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Glucose-sensitive conductances in rat pancreatic β-cells: contribution to electrical activity

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Abstract

The perforated patch technique was used to assess the relative contribution of K_{ATP} channel activity, assessed from input conductance (G_{input}), and volume-sensitive anion channel activity to the induction of electrical activity in single isolated rat pancreatic β-cells by glucose, 2-ketoisocaproate and tolbutamide. In cells equilibrated in the absence of glucose, the membrane potential was −71 mV and G_{input} 3.66 nS. Addition of 8 mM glucose resulted in depolarisation, electrical activity and a reduction in G_{input}, reflecting an inhibition of K_{ATP} channels. Cells equilibrated in 4 mM glucose had a membrane potential of −59 mV and a G_{input} of 0.88 nS. In this case, a rise in glucose concentration to 8–20 mM again resulted in depolarisation and electrical activity, but caused a small increase in G_{input}. 2-Ketoisocaproate also evoked electrical activity and an increase in G_{input}, whereas electrical activity elicited by addition of tolbutamide was accompanied by reduced G_{input}. Increasing the concentration of glucose from 4 to 8–20 mM generated a noisy inward current at −70 mV, reflecting activation of the volume-sensitive anion channel. The mean amplitude of this current was glucose-dependent within the range 4–20 mM. Addition of 2-ketoisocaproate or a 15% hypotonic solution elicited similar increases in inward current. In contrast, addition of tolbutamide failed to induce the inward current. It is concluded that K_{ATP} channel activity is most sensitive to glucose within the range 0–4 mM. At higher glucose concentrations effective in generating electrical activity, activation of the volume-sensitive anion channel could contribute towards the nutrient-induced increase in G_{input}.

Keywords: Islet; Pancreatic β-cell; Glucose; Electrical activity; Input; Conductance; K_{ATP} channel; Anion channel

1. Introduction

The stimulation of insulin secretion by nutrients, such as glucose, and by sulfonylureas is intimately associated with electrical activity consisting of bursts of Ca^{2+}-dependent action potentials (see [1,2] for reviews). Glucose-induced electrical activity is known to require metabolism of the sugar and the generation of one or more signals which couple glucose metabolism to depolarisation of the plasma membrane. Depolarisation results in opening of voltage-sensitive Ca^{2+} channels, Ca^{2+} entry into the cytosol and exocytosis. The exact nature of this coupling mechanism has not yet been fully elucidated. However, the consensus model is that a rise in intracellular [ATP] or the ATP/ADP ratio inhibits the activity of K_{ATP} channels, thus producing a net depolarisa-

Abbreviations: DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; KIC, 2-ketoisocaproate

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tion of the β-cell membrane [3,4]. Thus, addition of glucose to β-cells has been shown to inhibit K_{ATP} channel activity in cell-attached patches [3,5–8]. Similarly, glucose-induced electrical activity in mouse β-cells has been shown to be accompanied by a reduction in whole-cell K_{ATP} conductance, assessed by measuring G_{input} by means of the perforated patch recording technique [9]. However, the majority of these studies have investigated the effects on K_{ATP} channel activity of adding glucose to cells previously equilibrated in the absence of the sugar; there is relatively little information available regarding the glucose concentration-dependency of the channel. It should also be borne in mind that inhibition of K_{ATP} channels requires an inward (depolarising) conductance in order to depolarise the cells. The identity of this inward current is at present open to question.

A volume-sensitive anion conductance has recently been described in insulin-secreting cells which could fulfill such a role [10,11]. Activation of this conductance by exposure to a 15% reduction in extracellular osmolarity is manifest as a noisy inward current in rat β-cells voltage-clamped at −65 mV [12]. A similar pattern of inward current noise is associated with electrical activity elicited by glucose or 2-ketoisocaproate (KIC) [12], suggesting that nutrient stimuli can also activate the volume-sensitive anion channel. This suggestion is supported by the finding that the inward current, electrical activity and insulin release evoked by hypotonic solutions [13] or nutrient stimuli [12] are sensitive to inhibition by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), two inhibitors of the volume-sensitive anion channel [10,11]. It is also supported by the demonstration that glucose causes β-cell swelling to a degree sufficient to activate the volume-sensitive anion channel [14], whilst channel activity is increased by a rise in intracellular [ATP], even in the absence of cell swelling [15].

