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# Rapid Report

# Glucose-dependent regulation of rhythmic action potential firing in pancreatic $\beta$-cells by $K_{ATP}$-channel modulation

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**The regulation of a $K^+$ current activating during oscillatory electrical activity ($I_{K,slow}$) in an insulin-releasing $\beta$-cell was studied by applying the perforated patch whole-cell technique to intact mouse pancreatic islets. The resting whole-cell conductance in the presence of 10 mM glucose amounted to 1.3 nS, which rose by 50 % during a series of 26 simulated action potentials. Application of the $K_{ATP}$-channel blocker tolbutamide produced uninterrupted action potential firing and reduced $I_{K,slow}$ by ~50 %. Increasing glucose from 15 to 30 mM, which likewise converted oscillatory electrical activity into continuous action potential firing, reduced $I_{K,slow}$ by ~30 % whilst not affecting the resting conductance. Action potential firing may culminate in opening of $K_{ATP}$ channels by activation of ATP-dependent $Ca^{2+}$ pumping as suggested by the observation that the sarco-endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) inhibitor thapsigargin (4 $\mu$M) inhibited $I_{K,slow}$ by 25 % and abolished bursting electrical activity. We conclude that oscillatory glucose-induced electrical activity in the $\beta$-cell involves the opening of $K_{ATP}$-channel activity and that these channels, in addition to constituting the glucose-regulated $K^+$ conductance, also play a role in the graded response to supra-threshold glucose concentrations.**

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Exposure of pancreatic $\beta$-cells to glucose and other stimulators of insulin secretion, is associated with the appearance of electrical activity (Henquin & Meissner, 1984; Ashcroft & Rorsman, 1989). Over the physiological range of glucose concentrations, this electrical activity consists of oscillations in membrane potential between depolarised plateaux, on which bursts of action potentials are superimposed, separated by repolarised electrically silent intervals. The oscillations in electrical activity are accompanied by changes in the cytoplasmic $Ca^{2+}$ concentration (Santos *et al.* 1991), which in turn give rise to brief pulses (~10 s) of insulin secretion (Gilon & Henquin, 1992; Bergsten, 1995; Barbosa *et al.* 1998). In addition to these rapid bouts of insulin secretion, there is also evidence for a much slower (10 min) oscillatory rhythm both systemically (Lang *et al.* 1979); and in isolated $\beta$-cells (Grapengiesser *et al.* 1991). The relationship between the rapid and slow oscillations is currently unclear.

Although the significance of the membrane oscillations is undisputed, the underlying mechanism remains elusive (review: Sherman, 1996). A problem has been the failure of isolated $\beta$-cells maintained in tissue culture (the standard preparation for patch-clamp experiments) to generate the characteristic bursting pattern (Smith *et al.* 1990; Ämmälä *et al.* 1991; Larsson *et al.* 1996; Kinard *et al.* 1999). We have developed a technique, which enables patch-clamp recordings on $\beta$-cells in intact mouse islets (Göpel *et al.* 1999*a*) and have thereby documented a $K^+$ current, which develops gradually during simulated electrical activity ($I_{K,slow}$; Göpel *et al.* 1999*b*). However, the mechanisms by which tolbutamide and high glucose concentration abolish oscillatory electrical activity and the significance of $I_{K,slow}$ in this context remains enigmatic. A recent study on isolated mouse $\beta$-cells, that did not exhibit any spontaneous membrane potential oscillations, revealed a glucose- and tolbutamide-sensitive current reminiscent of $I_{K,slow}$ (Rolland *et al.* 2002) but the magnitude of the current was small (0.2 nS) and its significance for bursting electrical activity in the intact islet is therefore unclear.

