Cell-attached recordings of the volume-sensitive anion channel in rat pancreatic β-cells

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Abstract

The cell-attached configuration of the patch-clamp technique was used to study the volume-sensitive anion conductance in isolated rat pancreatic β-cells at the single-channel level. In unstimulated cells, current level was close to zero. Exposure of cells to a 33% hypotonic solution resulted in the generation of an inward current at 0 mV pipette potential. A similar inward current was elicited by a rise in glucose concentration or by addition of α-ketoisocaproate. In contrast, the sulphonylurea tolbutamide was ineffective. The inward current evoked by hypotonic solutions consisted of occasional discreet channel events interspersed with periods of current noise which could not be clearly resolved into unitary channel events. Stimulation with glucose resulted in a predominantly noisy pattern of current. With a reduced [Cl$^-$] pipette solution, regular channel openings could be resolved in the presence of a stimulatory glucose concentration, with a calculated conductance of 215 pS. Channel activity could also be recorded in excised inside-out patches, though rapid 'rundown' occurred under such conditions. It is concluded that hypotonic solutions and glucose activate the volume-sensitive anion channel in the cell-attached configuration by increasing channel open probability. This generates an inward current in non-voltage-clamped cells. The channel showed complex kinetics which depended in part upon extracellular [Cl$^-$]. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Pancreatic β-cell; Chloride channel; Cell volume

1. Introduction

A volume-sensitive anion conductance has been described in insulin-secreting cells [1–3]. Activation of this conductance in intact, isolated rat β-cells by exposure to a 30% reduction in extracellular osmolarity generates a sustained inward current leading to depolarisation, electrical activity and insulin release [4]. A reduction in osmolarity of 10–15% is sufficient

to elicit these effects [5,6]. However, activation of the conductance by these smaller changes in osmolarity is manifest as a pattern of rapid transient inward currents or 'noise', rather than a sustained current. A similar increase in whole-cell current noise has also been observed in β-cells during stimulation with glucose and α-ketoisocaproate [5]. The inward current elicited by either hypotonic solutions [4] or nutrient stimuli [5] is sensitive to inhibition by 4,4′-diisothio-cyanatostilbene-2,2′-disulphonic acid (DIDS), an inhibitor of the volume-sensitive anion channel [3]. These observations raise the possibility that nutrient stimuli might be able to activate the volume-sensitive anion conductance. This possibility is further sup-

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ported by the recent demonstration that glucose causes β-cell swelling to an extent sufficient to activate the conductance [6].

The present study demonstrates that an inward current can be demonstrated in cell-attached patches in β-cells exposed to hypotonic solutions or nutrient secretagogues, similar to the current previously observed in whole-cell recordings. A preliminary characterisation of the kinetics of this current has also been carried out.

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 by a brief (3–4 min) incubation in Ca^{2+}-free medium consisting of (in mmol/l) 135 NaCl, 5 KCl, 1 $MgSO_4$, 4 glucose, 1 EGTA and 10 HEPES-NaOH (pH 7.4). Cells were re-suspended in HEPES-buffered RPMI medium (Gibco, Paisley, UK), plated onto 30-mm-diameter polystyrene dishes and cultured for 2–7 days in humidified air at 37°C. Single β-cells, identified by their size and typical granular appearance, were used for all recordings. Cells were superfused at a rate of approximately 2 ml/min with a solution consisting of (in mmol/l) 135 NaCl, 5 KCl, 1 $MgSO_4$, 1 NaH_2PO_4, 1.2 $CaCl_2$, 10 HEPES-NaOH (pH 7.4) and glucose or α-ketoisocaproate at the required concentration. When cells were exposed to hypotonic solutions, 100 mmol/l mannitol was substituted for 50 mmol/l NaCl and a 33% hypotonic solution made by removal of mannitol.

Cell-attached recordings (gigaohm seal resistance) were made using a List EPC-7 amplifier. The pipette solution was designed to eliminate Na^+, K^+ and Ca^{2+} currents. The basic solution consisted of 140 CsCl, and 10 HEPES-CsOH (pH 7.4). In some experiments, a low $[Cl^-]$ pipette solution was used, containing 30 Cs_3citrate, 40 CsCl and 10 HEPES-CsOH. Relatively large diameter patch pipettes were used (approximately 1 MΩ) in order to optimise the number of patches containing active channels. All experiments were carried out at 30–32°C. Current recordings were filtered at 3 kHz and stored on DAT tapes for subsequent analysis. Two distinct approaches were used in order to analyse 'noisy' cur-

rents. The first approach utilised fluctuation or noise analysis (for example, see [7]). The recordings were filtered at 300 Hz via an 8-pole Bessel filter, transferred in 10-s segments to the hard disc of a Pentium computer and sampled at 512 Hz [7]. Mean whole-cell current and current variance were measured using pClamp software (version 6.0.3; Axon Instruments, Foster City, CA, USA). Analysis of current noise was performed by non-linear regression analysis using 'Graphpad' software. Current variance was plotted against mean current (following subtraction of leak current – see below) and the data fitted to the equation

$$\sigma^2 = iI - I_2/N \tag{1}$$

where σ^2 is the variance, I the mean current, i the unitary conductance and N the number of active channels (or subconductance levels).