The aim of the present study was to investigate changes in G_{input} as an index of whole-cell K_{ATP} channel activity, and activity of the volume-sensitive anion in rat pancreatic β-cells over a range of glucose concentrations in order to assess the relative contribution of these glucose-sensitive conductances to the modulation of electrical activity. The effects of KIC, another nutrient stimulus, and tolbutamide, which depolarises the β-cell by direct inhibition of the K_{ATP} channel [16,17], have also been investigated.

2. Materials and methods

Pancreatic islets were prepared from Sprague–Dawley rats (300–350 g; either sex) by collagenase digestion. Islets were dispersed into single cells and small clusters by a brief (3–4 min) 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 RPMI medium (Gibco, Paisley, UK) containing 5% (v/v) foetal 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 NaCl, 135; KCl, 5; $MgSO_4$, 1; NaH_2PO_4, 1; $CaCl_2$, 1.2; HEPES-NaOH, 10 (pH 7.4) and glucose at the required concentration. In some experiments, cells were exposed to a 15% hypotonic solution by withdrawal of 45 mM mannitol (substituted in the original medium for 22.5 mM NaCl).

β-Cell membrane potential and whole-cell currents were recorded using the 'perforated patch' technique [18] in current-clamp and voltage-clamp modes, respectively. Single β-cells, identified by their size and typical granular appearance, were used for all recordings in order to avoid contaminating currents from adjacent electrically coupled cells. In order to ascertain that cells were not coupled, the patch was removed by suction at the end of each experiment, so that any intact adjoining cells would be visualised. Only cells which had a low resting membrane potential (−55 mV or lower) in sub-threshold concentrations of glucose (0 or 4 mM), and which exhibited electrical activity in response to stimulation with nutrients or tolbutamide were used in this study. The pipette solution consisted of 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 8–13 pF. As discussed previously [12], this relatively large cell size is characteristic of rat β-cells maintained in medium-term culture. Currents were recorded using a List EPC-7 amplifier (List, Darmstadt, Germany)

under voltage-clamp conditions at a holding potential of −70 mV. G_{input} was measured by voltage-clamping the cells at −70 mV and applying 200-ms pulses of ±10 mV at 2-s intervals, essentially as described previously [9]. G_{input} was calculated from the average amplitudes of the resultant 'square' current excursions during 30-s segments of recording filtered at 100 Hz via an eight-pole Bessel filter (see Fig. 1) using 'Fetchan' software (pClamp 6; Axon Instruments, Foster City, CA, USA). Activity of the volume-sensitive anion channel was estimated, again using pClamp6 software, from the mean amplitude of the whole-cell inward current at a constant holding potential of −70 mV in 30 s. segments of recording filtered at 300 Hz. Inward (negative) currents are shown as downward deflections in all cases. 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). All chemicals were obtained from Sigma, Poole, UK.

3. Results

3.1. Low glucose concentrations reduce G_{input} in rat β-cells

The first part of the study was designed to investigate changes in G_{input} in relation to electrical activity in rat pancreatic β-cells. Cells equilibrated for 10 min. in the absence of glucose had a membrane potential of −71.2±2.4 mV, and a G_{input} of 3.66±0.91 nS (both $n=7$). This is in broad agreement with the corresponding values of −70 mV and 5.1 nS previously reported for mouse β-cells under similar conditions [9]. As shown in Fig. 1, addition of a stimulatory concentration of glucose (8 mM) resulted in a depolarisation leading to electrical activity. This effect was accompanied by a marked reduction ($P<0.02$) in G_{input} to a value of 0.90±0.06 nS ($n=5$), presumably reflecting inhibition of K_{ATP} channels [9]. Withdrawal of glucose resulted in repolarisation accompanied by a gradual increase in G_{input}.

