Small-Conductance Calcium-Activated K⁺ Channels Are Expressed in Pancreatic Islets and Regulate Glucose Responses

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Glucose-stimulated insulin secretion is associated with transients of intracellular Ca²⁺ concentration [Ca²⁺]i in the pancreatic β-cell. We identified the expression and function of specific small-conductance Ca²⁺-activated K⁺ (SK) channel genes in insulin-secreting cells. The presence of mRNA for SK1, -2, -3, and -4 (intermediate-conductance Ca²⁺-activated K⁺ 1 [IK1]) channels was demonstrated by RT-PCR in rodent islets and insulinoma cells. SK2 and -3 proteins in mouse islets were detected by immunoblot and immunocytochemistry. In the tTA-SK3 tet-off mouse, a normal amount of SK3 protein was present in islets, but it became undetectable after exposure to doxycycline (DOX), which inhibits the transcription of the tTA-SK3 gene. The SK/IK channel blockers apamin, dequalinium, and charybdo-toxin (ChTX), charybdotoxin; DEQ, dequalinium; DOX, doxycycline; ICM, islet complete medium; IK, intermediate-conductance Ca²⁺ channel; KATP, ATP-sensitive K⁺ channel; KRBB, Krebs-Ringer bicarbonate buffer; NDS, normal donkey serum; sAHP, slow afterhyperpolarization; SK, small-conductance K⁺ channel; TEA, tetraethylammonium (TEA) and charybdo-toxin (ChTX) sensitive, the maxi-KCa (BKCa, slo)–like channel. Although prominent in both isolated islet β-cells and an insulinoma cell line (17), it was shown not to play a significant role in glucose-induced electrical activity in pancreatic β-cells (18).

Characteristics of another type of KCa channel expressed in β-cells resemble the small-conductance KCa (SK) channels (5,16,19,20). These channels are K⁺-selective and are activated by an increase in the level of [Ca²⁺]i, such as occurs during trains of action potentials. In neurons, the activation of SK channels causes long-lasting membrane hyperpolarization, termed the slow afterhyperpolarization (sAHP) (21). In pancreatic β-cells, the contribution of SK currents to repolarization has not previously been investigated. Here, we examine the expression of specific SK channels in pancreatic islets and insulin-secreting cells and their role in the regulation of glucose-induced [Ca²⁺]i oscillations. To specifically study the role of SK3 channels, we used the SK3-tTA tet-off transgenic mouse constructed by Bond et al. (22).

RESEARCH DESIGN AND METHODS

Isolation and culturing of mouse islets of Langerhans and islet cells. Islets were isolated from the pancreas of 1- to 5-month-old C57BL/6 mice (Jackson Laboratories) using collagenase digestion and Ficoll gradients as previously described (12). Islets were cultured in RPMI-1640 medium supplemented with 10% FCS, 11.6 mmol/l glucose, 100 IU/ml penicillin, and 100 μg/ml streptomycin (islet complete medium [ICM]) for 2–5 days. To obtain dissociated islet Ca²⁺ concentration ([Ca²⁺]i) and thereby initiating insulin secretion (5–8).

The pattern of electrical activity in glucose-stimulated β-cells consists of oscillations in membrane potential (9,10). Oscillatory depolarizations are tightly coupled with Ca²⁺ transients and are regulated by the interplay of a variety of ion channel types, including various potassium (K⁺) channels. Several hypotheses have been proposed to explain the bursting pattern. These include periodic inactivation of Ca²⁺ channels, oscillations in KATP activity, periodic opening of the Ca²⁺ store–operated channels, or activity of delayed rectifiers (1,10,11–13). The idea that oscillations are brought about by interplay between KATP and Ca²⁺-activated K⁺ (KCa) channels has been revisited several times over the past 20 years (1,5,14–16).

Several groups of investigators have demonstrated KCa channel activities in pancreatic islet β-cells and insulin secreting cell lines. There are at least two distinct types of KCa channels in β-cells. One of them is highly tetraethyl-ammonium (TEA) and charybdo-toxin (ChTX) sensitive, the maxi-KCa (BKCa, slo)–like channel. Although prominent in both isolated islet β-cells and an insulinoma cell line (17), it was shown not to play a significant role in glucose-induced electrical activity in pancreatic β-cells (18).