In the second approach, current–amplitude histograms were constructed for 30-s segments of recording and fitted to Gaussian distributions by the 'least squares' method using pClamp software. Inward (negative) currents are shown as downward deflections in all cases; the zero current or closed channel levels are represented by dotted lines. When current–voltage plots were made, lines of 'best fit' were fitted by linear regression.

Collagenase (type 4) was obtained from Worthington (Cambridge Biosciences, Cambridge, UK). All chemicals were obtained from the Sigma, Poole, UK.

3. Results

Typical cell-attached patch recordings from single rat β-cells are shown in Fig. 1. In unstimulated cells, the background or leak current across the cell-attached patch at a pipette potential (V_p) of 0 mV was -0.53 ± 0.17 pA ($n = 25$). In 6/25 cells, exposure of cells to a 33% hypotonic solution resulted in the appearance of a noisy inward current. Similar currents were observed in cells upon raising the glucose concentration from 4 to 20 mmol/l (5/25 cells) or upon addition of 15 mmol/l α-ketoisocaproate (4/13 cells). However, the addition of 100 μmol/l tolbutamide, either to the bath or pipette solution, failed to induce any significant change in cell-attached patch current in 10/10 cells. Activation of the current by
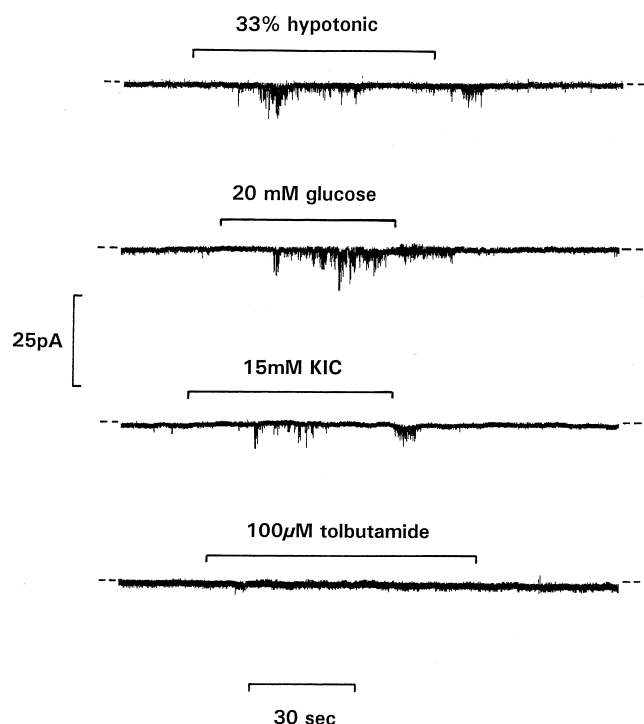
33% hypotonic

20 mM glucose

25pA

15mM KIC

100µM tolbutamide

30 sec

Fig. 1. Activation of the inward current in cell-attached patches. Cell-attached recordings from single rat β-cells. Effects of exposure to a 33% hypotonic solution (by removal of 100 mmol/l mannitol), a rise in glucose concentration from 4 to 20 mmol/l, addition of 15 mmol/l α-ketoisocaproate and of 100 µmol/l tolbutamide. The pipette potential was 0 mV in all cases. The zero current level is denoted by the dotted lines. Each trace is representative of those from a total of 4 to 10 similar recordings.

hypotonic solutions or by nutrient stimuli was reversible upon restoration of the control bath solution. The inward current could be detected in approximately 20% of cell-attached patches, whether in response to hypotonic solutions or nutrient stimuli. This figure is comparable with an earlier report of cell-attached recordings of a volume-sensitive anion channel in epithelial cells [8].

Fig. 2 shows a typical example of noise analysis performed on the noisy inward current evoked by 20 mmol/l glucose. The mean unitary current amplitude (i) was no different between cells stimulated with glucose or a hypotonic solution, the pooled value being 1.35 ± 0.21 pA ($n = 9$). The mean value of 'N', representing either channel number or subconductance states of a single channel, was 3.00 ± 0.28.

