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Evidence that Glucose-Induced Electrical Activity in Rat Pancreatic β-Cells Does Not Require K_{ATP} Channel Inhibition

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Abstract. K_{ATP}-channel activity, recorded in cell-attached patches from isolated rat pancreatic β-cells, was found to be maximally inhibited in the presence of a substimulatory concentration (5 mM) of glucose, with no further effect of higher, stimulatory glucose concentrations. K_{ATP} channel-independent effects of glucose on electrical activity were therefore investigated by incubating cells in the presence of a supramaximal concentration of tolbutamide. Addition of tolbutamide (500 μM) to cells equilibrated in the absence of glucose resulted in a rapid depolarization and electrical activity followed by a gradual repolarization and disappearence of electrical activity. Repolarization was not due to desensitization of K_{ATP} channels to the sulfonylurea, but was probably the result of activation of another K^+ conductance. The subsequent application of 16 mM glucose in the continued presence of tolbutamide depolarized the cells again, leading to renewed electrical activity. Input conductance of the cells was markedly reduced by tolbutamide, reflecting K_{ATP}-channel inhibition, but was not significantly affected by the addition of glucose in the presence of the drug.

In cells voltage-clamped at −70 mV, addition of glucose in the presence of tolbutamide generated a noisy inward current, probably representing activation of the volume-sensitive anion channel. K_{ATP} channel-independent activation of electrical activity by glucose was inhibited by the anion channel inhibitor 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid. It is concluded that the induction of electrical activity in rat pancreatic β-cells does not require inhibition of K_{ATP} channels. The K_{ATP} channel-independent mechanism could involve, at least in part, activation by glucose of the volume-sensitive anion channel.

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Introduction

Glucose-induced insulin release from the pancreatic β-cell is associated with a characteristic pattern of Ca^{2+}-dependent electrical activity (*see* Ashcroft & Rorsman, 1989; Rorsman, 1997 for reviews). In essence, a rise in glucose concentration depolarizes the β-cell, resulting in activation of voltage-dependent Ca^{2+} channels, which is manifest as a distinctive pattern of repetitive spiking. This effect of glucose requires metabolism of the sugar in the β-cell and the generation of one or more intracellular metabolic signal(s). The precise identity of the coupling mechanism between glucose metabolism and β-cell depolarization is unknown. However, the 'Concensus model' proposes that depolarization is due to closure of K_{ATP} channels, possibly due to an increase in intracellular [ATP] (Rorsman & Trube, 1985) and/or ATP/ADP ratio (Dunne & Petersen, 1986) resulting from increased metabolic flux. Hypoglycaemic sulfonylureas are thought to stimulate insulin release by a direct inhibition of the K_{ATP} channel (Sturgess et al., 1985).

Whilst the activity of K_{ATP} channels is undoubtedly sensitive to changes in glucose concentration, there is increasing evidence that glucose can influence insulin release by one or more K_{ATP} channel-independent mechanisms. Thus, glucose has been shown to induce a rapid and pronounced stimulation of insulin release even when K_{ATP} channel activity is blocked by tolbutamide (Best, Yates & Tomlinson, 1992; Straub et al., 1998). Using an alternative experimental approach, it has been shown that glucose

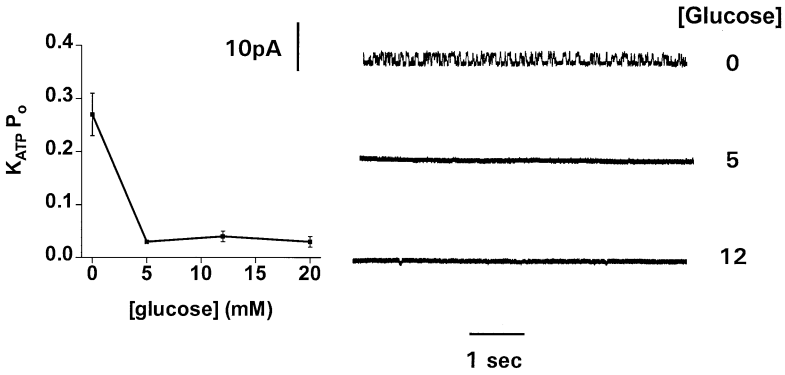

Fig. 1. Cell-attached recordings of K_{ATP}-channel activity in rat pancreatic β-cells: inhibition by glucose. *Left panel*: Effects of different glucose concentrations on channel mean open probability (K_{ATP} P_o). Values are means ± SEM from 9–16 cells. *Right panel*: Recordings from a typical cell-attached patch, in this case containing one active channel.

can also evoke insulin release when K_{ATP} channels are opened by diazoxide (Gembal, Gilon & Henquin, 1992; Gembal et al., 1993; Straub et al., 1998; Westerlund et al., 2001). It is generally believed that these K_{ATP} channel-independent actions of glucose require metabolism of the sugar, are independent of protein kinase A or C activation, and are probably 'non-ionic', that is, exerted at points distal to changes in membrane potential and Ca^{2+} entry into the cell (Komatsu et al., 1997, Aizawa et al., 1998, Henquin, 2000).

