Overnight Culture Unmasks Glucose-induced Insulin Secretion in Mouse Islets Lacking ATP-sensitive K⁺ Channels by Improving the Triggering Ca²⁺ Signal

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A current model ascribes glucose-induced insulin secretion to the interaction of a triggering pathway (KATP channel-dependent Ca²⁺ influx and rise in cytosolic [Ca²⁺]), and an amplifying pathway (KATP channel-independent augmentation of secretion without further increase of [Ca²⁺]). However, several studies of sulfonylurea receptor 1 null mice (Sur1KO) failed to measure significant effects of glucose in their islets lacking KATP channels. We addressed this issue that challenges the model. Compared with controls, fresh Sur1KO islets showed slightly elevated basal [Ca²⁺] and insulin secretion. In 15 mM glucose, the absolute rate of secretion was ~3-fold lower in Sur1KO than control islets, with only poor increase above base line. Overnight culture of Sur1KO islets in 10 mM glucose (not in 5 mM) augmented basal insulin secretion and considerably improved the response to 15 mM glucose, which reached higher values than in control islets, in which culture had little impact. Glucose stimulation during KCl depolarization showed that the amplifying pathway is functional in fresh and cultured Sur1KO islets. The differences in insulin secretion between fresh and cultured Sur1KO islets and between Sur1KO and control islets were not attributable to differences in insulin content, glucose oxidation rate, or synchronization of [Ca²⁺] oscillations. The unmasking of glucose-induced insulin secretion in β-cells lacking KATP channels is paradoxically due to improvement in the production of a triggering signal (elevated [Ca²⁺]). The results show that KATP channels are not the only transducer of glucose effects on [Ca²⁺] in β-cells. They explain controversies in the literature and refute arguments raised against the model implicating an amplifying pathway in glucose-induced insulin secretion.

A current model of the pancreatic β-cell ascribes the control of insulin secretion by glucose and other nutrients to the interaction of two signaling pathways set in operation by an acceleration of metabolism (1–3). The triggering pathway depends on ATP-sensitive K⁺ channels (KATP channels)² whereas the amplifying pathway is independent of these channels. In the triggering pathway, closure of KATP channels leads to membrane depolarization, Ca²⁺ influx through voltage-dependent Ca²⁺ channels, and a rise in the cytosolic Ca²⁺ concentration ([Ca²⁺]), which triggers exocytosis of insulin granules (4–9). The amplifying pathway requires that the triggering signal is produced but augments the secretory response without increasing [Ca²⁺], further (1, 10–13).

This model of a dual regulation of insulin secretion has been developed on the basis of experiments using pharmacological tools to prevent metabolized secretagogues from acting on KATP channels (10, 14, 15) by either closing them completely with sulfonylureas (to produce a rise in [Ca²⁺]), or holding them open with diazoxide during depolarization with KCl (to produce the triggering signal). The generation of mice lacking KATP channels in their β-cells because of a knock-out of the regulatory subunit, the high affinity sulfonylurea receptor (SUR1-ABCC8), provided alternative means to assess the validity of the model (16, 17). Several studies from distinct laboratories initially reported that Sur1KO β-cells were depolarized and displayed elevated [Ca²⁺], in low glucose, as expected, but that basal insulin secretion was not increased, and high glucose virtually failed to stimulate insulin secretion (16–20). In contrast to these unanticipated negative results, we have observed elevation of basal insulin secretion and strong secretory response to glucose alone in perifused islets from either adult or two-week-old Sur1KO mice (21, 22). Glucose-induced insulin secretion by incubated Sur1KO islets has also been observed in two other laboratories (23, 24).

These puzzling discrepancies have not been accounted for and continue to cast doubt on the validity of the model explaining the control of insulin secretion by triggering and amplifying pathways (25). In our attempts to identify the possible causes of disagreement, we noticed several differences in experimental approaches and were struck by the fact that glucose was reported to be ineffective in the perifused pancreas and perifused freshly isolated islets (16–20). The use of preparations submitted to sometimes markedly different ex vivo treatments before comparing insulin secretion and [Ca²⁺], is another confounding parameter. In the present study, we sought to identify the possible reasons for these discrepancies and discovered

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²The abbreviations used are: KATP channel, ATP-sensitive potassium channel; SUR1, sulfonylurea receptor 1; [Ca²⁺], cytosolic Ca²⁺ concentration; KO, knock-out.
that, although freshly isolated islets are indeed poorly responsive to glucose, overnight culture unmasks large effects of the sugar. Unexpectedly, however, these effects on insulin secretion do not involve improvements in glucose metabolism or the amplifying pathway but improvements in the production of the triggering Ca^{2+} signal, despite the lack of K_{ATP} channels.

