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Glucose Recruits K_{ATP} Channels via Non-Insulin-Containing Dense-Core Granules

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SUMMARY

β cells rely on adenosine triphosphate-sensitive potassium (K_{ATP}) channels to initiate and end glucose-stimulated insulin secretion through changes in membrane potential. These channels may also act as a constituent of the exocytotic machinery to mediate insulin release independent of their electrical function. However, the molecular mechanisms whereby the β cell plasma membrane maintains an appropriate number of K_{ATP} channels are not known. We now show that glucose increases K_{ATP} current amplitude by increasing the number of K_{ATP} channels in the β cell plasma membrane. The effect was blocked by inhibition of protein kinase A (PKA) as well as by depletion of extracellular or intracellular Ca^{2+}. Furthermore, glucose promoted recruitment of the potassium inward rectifier 6.2 to the plasma membrane, and intracellular K_{ATP} channels localized in chromogranin-positive/insulin-negative dense-core granules. Our data suggest that glucose can recruit K_{ATP} channels to the β cell plasma membrane via non-insulin-containing dense-core granules in a Ca^{2+}- and PKA-dependent manner.

INTRODUCTION

Molecular biological and electrophysiological studies have revealed that the adenosine triphosphate-sensitive potassium (K_{ATP}) channel consists of potassium inward rectifier 6 (Kir6) and sulfonylurea receptor (SUR) subunits (Aguilar-Bryan and Bryan, 1995, 1999; Clement et al., 1997; Inagaki et al., 1995; Seino and Miki, 2003). In the pancreatic β cell, the K_{ATP} channel complex comprises the SUR1 and Kir6.2 isoforms in a hetero-octameric complex with a 4:4 ratio of Kir6.2 and SUR1 subunits (Aguilar-Bryan and Bryan, 1995, 1999; Clement et al., 1997; Inagaki et al., 1995; Seino and Miki, 2003). Kir6.2 subunits form the pore of the K_{ATP} channel, while SUR1 subunits are grouped symmetrically around this central pore formation and act as regulatory subunits (Aguilar-Bryan and Bryan, 1995, 1999; Clement et al., 1997; Inagaki et al., 1995; Seino and Miki, 2003). These two K_{ATP} channel subunits, like other membrane proteins, recycle to maintain a certain number of functional K_{ATP} channels in the plasma membrane (Beguin et al., 1999; Hu et al., 2003).

The ATP/ADP ratio-dependent closure or opening behavior of K_{ATP} channels alters the β cell membrane potential and consequently opens or shuts voltage-gated Ca^{2+} channels, resulting in corresponding insulin secretory responses following the rise or fall in glucose (Yang and Berggren, 2005a, 2005b, 2006). In addition, K_{ATP} channel proteins, and in particular SUR1 subunits, may also act as a constituent of the exocytotic machinery to mediate insulin exocytosis independent of their electrical function (Eliasson et al., 2003). Therefore, an appropriate number of K_{ATP} channels in the plasma membrane are required for the efficient glucose responsiveness of β cells and thereby glucose homeostasis (Nichols, 2006). However, the molecular mechanisms whereby the β cell plasma membrane maintains an appropriate number of K_{ATP} channels remain unknown.

It is well known that β cells from different species including mice, when subjected to initial glucose stimulation long enough to induce second-phase insulin secretion, release more insulin when re-exposed to glucose. Such glucose sensitization is referred to as β cell memory to glucose stimulation (Grill et al., 1978; Grodsky, 1972; Nesher and Cerasi, 2002; Straub and Sharp, 2002; Zawalich and Zawalich, 1996). However, it remains elusive how β cell memory to glucose stimulation occurs. It is intriguing that glucose may recruit K_{ATP} channels to the β cell plasma membrane to preserve the β cell glucose responsiveness and thereby β cell memory to glucose stimulation. In the present work, we describe a pathway whereby glucose recruits K_{ATP} channels to the β cell plasma membrane via chromogranin-positive/insulin-negative dense-core granules in a Ca^{2+}- and protein kinase A (PKA)-dependent manner.

RESULTS

Glucose Increases the Number of Electrically Functional K_{ATP} Channels

To evaluate whether high glucose increases the number of electrically functional K_{ATP} channels in the β cell plasma membrane, we examined effects of 1 hr incubation with

3 mM glucose (as a control) and 17 mM glucose on whole-cell K_{ATP} currents in pancreatic β cells. It is known that β cells exposed to higher glucose concentrations return to the resting state, in terms of electrical activity, only after about 1 hr when exposed again to low concentrations of the sugar. Moreover, incubation with 17 mM glucose requires about 1 hr to fully and physiologically activate glucose signal transduction, including first- and second-phase insulin secretion in the β cell (Larsson-Nyren and Sehlin, 1996, 2002). Therefore, 1 hr was chosen for glucose stimulation in the present experiments. Apparently there is a significant difference in the ATP/ADP ratio between cells treated for 1 hr with 3 versus 17 mM glucose. The majority of K_{ATP} channels in cells subjected to the high-glucose incubation are closed (Ashcroft and Rorsman, 1989). Before and during the recordings, we therefore dialyzed the cells with 0.3 mM ATP/ADP and exposed them to the K_{ATP} channel opener diazoxide to equalize experimental conditions and maximally open K_{ATP} channels (Bokvist et al., 1999; Zunkler et al., 1988). Cells treated with 17 mM glucose displayed larger whole-cell K_{ATP} currents evoked by 10 mV hyperpolarizing and depolarizing voltage pulses from a holding potential of −70 mV (Figures 1A and 1B). However, there was no significant difference in cell capacitance, reflecting plasma membrane area, between 3 and 17 mM glucose-treated cells (4.8 ± 0.3 versus 4.5 ± 0.3 pF; n = 28, $p > 0.05$). This indicates that the plasma membrane area after 1 hr of stimulation is unaltered due to a compensatory glucose-stimulated endocytosis following glucose-stimulated exocytosis.

To verify that the majority of the whole-cell currents obtained under our experimental conditions were indeed K_{ATP} currents, we exposed the glucose-incubated cells to 0.1 mM tolbutamide, a specific K_{ATP} channel blocker, in the presence of diazoxide. Figure 1A shows representative whole-cell K_{ATP} current traces registered in low- and high-glucose-incubated cells before and during exposure to tolbutamide. The majority of the whole-cell currents were blocked in the presence of tolbutamide. Compiled data illustrate that incubation with 17 mM glucose for 1 hr (n = 28) significantly enhanced whole-cell K_{ATP} current density compared to incubation with 3 mM glucose (n = 28) ($p < 0.05$) and that the specific K_{ATP} channel blocker tolbutamide blocked 90% of these whole-cell K_{ATP} currents (Figure 1B).

Although the enhancement of whole-cell K_{ATP} currents by 17 mM glucose incubation indicates an increase in the number of functional K_{ATP} channels, changes in K_{ATP} channel activity at the single-channel level cannot be completely ruled out. To characterize single K_{ATP} channel properties in glucose-incubated cells, cell-attached recording was performed in the presence of 0.3 mM diazoxide to optimally detect unitary K_{ATP} currents. Tolbutamide-blockable inward unitary K^+ currents with an amplitude of ~5 pA were considered as K_{ATP} currents under the present recording conditions (Figure 2A). Figure 2A shows a typical unitary K_{ATP} current trace recorded from a cell-attached patch on a cell treated with 3 mM glucose. The recorded channel alternated between the open and closed states. Addition of 0.1 mM tolbutamide effectively closed this channel. Subsequently, it induced biphasic currents, demonstrating that the K_{ATP} channels in the recorded cell were blocked to the extent that action potentials were generated (Figure 2A). Analysis of unitary K_{ATP} currents showed that 17 mM glucose incubation did not influence single K_{ATP} channel properties as compared to 3 mM glucose incubation (Figures 2B and 2C). There was no difference in either mean open time or open probability between treatment with high glucose and treatment with low glucose (Figures 2B and 2C).

To verify electrophysiologically whether glucose increases the number of K_{ATP} channels, inside-out patch

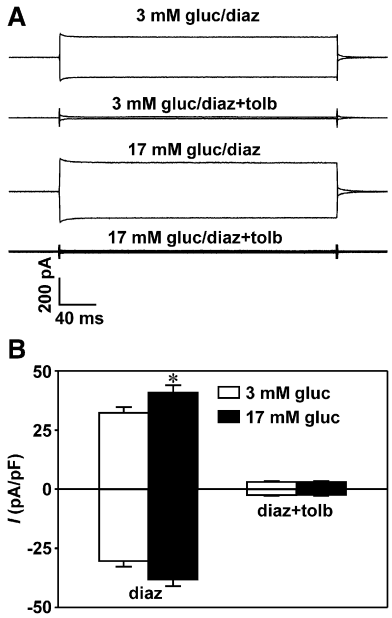

Figure 1. High Glucose Increases Whole-Cell K_{ATP} Channel Activity in the Pancreatic β Cell

Whole-cell K_{ATP} currents were registered in single β cells dialyzed with 0.3 mM ATP/ADP and exposed to 0.3 mM diazoxide. Gluc: glucose; diaz: diazoxide; tolb: tolbutamide.