In addition to the noisy inward current recorded during stimulation, discreet channel events ('square' openings) of variable duration could also be resolved

in several cells, particularly during stimulation with hypotonic solutions (Fig. 3). The large histogram peak, which represents 'background' (leak) current, has an amplitude of -0.70 pA whilst the discreet channel events, marked by arrows, had a relative average amplitude of -2.8 pA. In β-cells stimulated with glucose, the noisy pattern of inward current appeared to predominate and discreet channel events were less common (Fig. 4). This was reflected by the poorer resolution of the large and small histogram peaks, which had amplitudes of -0.72 and -3.1 pA, respectively, giving an estimated amplitude of 2.38 pA for the discreet channel openings.

Although activation of the current could be achieved by raising the concentration of glucose or by exposure to hypotonic solutions, the effects of hypotonic cell swelling are transient. Therefore, the majority of subsequent experiments were carried out using β-cells bathed in medium containing either a stimulatory (12 mmol/l) or substimulatory (4 mmol/l) concentration of glucose.

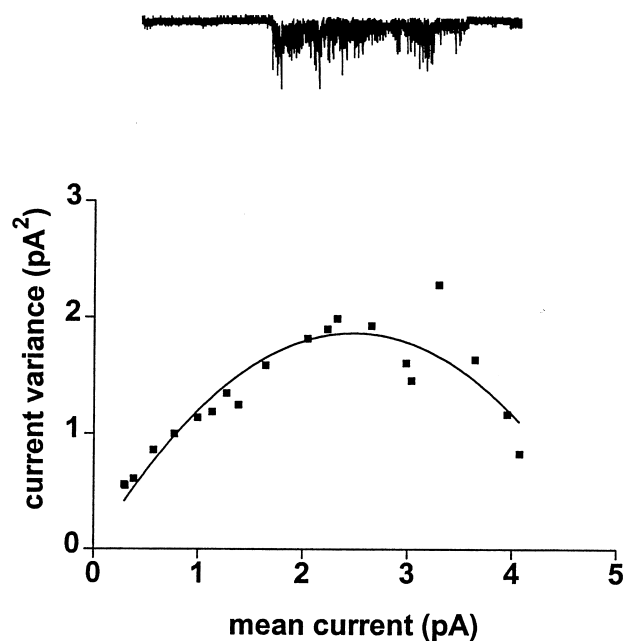The amplitude of the current was dependent upon

Fig. 2. Noise analysis of the glucose-induced inward current. Upper panel: cell-attached recording from a single rat β-cell during stimulation with 20 mmol/l glucose. The pipette potential was 0 mV. Lower panel: mean current in 10-s segments of the recording was plotted against current variance and fitted to Eq. 1 (see Section 2) by non-linear regression. For this particular cell, $i = -1.5$ and $N = 3.3$.
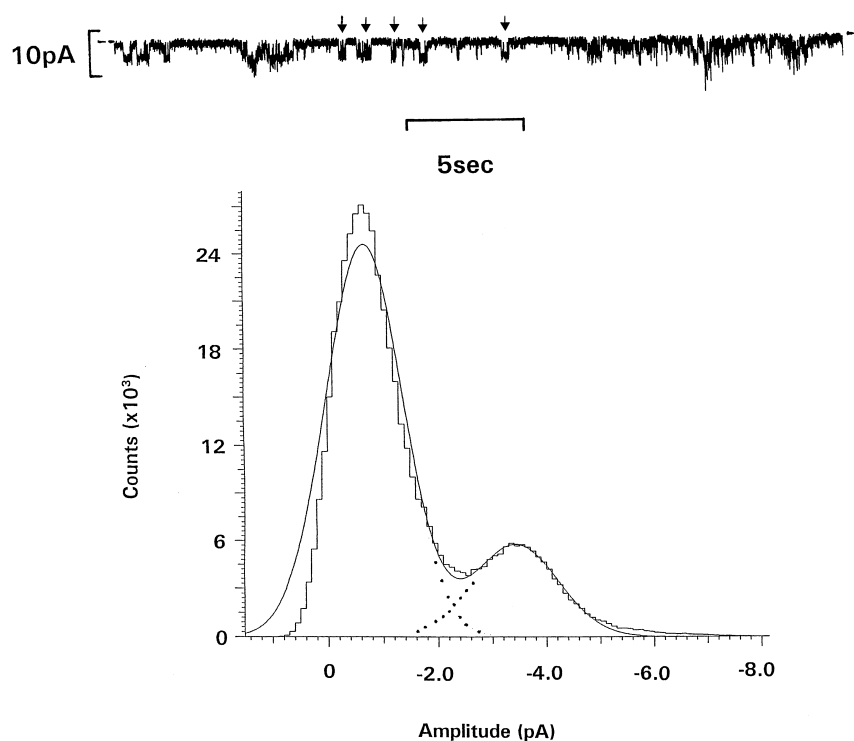
Fig. 3. Inward current evoked by exposure to a hypotonic solution. Upper panel: cell-attached recording of inward current in a rat β-cell during exposure to a 33% hypotonic solution. The discreet channel events referred to in the text are marked by arrows. The recording also shows the characteristic pattern of current noise. The dotted lines represent zero current. Lower panel: amplitude histogram taken from the above segment of recording. Gaussian functions were fitted by the 'least squares' method: the amplitudes of the two peaks are −0.7 and −3.5 pA.