In cells equilibrated for 10 min or longer in medium containing a substimulatory concentration of glucose (4 mM), the membrane potential was

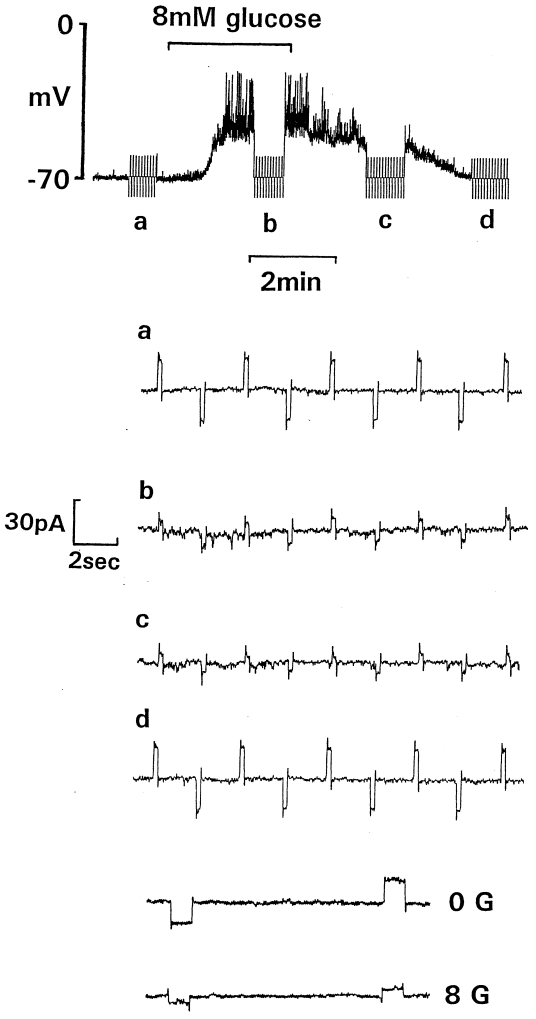

Fig. 1. Membrane potential recording (upper trace) from a single, isolated rat pancreatic β-cell using the perforated patch technique. The cell was incubated in the absence of glucose for approximately 10 min, and 8 mM glucose added for the period shown by the bar. At the points marked a, b, c and d, the amplifier was switched to voltage clamp, the cell held at −70 mV and subjected to 200-ms pulses of ±10 mV in order to measure G_{input}. The resultant current recordings are shown below. The bottom two traces are taken from traces a (0 G) and b (8 G) and expanded on a faster time scale. All recordings are typical of those from a total of five similar experiments.

−59.1±0.7 mV, and the corresponding G_{input} was 0.88±0.07 nS (both $n=36$). Both of these values were significantly ($P<0.01$ or less) different from those recorded in the absence of glucose, supporting the suggestion of a considerable degree of inhibition of K_{ATP} channel activity in the presence of subthreshold glucose concentrations [19]. In view of

the marked difference in G_{input} between cells incubated in the absence and presence of 4 mM glucose, experiments were performed to investigate the effect of withdrawal of 4 mM glucose on β-cell G_{input}. Following a 'lag' phase of approximately 2 min, withdrawal of glucose resulted in a gradual increase in G_{input} which was statistically significant at 5 min (Fig. 2). This effect was accompanied by an increasing outward current and presumably reflects activation of K_{ATP} channels.

3.2. Stimulatory glucose concentrations increase G_{input} in rat β-cells

All subsequent experiments were performed on cells equilibrated under 'physiological fasting' conditions, that is in the presence of a substimulatory concentration (4 mM) of glucose. A rise in glucose concentration to stimulatory levels again resulted in depolarisation and electrical activity (Fig. 3). However, under these conditions, electrical activity induced by a rise in glucose concentration was accompanied a modest increase in G_{input}. Such an effect was statistically significant with glucose concentrations of 16 or 20 mM (Fig. 5). The effects of KIC, another nutrient stimulus, on β-cell membrane potential and G_{input} were also investigated. In the presence of 4 mM

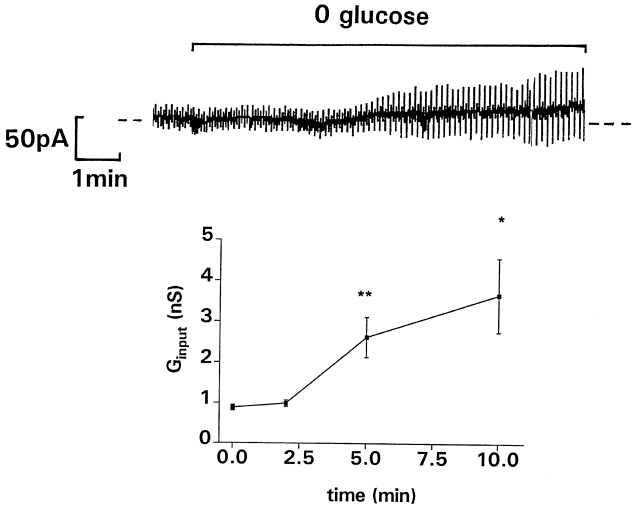

Fig. 2. Effect of withdrawal of 4 mM glucose on G_{input} in rat pancreatic β-cells. The upper trace is a typical response showing a parallel increase in outward current and in G_{input}. The cell was held at −70 mV and subjected to 200-ms pulses of ±10 mV. The dashed line represents zero current level. The lower panel shows mean ± S.E.M. from five cells. $*P < 0.05$; $**P < 0.01$.