In this study we have extended the characterisation of $I_{K,slow}$ in $\beta$-cells within intact islets. We demonstrate that $I_{K,slow}$ is a mosaic of $K_{ATP}$ channels (48 % of the current) and a tolbutamide-resistant $K^+$ conductance (52 %). Additionally, we provide evidence that $I_{K,slow}$ in bursting $\beta$-cells is modulated by glucose in a range of concentrations

associated with the conversion of bursting electrical activity into uninterrupted action potential firing. Finally, we outline a simple model by which electrical activity, via $Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels, may result in activation of $K_{ATP}$ channels and membrane repolarisation. Brief accounts of some of these observations have previously appeared in abstract form (Göpel *et al.* 1997, 2001; Galvanovskis *et al.* 2000).

## METHODS

All experiments were carried out on $\beta$-cells in intact islets isolated from Naval Medical Research Institute (NMRI) mice. The mice were stunned by a blow to the head and killed by cervical dislocation (procedures approved by the ethical committee at Lund University) and islets were isolated as described previously (Göpel *et al.* 1999*b*). The procedures for perforated patch whole-cell recordings and the criteria used for the functional identification of insulin-releasing $\beta$-cells have been described at length elsewhere (Göpel *et al.* 1999*b*). Pancreatic $\beta$-cells were functionally identified by the generation of bursting electrical activity in the presence of 10–15 mM glucose; the initial concentration of glucose was varied to elicit robust oscillatory electrical activity. Bursts of action potentials were simulated by a sequence of voltage-clamp pulses consisting of depolarisation to −40 from −70 mV for 5 s followed by a series of 26 100-ms voltage-clamp depolarisations between −40 and 0 mV (5 Hz). After the train, the cell was held at –40 mV for up to 20 s to facilitate the observation of outward $K^+$ currents. The standard extracellular medium consisted of (mM): 140 NaCl, 3.6 KCl, 2 $NaHCO_3$, 0.5 $NaH_2PO_4$, 0.5 $MgSO_4$, 5 Hepes (pH 7.4 with NaOH), 2.5 $CaCl_2$ and D-glucose as indicated. The pipette solution was composed of (mM): 76 $K_2SO_4$, 10 NaCl, 10 KCl, 1 $MgCl_2$ and 5 Hepes (pH 7.35 with KOH). Tolbutamide was purchased from Sigma and dissolved in DMSO to produce a stock solution with a concentration of 100 mM. All experiments were conducted at 32–35 °C. During the experiments the islets were superfused with extracellular medium at a rate of 1–2 ml $min^{-1}$. The effects of electrical activity on the membrane conductance is quoted relative to the pre-stimulatory level. Data are presented as mean values ± S.E.M. Statistical significances were evaluated using Student's *t* test.

## RESULTS

### $I_{K,slow}$ hyperpolarises the $\beta$-cell

We have previously proposed that the repolarisation that terminates the bursts of action potentials results from the activation of an outward $K^+$ current, which is turned on gradually during electrical activity ($I_{K,slow}$; Göpel *et al.* 1999*b*). Figure 1*A* and *B* provides a direct demonstration that $I_{K,slow}$ is sufficient to hyperpolarise electrically active glucose-stimulated $\beta$-cells. The experiment commenced in 10 mM glucose to allow recording of glucose-induced electrical activity to ascertain the identity of the cell. The amplifier was subsequently switched into the voltage-clamp mode and $I_{K,slow}$ was elicited by the train of voltage-clamp depolarisations. The current thus elicited is shown on an expanded time base in Fig. 1*B*. The amplifier was finally returned to the current-clamp mode to record the membrane potential. The sustained increase in $K^+$ conductance transiently hyperpolarised the $\beta$-cell (dashed horizontal line in Fig. 1*A*). In a series of 21 experiments conducted in the presence of 10 or 15 mM glucose, the most negative potential attained during the silent phase was −54 ± 2 mV under control conditions and −62 ± 2 mV ($P < 0.0001$) following the train. Electrical activity in the neighbouring cells spreading into the voltage-clamped cell via the gap junction, seen as upside-down burst-like current deflections (cf. Fig. 1*F* in Göpel *et al.* 1999*b*), was unaffected by the activation of $I_{K,slow}$. The small-amplitude bursts observed shortly after electrical stimulation (arrows) we likewise attribute to current spread from neighbouring cells via the gap junctions.

