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Simultaneous recordings of glucose dependent electrical activity and ATP-regulated K^+-currents in isolated mouse pancreatic β-cells

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Membrane potential and membrane currents were recorded from single mouse pancreatic β-cells using the perforated patch whole-cell recording technique at 30 °C. Single β-cells maintained in primary tissue culture exhibited glucose-dependent electrical activity similar to that reported for freshly isolated intact islets. The resting input conductance (5.1 ± 0.9 nS) was determined by ATP-regulated K^+ (K_{ATP}) channels as it was blocked by 1 mM tolbutamide. 8 mM glucose decreased the input conductance by 80%. The input conductance at −70 mV was of a similar value during the plateau phase and during the silent phase of electrical activity in 8 mM glucose. This suggests that oscillations of K_{ATP} channel activity do not underlie the slow waves.

Insulin secretion; ATP-regulated K^+-channels; Glucose; Nystatin

1. INTRODUCTION

The resting conductance of the insulin-secreting pancreatic β-cell is predominantly determined by K_{ATP} channels. These channels are closed by glucose metabolism which leads to membrane depolarization, the initiation of electrical activity and thus to insulin secretion [1]. Microelectrode recordings of the membrane potential from β-cells within freshly isolated intact islets of Langerhans have established that electrical activity consists of oscillations (slow waves) between a depolarized plateau, on which Ca^{2+}-dependent action potentials are superimposed, and a repolarized electrically silent interburst interval [2]. This type of glucose-dependent electrical activity has not been recorded from single isolated β-cells, which has led to the hypothesis that slow wave activity requires the presence of a number of β-cells electrically coupled together [3].

The origin of the slow waves remains obscure. One idea is that the interburst intervals may result from the periodic activation of a K^+-conductance, for example, activation of Ca^{2+}-activated K^+-channels [4] or K_{ATP} channels [5]. An alternative view is that Ca^{2+}-dependent inactivation of Ca^{2+} channels may somehow contribute to this process.

The standard whole-cell configuration is not suitable for studying the effect of glucose metabolism on whole-cell currents as cytosolic constituents are rapidly dialysed from the cell (a phenomenon known as washout). We have therefore used the perforated patch configuration of the patch clamp method, recently introduced by Horn and Marty [6] to investigate the basis of slow wave activity in single mouse β-cells. In this method, the patch pipette contains the pore-forming antibiotic nystatin, which incorporates into the patch membrane and forms a low resistance pathway which provides electrical access to the cell interior. The nystatin pores are sufficiently small to prevent the washout of second messenger systems in other cells [6]. We show here that glucose metabolism is also preserved.

2. MATERIALS AND METHODS

Pancreatic β-cells were isolated from NMRI-mice and maintained in primary tissue culture as previously described [7]. Cells were continuously superfused with an external solution consisting of (in mM): 138 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$ and 5 Hepes (pH 7.4 with NaOH). Glucose was added at the concentrations indicated in the text. All experiments were conducted at 29–31°C; at higher temperatures the seals became too fragile to allow long-term experiments. Membrane potential and whole-cell currents were recorded using the perforated patch whole cell method [6] with an EPC-7 patch clamp amplifier (List Electronic, Darmstadt, FRG) and stored on video tape for later analysis. The pipette solution contained (in mM) 10 KCl, 10 NaCl, 70 K_2SO_4, 7 $MgCl_2$, 5 Hepes (pH 7.35 with KOH). The pipettes were first tip-filled with the above solution and then back-filled with the same solution supplemented with 100 μg/ml nystatin in 0.2% DMSO. Perforation was monitored by the increase in our ability to compensate for the cell capacitance and by the decrease in the series resistance (G_s). Perforation was usually adequate for voltage-clamping ($G_s < 50$ MΩ) within 15 min of seal formation whereas the membrane potential could be recorded normally within a few minutes ($G_s < 150$ MΩ).