(A) The top two panels show sample whole-cell K_{ATP} current traces, generated by ±10 mV voltage pulses (300 ms, 0.5 Hz) from a holding potential of −70 mV, from a cell (4.1 pF capacitance) subjected to 1 hr of 3 mM glucose incubation in the absence (top panel) and presence (second panel) of 0.1 mM tolbutamide. The bottom two panels show sample whole-cell K_{ATP} current traces, generated by the same voltage protocol, from a cell (4.0 pF capacitance) subjected to 1 hr of 17 mM glucose incubation in the absence (third panel) and presence (bottom panel) of 0.1 mM tolbutamide.

(B) Summary graph of whole-cell K_{ATP} current density showing that 17 mM glucose-treated cells (left-hand black column, n = 28) displayed larger currents than 3 mM glucose-treated cells (left-hand white column, n = 28) in the absence of 0.1 mM tolbutamide. The majority of the whole-cell currents were blocked by tolbutamide. Moreover, there was no difference in the tolbutamide-insensitive current between 3 mM (right-hand white column, n = 23) and 17 mM glucose incubation (right-hand black column, n = 23). This confirms that non-K_{ATP} currents were not affected by glucose stimulation. Statistical significance was evaluated by unpaired Student's t test. *$p < 0.05$. In this and all other figures, error bars represent ±SEM.

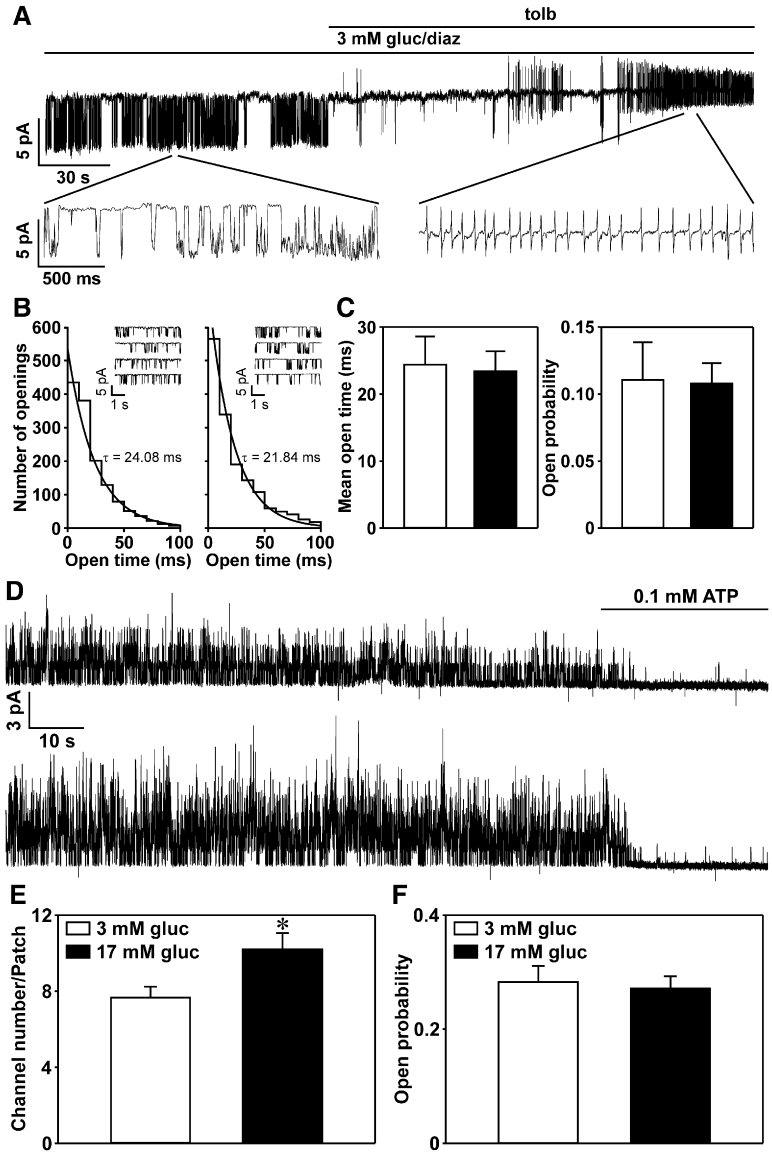

Figure 2. High Glucose Does Not Alter Properties of Single K_{ATP} Channels but Increases the Number of K_{ATP} Channels in the Pancreatic β Cell

Unitary K_{ATP} currents were obtained in cell-attached patches on β cells exposed to 0.3 mM diazoxide in the presence of 3 or 17 mM glucose and inside-out patches from β cells bathed with 3 or 17 mM glucose. Statistical significance was evaluated by unpaired Student's t test and Mann-Whitney U test. *$p < 0.05$.

(A) Sample trace showing that 0.1 mM tolbutamide effectively blocks unitary K_{ATP} currents and subsequently evokes biphasic currents derived from action potentials in a cell-attached patch on a β cell in the presence of 0.3 mM diazoxide following 1 hr of 3 mM glucose incubation. Two segments of the recording are expanded to illustrate unitary K_{ATP} (left lower panel) and action-potential-derived currents (right lower panel) with high temporal resolution.

(B) Examples of open time distributions of single K_{ATP} channels in cells subjected to 1 hr of 3 mM (left panel) or 17 mM (right panel) glucose incubation. Insets show original unitary K_{ATP} current traces. τ: open time constant.

(C) Summary graph showing that treatment with 17 mM glucose (black columns, n = 10) does not alter the mean open time (left panel) and open probability (right panel) of single K_{ATP} channels as compared to treatment with 3 mM glucose (white columns, n = 11, $p > 0.05$).

(D) Sample trace showing unitary K_{ATP} currents in an inside-out patch from a 3 mM glucose-treated cell (upper panel) and from a 17 mM glucose-treated cell (lower panel). The identity of K_{ATP} channels was confirmed by ATP inhibition.

(E) Summary graph showing that patches from 17 mM glucose-treated cells (black column, n = 26) harbor significantly more K_{ATP} channels than those from 3 mM glucose-treated cells (white column, n = 26).

(F) Summary graph showing that treatment with 17 mM glucose (black columns, n = 26) does not alter the open probability of K_{ATP} channels as compared to treatment with 3 mM glucose (white columns, n = 26, $p > 0.05$).

recordings were employed. A sample trace of unitary K_{ATP} currents in an inside-out patch from a 3 mM glucose-treated cell (Figure 2D, upper panel) displays lower levels of K_{ATP} conductance, reflecting a lower number of K_{ATP} channels, in comparison with a sample trace from a 17 mM glucose-treated cell (Figure 2D, lower panel). The identity of K_{ATP} channels shown in these two traces was confirmed by ATP inhibition (Figure 2D). Figure 2E illustrates that patches excised from 17 mM glucose-treated cells (black column, n = 26) contained significantly more K_{ATP} channels than those from 3 mM glucose-treated cells (white column, n = 26, $p < 0.05$). However, there was no significant difference in the open probability of K_{ATP} channels between 3 mM (Figure 2F, white column, n = 26) and 17 mM glucose-treated cells (black column, n = 26, $p > 0.05$).

Glucose Recruits K_{ATP} Channels to the Plasma Membrane in a Ca^{2+}- and PKA-Dependent Manner

To test for the molecular mechanisms whereby high glucose increases whole-cell K_{ATP} channel activity, we incubated cells for 1 hr with 17 mM glucose plus the Ca^{2+} chelator EGTA at 0.5 mM or the PKA inhibitor H-89 at 1 μM, or with 3 mM glucose plus insulin at 50 μU/ml. Whole-cell K_{ATP} current recordings registered in single β cells dialyzed with 0.3 mM ATP/ADP and exposed to 0.3 mM diazoxide show that treatment with EGTA or H-89 fully prevented 17 mM glucose-induced enhancement of whole-cell K_{ATP} channel activity. Figure 3A shows that whole-cell K_{ATP} currents in cells incubated with 17 mM glucose plus EGTA (n = 38) or H-89 (n = 39) were significantly lower than in cells incubated with 17 mM glucose alone (n = 38) ($p < 0.05$).