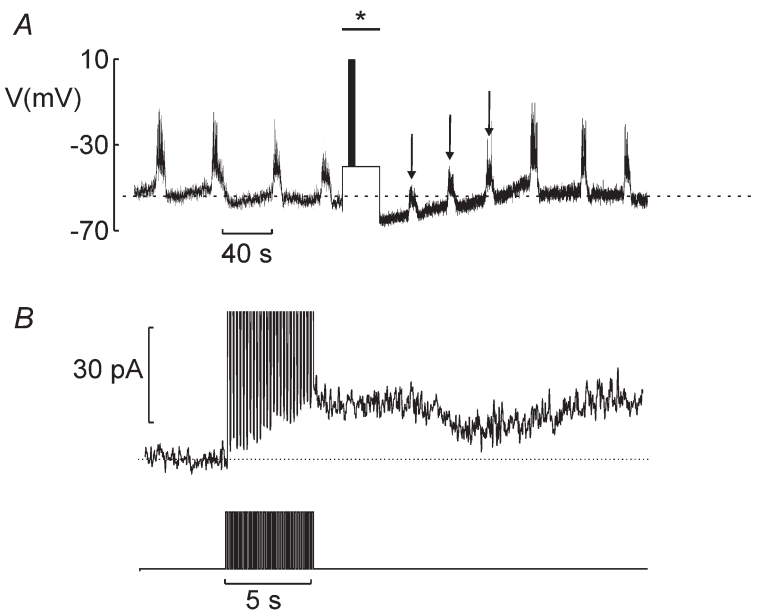


**Figure 1. Activation of $I_{K,slow}$ influences the $\beta$-cell membrane potential**

*A*, electrical activity recorded from a $\beta$-cell in an intact islet. During the period indicated by * and bar, the amplifier was switched into the voltage-clamp mode and the cell subjected to the train of simulated action potentials as indicated. The dashed horizontal line corresponds to the most repolarised potential during two successive bursts before going into the voltage-clamp mode. *B*, $I_{K,slow}$ (top) and stimulation protocol (lower) displayed on an expanded time base. The dotted lines indicate the pre-stimulatory current level.

### Tobutamide-sensitive and tolbutamide-resistant $I_{K,slow}$

Figure 2*A* shows bursting electrical activity recorded from a $\beta$-cell exposed to 10 mM glucose. All cells tested ($n = 15$) responded reversibly to 0.1 mM tolbutamide with the induction of continuous firing of action potentials. The action potentials seen in the presence of tolbutamide often tend to become grouped in short (2 s) bursts separated by brief and shallow (5 mV) repolarised intervals (Fig. 2*B*). At the time indicated by the letters a–c in Fig. 2*A*, the patch-clamp amplifier was switched from current- to voltage-clamp mode and electrical activity simulated by a train of voltage-clamp depolarisations. The evoked $I_{K,slow}$ responses are displayed in Fig. 2*C*. The peak $I_{K,slow}$ measured under control conditions at the end of the train of depolarisations to 0 mV averaged 61 ± 20 pA ($n = 15$). The effect of tolbutamide on electrical activity correlated with a reduction of $I_{K,slow}$ to 20 ± 2 pA ($n = 15$, $P < 0.05$). On average tolbutamide reduced $I_{K,slow}$ by 48 ± 7 % ($P < 0.001$). Thus, $I_{K,slow}$ reflects activation of both tolbutamide-sensitive channels (most likely $K_{ATP}$ channels) and $K_{ATP}$-insensitive $K^+$ channels. Following the removal of tolbutamide, the amplitude of $I_{K,slow}$ gradually returned to the control amplitude (Fig. 2*C*c). The effect of tolbutamide was very variable and ranged between zero (cf. Göpel *et al.* 1999*b*) and 89 %. Thus, some unidentified cellular process (such as cell metabolism) determines the magnitude of $K_{ATP}$ channel component of $I_{K,slow}$. The role of the tolbutamide-resistant current is unclear but it is apparently insufficient for the generation of bursting electrical activity. Consistent with previous observations (Göpel *et al.* 1999*a*), there is little effect of tolbutamide on resting conductance when applied in the presence of ⩾10 mM glucose (Fig. 2*C*; see current response when the membrane potential is stepped from −70 to −40 mV).