**EXPERIMENTAL PROCEDURES**

The study was approved by, and the experiments were conducted in accordance with, the guidelines of the Animal Research Committee of our institution.

*Solutions and Reagents*—The control medium used for islet isolation was a bicarbonate-buffered solution containing (in mM) 120 NaCl, 4.8 KCl, 2.5 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, and 24 NaHCO\textsubscript{3}. The medium was gassed with O\textsubscript{2}/CO\textsubscript{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 30 mM were compensated for by decreases in NaCl. Unless indicated otherwise, reagents were from Sigma or Merck AG (Darmstadt, Germany). Diazoxide (a gift from Schering-Plough, Brussels, Belgium) was added from freshly prepared stock solutions in 0.1 N NaOH.

*Animals*—Sur\textsubscript{1}KO mice, generated (16) and provided by J. Bryan (Houston, TX), were maintained in our animal facilities. Controls were C57Bl/6 mice originally obtained from Charles River Laboratories, Brussels, Belgium. All experiments were performed with female mice of a similar age (50 ± 1 week). The Sur\textsubscript{1}KO mice used in this study were slightly heavier than the controls (31.5 ± 0.6 g, n = 51 versus 27.8 ± 0.8 g, n = 25), but their morning blood glucose was similar (6.78 ± 0.23 mM versus 6.75 ± 0.20 mM).

*Preparation of Islets*—Islets from Sur\textsubscript{1}KO and control mice were aseptically isolated by collagenase digestion of the pancreas followed by hand selection (26). The islets were then either studied fresh (i.e. after 2 h of preincubation at 37 °C in control medium containing 10 mM glucose) or cultured for ~18 h in RPMI 1640 medium (Invitrogen) kept at 37 °C in a 95% air:5% CO\textsubscript{2} atmosphere. The culture medium contained 5 or 10 mM glucose, 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. Islets from the same preparations (same mice) were used to compare the insulin secretory responses and [Ca^{2+}]\textsubscript{i} formed with islets from four to nine different preparations.

*Measurements of Insulin Secretion and Islet Insulin Content*—Fresh and cultured islets were distributed in batches of 20–40 before being transferred into perfusion chambers (21). The islets were then perfused (flow rate of 1 ml/min) at 37 °C with test solutions, the composition of which is indicated at the top of each figure. 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 (27). At the end of the experiments, the islets were recovered and their insulin content was determined after extraction in acid-ethanol. The fractional insulin secretion rate was then calculated as the percentage of islet insulin content that was secreted per minute.

*Measurements of Islet [Ca^{2+}]\textsubscript{i}*—Fresh and cultured islets were loaded with the Ca\textsuperscript{2+} indicator Fura-PE3/AM (2 µM) (Teflabs, Austin, TX) for 2 h in control medium containing 10 mM glucose or 5 mM glucose for the islets cultured in 5 mM glucose. The loaded islets were placed into the perfusion chamber of a spectrofluorimetric system equipped with a camera and with which [Ca\textsuperscript{2+}]\textsubscript{i} was measured at 37 °C as described previously (28).

*Measurements of Glucose Oxidation*—Fresh and cultured islets were distributed in batches of 10 and incubated for 2 h at 37 °C in 50 µl of control medium containing 1 or 15 mM glucose supplemented with 1 µCi of [U-\textsuperscript{14}C]glucose (Amersham Biosciences). Oxidation of glucose was calculated from the production of [\textsuperscript{14}C]CO\textsubscript{2}. Technical aspects of the method have been described previously (29).

*Presentation of Results*—All experiments have been performed with islets from four to nine different preparations. Results are presented as means ± S.E. The statistical significance of differences between the means for Sur\textsubscript{1}KO and control islets was assessed by unpaired Student’s t test.