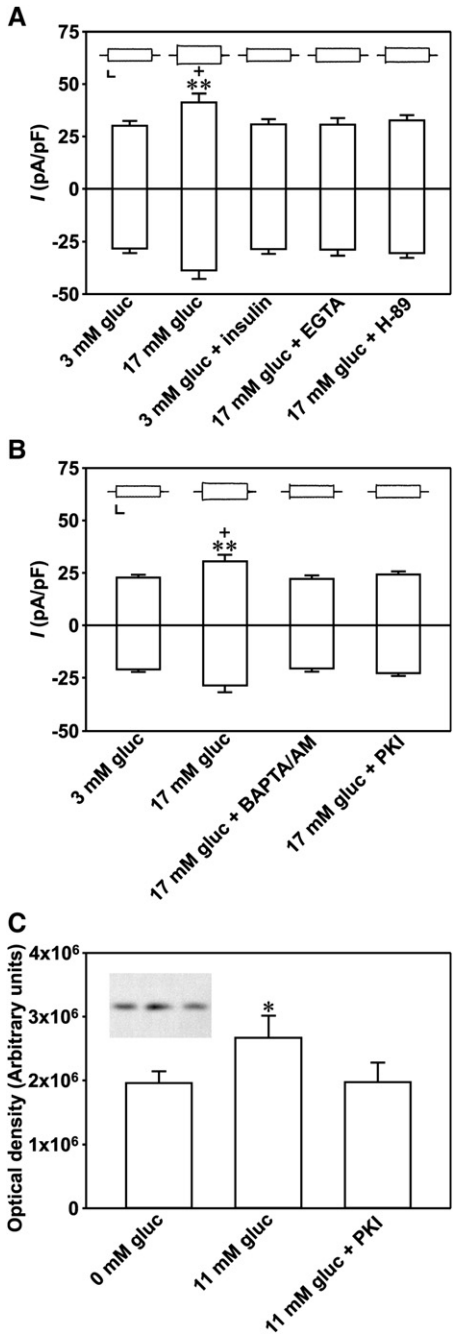

Figure 3. High-Glucose-Induced Recruitment of K_{ATP} Channels to the β Cell Plasma Membrane Depends on Intracellular Ca^{2+} and PKA Activity

Statistical significance was evaluated by one-way ANOVA followed by least significant difference test.

(A and B) Whole-cell K_{ATP} current recordings were registered in single β cells dialyzed with 0.3 mM ATP/ADP and exposed to 0.3 mM diazoxide. Insets depicted in summary graphs show original whole-cell K_{ATP} current traces, generated by ±10 mV voltage pulses (300 ms, 0.5 Hz) from a holding potential of −70 mV, from corresponding groups below. Calibration bars are 200 pA and 50 ms. Gluc: glucose; PKI: protein kinase A inhibitor 14-22 amide.

(A) Summary graph showing that 17 mM glucose-treated cells (n = 38) displayed larger currents than 3 mM glucose-treated cells in the absence (n = 39) or presence of insulin (50 μU/ml, n = 39) and 17 mM glucose-treated cells in the presence of 0.5 mM EGTA (n = 38) or 1 μM H-89 (n = 39). **p < 0.01 versus 3 mM glucose; +p < 0.05 versus 3 mM glucose plus insulin (50 μU/ml), 17 mM glucose plus 0.5 mM EGTA, and 17 mM glucose plus 1 μM H-89.

(B) Summary graph showing that 17 mM glucose-treated cells (n = 23) exhibited larger currents than 3 mM glucose-treated cells (n = 24) or 17 mM glucose-treated cells in the presence of 0.1 mM BAPTA/AM (n = 23) or 20 μM PKI (n = 23). **p < 0.01 versus 3 mM glucose and 17 mM glucose plus 0.1 mM BAPTA/AM; +p < 0.05 versus 17 mM glucose plus 20 μM PKI.

(C) High glucose promotes K_{ATP} channel Kir6.2 translocation to the plasma membrane of insulin-secreting RINm5F cells. Histogram illustrates that the intensity of the Kir6.2 immunoreactivity in the plasma membrane of 11 mM glucose-treated cells is significantly higher than in cells treated with 0 mM glucose or 11 mM glucose plus 20 μM PKI. The inset immunoblot shows the Kir6.2 immunoreactivity in plasma membrane proteins obtained with biotinylation purification from RINm5F cells treated with 0 mM glucose (left band), 11 mM glucose (middle band), and 11 mM glucose plus 20 μM PKI (right band), respectively. n = 4; *p < 0.05 versus 0 mM glucose and 11 mM glucose plus 20 μM PKI.

There was no significant difference in K_{ATP} currents between treatments with 3 mM glucose and 17 mM glucose plus EGTA or H-89 (p > 0.05). Furthermore, incubation with insulin in the presence of 3 mM glucose produced no effect on whole-cell K_{ATP} channel activity. As shown in Figure 3A, there was no significant difference in K_{ATP} currents between treatment with 3 mM glucose alone and treatment with 3 mM glucose plus insulin (p > 0.05).

To confirm intracellular Ca^{2+} and PKA dependency of the effect of glucose on whole-cell K_{ATP} channel activity, we also examined the influence of 1 hr incubation with 17 mM glucose plus the membrane-permeable Ca^{2+} chelator BAPTA/AM at 0.1 mM concentration or the myristoylated PKA inhibitor 14-22 amide (PKI), a membrane-permeable specific PKA inhibitor, at 20 μM concentration. As shown in Figure 3B, addition of BAPTA/AM (n = 23) or PKI (n = 23) efficiently abolished 17 mM glucose-induced enhancement of whole-cell K_{ATP} channel activity in β cells dialyzed with 0.3 mM ATP/ADP and exposed to 0.3 mM diazoxide. K_{ATP} currents in cells incubated with 17 mM glucose plus BAPTA/AM or PKI did not differ significantly from those in cells treated with 3 mM glucose alone (p > 0.05) but were significantly decreased in comparison with those in cells incubated with 17 mM glucose (p < 0.01 for 17 mM glucose plus 0.1 mM BAPTA/AM and p < 0.05 for 17 mM glucose plus 20 μM PKI).

Increases in both the activity of whole-cell K_{ATP} channels and the levels of unitary K_{ATP} conductance with unaltered single-channel properties could be due to activation of silent K_{ATP} channels already existing in the plasma membrane and/or an increase in K_{ATP} channel incorporation into the plasma membrane. To test for the possible recruitment of K_{ATP} channel subunits from intracellular compartments to the plasma membrane by high-glucose stimulation, we isolated plasma membrane proteins employing a biotinylation approach. RINm5F cells, representing a homogeneous population of insulin-secreting cells, were used for biotinylation purification of β cell plasma

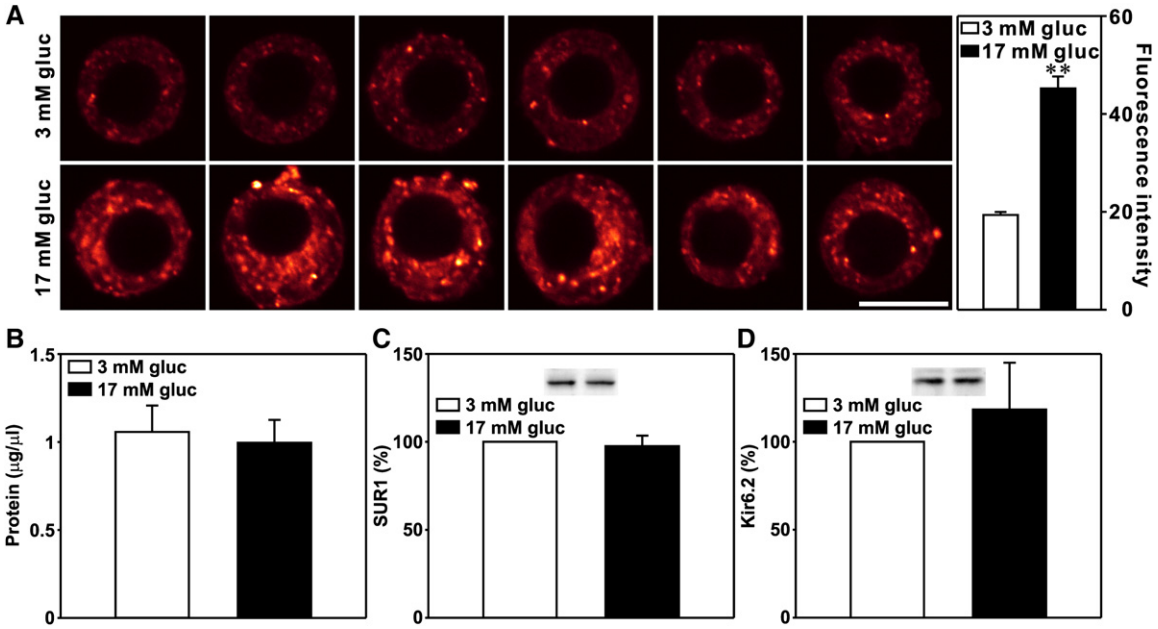

Figure 4. High Glucose Enhances Endocytosis but Does Not Alter the Expression of Total Proteins or K_{ATP} Channel Kir6.2/SUR1 in Pancreatic β Cells

Statistical significance was evaluated by unpaired Student's t test. **$p < 0.01$.

(A) Images at left are representative confocal images showing that FM1-43 accumulation in cells incubated for 1 hr with 3 mM glucose (upper row) was less pronounced than in cells incubated with 17 mM glucose (lower row). Panel at right shows quantification of total FM1-43 accumulation in pancreatic β cells, indicating that 1 hr incubation with 17 mM glucose (black column, n = 52) significantly enhanced FM1-43 accumulation as compared to 1 hr incubation with 3 mM glucose (white column, n = 46). Scale bar = 10 μm.