### High glucose concentrations abolish the burst pattern by reducing $I_{K,slow}$

Given that a significant fraction of $I_{K,slow}$ flows through $K_{ATP}$ channels and that the $K_{ATP}$-channel blocker tolbutamide shares the ability of the sugar to produce continuous spiking (Fig. 2), it seemed possible that glucose modulates

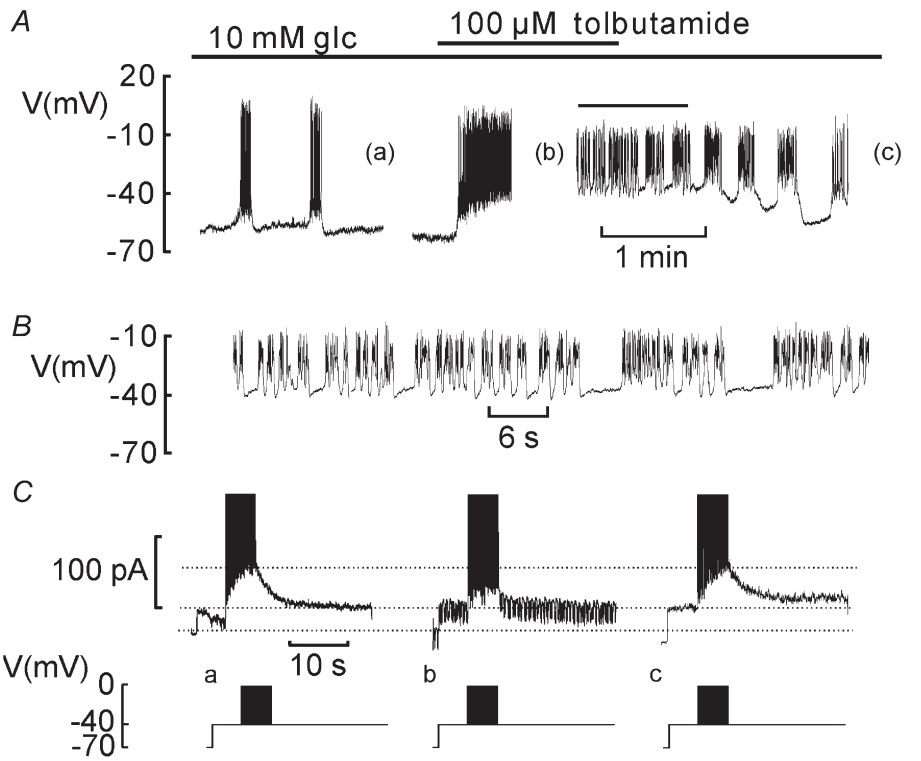


**Figure 2. Tolbutamide-sensitive component of activity-dependent $K^+$ current in bursting $\beta$-cells**

*A*, electrical activity recorded in the presence of 10 mM glucose alone (glc; left), in the presence of 0.1 mM tolbutamide (middle) and after withdrawal of the drug (right). At the times indicted by (a), (b) and (c), the membrane potential recording was interrupted to record $I_{K,slow}$ voltage-clamp currents. *B*, electrical activity in the presence of tolbutamide on an expanded time base. The expanded segment has been indicated in *A* by the horizontal line. *C*, lower, voltage-clamp stimulation protocol. *C*, upper, $I_{K,slow}$ recorded in the presence of 10 mM glucose alone (a), in the presence of 10 mM glucose and 0.1 mM tolbutamide (b) and following wash-out of tolbutamide and the resumption of oscillatory electrical activity (c). The horizontal dotted lines indicate (from top to bottom) the peak current amplitude at 10 mM glucose, the pre-stimulatory current level recorded at – 40 mV and the holding current at – 70 mV.