**RESULTS**

Glucose-induced Insulin Secretion in Sur\textsubscript{1}KO Islets Is Concentration-dependent—Control and Sur\textsubscript{1}KO islets were cultured for ~18 h in 10 mM glucose before being perfused with a medium containing increasing concentrations of glucose (Fig. 1). In control islets, basal insulin secretion (in 1 mM glucose) was low and not augmented by 5 mM glucose. Stimulation with 10 mM glucose induced biphasic insulin secretion, and further increases were produced by 15 and 20 mM glucose. In Sur\textsubscript{1}KO islets, basal insulin secretion was high and, although it

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**FIGURE 1.** Concentration dependence of glucose-induced insulin secretion in control (open circles) and Sur1KO islets (filled circles) after culture in 10 mM glucose. The concentration of glucose (G in mM) was increased stepwise as indicated, except for one group of Sur1KO islets perfused with 10 mM glucose throughout (G1, small filled circles). Values are means ± S.E. for six experiments with different islet preparations, except in the group of stimulated Sur1KO islets, where n = 9.
declined with time, remained much higher than in controls after 2 h of perifusion with only 1 mM glucose. Stimulation with 5 mM glucose produced an initial decrease in insulin secretion followed by a sustained elevation above the base line (Fig. 1). The secretory rate markedly augmented in response to 10 mM glucose and, in 6 of 9 experiments, a further small increase was produced by 15 mM glucose to reach a maximum of 4–5-fold base-line secretion (Fig. 1). Experiments with longer periods of stimulation also showed that the maximum effect of glucose was reached between 10 and 15 mM in \( \text{Sur1} \) KO islets (data not shown). These results confirm that glucose induces insulin secretion in cultured adult \( \text{Sur1} \) KO islets (21) and establish that this effect is concentration-dependent with a shift to the left of the glucose-response curve as compared with control islets.

Influence of Culture on Glucose-induced Insulin Secretion in \( \text{Sur1} \) KO Islets—Overnight culture had a profound impact on the insulin secretory capacity of \( \text{Sur1} \) KO islets (Fig. 2A). After culture in the presence of 10 mM glucose, abrupt stimulation with 15 mM glucose evoked a large and reversible increase in insulin secretion above the already elevated base line in 1 mM glucose (Fig. 2A). When \( \text{Sur1} \) KO islets were studied fresh, their basal insulin secretory rate during perifusion with 1 mM glucose was \(~\)4-fold lower than in islets from the same preparations cultured in 10 mM glucose (Fig. 2A) but still much higher than in fresh control islets (0.012 \pm 0.001 versus 0.001 \pm 0.0002%/min). This elevation was sustained for \( \sim\)2 h of perifusion with 1 mM glucose (Fig. 2C) and could be corrected by chelation of extracellular \( \text{CaCl}_2 \) with EGTA (data not shown).
Glucose Stimulation of Sur1KO Islets

FIGURE 3. Characteristics and synchrony of glucose-induced [Ca²⁺]ᵢ oscillations in fresh and cultured Sur1KO islets. Shown are three representative islets from the same preparation studied fresh (A) or after ~18 h of culture in 5 mM glucose (C) or 10 mM glucose (D). The concentration of glucose in the perfusion medium was changed between 1 and 15 mM as indicated. B shows the synchrony of [Ca²⁺]ᵢ oscillations occurring in the four non-contiguous regions of another fresh Sur1KO islet, as schematized in the inset. This analysis is representative of similar one done in 12 islets from 5 preparations.

Stimulation of fresh Sur1KO islets with 15 mM glucose consistently increased insulin secretion, but this effect was of relatively small amplitude (initial doubling) and not well sustained (Fig. 2C). On cessation of the stimulation, a paradoxical increase in insulin secretion consistently occurred.

To test whether the unmasking of a large insulin secretory response after overnight culture required specific culture conditions, Sur1KO islets were cultured in 5 mM instead of 10 mM glucose. This resulted in a ~50% decrease in “basal” insulin secretion compared with fresh islets and unmasked a biphasic increase in secretion in response to 15 mM glucose (Fig. 2C). Although the absolute rates of insulin secretion were markedly different, high glucose induced a similar 3.0–3.5-fold increase in insulin secretion in Sur1KO islets after culture in 5 and 10 mM glucose, which contrasts with the ~50% increase in fresh islets. The differences in insulin secretion by the three groups of islets cannot be attributed to differences in insulin content (Fig. 2A, inset).