V_p (Fig. 5). The V_p at which zero current was measured was not significantly different between cells stimulated with glucose or hypotonic solution, the pooled value being -4.6 ± 0.9 mV ($n = 11$). It should be noted that the pipette potential at which zero current through the channel is measured will be influenced by the intracellular concentrations of [Cl⁻] and other anions and by the cell membrane potential. Since these variables are not known with certainty, the reversal potential of the current cannot be readily related to the value of E_{Cl} from cell-attached recordings. However, for a Cl⁻-selective channel, a reduction in extracellular [Cl⁻] would be predicted to cause a negative shift in zero-current pipette potential. Experiments were therefore carried out to examine the effects on channel activity and kinetics of a reduction in extracellular (pipette) [Cl⁻] from 142.4 to 40 mmol/l whilst maintaining a high cation (Cs⁺) concentration. Under these conditions, it was found unexpectedly that channel activity could be resolved consistently into discreet square channel openings with greatly reduced current noise, enabling a more precise study of the kinetics of the channel underlying the current. Fig. 6 shows an example of a recording from a cell in the presence of 12 mmol/l glucose with a low [Cl⁻] pipette solution. In this case, the cell-attached patch contained two active channels (or one channel with two subconductance states) with a single-channel conductance of 222 pS. From a total of five such recordings, the mean conductance was 215 ± 4 pS. As was the case with the glucose-induced inward current recorded with a high [Cl⁻] pipette solution (e.g. see Figs. 1, 2 and 4), single-channel openings occurred in irregular 'bursts' of variable duration interspersed with relatively silent periods. The measurement of channel open probability in 30-s segments of recording gave a mean value of 0.25 ± 0.03 ($n = 5$) at $V_p = 0$ mV. This value was reduced at positive (hyperpolarising) potentials (0.09 ± 0.02 at $+30$ mV; $P < 0.02$) and increased at
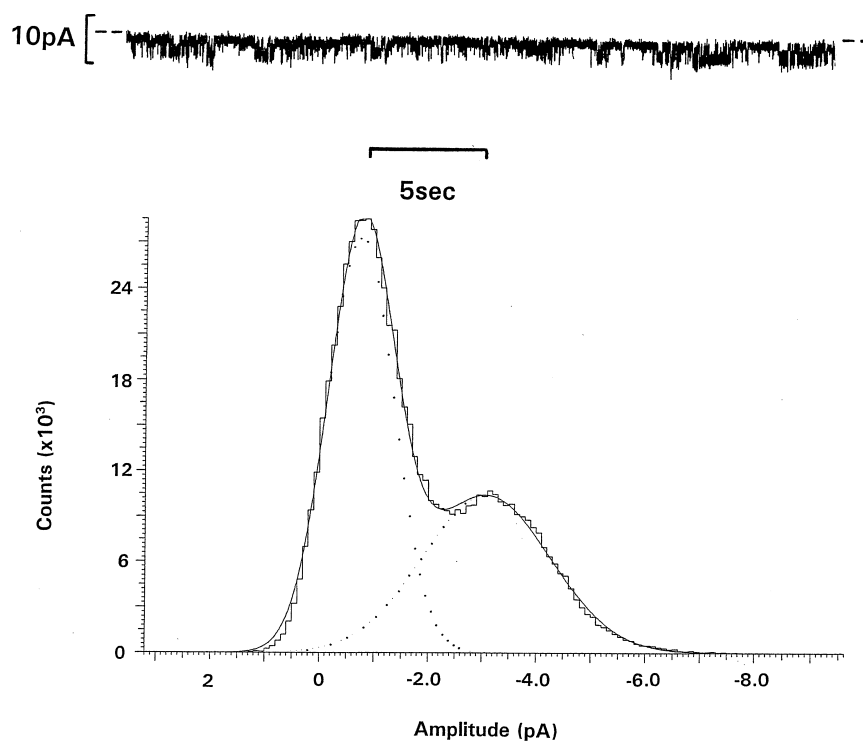
Fig. 4. Inward current evoked by a rise in glucose concentration. Upper panel: cell-attached recording of inward current in a rat β-cell during exposure to 12 mmol/l glucose. The dotted lines represent zero current. Lower panel: amplitude histogram taken from the above segment of recording. Gaussian functions were fitted by the 'least squares' method: the amplitudes of the two peaks are −0.72 and −3.1 pA.
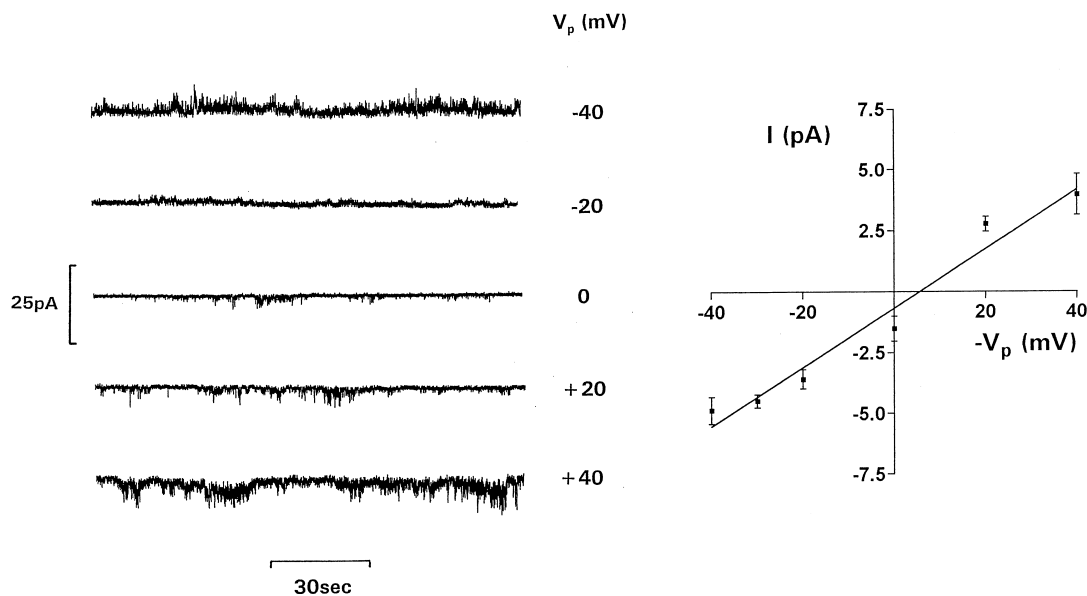