The purpose of the present study was to investigate whether K_{ATP} channel inhibition is a prerequisite for glucose-induced electrical activity in rat pancreatic β-cells. It is demonstrated that K_{ATP}-channel activity is maximally inhibited in the presence of a substimulatory concentration of glucose. It is also shown that glucose can cause depolarization and electrical activity in the β-cell under conditions where K_{ATP} channel activity is already maximally inhibited by a supra-maximal concentration of the sulfonylurea tolbutamide. This finding strongly suggests the existence of other ionic mechanisms through which glucose metabolism can regulate β-cell electrical and hence secretory activity.

Materials and Methods

Rat pancreatic islets were isolated by collagenase digestion and dispersed into single cells and small clusters by a brief incubation in Ca^{2+}-free medium consisting of (mM) NaCl, 135; KCl, 5; $MgSO_4$, 1; glucose, 4; EGTA, 1 and HEPES-NaOH, 10 (pH 7.4). Cells were suspended in HEPES-buffered MEM medium (Gibco, Paisley, Scotland) containing 5% (v/v) fetal calf serum, plated onto 30-mm diameter polystyrene dishes and cultured for 2–7 days in humidified air at 37°C. Cells were superfused at a rate of approximately 2 ml/min with a solution consisting of (mM) NaCl, 135; KCl, 5; $MgSO_4$, 1; NaH_2PO_4, 1; $CaCl_2$, 1.2; HEPES-NaOH, 10 (pH 7.4) containing test substances at the required concentration.

K_{ATP}-channel activity was recorded in cell-attached patches. The pipette solution contained (mM) KCl, 140; $MgCl_2$, 2 and HEPES-KOH, 10 (pH 7.4) with a pipette potential of 0 mV such that channel activation generated an inward current. Channel-open probability was assessed from 30-sec segments of recording using pClamp 6 software (Axon Instruments, Foster City, CA,). β-cell membrane potential and whole-cell currents were recorded using the perforated-patch technique (Rae et al., 1991) in current-clamp and voltage-clamp modes, respectively, with a List EPC-7 amplifier (List, Darmstadt, Germany). The pipette solution consisted of (mM) K_2SO_4 60; KCl, 10; NaCl, 10; HEPES-NaOH, 10 (pH 7.2) and amphotericin B (240 μg/ml). Series resistance was < 25 MΩ and whole-cell capacitance, within the range 8–12 pF. Whole-cell K^+ conductance was measured as outward current in cells voltage-clamped at −40 mV. Inward current was measured at a holding potential of −70mV. This current has been previously described both in whole-cell (Best, 1997, 2000) and cell-attached (Best, 1999) recordings from nutrient-stimulated rat β-cells. The reversal potential of the current, the fact that it can be evoked by hypotonic cell swelling in addition to nutrient stimuli, and the sensitivity of the current to anion channel-inhibitors have led to the suggestion that the current represents activation of the volume-sensitive anion channel (VSAC; Best, 1997; 1999; 2000). This channel has been extensively characterized at the whole-cell level (Kinard & Satin, 1995; Best, Sheader & Brown, 1996; Best, Miley & Yates, 1996; Best, Speake & Brown, 2001). Input conductance (G_{input}) was measured by voltage-clamping the cells at −70 mV and applying 200-msec pulses of ±10 mV at 2-sec intervals, essentially as described previously (Smith, Ashcroft & Rorsman, 1990; Best, 2000). G_{input} was calculated from the average amplitudes of the resultant square current excursions during 30-sec segments of recording filtered at 100 Hz via an 8-pole Bessel filter. For all experiments, single isolated β-cells were used in order to avoid contaminating currents from adjacent electrically-coupled cells. Only cells that had a resting membrane potential of −60 mV or more negative in the absence of glucose, and which exhibited electrical activity in response to stimulation with glucose or tolbutamide, were used in this study. All experiments were carried out at 30–32°C. Statistical significance was ascribed using Student's *t*-test.

Collagenase (type 4) was obtained from Worthington (Cambridge Biosciences, Cambridge, UK). Tolbutamide, tetraethylammonium chloride and all other chemicals were obtained from Sigma Chemical, Poole, UK.