Influence of Culture on Glucose-induced [Ca²⁺]ᵢ, Changes in Sur1KO Islets—Fig. 2, B and D, and Fig. 3 compare [Ca²⁺]ᵢ in fresh and cultured Sur1KO islets. On average, basal [Ca²⁺]ᵢ was highest after culture in 10 mM glucose, lowest after culture in 5 mM glucose, and intermediate in fresh islets (Fig. 2B), which can explain the differences in basal insulin secretion (Fig. 2A). This elevation of [Ca²⁺]ᵢ in Sur1KO islets perfused with 1 mM glucose was due to the occurrence of oscillations that were regular or rare in fresh islets (Fig. 3, A and B), infrequent after culture in 5 mM glucose (Fig. 3C), and frequent after culture in 10 mM glucose (Fig. 3D). Upon stimulation with 15 mM glucose, average [Ca²⁺]ᵢ in Sur1KO islets initially decreased before increasing rapidly with an overshoot above prestimulatory values (Fig. 2B). However, as shown in Fig. 2D (blow-up of Fig. 2B), this overshoot was transient. Steady-state (35–50 min) [Ca²⁺]ᵢ, was not stimulated by high glucose in fresh islets (111 ± 10 nM versus 91 ± 8 nM, p = 0.06). There was also no significant difference between average steady-state [Ca²⁺]ᵢ in Sur1KO islets perfused with low or high glucose after culture (not shown).

The major acute effect of glucose on [Ca²⁺]ᵢ in Sur1KO islets was not quantitative but qualitative (Fig. 3). Compared with 1 mM glucose, 15 mM glucose increased the frequency and decreased the duration of [Ca²⁺]ᵢ oscillations in Sur1KO islets. This effect was consistent in either fresh or cultured islets. However, in fresh Sur1KO islets, high glucose did not cause any long elevation of [Ca²⁺]ᵢ after the initial drop (Fig. 3A), even when the stimulation was preceded by several minutes of low and stable [Ca²⁺]ᵢ (Fig. 3B). This contrasts with the biphasic [Ca²⁺]ᵢ response elicited in cultured Sur1KO islets, in which one long elevation of [Ca²⁺]ᵢ followed the initial drop (Fig. 3, C and D). Characteristically, this first phase [Ca²⁺]ᵢ increase was of low amplitude in Sur1KO islets cultured in 5 mM glucose (Fig. 3C). The oscillations of [Ca²⁺]ᵢ, observed in low and high glucose were well synchronized throughout Sur1KO islets, either fresh (Fig. 3B) or cultured (not shown).

Influence of Culture on Insulin Secretion and [Ca²⁺]ᵢ in Control Islets—To determine whether the culture-induced changes described above are peculiar to Sur1KO islets or nonspecific, we compared similarly treated control islets (Fig. 4). When these islets were studied under our standard conditions (~18 h of culture in 10 mM glucose), they displayed their usual biphasic [Ca²⁺]ᵢ and insulin secretory responses to glucose (21, 28).

In fresh control islets, high glucose also caused an initial drop in [Ca²⁺]ᵢ, followed by a first phase increase and oscillations during steady-state stimulation (Fig. 4C). All of these changes were well synchronized throughout the islet (data not shown). Basal [Ca²⁺]ᵢ was marginally higher in fresh islets than in islets cultured in 10 mM glucose (76 ± 1 versus 57 ± 1 nM, p < 0.001), but the increase produced by 15 mM glucose was of a smaller amplitude (Fig. 4B). Insulin secretion was characterized by
smaller first and second phases with disappearance of the difference after ~30 min of stimulation (Fig. 4A).

After culture in a lower glucose concentration (5 instead of 10 mM), the acute changes in [Ca^{2+}] were altered as previously reported (28). The first phase rise was decreased in duration and amplitude, and the second phase of [Ca^{2+}] rise was smaller (Fig. 4B). Insulin secretion was biphasic with much smaller first and second phases than in islets cultured in 10 mM glucose (Fig. 4A). The differences in insulin secretion by the three groups of islets are not attributable to differences in islet insulin content, which, when compared with fresh islets, was not modified after culture in 5 mM glucose and decreased by ~25% after culture in 10 mM glucose (Fig. 4A, inset).

In each of the three groups of control islets, basal [Ca^{2+}] was lower than in the corresponding group of Sur1KO islets, whereas [Ca^{2+}] was higher during steady-state stimulation with 15 mM glucose (compare Figs. 4B and 2B). For example, stimulated [Ca^{2+}] in fresh control islets (Fig. 4B, thick line) was similar to stimulated [Ca^{2+}] in Sur1KO islets after culture in 10 mM glucose (Fig. 2B, medium line) (200 ± 12 nM versus 190 ± 14 nM).