Fig. 5. Current–voltage relationship of glucose-induced current. Left panel: segments of cell-attached recording from a rat β-cell during exposure to 12 mmol/l glucose at different pipette potentials (V_p). Right panel: current–voltage relationship. Mean current amplitude for 30-s segments of recording was plotted against pipette potential. Each point represents the mean ± S.E.M. derived from five cells.
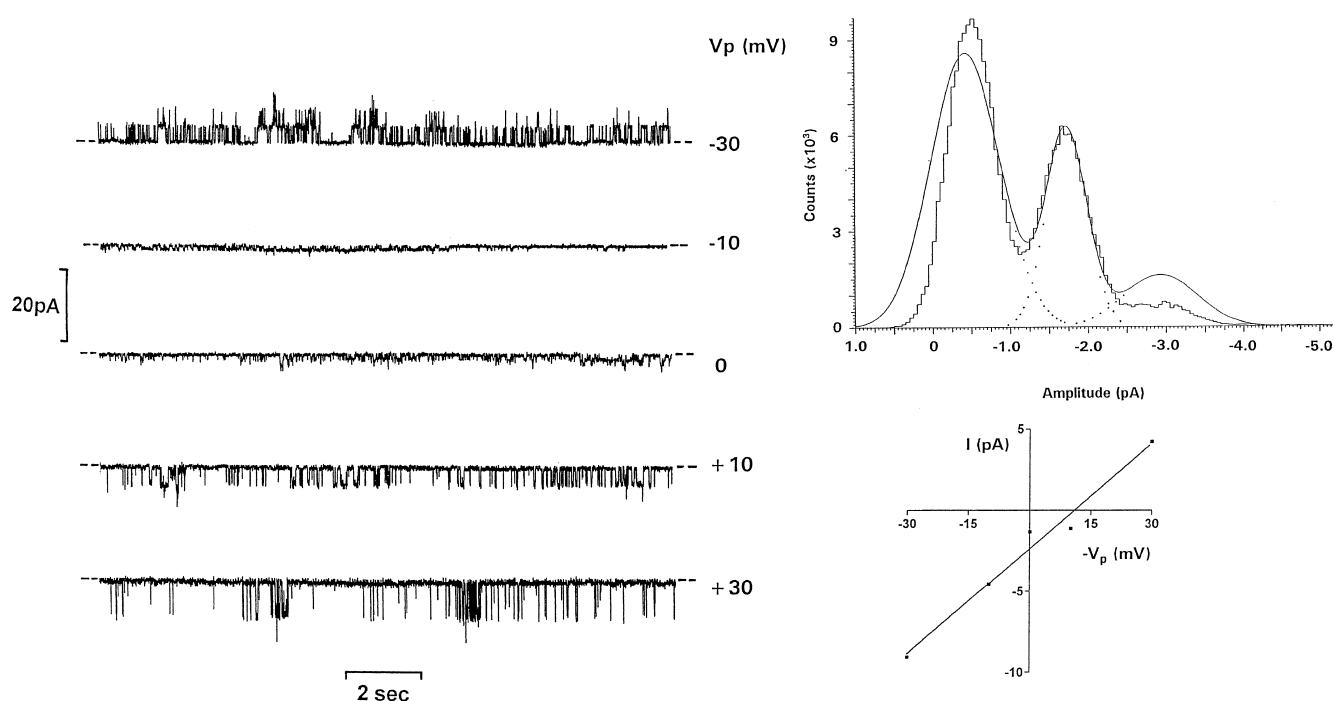
Fig. 6. Cell-attached recordings of discreet channel events during glucose stimulation. Cells were incubated in the presence of 12 mmol/l glucose. The patch pipette contained low (40 mmol/l) [Cl⁻]. Left panel: recordings of channel activity at different pipette potentials (Vp). Upper right panel: amplitude histogram obtained from the recording at 0 mV. The amplitudes of the three peaks are -0.45, -1.74 and -2.95 pA. Lower right panel: current–voltage relationship for this cell. Amplitude of channel events was plotted against pipette potential. The data in this figure are representative of those from a total of five cells.