Results

The first series of experiments examined the concentration dependence of K_{ATP} channel inhibition by glucose. K_{ATP}-channel activity could be recorded in cell-attached patches from β-cells equilibrated for 10 minutes or more in the absence of glucose (Fig. 1, *right panel*). Under such conditions, the mean open probability (P_o) was 0.27 ± 0.04 ($n = 16$), a value very close to that reported previously (Ashcroft, Ashcroft & Harrison, 1988). After approximately 5

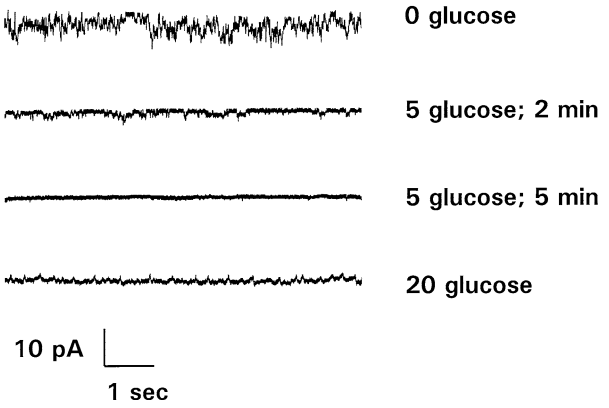

Fig. 2. Cell-attached recordings of K_{ATP}-channel activity in a rat pancreatic β-cell: time-dependence of inhibition by glucose. Channel activity was reduced in terms of current amplitude and open probability within 2 minutes of addition of 5 mM glucose and effectively inhibited after 5 minutes exposure to glucose. An increase in glucose concentration to 20 mM did not further affect channel activity but evoked small, upward action currents.

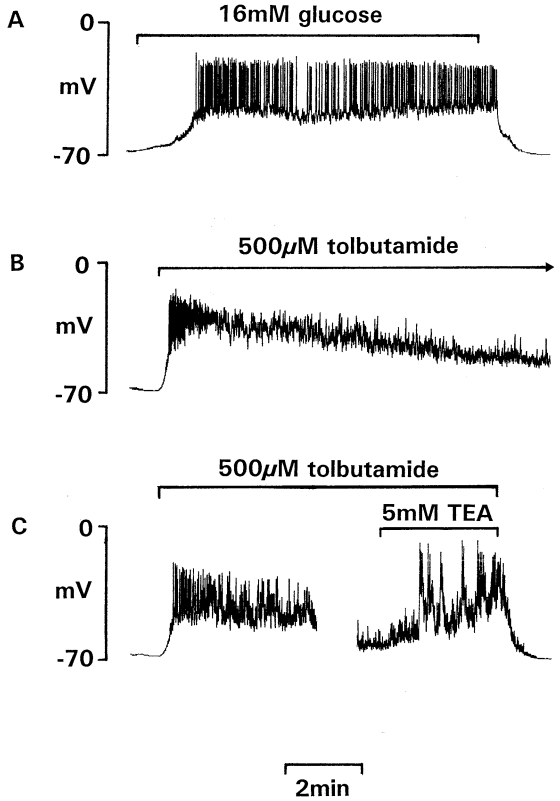

Fig. 3. Electrical activity in isolated rat pancreatic β-cells evoked by glucose and tolbutamide. (*A*) Effect of addition of 16 mM glucose to a cell previously equilibrated in the absence of the sugar. (*B*) Effect of a maximal concentration of tolbutamide (500 μM) in the absence of glucose. (*C*) Effect of tolbutamide followed by. 5 mM tetraethylammonium chloride (TEA). The gap in the lower trace represents a period of 5 min, during which the cells gradually repolarized in the continued presence of tolbutamide. Each recording is representative of at least 5 similar experiments.

minutes in the presence of a sub-stimulatory concentration of glucose (5 mM), K_{ATP}-channel activity was found to be maximally inhibited (P_o = 0.03 ± 0.001; n = 15). A subsequent increase in glucose concentration to 12 or 20 mM, levels previously shown to induce electrical activity in rat β-cells (Miley et al., 1997), had no further effect on channel activity (Fig. 1). Thus, the P_o of K_{ATP} channels was minimal in the presence of 5 mM glucose and was not further affected by raising glucose concentration to levels that would be expected to induce electrical and secretory activity. The inhibition of K_{ATP} channel activity by 5 mM glucose was time-dependent (Fig. 2). This particular cell-attached patch contained 3–4 active channels with a net open-probability (*NP*) of 0.74 in the absence of glucose. After 2 minutes exposure to 5 mM glucose, K_{ATP}-channel activity was still evident, but *NP* was reduced to 0.23. In contrast to a previous report (Ashcroft et al., 1988), exposure to glucose also appeared to reduce current amplitude, in this particular case by approximately 50% (Fig. 2). The reason for this is not clear, but it could in part be due to the fact that the cell membrane potential is higher in the presence of 5 mM glucose than in the complete absence of the sugar (−59 mV *vs.* −71 mV; Best, 2000). A more positive membrane potential would be expected to reduce the driving force for K^+ entry. Following 5 minutes exposure to 5 mM glucose, K_{ATP} channel activity was maximally inhibited (*NP* = 0.02; Fig. 2). Again, application of a stimulatory glucose concentration (20 mM) did not further affect channel activity, but was often found, as in the cell shown in Fig. 2, to generate small outward K^+ action currents arising from rapid depolarizing action potentials.