The Amplifying Pathway in Fresh Sur1KO and Control Islets—We have previously reported that the amplifying pathway is operative in Sur1KO islets cultured in 10 mM glucose (21, 22), but malfunction of the pathway could contribute to the poor effect of glucose on insulin secretion in fresh Sur1KO islets. To test this hypothesis, Sur1KO islets, either fresh or cultured in 10 mM glucose, were depolarized with 30 mM KCl in the presence of 100 μM diazoxide, which caused marked increases in [Ca^{2+}], and insulin secretion (Fig. 5, A and B). Raising the concentration of glucose from 1 to 15 mM induced a transient decrease in [Ca^{2+}], followed by a return to the steady state (Fig. 5B). Simultaneously, the insulin secretory rate was amplified 2.5–3-fold. Both [Ca^{2+}] and insulin secretory changes were essentially parallel in fresh islets and after culture (Fig. 5, A and B).

In a second protocol, the experiments were started in 1 or 15 mM glucose before depolarization with 30 mM KCl (Fig. 5, C–F). In fresh Sur1KO islets, KCl-induced insulin secretion was ~6.5-fold larger in 15 than 1 mM glucose (Fig. 5C), although the increase in [Ca^{2+}] was only marginally larger (Fig. 5D). In Sur1KO islets cultured in 10 mM glucose, the small difference in [Ca^{2+}] persisted, and the amplification of insulin secretion by high glucose was again large (~3-fold) (data not shown). In control islets, the initial [Ca^{2+}] and insulin secretory rates were similar in 1 and 15 mM glucose, because K_{ATP} channels were held open by the presence of diazoxide. KCl induced a virtually identical rise in [Ca^{2+}] at the two glucose concentrations but produced a 3-fold larger increase in insulin secretion in 15 mM glucose (Fig. 5, E and F). When similar experiments

10 mM glucose (Cult G5 and Cult G10). The concentration of glucose in the perfusion medium was changed between 1 and 15 mM as indicated. The inset in A shows the insulin content of the three groups of islets at min 0 of the experiments. * denotes a significant difference (p < 0.05) versus fresh islets. C shows [Ca^{2+}] changes in a representative fresh control islet. Values are means ± S.E. for 9 experiments of insulin secretion and 30 islets from 5 preparations for [Ca^{2+}].
were done with control islets cultured in 10 mM glucose, the amplifying action of glucose on insulin secretion was ~2-fold (data not shown).

Taken together, these experiments indicate that the amplifying pathway of insulin secretion functions well in fresh 
*Sur1* KO islets as well as in cultured 
*Sur1* KO islets and in fresh or cultured control islets. It is also noteworthy that KCl raised [Ca^{2+}]_c to lower levels in 
*Sur1* KO than control islets under all tested conditions.

**Influence of Culture on Glucose Oxidation by Control and 
*Sur1* KO Islets**—As shown in Table 1, glucose oxidation by incubated islets was much faster in the presence of 15
than 1 mM glucose. This increase amounted to ~20-fold in control islets and ~15-fold in 
*Sur1* KO islets, a difference largely attributable to a higher basal oxidation rate in 
*Sur1* KO islets. Culture only had a small impact on glucose oxidation. Compared with fresh islets, oxidation was increased in control-cultured islets. In 
*Sur1* KO islets, oxidation was decreased after culture in 5
mM glucose and increased, at least in high glucose, after culture in 10
mM glucose (Table 1). However, this increase (by 13%) is not commensurate with the huge changes in glucose-induced insulin secretion. Overall, these results indicate that changes other than glucose metabolism underlie the influence of culture on the secretory function of control and 
*Sur1* KO islets.

**DISCUSSION**

Our study shows that overnight culture of 
*Sur1* KO islets unmasks the ability of glucose to strongly stimulate insulin secretion in 
*β*-cells that lack K<sub>ATP</sub> channels because of a knock-out of the regulatory subunit
SUR1. Unexpectedly, this unmasking involved improvement of the triggering Ca^{2+} signal. By explaining most controversies on the behavior of 
*Sur1* KO 
*β*-cells, our results refute arguments raised against the physiological relevance of a K<sub>ATP</sub> channel-independent control of insulin secretion and provide further support for the model implicating a dual regulation of 
*β*-cell secretory function by triggering and amplifying pathways.