(B–D) Quantification of total protein concentration and relative abundance of total SUR1 and Kir6.2 subunits in pancreatic β cell homogenates subjected to 1 hr incubation with 3 mM (white columns, n = 6) or 17 mM glucose (black columns, n = 6). There was no significant difference in the total protein concentration (B) and relative abundance of total SUR1 (C) and Kir 6.2 subunits (D) between low- and high-glucose incubation. Insets show representative immunoblots of β cell homogenates subjected to 1 hr incubation with 3 mM (left lanes) and 17 mM glucose (right lanes) and probed with anti-SUR1 (C) and anti-Kir6.2 antibodies (D).

membrane proteins. Immunoblotting analysis of such β cell plasma membrane proteins revealed significant recruitment of the K_{ATP} channel pore-forming subunit Kir6.2 from intracellular compartments to the plasma membrane following glucose incubation. As shown in Figure 3C, treatment with 11 mM glucose significantly increased the Kir6.2 immunoreactivity in the plasma membrane as compared to incubation with 0 mM glucose ($p < 0.05$). Furthermore, addition of the membrane-permeable specific PKA inhibitor PKI at 20 μM effectively ablated the 11 mM glucose-induced increase in Kir6.2 immunoreactivity. The Kir6.2 immunoreactivity obtained from cells treated with 11 mM glucose plus 20 μM PKI was similar to that obtained with 0 mM glucose alone ($p > 0.05$).

Glucose Facilitates β Cell Endocytosis

The increase in plasma membrane K_{ATP} channels by glucose stimulation may also result from reduced endocytosis of the plasma membrane. To examine this possibility, β cell endocytosis was measured following 17 mM glucose incubation. Imaging experiments showed that there was two times more accumulation of the endocytotic marker dye FM1-43 in cells incubated with 17 mM glucose compared to cells incubated with 3 mM glucose (Figure 4A). This demonstrates that 17 mM glucose facilitates β cell endocytosis. Hence, suppression of endocytosis cannot explain our findings.

Glucose Does Not Alter Expression of Total Proteins or K_{ATP} Channel Kir6.2/SUR1

Although immunoblot analysis in combination with isolation of plasma membrane proteins clearly shows a shift in Kir6.2 subunits from intracellular compartments to the plasma membrane, one may still question whether high glucose stimulates the expression of K_{ATP} channel Kir6.2 and SUR1 subunits. To address this issue, we quantified the abundance of total proteins, Kir6.2, and SUR1 in the homogenate of islets exposed to 3 or 17 mM glucose for 1 hr. Figures 4B–4D show that incubation with 17 mM glucose for 1 hr (n = 6) did not significantly alter the abundance of total proteins, Kir6.2, or SUR1 as compared with incubation with 3 mM glucose for 1 hr (n = 6) ($p > 0.05$).

K_{ATP} Channels Localize in Chromogranin-Positive/Insulin-Negative Granules

To determine which subcellular organelles carry intracellular K_{ATP} channels to the β cell plasma membrane, we employed confocal microscopy in conjunction with

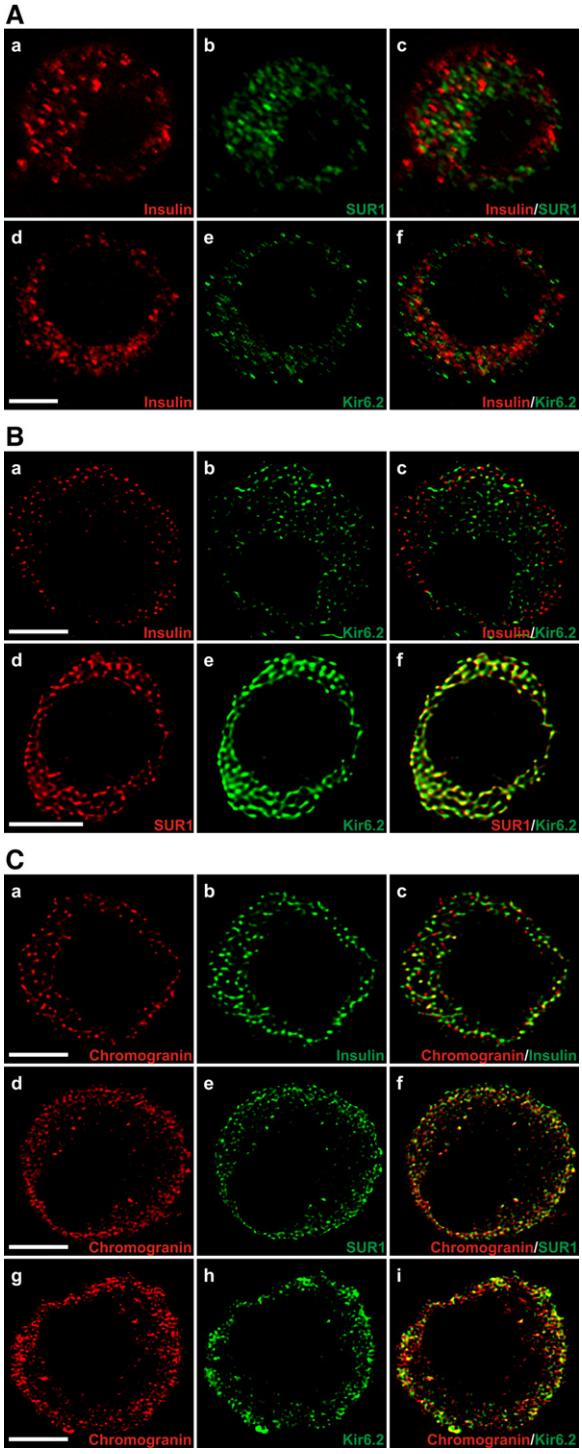

Figure 5. K_{ATP} Channels Localize in Chromogranin-Positive/Insulin-Negative Structures in Pancreatic β Cells

Deconvoluted confocal immunofluorescence images of cultured pancreatic β cells labeled with either monoclonal or polyclonal anti-insulin antibody together with anti-SUR1, anti-Kir6.2, or anti-chromogranin antibody; with anti-SUR1 antibody together with anti-Kir6.2 antibody; or with anti-chromogranin antibody together with anti-SUR1 or anti-Kir6.2 antibody.

(A) A cell incubated with a mixture of anti-insulin and anti-SUR1 antibodies exhibits intense insulin-like immunofluorescence (insulin-LI) (a) and moderately intense SUR1-LI (b) in granule-like structures. A cell incubated with a mixture of anti-insulin and rabbit anti-Kir6.2 antibodies exhibits intense insulin-LI (d) and moderately intense Kir6.2-LI (e) in granule-like structures. The subcellular distribution of insulin-LI (a and d) is significantly different from that of SUR1-LI (b) and Kir6.2-LI (e). No appreciable overlay of insulin-LI with either SUR1-LI (c) or Kir6.2-LI (f) is seen.

(B) A cell labeled with a mixture of anti-insulin and goat anti-Kir6.2 antibodies shows intense insulin-LI (a) and moderately intense Kir6.2-LI (b) in granule-like structures. There is a significant difference in subcellular distribution pattern between insulin-LI (a) and Kir6.2-LI (b). Kir 6.2-LI is not overlaid with insulin-LI (c). A cell subjected to incubation with a mixture of rabbit anti-SUR1 and goat anti-Kir6.2 antibodies displays moderately intense SUR1-LI (d) and Kir6.2-LI (e) in granule-like structures. SUR1-LI (d) distributes very similarly to Kir6.2-LI (e) in the cell and overlaps considerably with Kir6.2-LI (f).

(C) A cell incubated with a mixture of anti-chromogranin and anti-insulin antibodies presents intense chromogranin-LI (a) and insulin-LI (b) in granule-like structures. Chromogranin-LI (a) and insulin-LI (b) spread in a similar manner in the cell. However, chromogranin-LI is only partly colocalized with insulin-LI (c). A cell labeled with a mixture of anti-chromogranin and goat anti-SUR1 antibodies exhibits intense chromogranin-LI (d) and moderately intense SUR1-LI (e) in granule-like structures. SUR1-LI (e) distributes similarly to chromogranin-LI (d). There is a substantial colocalization of SUR1-LI and chromogranin-LI (f) in the cell. A cell subjected to incubation with a mixture of anti-chromogranin and goat anti-Kir6.2 antibodies displays intense chromogranin-LI (g) and moderately intense Kir6.2-LI (h) in granule-like structures. The distribution pattern of Kir6.2-LI (h) is very similar to that of chromogranin-LI (g), and they colocalize substantially in the cell (i). These experiments were performed three times. Scale bars = 4 μm.

deconvolution analysis to localize immunofluorescence-labeled insulin, chromogranin, Kir6.2, and SUR1 subunits in cultured pancreatic β cells. We also used immunogold labeling and electron microscopy to reveal ultrastructural localization of SUR1 subunits in pancreatic β cells.