The above findings suggest that modulation by glucose of K_{ATP} -channel activity may be minimal over the range of glucose effective in generating electrical and secretory activity in the β-cell. The next series of experiments therefore investigated the role of the K_{ATP} channel in modulating β-cell membrane potential and electrical activity. In cells equilibrated for a period of approximately 5–10 minutes in the absence of glucose, the resting membrane potential was −65.5 ± 0.7 mV (n = 13). Addition of 16 mM glucose caused a gradual depolarization of the membrane potential to a plateau point of −45 to −50 mV, at which electrical activity was evoked, consisting of rapid repetitive spiking (Fig. 3*A*). Electrical activity persisted throughout the period of exposure to the high glucose concentration, repolarization only occurring when glucose was removed from the superfusate. Figure 3*B* shows the effect of K_{ATP} channel inhibition using a supramaximal concentration of tolbutamide (500 μM) in cells equilibrated in the absence of glucose. Application of tolbutamide resulted in a rapid depolarization leading to electrical activity.

However, this effect was followed by a period of 8–15 minutes during which the cells gradually repolarized, with a concomitant reduction in electrical activity, despite the continued presence of the sulfonylurea. The newly established membrane potential in the presence of tolbutamide was −55.2 ± 0.9 mV (n = 13). A similar experiment is shown in Fig. 3*C*. Tolbutamide-induced electrical activity was again followed by a gradual repolarization of the membrane potential. In this case, the subsequent application of tetraethylammonium chloride (TEA; 5 mM) in the continued presence of tolbutamide depolarized the cell, leading to a renewed period of electrical activity. A similar though less rapid depolarization was also observed with 100 nM iberiotoxin in the presence of tolbutamide (*not shown*). Upon removal of the K^+-channel blockers from the medium, the cell repolarized to resting levels (−65 to −70 mV). Neither TEA nor iberiotoxin affected β-cell membrane potential in the absence of tolbutamide or glucose (*not shown*).

The effect of prolonged exposure of β-cells to tolbutamide was next examined on K_{ATP} channel activity using two complementary approaches. Figure 4*A* shows a recording of whole-cell K^+ conductance in a cell voltage-clamped at −40 mV. In the absence of glucose, a marked outward current was apparent. The application of 500 μM tolbutamide rapidly inhibited this current, indicating that it was due almost entirely to K_{ATP} channel activity. This inhibition persisted throughout long-term (∼14 min) exposure to the sulfonylurea, such that there was no evidence of desensitization of channel activity. Inhibition of the current was reversible upon withdrawal of the drug. The lack of K_{ATP}-channel desensitization to tolbutamide was confirmed in cell-attached recordings. Figure 4*B* shows a recording from a cell-attached patch containing 3–5 active channels in the absence of glucose. Again, addition of tolbutamide caused a rapid inhibition of K_{ATP} channel activity, which persisted throughout exposure to the sulfonylurea.

The next series of experiments examined whether glucose could cause depolarization and electrical activity in cells where K_{ATP} channel activity was inhibited by tolbutamide. The recording from one such experiment is shown in Fig. 5. The addition of tolbutamide in the absence of glucose again depolarized the cell and generated electrical activity. In this particular cell, this consisted of a regular bursting pattern rather than continuous spiking. However, in common with all other cells studied, the intensity of the electrical activity gradually diminished and ceased, in this case after approximately eight minutes. At this point, application of 16 mM glucose in the continued presence of sulfonylurea was found to depolarize the cell, leading to renewed electrical activity. A similar, though less pronounced effect was observed with 7 mM glucose (*not shown*). Repolarization