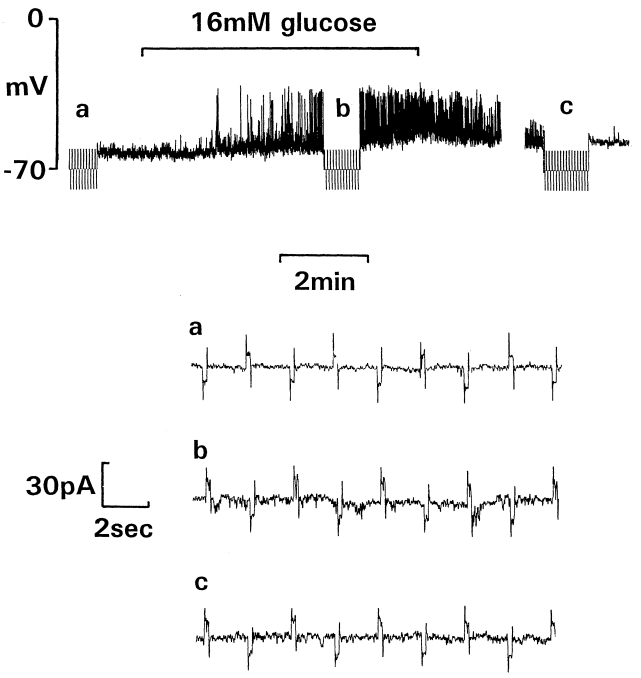

Fig. 3. Membrane potential recording (upper trace) from a single, isolated rat pancreatic β-cell using the perforated patch technique. The concentration of glucose was raised from 4 to 16 mM for the period shown by the bar. At the points marked a, b and c, the amplifier was switched to voltage clamp, the cell held at −70 mV and subjected to 200-ms pulses of ±10 mV in order to measure G_{input}. The resultant current recordings are shown in the lower traces. The gap in the upper recording represents a period of approximately 3 min' recovery from exposure to high glucose. The recordings are typical of those from a total of 12 similar experiments.

glucose, addition of 12 mM KIC depolarised the cells and induced electrical activity in a manner comparable to increased glucose concentrations (Fig. 4, left panels). Again, this effect was accompanied by a modest, but significant, increase in G_{input} (Fig. 5), as found with glucose stimulation. In contrast, electrical activity induced by 0.1 mM tolbutamide in the presence of 4 mM glucose was associated with a small, but statistically significant reduction in G_{input} (Fig. 4, right panels; Fig. 5), probably reflecting the direct inhibition of K_{ATP} channels by the sulfonylurea.

3.3. Glucose-induces inward current noise in rat β-cells

As can be seen in Figs. 1, 3 and 4, the current

recordings during stimulation with either glucose or KIC were characterised by periods of noisy inward current. This current has been reported previously in β-cells during exposure to stimulatory concentrations of nutrients or during hypotonically induced cell swelling, suggesting that it represents activation of the volume-sensitive anion channel ([12] and see below). The next part of the study was therefore designed to investigate changes in the amplitude of this anion channel current over a range of glucose concentrations and in response to KIC and tolbutamide. Fig. 6 shows typical current recordings in single isolated rat β-cells voltage-clamped at −70 mV. The inward current could be elicited by raising the glucose concentration from 4 to 16 mM (Fig. 6A). This current persisted in the absence of extracellular Ca^{2+} (Fig. 6B), providing further confirmation that the current does not represent action currents arising from electrically coupled cells. The glucose-induced inward current was also observed when K_{ATP} channel activity was inhibited by the presence of 100 μM tolbutamide (Fig. 6C). A similar pattern of inward current could be elicited by addition of 12 mM KIC or by exposure to a 15% hypotonic solution (Fig. 7). A rise in glucose concentration from 4 to 8 mM was consistently sufficient to elicit inward current noise, producing a significant ($P < 0.01$) increase in mean current amplitude (Fig. 8). Further rises in the concentration of glucose to 12, 16 and 20 mM resulted in a progressive increase in mean current amplitude (Fig. 8). The mean current amplitude in cells stimulated with 20 mM glucose was comparable to that seen during stimulation with 12 mM KIC or exposure to the hypotonic solution (Fig. 8). Tolbutamide failed to activate the inward current (Figs. 6–8), consistent with a previous study [12].

