimodipine and thapsigargin) (46). The phenomenon that we report here is completely different. First, the combination of glucose and forskolin does not increase insulin secretion by control mouse islets in the presence of diazoxide (22). Second, all effects of glucose on insulin secretion by adult or 2-week-old Sur1KO islets are abrogated either by extracellular Ca^{2+} omission or inhibition of Ca^{2+} channels. Our results...
do not suggest the existence of a triggering signal other than cytosolic Ca\(^{2+}\).

In normal \(\beta\)-cells, glucose controls the triggering of \([Ca^{2+}]_c\) signal through its action on K\(_{ATP}\) channels. It is thus surprising that high glucose can transiently raise \([Ca^{2+}]_c\) beyond the already elevated basal level in adult Sur1KO islets (22) and that this effect is of even greater magnitude and duration in 2-week-old Sur1KO islets. Glucose also influenced the pattern of \([Ca^{2+}]_c\) changes in Sur1KO islets. Despite the absence of K\(_{ATP}\) channels, adult (17, 22) and 2-week-old (this study) Sur1KO islets display \([Ca^{2+}]_c\) oscillations that reflect influx of Ca\(^{2+}\) during cyclic depolarizations of \(\beta\)-cells. Thus, microelectrode recordings have shown that oscillations of membrane potential occur in adult Sur1KO \(\beta\)-cells (47). Their frequency increases with the glucose concentration as does the frequency of the \([Ca^{2+}]_c\) oscillations that we observe in Sur1KO islets. This implies that a metabolic sensor other than K\(_{ATP}\) channels can influence the time course of the triggering signal.

One major and totally unanticipated finding of the present study is that glucose also exerts a long term influence on \(\beta\)-cells lacking K\(_{ATP}\) channels, which steadily lowers basal \([Ca^{2+}]_c\), in 1 mM glucose and unmask a depolarization-mediated biphasic rise of \([Ca^{2+}]_c\) and insulin secretion by high glucose. The results presented in Figs. 5 and 6 show that, after culture of 2-week-old Sur1KO islets in 5 mM glucose, high glucose can cause depolarization of islet cells and stimulate Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels, thereby triggering biphasic insulin secretion. This intriguing observation raises the questions of how overnight exposure to 5 mM glucose can cause \(\beta\)-cell repolarization and how subsequent acute exposure to 15 mM glucose can cause depolarization in the absence of K\(_{ATP}\) channels. We did not find evidence that either phenomenon is dependent on Na\(^+\) or Cl\(^-\) fluxes or sodium pump activity. Small conductance Ca\(^{2+}\)-activated potassium channels (48) underlying I\(_{K_{\text{slow}}}\) in normal \(\beta\)-cells (49) are sensitive to metabolism, namely the current decreases when the glucose concentration increases (50, 51). An activation of I\(_{K_{\text{slow}}}\) theoretically could underlie repolarization of 2-week-old Sur1KO \(\beta\)-cells with eventual lowering of \([Ca^{2+}]_c\), as observed after culture of the islets in 5 instead of 10 mM glucose. Conversely, an inhibition of I\(_{K_{\text{slow}}}\) by glucose could explain the acute depolarization by high glucose. However, UCL 1684, an inhibitor of I\(_{K_{\text{slow}}}\) in adult normal \(\beta\)-cells, was ineffective in our model. In contrast, BaCl\(_2\), a non-specific potassium channel inhibitor (43), depolarized the islets and increased \([Ca^{2+}]_c\) and insulin secretion in 1 mM glucose. Another plausible explanation would be that glucose metabolism directly modulates voltage-dependent Ca\(^{2+}\) channels, as reported by two studies using normal mouse or rat \(\beta\)-cells (52, 53). Inhibiting ATP production by sodium azide decreased the amplitude of Ca\(^{2+}\) spikes in adult Sur1KO \(\beta\)-cells (47), but the interpretation of this observation is complicated by other effects of azide, including release of Ca\(^{2+}\) from intracellular stores (22). A cAMP-mediated modulation of Ca\(^{2+}\) channels is also unlikely, because the effect of high glucose was not consistently accompanied by an increase in cAMP and was not mimicked by acute or sustained elevations of cAMP. Taken together, our results indicate that metabolism can regulate Ca\(^{2+}\) influx by acting on a target other than K\(_{ATP}\) channels in Sur1KO \(\beta\)-cells.

The fact that glucose can promote Ca\(^{2+}\) influx in \(\beta\)-cells and produce the triggering signal for insulin secretion in Sur1KO \(\beta\)-cells must be put into perspective. These results do not detract from the preeminence of the K\(_{ATP}\) channel-dependent pathway by which glucose and other nutrients trigger insulin secretion in normal \(\beta\)-cells and need not imply that a similar mechanism exists in normal \(\beta\)-cells. Adaptations to the lack of K\(_{ATP}\) channels are possible even at a young age. If a similar mechanism does exist in the presence of functional K\(_{ATP}\) channels its role needs to be established. On the other hand, its contribution could become important when K\(_{ATP}\) channels are defective, for instance in certain forms of persistent hyperinsulinism. Children with a diffuse form of congenital hyperinsulinism due to mutations of Sur1 (ABCC8) are insensitive to diazoxide or tolbutamide, as expected, but increase their plasma insulin levels in response to intravenous glucose (54, 55). The amplifying pathway, operative in \(\beta\)-cells of these patients (56), may partially explain this response. However, it is striking that glucose evoked an unexpected, acute insulin response, resembling a first phase (54). We suggest the underlying mechanism might be the same as that involved in our 2-week-old Sur1KO islets.

Sur1KO mice were originally produced as a model of human congenital hyperinsulinism with hypoglycemia. Unlike affected children, except for a few hours after birth (16), the mice are not hypoglycemic. The average plasma glucose was 5.7 mM in our 2-week-old pups, and we speculate that living with a blood glucose concentration close to 5 mM leads to down-regulation of the insulin-secretory capacity of Sur1KO \(\beta\)-cells as does culture of Sur1KO islets in 5 versus 10 mM glucose.

In conclusion, our study of 2-week-old Sur1KO islets demonstrates that glucose can achieve acute and long term regulation of insulin secretion from \(\beta\)-cells lacking K\(_{ATP}\) channels, not only via the amplifying pathway but also through a K\(_{ATP}\) channel-independent control of the triggering Ca\(^{2+}\) signal. The contribution of this novel mechanism to insulin secretion by normal subjects and patients with congenital hyperinsulinism needs to be established to assess whether it represents a potentially interesting therapeutic target.

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