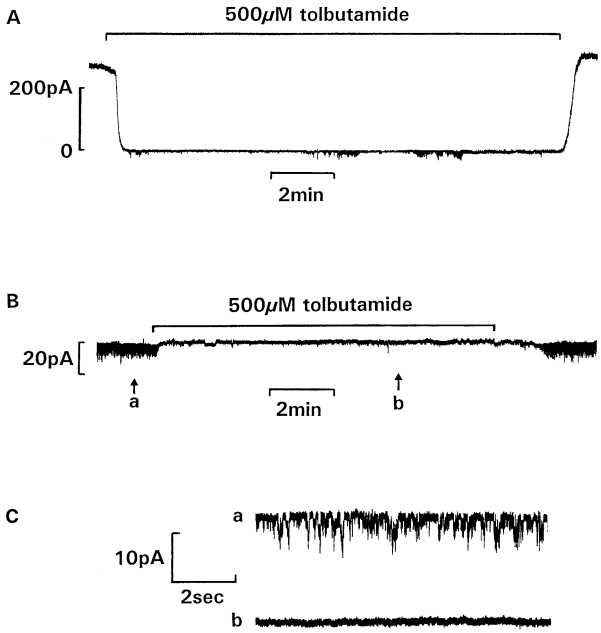

Fig. 4. Prolonged inhibition of β-cell K_{ATP}-channel activity by tolbutamide. (*A*) Perforated-patch recording of whole-cell K^+ conductance in a cell voltage-clamped at −40 mV in the absence of glucose. (*B*) Recording from a cell-attached patch containing 3–5 active K_{ATP} channels in the absence of glucose. (*C*) Channel activity at the times indicated by arrows *a* and *b*, presented at highest time- and current resolution. In both cases *A* and *B*, the cells were continuously exposed to tolbutamide for the period indicated. Each recording is representative of at least 4 similar experiments.

occurred upon removal of the sugar from the superfusate. Figure 6 shows a similar experiment, but in this case, the amplifier was switched to voltage-clamp mode, the cell held at −70 mV and subjected to voltage pulses of ±10 mV in order to measure G_{input} during certain periods of the experiment (marked *a*, *b*, *c*, *d*). In cells equilibrated in the absence of glucose, the resting membrane potential of approximately −67 mV corresponded to a relatively high G_{input} (1.2 nS; period *a*) presumably reflecting high activity of K_{ATP} channels (Smith, Ashcroft & Rorsman, 1990; Best, 2000). Addition of tolbutamide depolarized the cell and induced electrical activity, an effect associated with reduced G_{input} (0.3 nS; period *b*), indicating K_{ATP}-channel inhibition by the drug. After the subsequent repolarization of the cell in the continued presence of tolbutamide, G_{input} remained at a similarly low value (period *c*), consistent with the stable inhibition of K_{ATP} channel activity shown in Fig. 4. No significant change in G_{input} was found to accompany electrical activity evoked by the addition of glucose in the presence of tolbutamide (period *d*). Thus, the pronounced inhibition of whole-cell K_{ATP} conductance by tolbutamide was clearly apparent from measurement of G_{input}, but such measurements

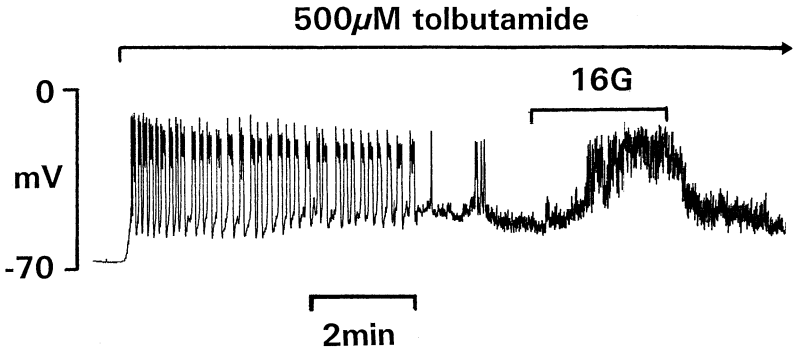

Fig. 5. Glucose induces electrical activity in rat β-cells when K_{ATP} channels are blocked by tolbutamide. Initially, tolbutamide was added to the cell in the absence of glucose. The cell was subsequently exposed to 16 mM glucose (*16G*) for the period marked in the continued presence of tolbutamide. The recording is representative of at least 5 similar experiments.