**Controversies in the Literature**—In previous studies of 
*Sur1* KO 
*β*-cells, insulin secretion and [Ca^{2+}]_c have only rarely (21, 22) been studied under reliably comparable conditions (distinct preparations, such as intact islets or single cells, were studied fresh or after culture). This inconsistency is a major confounding factor that may contribute to paradoxical dissociations between the two parameters. Indeed, we have shown that

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**FIGURE 5.** Influence of glucose on insulin secretion (**A**, **C**, and **E**) and islet [Ca^{2+}]_c (**B**, **D**, and **F**) in the presence of a high concentration of KCl. Diazoxide (100 µM) was present in all solutions. **A** and **B**, 
*Sur1* KO islets studied fresh (filled circles and thick line) and after culture in 10 mM glucose (open circles and thin line). The concentration of glucose was changed and the stimulation with 30 mM KCl applied as indicated. Values are means ± S.E. for 5 experiments of insulin secretion and 24 islets from 4 preparations for [Ca^{2+}]_c. Fresh 
*Sur1* KO islets (C and D) and fresh control islets (E and F) were perfused with 1 mM glucose (open circles and thin lines) or 15 mM glucose (filled circles and thick lines) throughout and stimulated with 30 mM KCl as indicated. Values are means ± S.E. for 5 experiments of insulin secretion and 24–30 islets from 4–5 preparations for [Ca^{2+}]_c.
Glucose Stimulation of Sur1KO Islets

TABLE 1
Glucose oxidation by control and Sur1KO islets
Values are pmol/10 islets for 2 h.
Means ± S.E. for 18 (Sur1KO) and 20 (controls) batches of islets from four different preparations.

| Conditions      | Glucose 1          | Glucose 15          | Glucose 1           | Sur1KO islets |
|-----------------|--------------------|---------------------|---------------------|---------------|
|                 | mM                 | mM                  | mM                  | mM            |
| Fresh islets    | 14.6 ± 0.6 (× 22.1)| 322 ± 49            | 23.8 ± 0.9a (× 15.0)| 356 ± 47      |
| Cultured G5     | 17.9 ± 0.6a (× 20.2)| 361 ± 55            | 20.2 ± 0.9b (× 14.9)| 301 ± 45a     |
| Cultured G10    | 18.0 ± 0.7b (× 23.6)| 424 ± 66b           | 22.8 ± 1.2a (× 17.6)| 402 ± 54b     |

* p < 0.05 or less versus control islets under the same conditions.
* p < 0.05 or less versus fresh islets in the same column.

culture and glucose concentration during culture can profoundly influence [Ca2+]c and insulin secretion in Sur1KO islets.

The first controversy is about basal insulin secretion. All previous studies concurred with the predicted elevation of basal [Ca2+]c, in depolarized Sur1KO β-cells (17, 19–22, 30, 31), and we showed that this increase is due to a continuous influx of extracellular Ca2+ (21). Interestingly, these [Ca2+]c measurements were all done in islets or dispersed cells cultured for 1–4 days. There was thus an apparent discrepancy between an elevation of basal [Ca2+]c (cultured cells) and a low, normal, basal insulin secretion rate (fresh islets or perfused pancreas) (16, 17, 19). On the other hand, a high basal insulin secretory rate was almost (18) consistently found with cultured Sur1KO islets (20–23, 31). The following explanation is provided by the present study. Base-line [Ca2+]c and insulin secretion are much less elevated in fresh Sur1KO islets than after culture in 10 mM glucose so that the elevation of base-line secretion in fresh preparations has escaped detection, except in one study measuring cumulative insulin secretion by islets incubated for 1 h (24).

Another controversy is about glucose-induced insulin secretion. The perfused pancreas of Sur1KO mice was reported to secrete only a very small amount of insulin in response to a stimulation by glucose alone (17, 32). Freshly isolated Sur1KO islets were found to be unresponsive (19) or to increase their insulin secretory rate only slightly when challenged by 15–17 mM glucose (16, 24), much less than did control islets. In our hands, 15 mM glucose consistently increased insulin secretion in fresh Sur1KO islets, but this effect was smaller than in control islets in absolute terms and mainly in relative terms (fold-change above the base line). There is thus fair agreement that the insulin secretory response to glucose is unpredictably small in fresh Sur1KO β-cells.