negative (depolarising) potentials (0.44 ± 0.03 at -30 mV; $P < 0.01$). In cells incubated in the presence of a substimulatory concentration of glucose (4 mmol/l), open probability was < 0.01 in 9/9 cells. In cells stimulated with 12 mmol/l glucose, channel current reversal was measured at V_p -13.7 ± 0.4 mV ($n = 5$), representing a significant ($P < 0.001$) negative shift in reversal potential from that measured with high pipette [Cl⁻]. The magnitude of this shift in reversal potential (-9.1 mV) is less than that which would be predicted for a 'pure' Cl⁻ channel, probably due to the fact that the channel has a significant permeability not only to other anions (possibly including citrate), but also to cations [3].

In three cells in which glucose-stimulated channel activity could be detected in cell-attached patches, a similar pattern of channel openings could be recorded in inside-out patches excised from these cells into the bath solution (142.4 mmol/l Cl⁻). In the example shown in Fig. 7, channel activity at V_p from 0 to +40 mV consisted of openings of varying

duration which, at more positive potentials, were punctuated by rapid, brief closures. Patch excision appeared to result in an increase in channel open probability to 0.62 ± 0.1 ($n = 3$) at +20 mV. However, channel activity in excised patches was invariably found to be short-lived and could only be recorded for 2–3 min following excision, possibly due to the removal of ATP or other cellular components. In the recording shown in Fig. 7, there was evidence of two active channels or subconductance states. The estimated single-channel conductance was 127 ± 9 pS ($n = 3$) at +20 mV, suggesting that excision of the patch resulted in a reduction in channel conductance, again possibly due to the removal of ATP or other modulatory factors or to the change in Cl⁻ gradient. The reversal potential for this channel in excised patches was calculated as -13 ± 1 mV ($n = 3$). The deviation of this value from the E_{Cl} of -33 mV was observed to a similar extent in whole-cell recordings and again probably reflects the cation permeability of the channel [3].
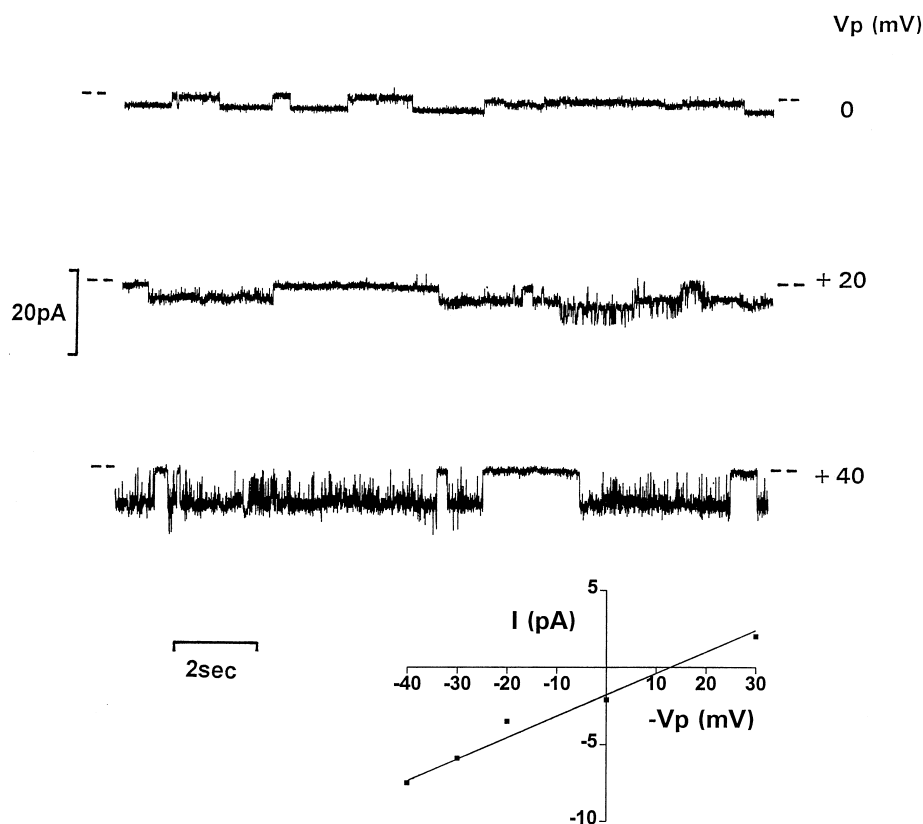
Fig. 7. Channel activity in an excised inside-out patch. The patch was excised from a cell incubated in the presence of 12 mmol/l glucose after recording channel activity in the cell-attached configuration (see Fig. 5). The pipette and bath solutions contained low (40 mmol/l) and high (142.4 mmol/l) [Cl$^-$], respectively ($E_{Cl} \sim -33$ mV). Upper panel: recordings of channel activity at different pipette potentials (V_p). Lower panel: current–voltage relationship for this patch. Amplitude of channel events was plotted against pipette potential. The data in this figure are representative of those from a total of three patches.