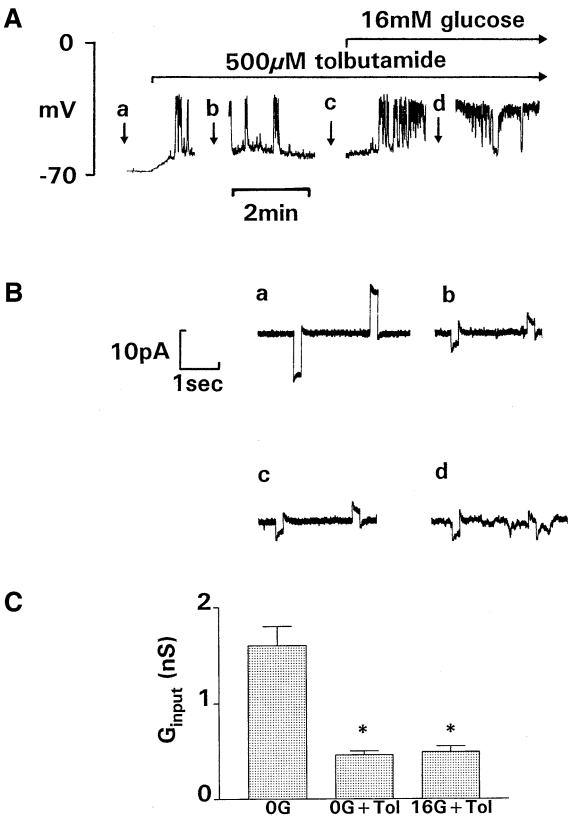

Fig. 6. Membrane potential recordings and input conductance (G_{input}) from isolated rat pancreatic β-cells. (*A*) A similar experiment to that shown in Fig. 3 except that, at the points marked *a*, *b*, *c*, *d*, the amplifier was switched to voltage-clamp, and the cell exposed to 200-msec pulses of ±10 mV from a holding potential of −70 mV in order to measure G_{input}. The resultant current traces are shown in (*B*). These periods *a–d* were of 1 min duration with the exception of period *b* which was approximately 8 mins. Each recording is representative of at least 5 similar experiments. (*C*) Mean values ± SEM of G_{input} in the absence of glucose (*OG*; $n = 9$), absence of glucose but presence of 500 μM tolbutamide (*OG + Tol*; $n = 12$) and 16 mM glucose plus 500 μM tolbutamide (*16G + Tol*; $n = 11$). $^*p < 0.001$ compared to *OG*.

were not sufficiently sensitive to detect the smaller changes in whole-cell conductance associated with activation/inactivation of other membrane conductances, namely those associated with the repolarization and subsequent glucose-induced electrical activity in the presence of tolbutamide (Fig. 6, lower panel).

Electrical activity evoked by glucose in the presence of tolbutamide was associated with a pattern of noisy inward current (Fig. 6, period *d*), probably reflecting activation of the VSAC. Experiments were therefore carried out to investigate whether VSAC activation could contribute towards the K_{ATP} channel-independent β-cell depolarization by glucose. As illustrated in Fig. 7*A*, glucose-induced electrical activity in cells equilibrated for 10–30 minutes in the presence of tolbutamide was sensitive to inhibition by the anion channel inhibitor 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid (DIDS). Figure 7*B* (period *b*) shows more clearly the noisy inward current referred to above in cells voltage-clamped constantly at −70 mV during stimulation with glucose in the presence of tolbutamide.

Discussion

It is well established that β-cell K_{ATP} channel activity, assessed either directly (Arkhammar et al., 1987; Ashcroft et al., 1988) or from G_{input} measurements (Best, 2000), is maximal in the prolonged absence of glucose, and is most sensitive to inhibition by glucose within the substimulatory range of concentrations. Consistent with previous reports (Arkhammar et al., 1987; Ashcroft et al., 1988), the present data indicate that channel-open probability is low in the presence of 5 mM glucose. However, the suggestion in these earlier studies that K_{ATP}-channel activity could be further reduced to a small degree by stimulatory glucose concentrations (10–20 mM) could not be substantiated by the present findings. The reasons for this apparent discrepancy between the present and earlier reports could be related to the duration of exposure of the cells to glucose. Thus, the present study showed that a variable delay occurred upon exposure of cells to glucose before maximal channel inhibition became apparent. For example, in the case of the cell shown in Fig. 2, K_{ATP} channel activity