4. Discussion

The input conductance (G_{input}) of the unstimulated β-cell is dominated by K_{ATP} channel activity [1,20] and has been used to assess the whole-cell K_{ATP} conductance in intact β-cells using the perforated patch

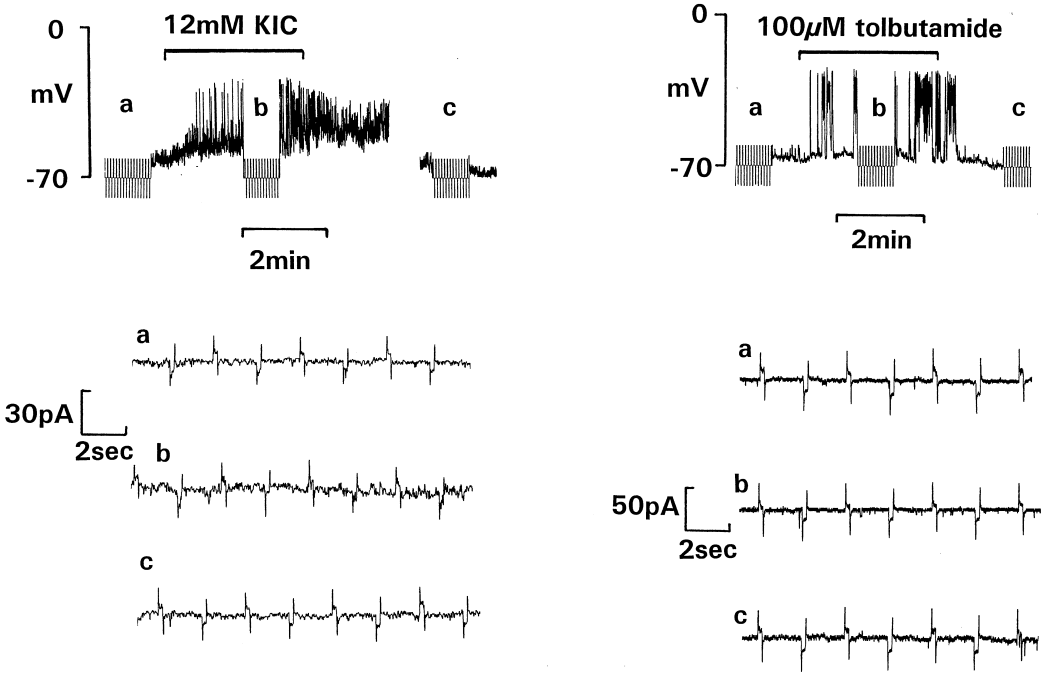

Fig. 4. Membrane potential recordings (upper traces) from single, isolated rat pancreatic β-cells using the perforated patch technique. Effects of 12 mM 2-ketoisocaproate (KIC; left panel) and 100 μM tolbutamide (right panel), both in the presence of 4 mM glucose. In both cases, the amplifier was switched to voltage clamp at the points marked a, b and c, the cell held at −70 mV and subjected to 200-ms pulses of ±10 mV in order to measure G_{input}. The resultant current recordings are shown in the lower traces. The gap in the upper recording represents a period of approximately 3.5 min' recovery from exposure to KIC. The recordings are typical of those from a total of five and seven similar experiments, respectively.