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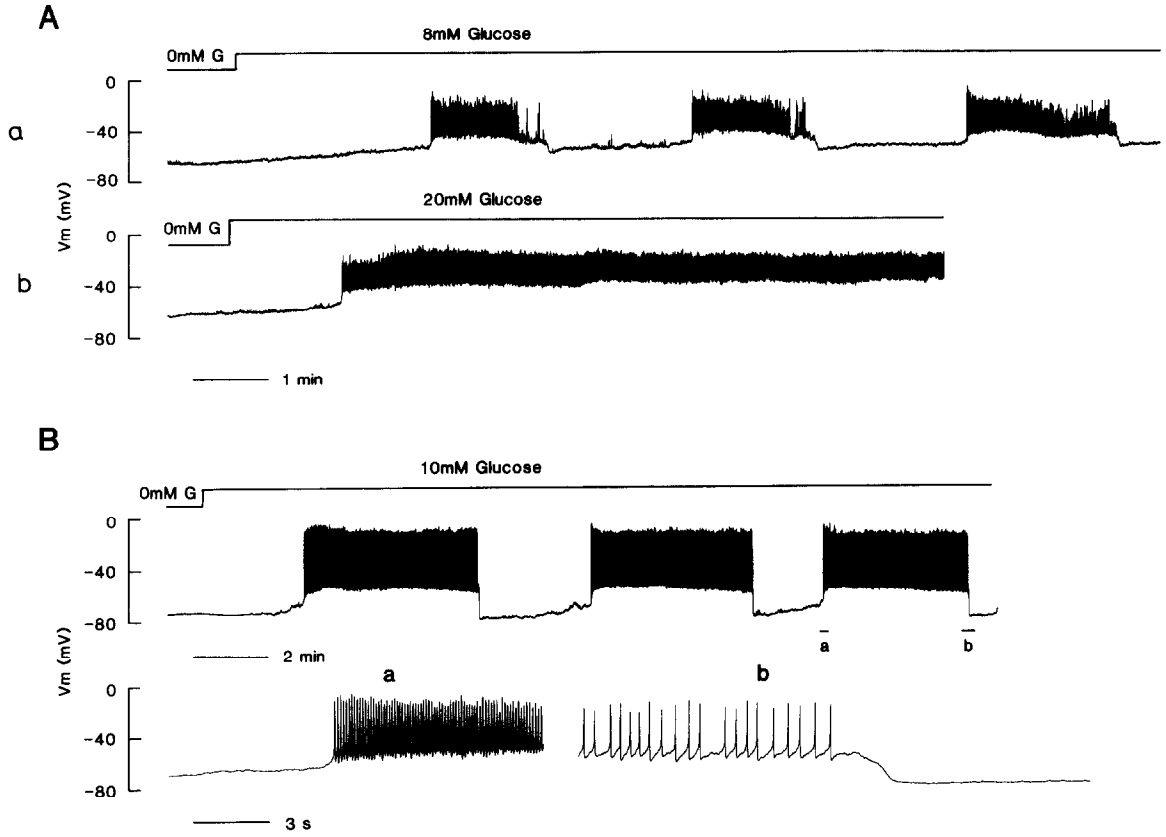

Fig.1. Electrical activity elicited by glucose in isolated mouse pancreatic β-cells. (A) Membrane potential recorded in response to 8 mM (a) and 20 mM glucose (b) from the same β-cell within a cluster of 64 μm diameter (about 5 cells) at 29°C. (B top) Membrane potential recorded in 0 mM and 10 mM glucose from a single β-cell at 31°C. (B bottom; a,b) Action potentials displayed at a faster time base from the parts of the record marked a and b of the recording above.

3. RESULTS

Fig.1A shows a current-clamp recording of membrane potential from a mouse pancreatic β-cell within a small cluster. In the absence of glucose the cell was electrically silent with a resting membrane potential of −62 mV. The addition of 8 mM glucose (fig.1Aa) resulted in a slow depolarization and the initiation of electrical activity (slow waves): the latter consisted of groups of action potentials (bursts) separated by electrically silent intervals. In this cell, each burst had a duration of about 1.5 min and an interburst interval of similar length. Continuous electrical activity was produced when the glucose concentration was increased to 20 mM (fig.1Ab). The burst duration recorded at intermediate glucose concentrations (8–20 mM) showed considerable variability between cells: fig.1B illustrates an example of bursts with a duration of 4 min elicited by 10 mM glucose from a single β-cell. Fig.1B (bottom a,b) also shows that the action potential frequency declined through the burst and that each burst is terminated by a rapid hyperpolarization. Similar results are found in microelectrode recordings from intact islets.

These results demonstrate that perforated patch recording configuration preserves both the metabolism of the β-cell and its ability to respond to glucose. They further demonstrate that clusters of 2–3 β-cells, and indeed single β-cells, are capable of producing slow wave activity.

It is well established that the input conductance of the unstimulated β-cell is dominated by the K_{ATP} channel [1]. Fig.2 illustrates the changes in membrane potential and membrane current recorded from the same cell in response to increasing the glucose from 0 to 8 mM. In the absence of glucose the membrane potential was −70 mV and the input conductance (measured from the current responses to alternate 10 mV hyperpolarizing and depolarizing voltage steps from a holding potential of −72 mV) was 2.0 nS (fig.2Ca). This conductance is mainly due to K_{ATP} channels as it was almost completely inhibited by tolbutamide (data not shown), a specific blocker of this channel in β-cells [1]. In support of the hypothesis that the K_{ATP} channel is subject to considerable inhibition at rest, the input conductance was 5.1 ± 0.9 nS (n = 18), much less than that measured during standard whole-cell experiments at 20°C after the washout of internal