In the characterization of cultured β cells, the specificity of both monoclonal and polyclonal antibodies against insulin was examined in permeabilized and fixed cells incubated with the primary antibody per se, the primary antibody preabsorbed with bovine insulin, and the primary antibody-omitted solution. The cells incubated with the primary antibody exhibited intense insulin-like immunoreactivity (insulin-LI), which was granular in appearance. However, no specific staining was observed in cells incubated with the primary antibody preabsorbed with bovine insulin or the primary antibody-omitted solution (data not shown). Double immunofluorescence labeling of insulin with Kir6.2 or SUR1 revealed that incubation with the mixture of anti-insulin and anti-SUR1 or anti-Kir6.2 antibodies (from the Seino laboratory, Kobe University Graduate School of Medicine) gave moderately intense Kir6.2-LI and SUR1-LI with clear granule-like structures; these, however, were not colocalized with insulin-LI (Figure 5A). Figure 5A shows a significant difference in the subcellular distribution pattern between insulin- and SUR1- or Kir6.2-LI. To further verify the subcellular distribution of insulin- and Kir6.2-LI, a commercial anti-Kir6.2 antibody was employed. As shown in Figures 5Ba–5Bc, Kir6.2-LI did not colocalize with insulin-LI. Lack of colocalization of either SUR1 or Kir6.2 with insulin made it necessary to ensure that the two associated K_{ATP} channel subunits SUR1

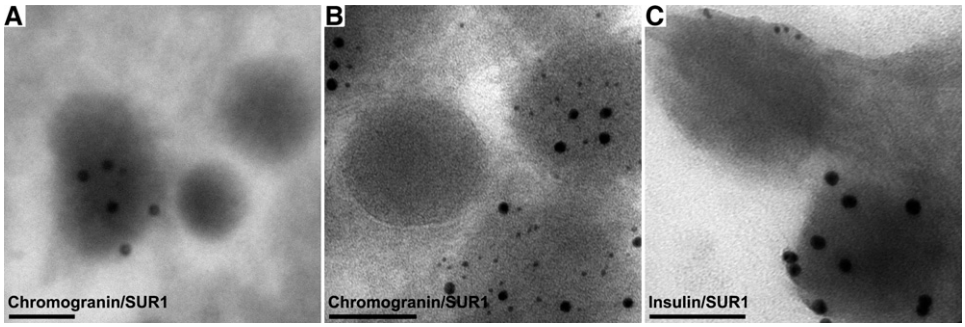

Figure 6. SUR1 Localizes in Chromogranin-Positive/Insulin-Negative Dense-Core Secretory Granules, but Not in Insulin-Containing Granules

Immunoelectron micrographs of pancreatic β cells double-labeled with anti-chromogranin and anti-SUR1 subunit antibodies or anti-insulin and anti-SUR1 subunit antibodies. Immunogold particles (3 nm) corresponding to SUR1 subunits were visualized in dense-core secretory granules in wild-type β cells (B), but not in *sur1* knockout β cells (A). However, immunogold particles (10 nm) representing chromogranin were revealed in dense-core secretory granules in both *sur1* knockout (A) and wild-type β cells (B). Importantly, SUR1 (3 nm) and chromogranin immunogold particles (10 nm) are situated in the same dense-core secretory granules (B), but SUR1 (3 nm) and insulin immunogold particles (10 nm) localize in two different dense-core secretory granules in wild-type β cells (C). Scale bars = 0.1 μm.

and Kir6.2 could be colocalized with the anti-SUR1 and anti-Kir6.2 antibodies used. Figures 5Bd–5Bf show that these two subunits were indeed highly colocalized.

To clarify the identity of intracellular K_{ATP} channel-containing granules, cultured β cells were double-labeled with anti-chromogranin and anti-insulin antibodies or anti-chromogranin and anti-SUR1 or anti-Kir6.2 antibodies. Only a portion of insulin-positive granules contained chromogranin (Figures 5Ca–5Cc). Furthermore, a substantial portion of SUR1-containing granules displayed chromogranin-LI (Figures 5Cd–5Cf). Likewise, a considerable portion of Kir6-2-containing granules exhibited chromogranin-LI (Figures 5Cg–5Ci).

To localize SUR1 subunits at the ultrastructural level, immunogold labeling was performed in wild-type and *sur1* knockout islet cells. Immunogold particles corresponding to SUR1 subunits were visualized in chromogranin-positive dense-core granules in wild-type islet cells (Figure 6B), but not in *sur1* knockout islet cells (Figure 6A). Furthermore, as shown in Figure 6C, SUR1 subunits labeled with smaller gold particles are situated in insulin-negative dense-core granules. Insulin labeled with larger gold particles appears in another type of dense-core granules. SUR1 subunits and insulin did not colocalize in the same granules (see also Figure S1 in the Supplemental Data available with this article online).

There is discrepancy between our observations and a report by Geng et al. (2003) indicating that the insulin secretory granule is the major site of K_{ATP} channels in the β cell. There are several possible explanations for such a discrepancy. First, the crossreaction of the antibody used against insulin with proinsulin should be seriously considered. It is well known that the transport vesicles from the rough endoplasmic reticulum contain unsorted proteins (Figure 7). Therefore, the transport vesicles in the β cell could be labeled with not only the anti-SUR1 and/or anti-Kir6.2 antibodies but also the antibody recognizing proinsulin. The previous report did not rule out this possibility (Geng et al., 2003). Second, a difference in data analysis could also result in the discrepancy between our observations and those of Geng et al. (2003). We randomly analyzed immunostained cells, whereas the authors of the previous report chose cells exhibiting small uniform immunofluorescent puncta. Third, our deconvolution analysis definitely excludes the possibility that fluorescence spreading results in artificial overlapping of labeled Kir6.2 or SUR1 subunits with labeled insulin. Finally, overexpression of Kir6.2-GFP and SUR1-GFP as well as labeling with the fluorescence-labeled sulfonylurea compounds green glibenclamide BODIPY FL and red glibenclamide TR were used as additional techniques to address localization of K_{ATP} channels in insulin secretory granules in the Geng et al. (2003) study. Overexpression can cause spillover of overexpressed proteins into abnormal subcellular compartments and pathways where endogenous proteins do not normally reside. Furthermore, GFP tagging can severely interfere with tagged protein localization and trafficking by masking signal sequences encoded in these proteins that control their distribution and trafficking. Sulfonylurea binding sites can be located at some other molecules in addition to K_{ATP} channel SUR1 subunits (Barg et al., 1999; Deeley et al., 2006). Here, we rely on techniques characterizing endogenous K_{ATP} channels to draw our conclusions.

DISCUSSION

The present study shows that glucose stimulation efficiently recruits functional K_{ATP} channel Kir6.2 and SUR1 subunits to the β cell plasma membrane. Our electrophysiological data indicate that the increased whole-cell K_{ATP} channel activity promoted by high glucose is due to an elevated number of K_{ATP} channels rather than an enhanced function of channels already existing in the plasma membrane. This is strongly supported by the fact that high-glucose incubation significantly increased the number of

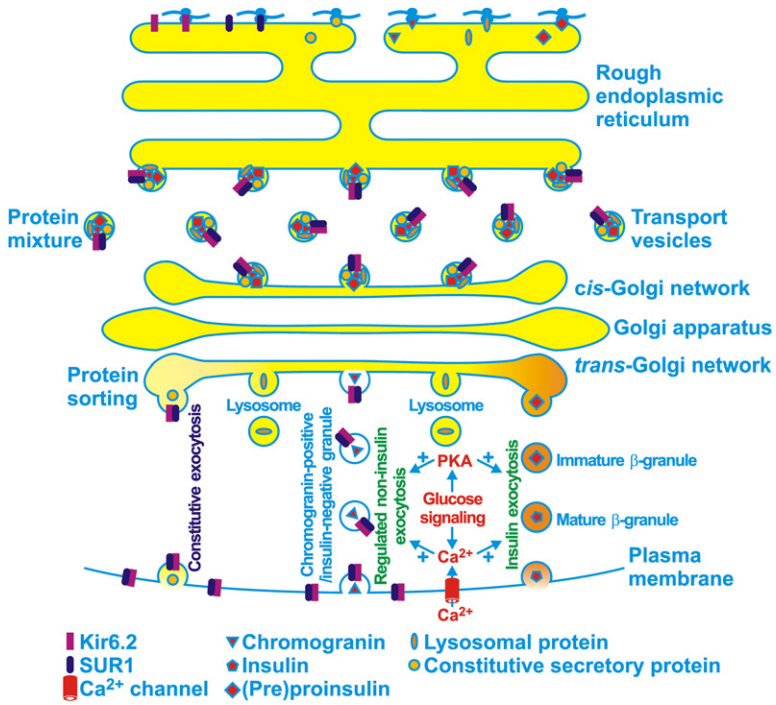

Figure 7. Schematic Diagram of Glucose-Induced Recruitment of SUR1 and Kir6.2 Subunits

Glucose stimulation not only triggers insulin exocytosis but also promotes K_{ATP} channel SUR1 and Kir6.2 subunit recruitment via chromogranin-positive/insulin-negative dense-core secretory granules to the β cell plasma membrane in a Ca^{2+}- and PKA-dependent manner. This model is supported by (1) localization of intracellular K_{ATP} channels in the chromogranin-positive/insulin-negative dense-core secretory granules, (2) translocation of intracellular K_{ATP} channels to the plasma membrane by glucose stimulation, and (3) ablation of glucose-induced K_{ATP} channel translocation by inhibition of PKA as well as elimination of extracellular or intracellular Ca^{2+}.