In pancreatic β-cells, glucose-stimulated insulin secretion is linked to the generation of electrical activity. Increases in cytosolic ATP, derived from glucose metabolism, and the concomitant decrease in ADP result in the blocking of ATP-sensitive K⁺ (KATP) channels (1–4). The resultant membrane depolarization activates voltage-dependent L-type Ca²⁺ channels and triggers intracellular Ca²⁺ release, elevating cytoplasmic 

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[Ca²⁺]i, intracellular Ca²⁺ concentration; CCD, charged-coupled device; ChTX, charybdo-toxin; DEQ, dequalinium; DOX, doxycycline; ICM, islet complete medium; IK, intermediate-conductance Ca²⁺–activated K⁺ channel; KATP, ATP-sensitive K⁺ channel; KCa, Ca²⁺–activated K⁺ channel; KRBB, Krebs-Ringer bicarbonate buffer; NDS, normal donkey serum; sAHP, slow afterhyperpolarization; SK, small-conductance K⁺ channel; TEA, tetraethylammonium.

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cells, islets were incubated 5–20 min in trypsin/EDTA solution (Sigma), vigorously shaken to dissociate the cells, and plated on glass coverslips in ICM.

Measurement of [Ca$^{2+}$]. Mouse or rat pancreatic islets and βTC3 mouse insulinoma cells were loaded with fura-2 for 25 min at 37°C in Krebs-Ringer bicarbonate buffer (KRBB) containing (in mmol/l) 119 NaCl, 4.7 KCl, 1.8 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, and 25 NaHCO$_3$ supplemented with 5 μmol/l acetoxymethyl ester of fura-2 (Molecular Probes, Eugene, OR). Fluorescence imaging was performed using a charged-coupled device (CCD) camera–based imaging system (Hamamatsu, Hamamatsu City, Japan) and MetaFlour software (Universal Imaging, Downingtown, PA). During imaging experiments, cells or islets were kept at 37°C and constantly perfused with appropriate KRBB-based solutions of glucose and SK channel-inhibitors at a flow rate of 2.5 ml/min. [Ca$^{2+}$] was expressed as the ratio of fluorescence intensity at excitation wavelengths 340 and 380 nm (F340/F380). Calibration of [Ca$^{2+}$] measurements in fura-2 was performed using a fura-2 calcium imaging calibration kit (Molecular Probes). The F340/F380 was linearly related to [Ca$^{2+}$] in the range that was encountered in our imaging experiments (F340/F380 from 0.4 to 3.0 and [Ca$^{2+}$] from 0.15 to 1.35 μmol/l).

RT-PCR. RNA from rat islets, mouse islets, MIN6 cells, βTC3 cells, and rat brain was prepared using TRIzol (Invitrogen) and reverse-transcribed using oligo-(dT) primers with Superscript II (Invitrogen) using protocols supplied by the manufacturers. PCR was performed using Taq polymerase (Qiagen) with “solution Q” additive (Qiagen) and hot start. Specific primers for RT-PCR were: for SK1, sense 5'-CTGTTGGGAGGGCTGTCTG-3' and antisense 5'-CCGAAACCGCCTGTGCTG-3'; for SK2, sense 5'-ATGGCCCTTCCACACACTGTC-3' and antisense 5'-TGCCACTACGGCTACACAGG-3'; for SK3, sense 5'-CAAGAACCTGGCCACACTGACG-3' and antisense 5'-CAGGCGTGACTCCTCCTACTCT-3'. The general prot ool for amplification was 95°C for 30 s, 56–62°C for 30 s, and 72°C for 30 s, for 35 cycles.

PCR products were analyzed in agarose gels and identified by Southern hybridization with SK-specific probes (full-length cDNAs were kindly provided by Dr. L. Kaczmarek of Yale University), and the amplicons were gel-purified and sequenced.

Statistical analysis. Statistical analysis was performed by t test using Microsoft Excel (Microsoft) or SigmaPlot (SPSS). The results were presented as mean ± SE. The differences between groups were considered statistically significant at a P value of 0.05.