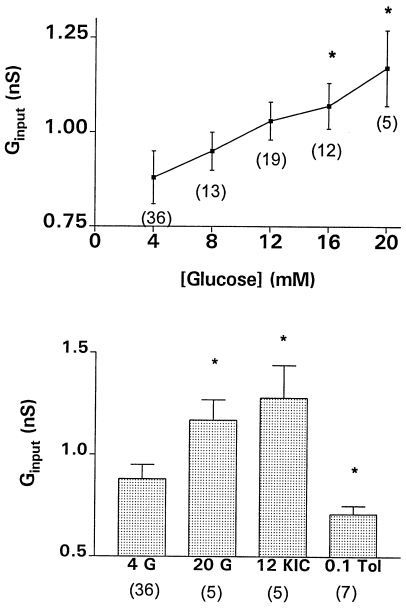

Fig. 5. Input conductance (G_{input}) in rat pancreatic β-cells. Upper panel: effect of increasing glucose concentration within the range 4–20 mM. Bottom panel: 4 mM glucose (4 G), 20 mM glucose (20 G), 12 mM 2-ketoisocaproate (12 KIC) and 0.1 mM tolbutamide (0.1 Tol), the latter two in the presence of 4 mM glucose. Each point is the mean ± S.E.M. The figures in parentheses represent the number of replicates. $*P < 0.05$ compared to 4 mM glucose control.

technique [9,21]. However, these studies examined the effect of adding stimulatory concentrations of glucose (8–20 mM) to cells previously equilibrated in the absence of the sugar. In these cases, electrical activity evoked by the addition of glucose was accompanied by a marked reduction in G_{input}, a finding confirmed in the present study and which is probably due to inhibition by glucose of K_{ATP} channels. However, the present report also demonstrates that G_{input} in cells equilibrated in the absence of glucose is considerably higher than in cells incubated in the presence of a subthreshold glucose concentration (in this case 4 mM) which would be expected under physiological fasting conditions. A similar phenomenon has been demonstrated in β-cells using the cell-attached recording technique, where application of substimulatory concentrations of glucose (2 and 5 mM) resulted in a dramatic reduction in channel open probability [7]. It has also been demonstrated in the present study that, upon withdrawal of glucose from the incubation medium, a progressive increase in G_{input} follows a short lag phase, possibly representing the time taken for intracellular ATP concentration (or ATP/ADP ratio) to fall to a level at which K_{ATP} channel opening occurs.

Whilst addition of a substimulatory concentration of glucose had the predicted effect of reducing G_{input}, raising the glucose concentration from 4 mM to levels at which electrical activity was induced (8–20 mM) was found to produce a small, concentration-dependent increase in G_{input}. An essentially similar effect was observed with KIC, another nutrient-type stimulus, although not with tolbutamide which directly inhibits K_{ATP} channel activity. This effect of raised glucose concentrations and KIC might, at first sight, be surprising, given that glucose has been reported to cause a concentration-dependent reduction in K_{ATP} channel activity in cell-attached patches, at least within the range 0–10 mM [7]. KIC has also been shown to inhibit β-cell K_{ATP} channel activity under similar conditions, albeit in the absence of glucose [22]. However, it should be borne in mind that although the β-cell K_{ATP} conductance is thought to make a major contribution to G_{input} [1,9,20], the latter is essentially a measure of the net electrical

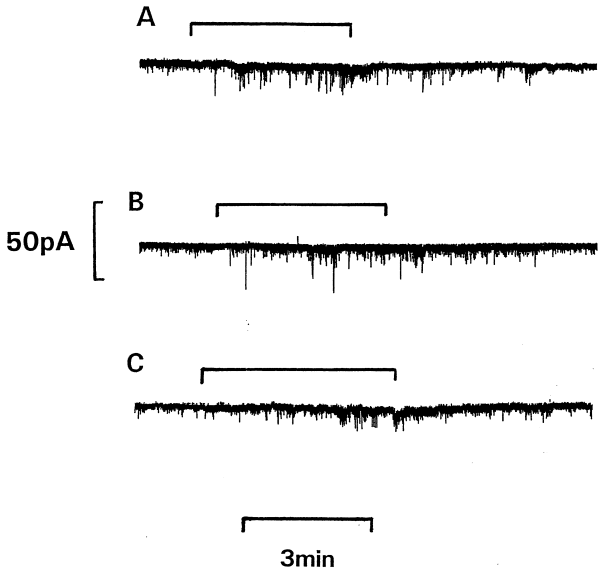

Fig. 6. Effect of a rise in glucose concentration from 4 to 16 mM on whole-cell current in single rat β-cells voltage-clamped at −70 mM under perforated patch conditions. (A) Control. (B) In the absence of extracellular Ca^{2+}. (C) In the presence of 100 μM tolbutamide. The glucose concentration was raised for the period indicated by the horizontal bars. The dashed lines represent the zero current level. Each trace is representative of at least three similar recordings.