$\beta$-cell electrical activity by a concentration-dependent reduction of the $K_{ATP}$ channel component of $I_{K,slow}$. Figure 3*A* shows a membrane potential recording from a $\beta$-cell in an intact islet initially exposed to 15 mM glucose. Elevating the concentration of the sugar to 30 mM reversibly converted oscillatory electrical activity into continuous action potential firing. At the times indicated (a and b), the amplifier was switched into the voltage-clamp mode to record $I_{K,slow}$. In the presence of 15 mM glucose, the amplitude of $I_{K,slow}$ was ~50 pA (Fig. 3*B*a). When the same protocol was repeated in the presence of 30 mM glucose, $I_{K,slow}$ only amounted to ~10 pA (Fig. 3*B*b). In a series of 11 experiments, peak $I_{K,slow}$ measured at 15 and 30 mM glucose averaged 45 ± 11 and 30 ± 8 pA, respectively ($P < 0.05$). The percentage decrease was 33 ± 10 %. The induction of uninterrupted action potential firing by high glucose was not associated with any reduction of the resting conductance (estimated from the current response when stepping from −70 to −40 mV), which averaged 0.97 ± 0.13 and 0.89 ± 0.13 nS ($n = 10$) at 15 and 30 mM glucose, respectively. We conclude that the $K_{ATP}$ channels are nearly fully inhibited in the resting state and that electrical activity leads to (partial) reactivation of these channels.

### Effects of thapsigargin and glucose on $I_{K,slow}$

Thapsigargin, an inhibitor of the sarco-endoplasmatic reticulum $Ca^{2+}$-ATPase (SERCA), shares the ability of glucose and tolbutamide to convert oscillatory electrical activity into continuous action potential firing (Fig. 4*A*, cf. Worley *et al.* 1994; Gilon *et al.* 1999). In a series of five experiments, exposure of islets, already exposed to 10 mM glucose, to thapsigargin (4 $\mu$M for 2 min) invariably increased burst duration and resulted in uninterrupted action potential firing in three (of five) cells. Accordingly, the fraction active phase (periods of electrical activity) averaged 37 ± 6 % in the presence of 10 mM glucose alone and increased to 88 ± 8 % ($n = 5$; $P < 0.001$) after exposure to thapsigargin. The latter effect associated with a moderate 24 ± 9 % ($n = 5$; $P < 0.02$) decrease in peak $I_{K,slow}$ (Fig. 4*B*), similar to the inhibition obtained when elevating glucose from 15 to 30 mM (Fig. 3). It was ascertained that the resting conductance, determined from the current response upon depolarisation from –70 to −40 mV, principally reflecting $K_{ATP}$ channel activity and gap-junction conductance, was not affected by thapsigargin (indicated by the two lower dotted lines Fig. 4*B*). In the five experiments in which the effects of thapsigargin on $\beta$-cell electrical activity was tested, the resting conductance