In contrast, in this and our previous studies (21, 22), a large increase in insulin secretion (>3-fold above the base line and higher than the absolute secretory rate in controls) was evoked by high glucose after overnight culture of Sur1KO islets in 10 mM glucose. A similar increase has been reported by one group using incubated islets (23), but no significant effect was reported by two other groups (18, 20, 31, 33). It is possible that the use of another culture medium with a lower glucose concentration (33) or extension of the islet culture for 3–4 days (20, 31) explains this persisting discrepancy. Nevertheless, these two groups observed stimulatory effects of glucose after activation of protein kinases A or C (18, 31). Admittedly, no general consensus has been reached yet, but we provide convincing evidence that a brief culture period under appropriate conditions unmasks a large and concentration-dependent response of Sur1KO islets to glucose. Islets lacking KATP channels are not intrinsically blind to glucose stimulation.

Mechanisms of the Unmasking—After overnight culture of Sur1KO islets in 10 mM glucose, basal and glucose-stimulated insulin secretion were both markedly increased, resulting in a 7–8-fold higher stimulated secretory rate than in fresh islets. This large difference in secretion was not attributable to differences in insulin content of the islets. We also rule out the trivial possibility of damage of our fresh Sur1KO islets with recovery during culture. If this were the case, [Ca2+]c responses would be irregular and show synchronized oscillations, and the responsiveness of fresh control islets would be impaired as well. Poor glucose metabolism in fresh Sur1KO islets is also unlikely. Thus, the absolute rate of glucose oxidation (in 15 mM glucose) was only 10% lower than after culture in 10 mM glucose, and the fold increase between low and high glucose was similar. Another possibility was that fresh Sur1KO islets remained under the influence of external inhibitory signals present in vivo. In this case, unmasking of glucose responsiveness by culture should be independent of the culture conditions. After overnight culture of Sur1KO islets in 5 rather than 10 mM glucose, acute stimulation by 15 mM glucose evoked a 3-fold increase in insulin secretion above a lower base line. This increase in the effect of glucose contrasted with the decrease observed in control islets cultured in 5 mM glucose, but it was only relative, the absolute amount of secreted insulin was not modified. Such a change cannot readily be explained by tapering off of extrinsic inhibitory influences.

To determine whether the mediocre insulin secretory response of fresh Sur1KO islets could be due to an inept metabolic amplification, this pathway was specifically tested under conditions where [Ca2+]c was augmented and controlled by KCl (1). The results unambiguously showed that the amplifying pathway was well functioning. Why, therefore, did glucose not evoke a larger secretion of insulin in fresh Sur1KO islets by activating the amplifying pathway in these β-cells where [Ca2+]c is elevated? This paradoxical failure finds its explanation in the interaction between the two signals. The amplifying pathway not only requires that [Ca2+]c be elevated to manifest itself, it also augments with the magnitude of the triggering signal (26, 34). The amplification is modest when the increase in [Ca2+]c is small, as in fresh Sur1KO islets stimulated by glucose alone, and becomes more effective at higher [Ca2+]c.
as in fresh Sur1KO islets stimulated by glucose during depolarization by high KCl.

The changes in [Ca\textsuperscript{2+}]\textsubscript{c} produced by culture stand out like the most probable mechanism of unmasking. Compared with fresh islets, culture of Sur1KO islets in 5 mM glucose lowered [Ca\textsuperscript{2+}]\textsubscript{c} and insulin secretion, at least in the basal state, whereas culture in 10 mM glucose increased basal and stimulated [Ca\textsuperscript{2+}]\textsubscript{c} and insulin secretion. We conclude that insulin secretion by fresh Sur1KO islets is low, because [Ca\textsuperscript{2+}]\textsubscript{c} is only moderately elevated in low glucose, despite the lack of K\textsubscript{ATP} channels, and only transiently increased upon stimulation by glucose. Culture in 10 mM glucose exerts a long-term effect on Ca\textsuperscript{2+} handling in these β-cells lacking K\textsubscript{ATP} channels, which leads to substantial elevation of [Ca\textsuperscript{2+}]\textsubscript{c}. As a result, insulin secretion augments under the influence of both a larger triggering signal and a greater efficacy of the amplification.