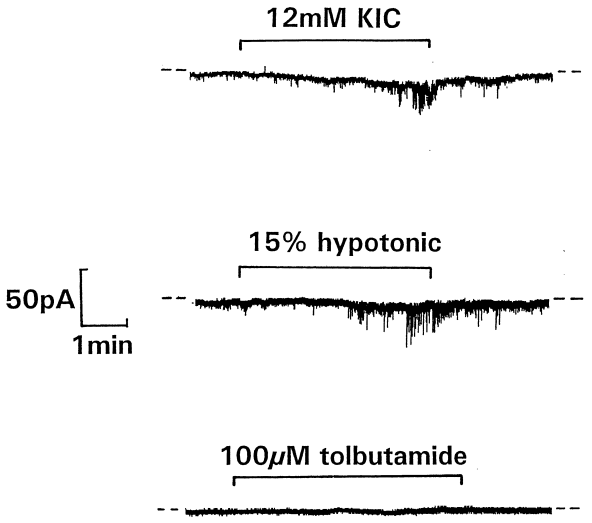

Fig. 7. Whole-cell currents in single rat β-cells voltage-clamped at −70 mV under perforated patch conditions. Effects of 12 mM 2-ketoisocaproate (KIC), a 15% hypotonic solution and 100 μM tolbutamide, all in the presence of 4 mM glucose. The dashed lines represent the zero current level. The recordings are representative of 12, 8, 5 and 4 similar experiments, respectively.

conductance of the cell membrane. Thus, the increase in G_{input} observed in the present study upon raising the glucose concentration above 4 mM does not necessarily imply that no further inhibition of K_{ATP} channels occurs under these conditions. However, this finding does suggest that, at concentrations of glucose above 4 mM, any reduction in G_{input} due to K_{ATP} channel closure is more than compensated for by an increase in some other conductance(s). Activation of Ca^{2+}-sensitive K^+ (K_{Ca}) and/or delayed-rectifier K^+ channels (K_{DR}) is thought to play a role in repolarisation of the action potential in the β-cell (see [1] for discussion of this topic). However, increased activity of these channels during electrical activity would be unlikely to make a major contribution towards the increased G_{input} observed during nutrient stimulation, since the cells are voltage-clamped close to E_K. In any case, an activation of net K^+ conductance by raised concentrations of glucose or by KIC would, of course, exert a hyperpolarising influence, which would not be consistent with the observed depolarisation leading to electrical activity. Thus, it seems likely that activation of an inward (depolarising) current makes a major contribution towards the nutrient-induced increase in G_{input}.

It has been previously reported that electrical activity in rat β-cells evoked by glucose or KIC, but not by tolbutamide, is associated with the generation of a noisy inward current, both in whole-cell [12] and cell-attached [23] recordings. A number of observations support the suggestion that this current represents activation of the volume-sensitive anion conductance. First, the current is sensitive to inhibition by DIDS and NPPB [12,13], two drugs previously shown to inhibit the volume-sensitive anion channel [10,11]. Second, as demonstrated in the present study, the characteristics of the current induced by stimulatory glucose concentrations or by KIC are essentially similar to that evoked by exposure to a modest (15%) hypotonic shock which induces a similar degree of cell swelling to that observed during glucose stimulation [14]. Moreover, it is unlikely in