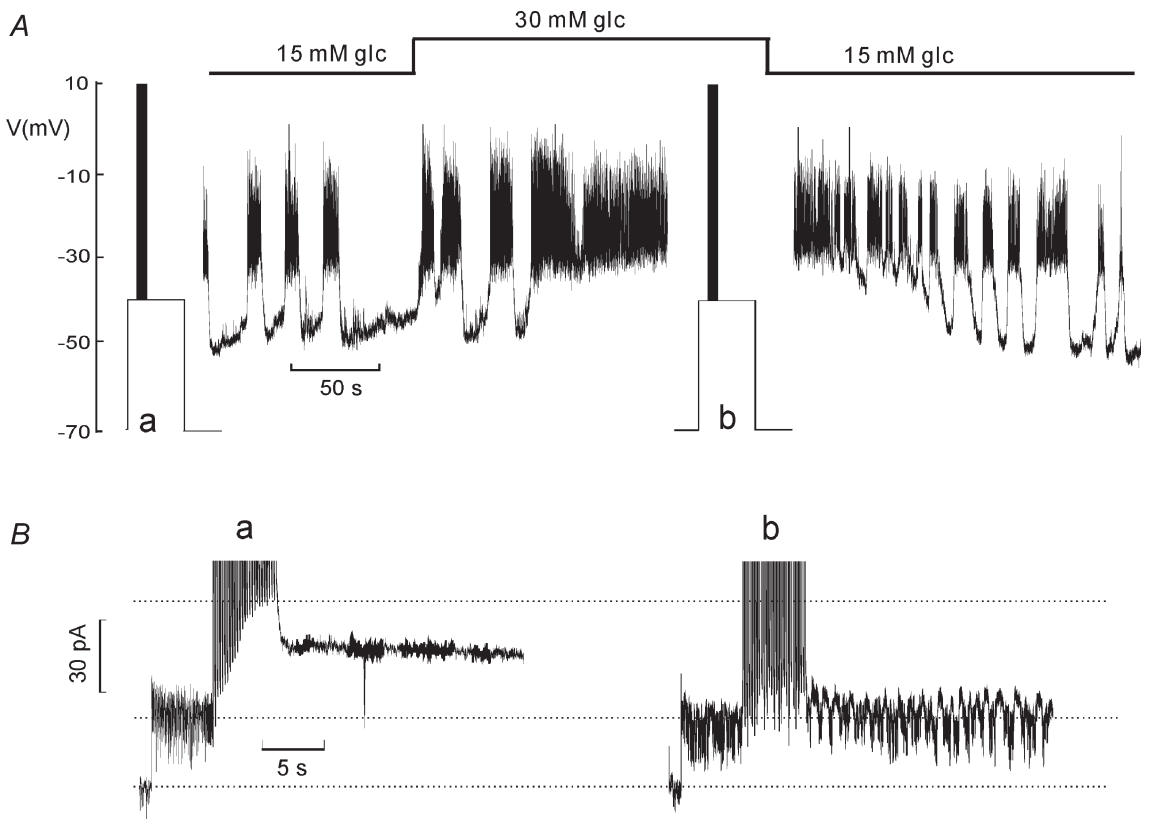


**Figure 3. Glucose modulation of $I_{K,slow}$ and $\beta$-cell electrical activity**

*A*, membrane potential recording. The glucose concentration (glc) was varied between 15 and 30 mM as indicated schematically above. At times indicated (a and b), the amplifier was switched from the current- to voltage-clamp mode to monitor $I_{K,slow}$. *B*, $I_{K,slow}$ recorded at 15 mM (a) and 30 mM (b) glucose. The horizontal dotted lines indicate (from top to bottom) the peak current amplitude at 15 mM glucose, the pre-stimulatory current level recorded at –40 mV and the holding current at –70 mV.

observed in the presence of the SERCA inhibitor averaged 109 ± 4 % of that seen in the absence of the compound making it unlikely that the stimulatory effect of thapsigargin on β-cell electrical activity results from closure of $K_{ATP}$ channels (i.e. a tolbutamide-like effect) or activation of a depolarising cationic conductance (cf. Worley *et al.* 1994).

## DISCUSSION

Glucose-induced insulin secretion is secondary to the induction of β-cell electrical activity. If we are to understand how glucose regulates insulin secretion in a concentration-dependent fashion, then it is essential to establish precisely how glucose modulates electrical activity in the pancreatic β-cell. Here we consider the generation of the β-cell bursts of action potentials at intermediate glucose concentrations and how glucose-dependent regulation of a $K^+$ current elicited by electrical activity ($I_{K,slow}$) may contribute to the graded insulin secretory response.