K_{ATP} channels in inside-out patches but did not change single K_{ATP} channel behavior in either inside-out or on-cell patches. The electrophysiological experiments are verified by evaluation of K_{ATP} channel pore-forming subunit Kir6.2 translocation by combining immunoblot analysis and biotinylation purification of plasma membrane proteins. The results demonstrate that glucose efficiently recruits Kir6.2 subunits to the β cell plasma membrane.

Increases in the number of functional K_{ATP} channels and the abundance of Kir6.2 subunit proteins in the β cell plasma membrane by glucose stimulation can be attributed to numerous possibilities. However, our data exclude the possible involvement of autocrine action of insulin, alteration in endocytosis, or changes in K_{ATP} channel gene expression as evidenced by the following findings. First, insulin was unable to mimic the effect of high glucose on K_{ATP} channel translocation. Second, as expected, high glucose significantly facilitated β cell endocytosis, which cannot explain the increase in K_{ATP} channels in the β cell plasma membrane. Third, there was no significant difference in the abundance of total proteins or protein levels of Kir6.2 and SUR1 subunits between high- and low-glucose treatments. Exclusion of these possibilities makes the role of exocytosis in the glucose-promoted K_{ATP} channel recruitment unambiguous.

Although exocytosis appears to mediate this glucose-promoted K_{ATP} channel recruitment, the specific secretory pathway involved is not clear. Dogmatically, it has been believed that neurons and endocrine and exocrine cells are equipped with only two types of secretory pathways: a regulated pathway for release of secretory molecules, such as neurotransmitters, hormones, and enzymes, and a constitutive pathway to renew integral membrane proteins and lipids. On the contrary, other types of cells were thought to possess only the constitutive pathway and are consequently called constitutive cells. However, experimental evidence has revealed that constitutive cells are also equipped with the regulated secretory pathway (Chavez et al., 1996; Coorssen et al., 1996). This pathway does not resemble the regulated pathway in neurons and endocrine and exocrine cells. The regulated secretory pathway in constitutive cells is indeed under the influence of $[Ca^{2+}]_i$ and activation of PKA and PKC (Chavez et al., 1996; Coorssen et al., 1996). However, it delivers integral membrane proteins instead of secretory molecules from the *trans*-Golgi network to the plasma membrane.

The β cell, as a specialized secretory cell, uses a highly differentiated regulated secretory pathway to serve its primary function, i.e., insulin secretion (Ashcroft and Rorsman, 1989; Molinete et al., 2000). As with all eukaryotic cells, a constitutive secretory pathway also operates in the β cell to renew its integral membrane proteins and lipids (Halban and Irminger, 1994). However, it is not known whether the β cell is also equipped with a regulated secretory pathway similar to that existing in constitutive cells and known to transport integral membrane proteins. Our data obtained by immunofluorescence labeling and confocal microscopy in combination with image deconvolution demonstrate that SUR1 and Kir6.2 subunits localize in abundant granule-like structures but do not colocalize with insulin. Interestingly, we found that a portion of insulin-containing granules are not equipped with the well-known regulated secretory granule marker chromogranin (Taupenot et al., 2003). More interestingly, Kir6.2 and SUR1 subunits are situated in the chromogranin-positive/insulin-negative granules. Importantly, our ultrastructural data

verify that that SUR1 localizes in chromogranin-positive/insulin-negative dense-core secretory granules, but not in insulin-containing granules. These results suggest that β cells are not likely to use the insulin secretory pathway to transport SUR1 or Kir6.2 subunits to the plasma membrane. The chromogranin-positive/insulin-negative dense-core granules equipped with K_{ATP} channels are unlikely to be classical constitutive vesicles since the latter bud off from the *trans*-Golgi network and immediately go to the plasma membrane in an unregulated manner. Therefore, the intracellular K_{ATP} channel-localizing granules represent regulated non-insulin-containing secretory granules (Figure 7).

It is reasonable to extrapolate from the present work that other neurosecretory cells, such as neurons and chromaffin cells, or even other islet cells may also be equipped with chromogranin-positive/transmitter- or hormone-negative secretory granules. Such secretory granules may transport K_{ATP} channels and other plasma membrane proteins, e.g., receptors, transporters, and other ion channels, to the plasma membrane to serve as a particular type of regulatory mechanism for plasma membrane protein density.

Interestingly, the increase in K_{ATP} channels in the plasma membrane caused by glucose stimulation is blocked by elimination of extracellular or intracellular Ca^{2+}. This glucose-promoted K_{ATP} channel recruitment is also ablated by inhibition of PKA, as evidenced by patch-clamp experiments and immunoblot analysis/biotinylation purification. These data indicate that the K_{ATP} channel-containing dense-core granules undergo regulated exocytosis dependent on $[Ca^{2+}]_i$ and PKA activity. Such regulated exocytosis in β cells resembles the regulated secretory pathway in constitutive cells since both recruit integral membrane proteins to the plasma membrane and are regulated by $[Ca^{2+}]_i$ and PKA phosphorylation. In accordance with our findings, previous evidence indicates that K_{ATP} channel trafficking is regulated by PKA phosphorylation. For example, PKA activation plays an important role in the regulation of the budding off of secretory granules from the *trans*-Golgi network (Muniz et al., 1997). The phosphorylation of SUR1 and Kir6.2 subunits has been demonstrated to regulate K_{ATP} channel trafficking (Beguin et al., 1999; Hu et al., 2003). The number of functional K_{ATP} channels in the β cell plasma membrane increases dramatically following PKA-mediated phosphorylation (Beguin et al., 1999).

It is well known that K_{ATP} channel translocation to the plasma membrane is mediated by constitutive exocytosis in nonsecretory cells (Zerangue et al., 1999). The present study suggests that pancreatic β cells also translocate K_{ATP} channels through a regulated exocytotic pathway following high-glucose stimulation. This pathway relies on chromogranin-positive/insulin-negative dense-core granules and is regulated by $[Ca^{2+}]_i$ and PKA activity. This mechanism responsible for the regulation of K_{ATP} channel density endows glucose with an additional function in the β cell—namely, to facilitate functional protein translocation by stimulating regulated non-insulin exocytosis.

The sensitivity of β cells to glucose stimulation depends critically on the copy number of plasma membrane K_{ATP} channels (Nichols, 2006). Indeed, opening β cell K_{ATP} channels results in β cell membrane hyperpolarization and, thereby, inhibition of insulin secretion (Koster et al., 2005). High glucose closes β cell K_{ATP} channels. It may seem that the glucose-promoted K_{ATP} channel recruitment to the plasma membrane should produce inhibitory or null effects on insulin secretion. However, functional consequences of K_{ATP} channel recruitment should be considered not only in the sustained period of high glucose but also in the transition phase from low to high and high to low glucose, with regard to both electrical and nonelectrical aspects. Hence, β cell K_{ATP} channel recruitment may be a prerequisite for proper β cell function. The resting membrane potential (approximately −70 mV) of β cells is determined by the high proportion of opened K_{ATP} channels and a very small fraction of unknown channels probably mediating Na^+ influx (Ashcroft and Rorsman, 1989). Therefore, the resting membrane potential of β cells is slightly more positive than K^+ equilibrium potential (approximately −75 mV). From the Goldman equation and the Nernst equation (Hille, 2001; Kandel et al., 2000), it can be predicted that the glucose-promoted increase in K_{ATP} channel number will result in a somewhat more hyperpolarized plasma membrane when the glucose concentration falls. This will result in less steady-state inactivation of voltage-gated Ca^{2+} channels and, thereby, a more pronounced insulin release subsequent to stimulation.

In addition, an increased number of K_{ATP} channels should make repolarization of the β cell plasma membrane more efficient at lowering glucose concentration, which is essential for glucose homeostasis. Therefore, the maintenance of an appropriate number of K_{ATP} channels in the β cell plasma membrane endows this cell with an efficient capability to respond electrically to changes in blood glucose concentration. Moreover, the K_{ATP} channel, and in particular the SUR1 subunit, not only couples glucose stimulation to insulin secretion through changes in β cell membrane potential but also is likely to serve as a constituent of the exocytotic machinery to mediate insulin exocytosis (Eliasson et al., 2003). It has been demonstrated that the complex interaction of SUR1 subunits, cAMP-GEFII, Piccolo, RIM2, Rab3, and $Ca_V1.2$ subunits plays an essential role in cAMP-dependent/PKA-independent insulin secretion (Ozaki et al., 2000; Shibasaki et al., 2004). *sur1* knockout β cells show much less cAMP-dependent/PKA-independent insulin secretion (Eliasson et al., 2003).

Physiologically, the glucose-promoted K_{ATP} channel recruitment likely functions as a feedback mechanism to electrically and nonelectrically control glucose-stimulated insulin secretion and thereby plays an important role in glucose homeostasis. It may also, at least in part, constitute the molecular mechanism of β cell memory to glucose stimulation operating in human, rat, and mouse β cells (Grill et al., 1978; Grodsky, 1972; Nesher and Cerasi, 2002; Straub and Sharp, 2002; Zawalich and Zawalich, 1996). Pathophysiologically, impairments of glucose-promoted K_{ATP} channel recruitment may be involved in the

development of diabetes since glucose-stimulated insulin secretion fails to operate properly in K_{ATP} channel knockout mice and since mice with a decreased number or complete loss of β cell K_{ATP} channels become diabetic following dietary stress (Koster et al., 2005; Miki et al., 1998; Remedi et al., 2004; Seghers et al., 2000; Shiota et al., 2002).