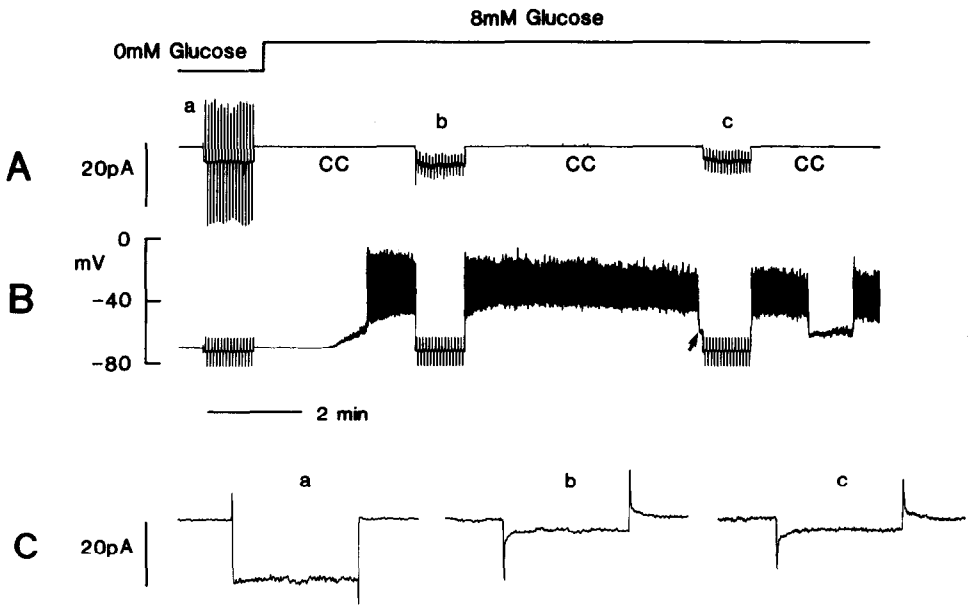

Fig.2. Effects of glucose on membrane current and membrane potential. Membrane current (A) and membrane potential (B) recorded from the same single cell in 0 mM glucose and subsequently in 8 mM glucose. The cell was voltage-clamped at a holding potential of −70 mV and ±10 mV pulses applied every 2 s. During the periods marked CC the amplifier was switched so that the cell was under current-clamp control. The arrow indicates spontaneous repolarization at the end of the burst. (C) Current elicited by a 200 ms hyperpolarizing step during the periods marked a, b and c in A. Temperature 30°C, C_{slow} 5.4 pF, G_s 25 MΩ.

ATP (about 20 nS; [7]). Raising the glucose concentration from 0 to 8 mM produced a slow depolarization to a membrane potential of −55 mV, at which action potentials were initiated (fig.2B). This depolarization was associated with an increase in voltage noise and a decrease in input conductance of 82% from 2 to 0.36 nS (fig.2Cb). There was no difference in the input conductance measured during the plateau (Cb) when compared to that measured during the silent phase (Cc).

4. DISCUSSION

In this paper we show that mouse β-cells maintained in primary tissue culture are capable of generating glucose-dependent electrical activity similar to that recorded from β-cells within intact, freshly isolated pancreatic islets. Previous studies have failed to record slow waves from single β-cells. This is not very surprising in the case of the whole-cell studies, since the metabolic machinery is washed out of the cell; it is less clear, however, why regular bursts of action currents are not recorded from cell-attached patches. Two possible explanations occur to us. First, our experiments were carried out at 30°C and not at room temperature. Secondly, current injection through the patch membrane might affect the burst and distort the pattern of electrical activity. Our studies show that models based on ion channel sharing between large groups of β-cells [3] are not required to explain the generation of the slow waves.

The duration of the bursts we observed was very variable, ranging between 1 and 4 min in 8–10 mM glucose at 30°C. This is considerably longer than the mean burst duration recorded from intact islets [2]. Although the burst frequency is consequently lower than reported for intact islets, it is comparable to the slow oscillations found in burst frequency [8]. Slow oscillations of similar frequency have also been observed in the cytoplasmic free Ca^{2+} concentration of single isolated β-cells in response to glucose stimulation [9]; our results support the idea that these oscillations reflect the entry of Ca^{2+} during bursts of action potential activity.

We have also investigated the origin of the slow waves. Since there is no difference in the magnitude of the resting K-conductance during the burst and during the interburst interval, it seems unlikely that oscillation of K_{ATP} channel activity produces the slow waves [5]. A similar argument mitigates against the involvement of oscillations in Ca-activated K-channel activity – or indeed any K-channel. We acknowledge, however, that we would be unable to resolve changes in K-current of less than 0.5 pA. Finally, we emphasize that although our data does not support the hypothesis that oscillations in K-channel activity underlie the slow waves, it

is entirely consistent with the idea that modulation of K_{ATP} channel activity is the mechanism by which an increase in external glucose increases the burst duration, and thereby insulin release.

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