Based on the effects of tolbutamide, we conclude $K_{ATP}$ channels contribute a significant fraction (48 %) of $I_{K,slow}$. What is the link between electrical activity and opening of $K_{ATP}$ channels? It has previously been demonstrated that $I_{K,slow}$ is dependent on $Ca^{2+}$ influx (Göpel *et al.* 1999*b*; Rolland *et al.* 2002). Although elevation of $[Ca^{2+}]_i$ has been postulated to stimulate mitochondrial metabolism and thus ATP production (Wollheim *et al.* 2000), the net effect of increased $Ca^{2+}$ entry during electrical activity appears to be a reduction of the intracellular ATP:ADP ratio (Detimary *et al.* 1998). This could theoretically result from either stimulated ATP hydrolysis or inhibited ATP production. Indeed, the elevation of $[Ca^{2+}]_i$ during electrical activity has been demonstrated to transiently depolarise the mitochondria and thereby (presumably) reduce ATP production (Krippeit-Drews *et al.* 2001). However, the observation that exposure of β-cells to thapsigargin, which increases basal $[Ca^{2+}]_i$ (Chow *et al.* 1995), often results in permanent depolarisation and uninterrupted firing of action potentials (Fig. 4 and Roe *et al.* 1994) is difficult to reconcile with such a concept. Nevertheless, we believe that the ability of thapsigargin to stimulate electrical activity, whilst not affecting the resting conductance nor the holding current, provides a critical clue to the mechanism underlying the grouping of β-cell action potentials to bursts. Under control conditions, $Ca^{2+}$ entry will be associated with rapid activation of $Ca^{2+}$ pumping leading to ATP hydrolysis and a decreased cytoplasmic ATP:ADP ratio (Detimary *et al.* 1998), culminating in the opening of $K_{ATP}$ channels and membrane repolarisation. By inhibition of SERCA, thapsigargin can be envisaged to exert an ATP-sparing action and under these conditions $Ca^{2+}$ entry is no longer associated with a rapid depletion of sub-membrane ATP. This scenario is supported by the report that up to > 40 % of the total $Ca^{2+}$-dependent ATPase activity in the β-cell is attributable to SERCA (Roe *et al.* 1994). In the presence of the SERCA inhibitor, $Ca^{2+}$ is removed solely by the plasma membrane $Ca^{2+}$-ATPase and the $Na^+$–$Ca^{2+}$ antiporter (Gall *et al.* 1999) resulting in a somewhat higher $[Ca^{2+}]_i$ than under control conditions. Importantly, the rate of ATP hydrolysis under these conditions is sufficiently reduced so that it can (partially) be balanced by concomitant ATP production.

Even if the $I_{K,slow}$ exhibits several features implicating it in the generation of bursting electrical activity in β-cells (gradual turn-on, slow deactivation and sensitivity to

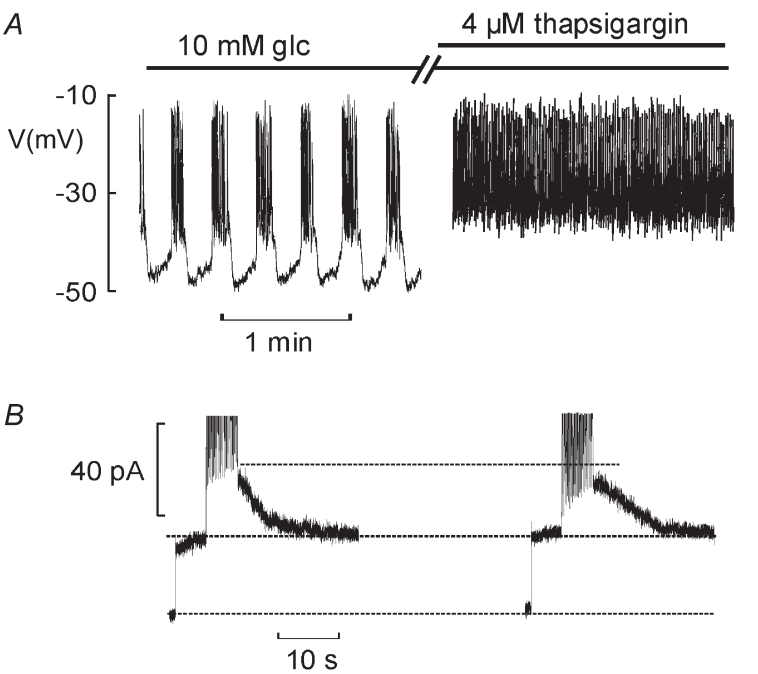


**Figure 4. Modulation of $I_{K,slow}$ and β-cell electrical activity by thapsigargin**

*A*, electrical activity recorded at 10 mM glucose before and after treatment of the islet for 2 min with 4 μM thapsigargin. *B*, $I_{K,slow}$ recorded under control conditions (left) and following treatment with thapsigargin (right). The horizontal lines indicate (from top to bottom) the peak amplitude observed under control conditions, the steady-state currents recorded at –40 mV pre- stimulation and the holding current at –70 mV.