Western blotting. Western blotting was performed with affinity-purified anti-rat SK2 and anti-rat SK3 (Alomone, Jerusalem). Protein extracts were prepared by extraction with SDS loading buffer (1% SDS, 30 mmol/l Tris HCl, pH 6.8, 5% β-mercaptoethanol, 5% glycerol, and 0.1% bromophenol blue) and heating at 70°C for 10 min. Proteins were separated in 8% gels and transferred onto polyvinylidene fluoride membrane (Whatman). For Western blotting, antibodies were diluted 1:300 in 3% nonfat milk (Carnation, IL) with PBS (Sigma), pH 7.4. For immunoa bsorption with antigenic peptides, appropriate antibodies were diluted 1:300 in PBS with 10 μg/ml of corresponding peptide for 1 h at room temperature. Immunoblotting. Purified mouse brain extract was prepared using TRIsol (Invitrogen) and reverse-transcribed using Superscript II (Invitrogen). RT-PCR was performed using mouse brain cDNA as a positive control. Negative controls were performed on samples of islet and βTC3 RNA without treatment with reverse transcriptase. Amplicons of the expected size were obtained with primers for all four genes (Fig. 1). The identity of the products was confirmed by hybridization with corresponding cDNA and/or sequencing.

Antibodies specifically recognizing SK3 and -2 were used to detect corresponding antigens in mouse islets by Western blot (Fig. 2). Anti-SK2 antibody strongly reacted with a protein fraction of 60 KDa. The 60-KDa size is close to the size of SK2, as deduced from the cDNA sequence. In the control experiment, SK2 peptide, which was used for the generation of anti-SK2 antibody, inhibited the binding of the antibody to the 60-KDa band, indicating that the binding was specific. For weaker bands, 68- and 100-KDa binding of antibody was also specific, indicating that these are either modifications of SK2 or unrelated cross-reacting proteins.

With anti-SK3, an immunoreactive band of ~70 KDa corresponding to the deduced size of SK3 protein was detected in mouse islets (Fig. 2) and MIN6 insulinoma using pairs of primers designed to amplify nonconserved regions of the genes. Amplification experiments were performed using mouse brain cDNA as a positive control. Negative controls were performed on samples of islet and βTC3 RNA without treatment with reverse transcriptase. Amplicons of the expected size were obtained for all four genes (Fig. 1). The identity of the products was confirmed by hybridization with corresponding cDNA and/or sequencing.

RESULTS Expression of the four genes SK1, -2, -3, and -4 was determined in mouse islets, rat islets, and βTC3 cell cDNA.
cells (not shown). SK3 peptide, which was used for the generation of anti-SK3 antibody, inhibited the binding of the antibody to the 70-KDa band. Additional evidence for the presence of SK3 protein was obtained in the experiments with tet-off SK3 mice (Fig. 8).

The same anti-SK2 and -SK3 antibodies were used to probe for SK2 and -3 expression in dissociated mouse islet cells (Fig. 3). SK2 and -3 immunoreactivity was detected in cells that were also positive for insulin. This staining was not detected in controls stained without primary antibody (not shown). The distribution of both proteins was mostly intracellular, occurring in a punctate pattern (Fig. 3A and D), which was only occasionally coincident with secretory granules, detected with anti-insulin antibodies (Fig. 3C and F). In addition to this pattern, anti-SK2 antibodies revealed an intense staining of either the nuclear membrane or an area near the nuclear membrane (Fig. 3D and F).

In the presence of elevated glucose levels, mouse islets often display oscillatory increases in [Ca^{2+}]_i that have been connected to various ion channel activities. To study the physiological role of K_{Ca} channels and SK1, -2, -3, and -4 (intermediate-conductance K_{Ca} 1 [IK1]), we examined the effects of SK/IK channel–blockers on the characteristics of [Ca^{2+}]_i oscillations in islets in the presence of glucose. We hypothesized that the inhibition of the K_{Ca} channels would delay the repolarization and prolong the depolarized phase of each oscillation, thus increasing the average [Ca^{2+}]_i.