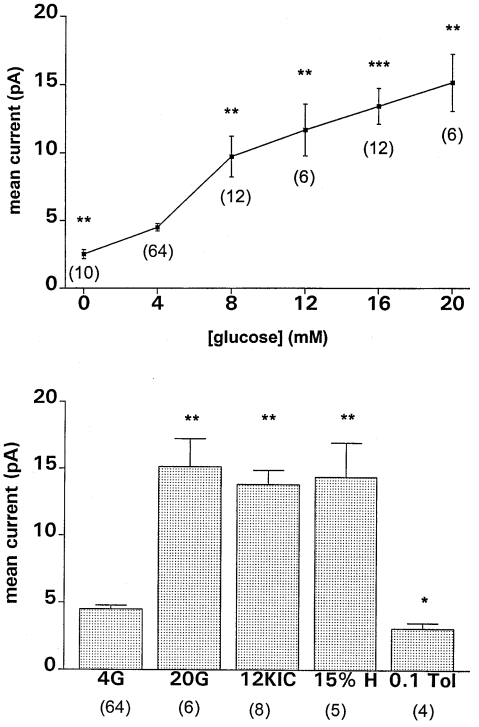

Fig. 8. Mean inward current amplitude in single rat β-cells measured from 30-s segments of recording. Cells were voltage-clamped at −70 mV under perforated patch conditions. Upper panel: effects of increasing glucose concentration within the range 0–20 mM. Lower panel: 4 mM glucose (4 G), 20 mM glucose (20 G), 12 mM 2-ketoisocaproate (12 KIC), a 15% hypotonic solution (15% H) and 0.1 mM tolbutamide (0.1 Tol), the latter three in the presence of 4 mM glucose. Each point is the mean ± S.E.M. The figures in parentheses represent the number of replicates. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to 4 mM glucose control.

cells voltage-clamped at −70 mV that the inward current could result from activation of Na^+, Ca^{2+} or K^+ conductances. Indeed, these conductances were eliminated in the previous cell-attached recordings by the use of a CsCl pipette solution [23].

Activation of the inward anion current during nutrient stimulation could contribute towards the observed increase in G_{input}. Upon raising the glucose concentration from 4 to 20 mM, an increase in G_{input} of 0.29 nS can be calculated from the data shown in Fig. 5. The corresponding increase in mean inward current (at −70 mV) was 10.64 pA (from Fig. 8). Assuming the reversal potential of the volume-sensitive anion channel to be approximately −16 mV under perforated patch conditions [13], this would represent an increase in conductance of 0.20 nS. Furthermore, both the increases in G_{input} and the amplitude of the inward current have been shown in the present study to be glucose dependent over the concentration range effective in modulating electrical and secretory activity in the β-cell.

The mechanism by which glucose activates the conductance is uncertain. However, the finding that glucose causes β-cell swelling, possibly due to the intracellular accumulation of glucose metabolites [14], raises the possibility that increased cell volume could couple glucose metabolism to activation of the volume-sensitive anion channel. In this context, it may be noted that 20 mM glucose and a 15% hypotonic solution, which cause a similar degree of cell swelling [14], also evoked a comparable increase in mean current amplitude. A further possibility is that anion channel activity is regulated by intracellular nucleotides [15]. It should also be noted that the activity of volume-regulatory mechanisms in general (certain ion channels and transporters) is sensitive not only to changes in cell volume, but also to intracellular ionic strength [24].

A question that arises from the present study is the respective functions of the K_{ATP} and volume-sensitive anion conductances in the β-cell response to nutrients. Whilst a role for the K_{ATP} channel in modulating nutrient-induced electrical activity cannot be ruled out, the observation that maximal changes in activity of this channel occur between 0 and 4 mM glucose might be more consistent with a pathophysiological role for this channel, hyperpolarising the cell and thus preventing insulin release during severe or prolonged hypoglycaemia. A similar mechanism is thought to exist in ventromedial hypothalamic neurones, where withdrawal of glucose causes hyperpolarisation due to ATP-sensitive K^+ channel activation [25]. Analogous protective mechanisms are also thought to exist in cardiac muscle [26], in several types of smooth muscle [27] and in inspiratory neurones [28]. In each of these cases, hypoxia appears to hyperpolarise the cell and thus prevent contraction by increasing K_{ATP} channel activity.

The present findings suggest that activation of the volume-sensitive anion channel during nutrient stimulation could play a major role in modulating electrical activity. The observation that blockers of this channel inhibit electrical activity in response to nutrients [12], but not sulfonylureas [29], would be consistent with this proposal. It should be emphasised that most, if not all, blockers of volume-sensitive anion channels, including DIDS and NPPB, are known to have poor selectivity for this type of channel, and the discovery or development of more selective inhibitors will be necessary to clarify this subject.

In conclusion, glucose elicits a dual effect on G_{input} in rat pancreatic β-cells. The marked reduction in G_{input} observed when the glucose concentration is increased within the range 0–4 mM probably reflects inhibition of K_{ATP} channel activity. In contrast, the more modest increases in G_{input} when glucose is raised above 4 mM to induce electrical activity are likely to represent activation of other channel types, possibly including the volume-sensitive anion channel. Such a mechanism could, at least in part, account for the K_{ATP} channel-independent regulation of electrical activity by glucose recently reported [30].

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