EXPERIMENTAL PROCEDURES

Islet Isolation and β Cell Culture

Islets of Langerhans were isolated from adult male and female mice, and single β cells from these islets were cultured as previously described (Juntti-Berggren et al., 2004).

Electrophysiology

Conventional whole-cell, on-cell, and inside-out patch-clamp techniques were used to record whole-cell and unitary K_{ATP} currents in single pancreatic β cells from 12 batches of isolations exposed to 0.3 mM diazoxide (Sigma). The cells were incubated with 3 mM glucose, 3 mM glucose plus insulin (50 μU/ml), 17 mM glucose, 17 mM glucose plus 0.5 mM EGTA, 17 mM glucose plus 1 μM H-89, 17 mM glucose plus 0.1 mM BAPTA/AM, or 17 mM glucose plus 20 μM PKI for 1 hr before recording. For whole-cell recording, electrodes were filled with a solution containing (in mM) 125 KCl, 30 KOH, 1 $MgCl_2$, 10 EGTA, 5 HEPES, 0.3 MgATP, 0.3 KADP (pH 7.2). In cell-attached experiments, electrodes were filled with a solution consisting of (in mM) 145 KCl, 10 NaCl, 1.1 $MgCl_2$, 2.5 $CaCl_2$, 10 HEPES (pH 7.2). For inside-out patch measurements, electrodes were filled with a solution containing (in mM) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES (pH 7.4). During conventional whole-cell and on-cell patch recordings, cells were bathed in the same extracellular solution containing (in mM) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES, and 3 or 17 glucose (pH 7.4). The bath solution for inside-out patch measurements contained (in mM) 125 KCl, 30 KOH, 1 $MgCl_2$, 10 EGTA, 5 HEPES (pH 7.15). Unitary K^+ currents were measured at 0 mV, whereas whole-cell K^+ currents were evoked by 10 mV hyperpolarizing and depolarizing voltage pulses (300 ms duration, 2 s pulse interval). Application of 0.1 mM tolbutamide (Sigma) or 0.1 mM MgATP (Sigma) was used to confirm whole-cell or unitary K_{ATP} currents. All recordings were made at room temperature (~22°C). Whole-cell currents were registered when they stabilized 4–6 min after addition of diazoxide or tolbutamide. The amplitude of whole-cell K^+ currents was normalized by the capacitance of the cells. Acquisition and analysis of data were performed using an Axopatch 200 amplifier and the software program pCLAMP (Axon Instruments).

Isolation of Plasma Membrane Proteins

RINm5F cells cultured in RPMI 1640 medium supplemented with 11 mM glucose were incubated for 1 hr in RPMI 1640 medium without glucose and then for another 1 hr in RPMI 1640 medium containing 0 mM glucose, 11 mM glucose, or 11 mM glucose plus 20 μM PKI. The cells were incubated with 2 mM Sulfo-NHS-LC-Biotin (Roche) for 50 min at 4°C. Subsequently, the cells were washed three times with PBS containing 100 mM glycine and homogenized in buffer containing 20 mM HEPES, 1 mM $MgCl_2$, 2 mM EDTA, and protease inhibitors (Roche). The homogenates were centrifuged at 1000 × g for 1 min to remove cell debris. NP-40 (2%) was added to the resultant supernatants. The samples were then incubated with streptavidin magnetic particles for 40 min at room temperature. The bead-bound proteins were eluted with SDS sample buffer containing 10% β-mercaptoethanol at 100°C for 12 min to obtain biotinylated plasma membrane proteins.

SDS-PAGE and Immunoblot Analysis

SDS-PAGE (Laemmli, 1970) was employed to analyze Kir6.2 and SUR1 subunits. The separated proteins were electroblotted to hydrophobic polyvinylidene difluoride membranes (Hybond-P, Amersham). The blots were incubated overnight at 4°C with rabbit polyclonal antibodies against Kir6.2 (1:2000) and SUR1 subunits (1:2000).

FM1-43 Imaging

Cultured β cells were incubated with 3 or 17 mM glucose medium for 20 min at 37°C. Subsequently, FM1-43 (Molecular Probes) was added to the medium at a concentration of 5 μM, and the cells were further incubated for 40 min. FM1-43 accumulation in the cells was measured with a Leica TCS SP2 confocal laser scanner equipped with a 488 nm Ar laser and connected to a Leica DM IRBE microscope (Leica Microsystems Heidelberg GmbH). FM1-43 was excited by a 488 nm laser line, and the resultant emission was captured using a Leica PL APO 100×/1.40 oil objective at 540–650 nm. Intracellular FM1-43 fluorescence intensity was quantified with Leica Confocal Software.

Immunocytochemistry and Confocal Microscopy

Cultured β cells were treated as previously described (Berggren et al., 2004; Lilja et al., 2001). Three sets of experiments were performed with cultured β cells. In the first set, cells were incubated with mouse monoclonal anti-insulin (1:100, BioGenex) or guinea pig polyclonal anti-insulin (1:200, Nordic BioSite). In the second set, cells were double-stained by combining rabbit polyclonal antibody with either Kir6.2 (1:200) or SUR1 (1:200) with monoclonal anti-insulin (1:100, BioGenex). In the third set, cells were double-labeled with goat polyclonal antibody to Kir6.2 (1:10, Santa Cruz Biotechnology) and rabbit polyclonal antibody to either SUR1 (1:200) or chromogranin (1:200, Abcam), goat polyclonal antibody to Kir6.2 (1:10, Santa Cruz Biotechnology) and guinea pig polyclonal antibody to insulin (1:200, Nordic BioSite), rabbit polyclonal antibody to chromogranin (1:200, Abcam) and guinea pig polyclonal antibody to insulin (1:200, Nordic BioSite), or goat polyclonal antibody to SUR1 (1:10, Santa Cruz Biotechnology) and rabbit polyclonal antibody to chromogranin (1:200, Abcam). The cells were thereafter incubated with goat anti-rabbit IgG coupled to fluorescein isothiocyanate (FITC) (1:100, Vector Laboratories), goat anti-mouse IgG coupled to Texas red (1:80, Vector Laboratories), goat anti-rabbit or anti-guinea pig IgG coupled to Alexa 488 (1:200, Molecular Probes) or Alexa 546 (1:200, Molecular Probes), or donkey anti-goat IgG coupled to Alexa 633 (1:200, Molecular Probes). Omission of the primary antibodies or incubation with nonimmune IgG from corresponding species was used as controls. Preabsorption of anti-insulin (1:200) with bovine insulin (1000 μg/ml) was also performed to evaluate the specificity of the anti-insulin.

The labeled specimens were visualized with a Leica TCS SP2 AOBS confocal laser scanner equipped with 488 nm Ar, 543 nm HeNe, and 633 nm HeNe lasers and connected to a Leica DM LFSA microscope as previously described (Berggren et al., 2004). Confocal images were processed and deconvoluted with Leica Confocal Software, Huygens Essential (Scientific Volume Imaging), and AQM Advance 6 (Kinetic Imaging).

Immunogold Labeling and Electron Microscopy

Islets were fixed in 3% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) overnight. Sectioning was performed according to Tokuyasu (1973). Basically, fixed islets were cut into 100 nm sections with a cryostat. Sections were placed on carbon-reinforced formvar-coated nickel grids and placed directly on drops of 0.1 M phosphate buffer containing 20 mM glycine for 30 min. The sections were incubated with anti-SUR1 antibody (1:400) together with either anti-insulin (1:200) or anti-chromogranin (1:200) antibody in 0.1 M phosphate buffer containing 0.1% gelatin and 0.1% bovine serum albumin overnight at room temperature. Subsequently, the sections were subjected to incubation with protein A-gold solution for the first labeling followed by incubation with secondary antibody for the second labeling (Wilcke et al., 1995). The grids were examined in a Tecnai 10 electron microscope at 80 kV, and digital images were acquired with a MegaView III camera (AnalySiS).

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/6/3/217/DC1/.

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REFERENCES

Aguilar-Bryan, L., and Bryan, J. (1999). Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr. Rev. *20*, 101–135.

Aguilar-Bryan, L., Nichols, C.G., Wechsler, S.W., Clement, J.P., Boyd, A.E., Gonzalez, G., Herrera-Sosa, H., Nguy, K., Bryan, J., and Nelson, D.A. (1995). Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science *268*, 423–426.

Ashcroft, F.M., and Rorsman, P. (1989). Electrophysiology of the pancreatic β-cell. Prog. Biophys. Mol. Biol. *54*, 87–143.

Barg, S., Renstrom, E., Berggren, P.O., Bertorello, A., Bokvist, K., Braun, M., Eliasson, L., Holmes, W.E., Kohler, M., Rorsman, P., and Thevenod, F. (1999). The stimulatory action of tolbutamide on Ca^{2+}-dependent exocytosis in pancreatic beta cells is mediated by a 65-kDa mdr-like P-glycoprotein. Proc. Natl. Acad. Sci. USA *96*, 5539–5544.