Apamin is a widely used inhibitor of SK2 and -3 (both with IC_{50} < 1 nmol/l) and, to a lesser degree, of SK1 (IC_{50} 3.3 nmol/l when expressed in mammalian cells) (23–25). Application of 100 nmol/l apamin after the addition of 12 mmol/l glucose augmented the glucose-induced [Ca^{2+}]_i response in mouse islets, increasing the average [Ca^{2+}]_i level by 15.26 ± 3.32% (P < 0.05, n = 10) (Fig. 4A). Apamin-dependent elevation of [Ca^{2+}]_i in islets did not occur in a nonstimulatory concentration of glucose (2 mmol/l) (not shown). Apamin was also able to increase [Ca^{2+}]_i levels in dissociated islet β-cells and in mouse insulinoma βTC3 cells (not shown).

It has been demonstrated previously that treatment of mouse islet cells with 5–20 mmol/l TEA increased the amplitude and frequency of [Ca^{2+}]_i oscillations (12). This response was attributed to inhibition of voltage-dependent K^+ channels and possibly other K^+ channels in β-cells. To measure the effect of apamin-sensitive K^+ channel inhibition in the absence of delayed rectifier or BK_{Ca} activity, we examined the effect of apamin in the presence of 10–20 mmol/l TEA. In these experiments, islets were subjected to a step increase in glucose concentration from 2 to 12 mmol/l and subsequent addition of 20 mmol/l TEA. This leads to a rise in [Ca^{2+}]_i, followed by [Ca^{2+}]_i oscillations. The addition of apamin (100 nmol/l) to islets in glucose (12 mmol/l) and TEA (20 mmol/l) produced an additional increase in the average [Ca^{2+}]_i level of 30.98 ± 7.18% (P < 0.05, n = 8). An increase in the average [Ca^{2+}]_i level also occurred in 14 mmol/l glucose with 20 mmol/l TEA (16.09 ± 1.76, P < 0.05, n = 8). The action of synthetic apamin peptide in this type of experiment was similar to that of natural apamin, with an increase in the average [Ca^{2+}]_i level of 19.03 ± 6.06% (P < 0.05, n = 7), which
makes the influence of other bee venom components on islet $[\text{Ca}^{2+}]_i$ physiology unlikely.

We also explored the effect of apamin on the frequency of $[\text{Ca}^{2+}]_i$ oscillations. We analyzed the experiments in which the $[\text{Ca}^{2+}]_i$ response to glucose was oscillatory. The average frequency of oscillations in experiments in which islets were stimulated with glucose (14 mmol/l) was $0.58 \pm 0.2$ oscillations/min ($n = 7$). The addition of apamin increased the frequency by $59.4 \pm 18.8\%$; however, this change was not statistically significant because of variability of oscillation frequency in different islets.

Two general types of oscillation patterns were observed with the action of TEA in islets, illustrated in Fig. 4B and C. In one of these (Fig. 4B), the addition of TEA to glucose-stimulated islets produced large oscillations of $[\text{Ca}^{2+}]_i$, with short plateaus sometimes containing superimposed spikes and an oscillation frequency of $0.58 \pm 0.09$ oscillations/min ($n = 6$). In these islets, the addition of apamin (100 nmol/l) resulted in an increase of $161.4 \pm 26.4\%$ ($P < 0.005$) in oscillation frequency.

In another type of islet response (Fig. 4C), the addition of TEA resulted in more rapid $[\text{Ca}^{2+}]_i$ oscillations ($5.64 \pm 0.77$ oscillations/min, $n = 7$), but this pattern was less ordered than the other one in Fig. 4B. The addition of 100 nmol/l apamin to this “fast” oscillating islet did not produce a statistically significant change in the frequency of oscillations.

Another inhibitor of SK-type $K_{Ca}$ channels, dequalinium (DEQ), at concentrations of 10 and 50 nmol/l was able to increase the $[\text{Ca}^{2+}]_i$ level in islets and islet cells (Fig. 5). The addition of 10 nmol/l DEQ to islets in 14 mmol/l glucose increased the $[\text{Ca}^{2+}]_i$ level by $11.8 \pm 1.8\%$ ($n = 8$), and 50 nmol/l DEQ increased the $[\text{Ca}^{2+}]_i$ level by $13.8 \pm 2.7\%$ ($n = 11$). The addition of DEQ (10 nmol/l) to islets in glucose (14 mmol/l) and TEA (20 mmol/l) increased the average $[\text{Ca}^{2+}]_i$ level by $106.14 \pm 7.65\%$ ($P < 0.05$, $n = 5$).