thapsigargin), it remains to be established how glucose both increases the plateau phase and shortens the silent intervals between two successive bursts. It should be noted that in β-cells *in situ*, the relationship between resting conductance and glucose concentration is hyperbolic with a $K_m$ around 5 mM with little further inhibition at glucose concentrations >10 mM (Göpel *et al.* 1999*a*). Increasing glucose to 30 mM has little effect on resting conductance (Fig. 3*B*), so the concentration-dependent modulation of β-cell electrical activity by glucose cannot simply be accounted for by changes of the resting $K_{ATP}$ channel activity. These considerations suggest that $I_{K,slow}$ itself must be regulated by glucose and we demonstrate here that its peak amplitude is decreased by one-third when glucose is elevated from 15 and 30 mM. Given that 48 % of $I_{K,slow}$ flows through $K_{ATP}$ channels, it seems justifiable to assume that inhibition of these channels account for the metabolic regulation of $I_{K,slow}$. Indeed, both the amplitude (15–20 pA) and deactivation kinetics (complete in < 5 s) of $I_{K,slow}$ observed at 30 mM glucose concentration are similar to the tolbutamide-resistant component of $I_{K,slow}$ (compare Figs. 2*C*b and 3*B*b) suggesting that glucose acts by suppressing the tolbutamide-sensitive component. We therefore propose that elevating glucose from an intermediate (e.g. 15 mM) to a maximally stimulatory concentration accelerates cellular metabolism sufficiently to balance the impact of stimulated $Ca^{2+}$-ATPase activity on the sub-membrane ATP:ADP ratio and thus prevents activation of the $K_{ATP}$ channels. Such a mechanism would easily account for the concentration-dependent increase in burst duration observed in response to increasing glucose concentrations and is perfectly compatible with the fact that β-cell oxidative metabolism and the amount of electrical activity increase in parallel (Atwater *et al.* 1979; Malaisse *et al.* 1984).

The finding that $K_{ATP}$ channels contribute to the cyclic increase in $K^+$ permeability occurring during oscillatory electrical activity has important functional implications. For example, the capacity of tolbutamide to produce continuous action potential firing becomes easy to understand if sulphonylurea-sensitive $K_{ATP}$ channels participate in the generation of the bursts. The ability of every β-cell within the intact islet to respond in a graded fashion to elevated glucose levels with enhanced electrical activity, which in turn accounts for the sigmoidal relationship between plasma glucose concentrations and insulin release observed systemically, can also be explained by a concentration-dependent inhibition of $K_{ATP}$ channels reactivated during electrical activity. Thus, the role of $K_{ATP}$ channels in the β-cell extends beyond merely serving as the glucose-regulated resting conductance, they also contribute to the progressive stimulation of electrical activity and insulin release by supra-threshold glucose levels, i.e. in a range of concentrations where little metabolic modulation of the $K_{ATP}$ channels was formerly believed to occur (Misler *et al.* 1986; Ashcroft *et al.* 1988; Göpel *et al.* 1999*b*). It remains to be explained why isolated β-cells lack bursting electrical activity but it is interesting that agents that increase intracellular cAMP levels (such as glucagon, normally secreted from neighbouring α-cells) induce both rapid membrane potential (Ämmälä *et al.* 1991) and $[Ca^{2+}]_i$ oscillations (Grapengiesser *et al.* 1991). It should therefore be considered whether the absence of rapid glucose-induced membrane potential oscillations in isolated β-cells is a consequence of the loss of paracrine signalling normally operating in the intact islet, which in turn may affect cell metabolism and/or the copy number of $Ca^{2+}$-ATPases and ion channels.

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