4. Discussion

Previous studies have demonstrated that exposure of single rat pancreatic β-cells to a 10% hypotonic challenge or to nutrient stimuli elicits an inward current at a holding potential of −70 mV under whole-cell perforated patch conditions [5]. The present study demonstrates that a current with similar characteristics can also be observed in cell-attached membrane patches. This, therefore, provides the first demonstration of an inward current induced by nutrient stimuli in non-voltage-clamped β-cells. As discussed previously [4,5], it is proposed that this current represents activation of the volume-sensitive anion conductance described in insulin-secreting cells [1–3]. This suggestion is reinforced by a number of findings from the present study. First, the current was activated by hypotonically induced cell swelling. Second, the inward current could be induced in the absence

of extracellular (pipette) Na$^+$, Ca^{2+} and K$^+$, and cannot therefore result from entry of these ions. As discussed above, the current cannot be readily identified from its reversal potential under cell-attached conditions, since neither the intracellular [Cl$^-$] nor the membrane potential are known with any accuracy during stimulation with hypotonic solutions or glucose. However, the finding that a reduction in pipette [Cl$^-$] caused a negative shift in the reversal potential of the current is consistent with an anion-selective channel. Furthermore, the zero-current potential of −13 mV obtained from the excised patch recordings is also comparable with that of −13.5 mV obtained from whole-cell measurements of the volume-sensitive anion conductance with a similar Cl$^-$ gradient [3].

Consistent with earlier observations from perforated patch whole-cell recordings [5], activation of the inward current in cell-attached patches could be

induced by an increase in glucose concentration or by application of α-ketoisocaproate, as well as by exposure to hypotonic solutions, whereas the sulphonylurea tolbutamide was ineffective. These findings provide further support for the hypothesis that the volume-sensitive anion conductance in β-cells can be activated by nutrient stimuli [5]. The mechanism underlying this activation is unknown, but could involve an increase in β-cell volume during nutrient stimulation [6]. In contrast to the effects of nutrient stimuli, the lack of effect of tolbutamide does not support the suggestion of Kinard and Satin [2] that the volume-sensitive anion conductance may be, like the K_{ATP} channel, modulated by the sulphonylurea receptor.