Similar experiments were performed with dissociated islet cells to determine whether the effect of DEQ is preserved after enzymatic islet dispersion. Only $\beta$-cells, which responded to an increase in glucose with an increase in $[\text{Ca}^{2+}]_i$, were analyzed. In dissociated islet cells ($n = 16$) stimulated with glucose (14 mmol/l) and TEA (20 mmol/l), sequential additions of 10 and 50 nmol/l of DEQ led to increases of $[\text{Ca}^{2+}]_i$ levels by $56.8 \pm 6.6\%$ ($P < 0.05$) and $289 \pm 19.9\%$ ($P < 0.05$), respectively, demonstrating the concentration-dependent action of DEQ in $\beta$-cells. This confirms that the effect of DEQ is not determined at the level of islet infrastructure, but it originates in $\beta$-cells and is not an indirect effect. Similarities in the effects of apamin and DEQ suggested that the SK channel inhibition was indeed the cause of changes in $[\text{Ca}^{2+}]_i$.

ChTX, a widely used inhibitor of the BK$_{Ca}$ and IK current, was used at a relatively high concentration (100 nmol/l, exceeding concentrations that have been shown to inhibit IK currents in different systems) (20,26–29). In the presence of elevated glucose (14 mmol/l) or in glucose (14 mmol/l) with TEA (20 mmol/l), ChTX was able to increase the level of $[\text{Ca}^{2+}]_i$ by $8.7 \pm 1.9\%$ ($P < 0.05$, $n = 9$) and $8.02 \pm 2.73\%$ ($P < 0.05$, $n = 6$), respectively. The detectable effect of ChTX on $[\text{Ca}^{2+}]_i$ responses in an islet is illustrated in Fig. 6 and can be explained by inhibition of IK1 (SK4) activity.

A recently described mouse model (SK3-tTA) permits experimental regulation of SK3 expression while retaining normal SK3 promoter function. We used this model to determine the physiological role of SK3 in mouse $\beta$-cells. In SK3-tTA mice, a tetracycline-based genetic switch (T) was inserted into the 5‘ untranslated region of the SK3...
gene so that subunit expression could be abolished by dietary DOX administration without interfering with the normal profile of SK3 expression (20). SK3 was present in pancreatic islets from homozygous tetracycline transactivator–regulated SK3 gene (T/T) mice (in slightly higher amount compared with wild type) (22), but it became undetectable after exposure to DOX (Fig. 7). T/T mice from transgenic mice (as in lane 1); transgenic mice homozygous for tetracycline transactivator–regulated SK3 gene (T/T) (lane 2); and T/T mice treated with 0.5 mg/ml dietary DOX (lane 3). A: Blots developed with anti-SK3 (Alomone). B: Blots developed with anti–glucose-6-phosphate dehydrogenase (Sigma-Aldrich).

FIG. 7. Modulation of SK3 expression in islets of tTA-SK3–targeted mice. Protein extracts of isolated pancreatic islets from: wild-type mice (lane 1); transgenic mice homozygous for tetracycline transactivator–regulated SK3 gene (T/T) (lane 2); and T/T mice treated with 0.5 mg/ml dietary DOX (lane 3). A: Blots developed with anti-SK3 (Alomone). B: Blots developed with anti–glucose-6-phosphate dehydrogenase (Sigma-Aldrich).

TABLE 1

<table>
<thead>
<tr>
<th>Isolet Type</th>
<th>[Ca^{2+}]_i peak</th>
<th>[Ca^{2+}]_i duration</th>
<th>[Ca^{2+}]_i frequency</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>2.65 mmol/l</td>
<td>1.56 sec</td>
<td>2.26 Hz</td>
</tr>
<tr>
<td>DOX-treated</td>
<td>2.81 mmol/l</td>
<td>1.78 sec</td>
<td>2.59 Hz</td>
</tr>
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were euglycemic, and glucose tolerance tests were not different in mice either with or without DOX administration (not shown).