**Comparison of Sur1KO and Control Islets**—After four days of culture in 10 mM glucose, acute stimulation with glucose induced smaller mitochondrial hyperpolarization and oxygen consumption in single islet cells and intact islets from Sur1KO than control mice (30, 31). Although this could suggest that glucose metabolism is impaired in islets lacking K\textsubscript{ATP} channels, the activity of glucokinase and the expression of genes of major metabolic enzymes were normal, leading the authors to conclude that a defect in glucose catabolism is not likely the cause of the defect in glucose-stimulated insulin secretion (31). Our observations of unimpaired oxidation of glucose by Sur1KO islets provide direct support to this conclusion.

The concentration-dependence of glucose-induced insulin secretion was shifted to the left in Sur1KO islets compared with control islets. A similar shift of the glucose dependence of insulin secretion exists in control islets treated with glibenclamide to close their K\textsubscript{ATP} channels (35). The explanation for both models is that the threshold for β-cell depolarization and generation of the triggering [Ca\textsuperscript{2+}]\textsubscript{c} rise at ~7 mM glucose no longer exists in the absence of functional K\textsubscript{ATP} channels. Lower glucose concentrations can already influence insulin secretion via the amplifying pathway.

One should not believe, however, that the acute increase in insulin secretion induced by glucose stimulation in Sur1KO islets is exclusively mediated by the amplifying pathway. We indeed confirm our previous observations (21, 22) that the initial drop in [Ca\textsuperscript{2+}]\textsubscript{c} produced by high glucose is followed by a transient rise above prestimulatory values, and we show that this rebound occurs in either fresh or cultured Sur1KO islets. During this initial period, the rapid increase in insulin secretion can thus be ascribed, at least partly, to an increase in the triggering signal. During steady-state stimulation, average [Ca\textsuperscript{2+}]\textsubscript{c} was not or only barely higher in stimulated than non-stimulated Sur1KO islets, which points to the amplifying pathway as the mediator of the increase in insulin secretion. The only reservation is that glucose changed the pattern of [Ca\textsuperscript{2+}]\textsubscript{c} oscillations, which became shorter and more frequent. These changes in [Ca\textsuperscript{2+}]\textsubscript{c} oscillations resemble those of the electrical activity measured with microelectrodes in fresh Sur1KO islets; the phases of depolarization with bursts of Ca\textsuperscript{2+} spikes were shorter and more frequent in high than low glucose (30). It has been reported that glucose-induced [Ca\textsuperscript{2+}]\textsubscript{c} oscillations are synchronized in chimeric islets containing ~30% normal β-cells and ~70% β-cells without functional K\textsubscript{ATP} channels (because of expression of a dominant negative transgene of Kir6.2) (36). We show here that the complete loss of K\textsubscript{ATP} channels in an islet does not alter the synchronization of glucose-induced [Ca\textsuperscript{2+}]\textsubscript{c} oscillations, a property endowed by gap-junction-mediated electrical coupling of β-cells (37).

It is thus evident that glucose can influence the Ca\textsuperscript{2+}-dependent oscillatory electrical activity (30) and the ensuing [Ca\textsuperscript{2+}]\textsubscript{c} oscillations (21) in adult β-cells lacking K\textsubscript{ATP} channels. Together with our recent report that glucose can promote Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels in two-week-old Sur1KO islets (22), these observations clearly show that a biophysical sensor other than K\textsubscript{ATP} channels can mediate the effects of glucose on the production of the triggering Ca\textsuperscript{2+} signal.

**CONCLUSIONS**

Freshly isolated Sur1KO islets only poorly secrete insulin in response to glucose, but this refractoriness is not an intrinsic defect of β-cells lacking K\textsubscript{ATP} channels. It is largely explained by an insufficient triggering Ca\textsuperscript{2+} signal that cannot be compensated for by a yet functional amplifying pathway but can be improved by overnight culture of the islets in 10 mM glucose. As a result of the culture, a large secretory response to glucose is unmasked. Overall, our observations do not provide evidence against, but additional support for, the models explaining glucose-induced insulin secretion by the interaction of triggering and amplifying pathways. They also show that K\textsubscript{ATP} channels are not the only possible transducers of glucose effects on [Ca\textsuperscript{2+}]\textsubscript{c} in β-cells. Future studies should establish whether this novel mechanism is only an adaptation to the lack of K\textsubscript{ATP} channels or also exists in normal β-cells.

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