As was the case in earlier whole-cell recordings [5], the noisy pattern of the current evoked by hypotonic solutions or nutrients was also a striking characteristic of the current recorded in cell-attached patches in the present study. The underlying reasons for this type of channel activity are uncertain, although several studies of volume-sensitive anion channels in other cell types have also reported noisy kinetics [8–10]. The noisy current pattern recorded was often accompanied by trains of discreet square channel openings, especially in cells exposed to a hypotonic solution. The relationship between these discreet events and the noisy current is not certain. It might be suggested at first sight, given the difference between unitary conductance estimated from analysis of the current noise (1.35 pA) and the amplitude of the discreet channel events (2.38–2.8 pA), that the two patterns of current represent two distinct channel types. However, a number of observations suggest that both types of current could both be due to activity of the volume-sensitive anion channel. For example, both types of current were evoked by hypotonic solutions and both currents reversed at the same pipette potential. Furthermore, the square channel openings were never recorded in the absence of the noisy current pattern. An unexpected though consistent finding in the present study was that reduction in extracellular (pipette) [Cl⁻] allowed a clearer resolution of discreet channel openings in stimulated cells. In considering the kinetics of the volume-sensitive anion channel under different conditions, it should be emphasised that the gating of this type of channel is known to be highly complex,

and that the kinetics of the channel can be affected profoundly and in an unpredictable manner by changes in intracellular and extracellular [Cl⁻] [11,12], by changes in ionic strength and by the nature of the permeant anionic species [13].

The ability to record discreet channel openings conferred by reducing pipette [Cl⁻] permitted a preliminary study of single-channel kinetics. The calculated single-channel conductance of 215 pS (with low [Cl⁻]$_o$) is considerably greater than the values reported for volume-sensitive anion channels in most other cell types (20–80 pS) (see [14,15] for reviews). In this respect, the β-cell channel resembles the 'maxi' volume-sensitive anion channel described in a number of tissues [16,17]. However, the volume-sensitive anion channel in insulin-secreting cells differs from the 'maxi' anion channel on the basis of halide selectivity and outward-rectification [2,3]. Indeed, on the basis of the unusual halide selectivity sequence Br > Cl ≫ I [2,3], the β-cell channel appears to be distinct from the volume-sensitive anion channels described in virtually all other cell types.

In the presence of a substimulatory concentration of glucose, open probability of the channel was effectively zero (< 0.01) at $V_p = 0$ mV. In contrast, exposure to a raised concentration of the hexose resulted in an increase in open probability to approximately 0.25. This finding would appear to be inconsistent with the suggestion of Jackson and Strange [18] that activation of the volume-sensitive anion channel consists of an increase in the number of active channels in the membrane brought about by a switching of channels from an 'off' state to an 'on' state, the latter with an open probability close to 1. However, it should be pointed out that this suggestion was derived from whole-cell and excised patch recordings from cells in which near-maximal channel activation was brought about by exposure to a large hypotonic challenge. It is possible that, in the intact β-cell stimulated with glucose, where changes in cell volume are relatively modest [6], the channel is modulated in a different manner. The open probability of the channel was found to depend upon the applied pipette potential, being increased and reduced, respectively, at depolarising and hyperpolarising potentials. A similar voltage-dependence has been reported for the osmotically activated channel

in opossum kidney cells [19] and is also a characteristic of the 'maxi' channel referred to above [14].

Activity of volume-sensitive anion channels has been recorded in cell-attached patches in a number of cell types [8,19,20]. In some cases, it has been reported that channel activity could only be observed if the channels were activated prior to seal formation [8], leading to the suggestion that tight seal formation may interfere with channel activation, possibly by impeding interactions between the lipid bilayer and the cytoskeleton (see [15] for discussion of this topic). This was not, however, the case in rat pancreatic β-cells, where the channel could be activated following seal formation, as previously reported in the case of opossum kidney cells [19].

The recording of volume-sensitive anion channel activity in excised patches has been reported both in outside-out [8,18,21] and inside-out [22,23] configurations. As noted earlier, a major limitation encountered in the present study in recording channel activity from inside-out patches was channel 'rundown', a phenomenon also reported in Ehrlich ascites tumour cells [22]. Further work will clearly be required to study this and other aspects of channel activity in excised patches.

In conclusion, activity of the volume-sensitive anion channel can be recorded in cell-attached patches from rat pancreatic β-cells. Channel activation shows complex kinetics and can be induced by exposure to hypotonic solutions or to nutrient secretagogues, a process which involves an increase in channel open probability. Activation of this anion-selective channel generates an inward current and could therefore be an important event in causing β-cell depolarisation which leads to electrical and secretory activity.

Acknowledgements

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