Islets from DOX-treated (depleted of SK3) and untreated T/T mice produced similar [Ca^{2+}]_i patterns when stimulated with glucose (14 mmol/l) or glucose/TEA (14/20 mmol/l) (not shown). However, there was a clear difference when both types of islets were treated with apamin. Islets from T/T mice that were not exposed to DOX (Fig. 8A) responded to apamin (100 mmol/l, in combination with glucose and TEA) with an 18.14 ± 2.65% increase in the average [Ca^{2+}]_i level (P < 0.05, n = 6) (Fig. 8A), similar to the wild-type mouse islets (Fig. 4) but not islets from DOX-treated T/T mice (Fig. 8B). Islets isolated from DOX-treated mice demonstrated just a trend of an increase in [Ca^{2+}]_i, that was not statistically significant (2.3 ± 1.56%, P = 0.22, n = 7) (Fig. 8B). Therefore, increased SK3 expression in T/T mouse islets leads to a detectable effect on islet physiology, whereas depletion of SK3 protein in islets leads to a substantial decrease in the [Ca^{2+}]_i response to apamin. This experiment shows a correlation between the expression of an apamin-sensitive SK3 channel and the apamin sensitivity of the [Ca^{2+}]_i response pattern.

DISCUSSION

In this report we demonstrate that three SK channels and the IK channel are expressed in rodent pancreatic islets and in insulinoma cells. mRNA for all four of these channels in islets and in insulinoma cells was identified by RT-PCR, and at least two proteins, SK2 and -3, were detected by reaction with specific antibodies. Antibodies for SK1 and -4 are not yet available.

Immunocytochemical staining has shown that both SK2 and -3 are expressed in insulin-secreting mouse islet β-cells. The staining pattern seen with anti-SK3 antibodies was similar to that demonstrated in rat hepatocytes with the same antiserum (30). The intracellular localization for SK2 was generally very similar to SK3, which might also reflect the similarity in the function of both proteins. The nuclear localization of SK2 presents a difference between the two proteins, and it suggests that future studies should address the possible functional expression of SK2 in this membrane compartment. Similar results of cellular distribution of SK2 have recently been reported in endothelial and smooth muscle cells (31).

Treatment of mouse islets with the SK1–3 channel inhibitor apamin modifies the [Ca^{2+}]_i response to glucose and TEA, increasing the general level of [Ca^{2+}]_i, and the frequency of [Ca^{2+}]_i oscillations. This implies that SK channels play a role in the regulation of the glucose-induced increase of [Ca^{2+}]_i in mouse β-cells. We attribute the apamin-induced increase in the general level of [Ca^{2+}]_i to the diminished density of repolarizing channels involved in the termination of the electrical activity. Another consequence of the SK channel inactivation would be a decrease in the duration of the time interval between the consecutive [Ca^{2+}]_i oscillations (the nadir). This could occur due to the lowered hyperpolarizing capacity of the remaining K^+ channels and therefore the increased sensitivity of the membrane potential to K_{ATP} closure. This could lead to a decreased spacing between consecutive
[Ca^{2+}]_i oscillations and therefore lead to an increased frequency of oscillations.

Studies of SK3 tet-off transgenic mice pointed to a correlation between expression of the SK3 channel and apamin sensitivity of the [Ca^{2+}]_i response pattern. The results allow us to assume that in wild-type mouse islets as well, the sensitivity of the glucose-induced [Ca^{2+}]_i responses to apamin is connected to the activity of apamin-sensitive SK1, -2, and -3 channels.

These experiments also imply that SK3 is the main determinant of apamin sensitivity in T/T mouse β-cells. Non–DOX-treated T/T mice are characterized by overexpression of SK3 (22), which could have had an effect on the balance of ion channel activities in the β-cell. Therefore, based on these results alone, we cannot conclude that the SK3 activity is also predominant among SK channels in the β-cells of wild-type mice. Additional experiments that would determine the representation of each of the SK channels in the β-cells, as well as studies of genetic manipulation upon other SK channels, will be needed in the future.