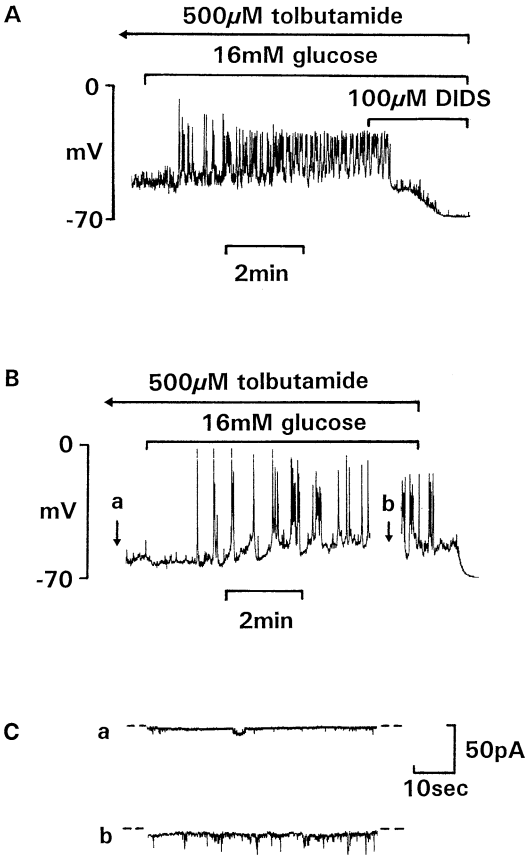

Fig. 7. Evidence that K_{ATP} channel-independent effects of glucose could involve activation of the volume-sensitive anion channel. (*A*) Effect of 100 μM DIDS on K_{ATP} channel-independent electrical activity evoked by 16 mM glucose. The cell was equilibrated in the presence of tolbutamide for 12 min prior to the addition of glucose. (*B*) Effect of 16 mM glucose on membrane potential in an isolated rat pancreatic β-cell previously equilibrated in the presence of tolbutamide for 10 min. During the periods marked *a*, *b*, the amplifier was switched to voltage-clamp, and the cell held at −70 mV in order to measure changes in inward whole-cell current. The resultant current traces are shown in (*C*). Periods *a* and *b* were approximately 60 sec in duration. Each recording is representative of at least 4 similar experiments.

persisted, albeit with reduced current amplitude and open probability, 2 minutes after addition of 5 mM glucose, maximal channel inhibition becoming apparent only after approximately 5 minutes exposure to glucose. It is possible that this delay in channel inhibition reflects the time required to reach maximal cellular ATP/ADP ratios following the addition of glucose. If this is the case, it is likely that the initial duration of exposure of the cell to zero glucose will also influence the time required for maximal channel inhibition following the addition of glucose.

The K_{ATP}-channel recordings suggested that glucose is likely to exert only a minor, if any, effect on channel activity over the concentration range effective in inducing electrical and secretory activity. It was therefore decided to investigate whether K_{ATP} channel activity was required for glucose-induced electrical activity by blocking channel activity with a supramaximal concentration of tolbutamide. This pharmacological knockout model has been previously used to study K_{ATP} channel-independent regulation of insulin release by glucose (Best et al., 1992). In initial experiments to study the effects of tolbutamide on β-cell membrane potential, an unexpected finding was that the sulfonylurea, in contrast to glucose, caused only a transient stimulation of electrical activity in single rat β-cells in the absence of glucose. This effect is analogous to that reported by Henquin (1998) who showed that a submaximal concentration of tolbutamide induced only a transient stimulation of electrical activity in the absence of glucose in intact mouse islets, and is consistent with the transient increase in cytosolic $[Ca^{2+}]$ and insulin release evoked by tolbutamide (Henquin, 1980; Martin, Reig & Soria, 1995; Best et al., 2000). The mechanism underlying the gradual repolarization in the continued presence of tolbutamide is unknown, and is not the main subject of the present study. However, the repolarization does not appear to involve reactivation of K_{ATP} channels due to desensitization of the sulfonylurea receptor. One possible explanation is an activation of other K^+ channel types, possibly including the "maxi" Ca^{2+}-dependent K^+ (BK_{Ca}) channel and delayed rectifying K^+ (K_{DR}) channel, both of which are present in pancreatic β-cells (Ashcroft & Rorsman, 1989). This possibility would be consistent with the finding that TEA, which is known to inhibit both BK_{Ca} and K_{DR} (Ashcroft & Rorsman, 1989), and iberiotoxin, a selective blocker of BK_{Ca} (Galvez et al., 1990), both caused a depolarization in the presence of tolbutamide. It should be pointed out that activation of these K^+ channels during prolonged exposure to tolbutamide was not apparent when measuring whole-cell K^+ conductance (*see* Fig. 4*A*). However, the cells were voltage-clamped for these measurements, thereby preventing changes in cell membrane potential and cytosolic $[Ca^{2+}]$. Thus, it is conceivable that activation of BK_{Ca} and/or K_{DR} could be brought about by depolarization of the cell membrane and/or a subsequent rise in cytosolic $[Ca^{2+}]$ (Ashcroft & Rorsman, 1989).