Beguin, P., Nagashima, K., Nishimura, M., Gonoi, T., and Seino, S. (1999). PKA-mediated phosphorylation of the human K_{ATP} channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. EMBO J. *18*, 4722–4732.

Berggren, P.O., Yang, S.N., Murakami, M., Efanov, A.M., Uhles, S., Kohler, M., Moede, T., Fernstrom, A., Appelskog, I.B., Aspinwall, C.A., et al. (2004). Removal of Ca^{2+} channel β_3 subunit enhances Ca^{2+} oscillation frequency and insulin exocytosis. Cell *119*, 273–284.

Bokvist, K., Olsen, H.L., Hoy, M., Gotfredsen, C.F., Holmes, W.F., Buschard, K., Rorsman, P., and Gromada, J. (1999). Characterisation of sulphonylurea and ATP-regulated K^+ channels in rat pancreatic α-cells. Pflugers Arch. *438*, 428–436.

Chavez, R.A., Miller, S.G., and Moore, H.P. (1996). A biosynthetic regulated secretory pathway in constitutive secretory cells. J. Cell Biol. *133*, 1177–1191.

Clement, J.P., Kunjilwar, K., Gonzalez, G., Schwanstecher, M., Panten, U., Aguilar-Bryan, L., and Bryan, J. (1997). Association and stoichiometry of K_{ATP} channel subunits. Neuron *18*, 827–838.

Coorssen, J.R., Schmitt, H., and Almers, W. (1996). Ca^{2+} triggers massive exocytosis in Chinese hamster ovary cells. EMBO J. *15*, 3787–3791.

Deeley, R.G., Westlake, C., and Cole, S.P. (2006). Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. Physiol. Rev. *86*, 849–899.

Eliasson, L., Ma, X., Renstrom, E., Barg, S., Berggren, P.O., Galvanovskis, J., Gromada, J., Jing, X., Lundquist, I., Salehi, A., et al. (2003). SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic β-cells. J. Gen. Physiol. *121*, 181–197.

Geng, X., Li, L., Watkins, S., Robbins, P.D., and Drain, P. (2003). The insulin secretory granule is the major site of K_{ATP} channels of the endocrine pancreas. Diabetes *52*, 767–776.

Grill, V., Adamson, U., and Cerasi, E. (1978). Immediate and time-dependent effects of glucose on insulin release from rat pancreatic tissue. Evidence for different mechanisms of action. J. Clin. Invest. *61*, 1034–1043.

Grodsky, G.M. (1972). A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. J. Clin. Invest. *51*, 2047–2059.

Halban, P.A., and Irminger, J.C. (1994). Sorting and processing of secretory proteins. Biochem. J. *299*, 1–18.

Hille, B. (2001). Ion Channels of Excitable Membranes, Third Edition (Sunderland, MA, USA: Sinauer).

Hu, K., Huang, C.S., Jan, Y.N., and Jan, L.Y. (2003). ATP-sensitive potassium channel traffic regulation by adenosine and protein kinase C. Neuron *38*, 417–432.

Inagaki, N., Gonoi, T., Clement, J.P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995). Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science *270*, 1166–1170.

Juntti-Berggren, L., Refai, E., Appelskog, I., Andersson, M., Imreh, G., Dekki, N., Uhles, S., Yu, L., Griffiths, W.J., Zaitsev, S., et al. (2004). Apolipoprotein CIII promotes Ca^{2+}-dependent β cell death in type 1 diabetes. Proc. Natl. Acad. Sci. USA *101*, 10090–10094.

Kandel, E.R., Schwartz, J.H., and Jessell, T.M. (2000). Principles of Neural Science, Fourth Edition (New York: McGraw-Hill).

Koster, J.C., Permutt, M.A., and Nichols, C.G. (2005). Diabetes and insulin secretion: the ATP-sensitive K^+ channel (K_{ATP}) connection. Diabetes *54*, 3065–3072.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature *227*, 680–685.

Larsson-Nyren, G., and Sehlin, J. (1996). Comparison of the effects of perchlorate and Bay K 8644 on the dynamics of cytoplasmic Ca^{2+} concentration and insulin secretion in mouse β-cells. Biochem. J. *314*, 167–173.

Larsson-Nyren, G., and Sehlin, J. (2002). Anion-selective amplification of glucose-induced insulin secretion. Acta Diabetol. *39*, 41–47.

Lilja, L., Yang, S.N., Webb, D.L., Juntti-Berggren, L., Berggren, P.O., and Bark, C. (2001). Cyclin-dependent kinase 5 promotes insulin exocytosis. J. Biol. Chem. *276*, 34199–34205.

Miki, T., Nagashima, K., Tashiro, F., Kotake, K., Yoshitomi, H., Tamamoto, A., Gonoi, T., Iwanaga, T., Miyazaki, J., and Seino, S. (1998). Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc. Natl. Acad. Sci. USA *95*, 10402–10406.

Molinete, M., Irminger, J.C., Tooze, S.A., and Halban, P.A. (2000). Trafficking/sorting and granule biogenesis in the β-cell. Semin. Cell Dev. Biol. *11*, 243–251.

Muniz, M., Martin, M.E., Hidalgo, J., and Velasco, A. (1997). Protein kinase A activity is required for the budding of constitutive transport vesicles from the trans-Golgi network. Proc. Natl. Acad. Sci. USA *94*, 14461–14466.

Nesher, R., and Cerasi, E. (2002). Modeling phasic insulin release: immediate and time-dependent effects of glucose. Diabetes *51*, S53–S59.

Nichols, C.G. (2006). K_{ATP} channels as molecular sensors of cellular metabolism. Nature *440*, 470–476.

Ozaki, N., Shibasaki, T., Kashima, Y., Miki, T., Takahashi, K., Ueno, H., Sunaga, Y., Yano, H., Matsuura, Y., Iwanaga, T., et al. (2000). cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat. Cell Biol. *2*, 805–811.

Remedi, M.S., Koster, J.C., Markova, K., Seino, S., Miki, T., Patton, B.L., McDaniel, M.L., and Nichols, C.G. (2004). Diet-induced glucose

intolerance in mice with decreased beta-cell ATP-sensitive K^+ channels. Diabetes *53*, 3159–3167.

Seghers, V., Nakazaki, M., DeMayo, F., Aguilar-Bryan, L., and Bryan, J. (2000). Sur1 knockout mice. A model for K_{ATP} channel-independent regulation of insulin secretion. J. Biol. Chem. *275*, 9270–9277.

Seino, S., and Miki, T. (2003). Physiological and pathophysiological roles of ATP-sensitive K^+ channels. Prog. Biophys. Mol. Biol. *81*, 133–176.

Shibasaki, T., Sunaga, Y., Fujimoto, K., Kashima, Y., and Seino, S. (2004). Interaction of ATP sensor, cAMP sensor, Ca^{2+} sensor, and voltage-dependent Ca^{2+} channel in insulin granule exocytosis. J. Biol. Chem. *279*, 7956–7961.

Shiota, C., Larsson, O., Shelton, K.D., Shiota, M., Efanov, A.M., Hoy, M., Lindner, J., Kooptiwut, S., Juntti-Berggren, L., Gromada, J., et al. (2002). Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. J. Biol. Chem. *277*, 37176–37183.

Straub, S.G., and Sharp, G.W. (2002). Glucose-stimulated signaling pathways in biphasic insulin secretion. Diabetes Metab. Res. Rev. *18*, 451–463.

Taupenot, L., Harper, K.L., and O'Connor, D.T. (2003). The chromogranin-secretogranin family. N. Engl. J. Med. *348*, 1134–1149.

Tokuyasu, K.T. (1973). A technique for ultracryotomy of cell suspensions and tissues. J. Cell Biol. *57*, 551–565.

Wilcke, M., Hultenby, K., and Alexson, S.E. (1995). Novel peroxisomal populations in subcellular fractions from rat liver. Implications for peroxisome structure and biogenesis. J. Biol. Chem. *270*, 6949–6958.

Yang, S.N., and Berggren, P.O. (2005a). β-Cell Ca_V channel regulation in physiology and pathophysiology. Am. J. Physiol. *288*, E16–E28.

Yang, S.N., and Berggren, P.O. (2005b). $Ca_V2.3$ channel and PKCλ: new players in insulin secretion. J. Clin. Invest. *115*, 16–20.

Yang, S.N., and Berggren, P.O. (2006). The role of voltage-gated calcium channels in pancreatic β-cell physiology and pathophysiology. Endocr. Rev. *27*, 621–676.

Zawalich, W.S., and Zawalich, K.C. (1996). Species differences in the induction of time-dependent potentiation of insulin secretion. Endocrinology *137*, 1664–1669.

Zerangue, N., Schwappach, B., Jan, Y.N., and Jan, L.Y. (1999). A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K_{ATP} channels. Neuron *22*, 537–548.

Zunkler, B.J., Lenzen, S., Manner, K., Panten, U., and Trube, G. (1988). Concentration-dependent effects of tolbutamide, meglitinide, glipizide, glibenclamide and diazoxide on ATP-regulated K^+ currents in pancreatic β-cells. Naunyn Schmiedebergs Arch. Pharmacol. *337*, 225–230.