Earlier reports from other laboratories claimed the lack of apamin effect on K^+ currents in islet cells (19,20), on β-cell membrane potential or on insulin secretion (32), either with or without glucose stimulation, which seemingly presents a disagreement with our results. This study is the first one where the [Ca^{2+}]_i response to glucose stimulation was assayed, rather than the plasma membrane electrical activity. It is possible that subtle changes in the activity of small-conductance KCa channels caused by apamin are amplified through their integrated effect on [Ca^{2+}]_i responses, thus deeming them detectable by our approach.

This is the first report in β-cells on the effects of DEQ, an established inhibitor of some KCa channels (33,34). We show that, similar to apamin, it augments the response to glucose, which is consistent with the possible inhibition of SK channels. For cloned SKCa1 and SKCa2 channels, the IC50 values were in the range of 1 μmol/l (33,34); therefore, these channels would be largely inhibited by 10 μmol/l DEQ. The increase in the [Ca^{2+}]_i level observed using 10 μmol/l DEQ is similar to the effect of apamin and is consistent with the inhibition of SK1, -2, or -3. However, it has also been shown that for IK (which is widely thought to be represented by the SK4 protein), the IC50 can be >10 μmol/l (34). It is likely, therefore, that the effect of 50 μmol/l DEQ in islets and dissociated cells is characteristic of inhibiting an IKCa-like channel.

Our results indicate that addition of the of ChTX (100 nmol/l) to islets incubated in glucose with TEA leads to the augmentation of the Ca^{2+} response. This suggests the involvement of a ChTX-sensitive activity other than BKCa (which is highly TEA-sensitive). These results are consistent with findings of Kozak et al. (20), who described a slow-activating KCa channel in mouse βTC3 cells (and a similar current in porcine islet β-cells) that was insensitive to TEA, 10 nmol/l ChTX, and apamin (200 nmol/l) but was sensitive to 100 nmol/l ChTX. Several IKCa conductances are blocked by ChTX, such as in human peripheral blood T-cells (26,35), HL60 granulocytes (28), erythrocytes (29), and Aplysia neurons (27). The long-standing idea of ChTX-sensitive currents not being involved in the glucose-generated electrical bursting pattern of islet cells was suggested by experiments where ChTX was used only at 20 nmol/l, which would mainly inhibit the BKCa-like current component.

The use of 100 nmol/l ChTX and 50 μmol/l DEQ allowed us to selectively probe for IKCa-like activity effects in the mouse islets, which are likely to be mediated by SK4 proteins (36–39). It is therefore possible that the SK4 channel in islet cells also contributes to the regulation of glucose responses.

In a number of reports, KCa channels with features similar to certain SK channels were described in islets and isolated β-cells (6,16,19,20), but their molecular identity has not been experimentally revealed. These reports in some ways contradict each other and do not demonstrate a clear and unambiguous picture of the possible set of KCa channels active in β-cells. It is also possible that KCa activities in β-cells are not limited to SK channels. Some apamin-insensitive conductances, such as apamin-insensitive KCa channels in β-cells (6,16,19,20) or saHP in neurons (24,25), cannot be explained by any known assortment of KCa channels and may suggest the expression of yet-identified channel genes or subunits. It is therefore possible that novel members of the SK family might be expressed in β-cells. Alternatively, the formation of the hetero-multimers could be a way to regulate their activity and sensitivity to drugs. It has been shown that some SK subunits can form heteromeric channels (37), whereas for others no data are yet available.

In this report we show that all four small-conductance KCa channels are expressed in pancreatic β-cells. Their characteristics and the potential ability of the subunits to interact suggest that SK channels could indeed play an important role in the regulation of islet [Ca^{2+}]_i oscillations and insulin secretion. As we have shown, decreased activity of KCa channels would decrease repolarizing currents and lead to an elevation of [Ca^{2+}]_i. This would in turn lead to an increase in the frequency or amplitude of oscillations, which was indeed observed in this study.

Taken together, our data support a role for SK channels, and SK3 in particular, in regulating glucose-induced changes in membrane potential, [Ca^{2+}]_i, and insulin secretion in mouse islets.

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