We and others have shown that glucose can stimulate insulin release in a K_{ATP} channel-independent manner (Best et al., 1992; Gembal et al., 1992; 1993; Komatsu et al., 1997; Straub et al., 1998; Aizawa et al., 1998; Westerlund et al. 2001), though it has been generally assumed that such effects are restricted to control of distal steps of the stimulus-secretion pathway; that is, independent of electrical activity and Ca^{2+} entry (Komatsu et al., 1997; Straub et al., 1998; Aizawa et al., 1998, Henquin, 2000). A major finding of the present study was that glucose could induce electrical activity in rat pancreatic β-cells un-

der conditions where K_{ATP} channel activity is blocked by a supramaximal concentration of tolbutamide. This action of glucose is not likely to be due to further inhibition of K_{ATP} channels, since the latter were shown to be maximally blocked under these conditions. Furthermore, glucose-induced insulin release under the conditions used in the present study was shown in a previous report to be accompanied by an increase in $^{86}Rb^+$ efflux (Best et al., 1992), which would again be inconsistent with an inhibition of K_{ATP}, or indeed any other K^+ channel activity by glucose. Thus, it is unlikely that the K_{ATP} channel-independent effect of glucose on electrical activity involves inhibition of any other K^+ channel types.

In the absence of glucose, β-cell K_{ATP} channel activity is high, and makes a major contribution to G_{input} (Ashcroft & Rorsman, 1989; Smith et al., 1990 and present data). Under such conditions, addition of tolbutamide causes a marked reduction in G_{input} (*see* Fig. 6), reflecting inhibition of K_{ATP}-channel activity (Sturgess et al., 1985). However, it is clear from the present study that measurement of G_{input} is not sufficiently sensitive to detect the small changes in membrane current responsible for repolarization of the cell membrane and in glucose-induced depolarization in the continued presence of tolbutamide; that is when K_{ATP} channel activity is minimal. Thus, it should be emphasized that G_{input} provides a relatively coarse estimate of total cell membrane electrical conductance, and that contribution of K_{ATP} channels to this conductance is most apparent when the cells are deprived of glucose.

The fact that K_{ATP}-channel activity is most sensitive to glucose within the substimulatory concentration range raises the possibility that a major patho-physiological function of this channel is to hyperpolarize the β-cell and prevent insulin release during hypoglycemia. This would not entirely preclude a role for K_{ATP} channels in the β-cell response to raised concentrations of glucose (i.e. >5 mM). However, the results of the present study indicate that K_{ATP} channel activity is virtually inhibited in the presence of 5 mM glucose, consistent with previous measurements of G_{input} (Best, 2000). As noted above, the membrane potential of β-cells is higher in the presence of 5 mM glucose than in the complete absence of the sugar, though not sufficiently so to induce electrical activity (Best, 2000) or insulin release (Malaisse et al., 1979; Ashcroft et al., 1988). This strongly suggests that at higher, stimulatory glucose concentrations, an additional ionic mechanism is active, which produces a further depolarization resulting in electrical and hence secretory activity. The nature of this ionic mechanism is unknown. However, evidence is provided in this study that K_{ATP} channel-independent depolarization of the β-cell by glucose could involve activation of the VSAC. Thus, the generation of electrical activity by glucose in the presence of tolbutamide was accompanied by a characteristic noisy inward current in cells voltage-clamped at −70 mV. As described above (*see* Methods), this current is thought to represent activation of the VSAC. Consistent with this, the K_{ATP} channel-independent induction of electrical activity by glucose was sensitive to inhibition by DIDS, at a concentration previously shown to inhibit the VSAC (Kinard & Satin, 1995; Best, Sheader & Brown, 1996) and to impair β-cell activation by glucose in the absence of tolbutamide (Best et al., 2000). In view of these latter findings, it should be borne in mind that DIDS, like virtually all other anion channel inhibitors, is poorly selective and has been shown to inhibit other ion channels including K_{ATP} (Proks, Jones & Ashcroft, 2001). However, such an effect would further depolarize the cell, and would not, therefore, explain the hyperpolarizing action of DIDS in this study. The K_{ATP} channel-independent effect of glucose on β-cell electrical activity could be evoked by as little as 7 mM of the sugar. This would also be consistent with activation of the VSAC, the activity of which increases in an approximately linear manner over the range 0–20 mM glucose (Best, 2000). The mechanism of activation of the VSAC by glucose is uncertain, but could involve increases in β-cell volume (Miley et al., 1997) and/or intracellular ATP levels (Miley, Brown & Best, 1999) in response to glucose metabolism.

In conclusion, the results of the present study suggest that glucose can induce electrical activity in rat pancreatic β-cells by a K_{ATP} channel-independent mechanism, possibly involving activation of the VSAC. Although this mechanism is unmasked using a pharmacological approach, it is likely to be of physiological importance, given that glucose-induced electrical activity can be inhibited by several blockers of the VSAC (Best, 1997; 2001; Best et al., 2000). This hypothesis is not at variance with the idea that glucose can exert additional K_{ATP} channel-independent influences over insulin release at steps distal to electrical activity.

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