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Inhibition of glucose-induced electrical activity in rat pancreatic β-cells by DCPIB, a selective inhibitor of volume-sensitive anion currents

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Abstract

We have investigated the effects of the ethacrynic acid derivative 4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid (DCPIB), an inhibitor of the volume-sensitive anion channel (VSAC), on electrical activity and insulin secretion in rat pancreatic β-cells. DCPIB inhibited whole-cell VSAC currents in β-cells with IC_{50} values of 2.2 and 1.7 μM for inhibition of outward and inward currents, respectively. DCPIB also inhibited the VSAC at the single channel level in cells activated by glucose. In intact cells, DCPIB caused a net increase in β-cell input conductance and evoked an outward current that was sensitive to inhibition by tolbutamide, suggesting K_{ATP} channel activation. However, no K_{ATP} channel activation was evident under conventional whole-cell conditions, suggesting that the drug might activate the channel in intact cells via an indirect mechanism, possibly involving nutrient metabolism. DCPIB suppressed glucose-induced electrical activity in β-cells, hyperpolarised the cell membrane potential at a substimulatory glucose concentration and prevented depolarisation when the glucose concentration was raised to stimulatory levels. The suppression of electrical activity by DCPIB was associated with a marked inhibition of glucose-stimulated insulin release from intact islets. It is concluded that DCPIB inhibits electrical and secretory activity in the β-cell as a combined result of a reciprocal inhibition of VSAC and activation of K_{ATP} channel activities, thus producing a marked hyperpolarisation of the β-cell membrane potential.

Keywords: DCPIB; Volume-sensitive anion channel; K_{ATP} channel; Islet; Pancreatic β-cell; Insulin release

1. Introduction

Glucose-induced insulin release is associated with a characteristic pattern of electrical activity in the pancreatic β-cell. A rise in glucose concentration causes a depolarisation of the β-cell membrane potential, leading to activation of voltage-dependent Ca^{2+} channels and producing a characteristic pattern of 'spiking' action potentials (see Ashcroft and Rorsman, 1989; Rorsman, 1997 for reviews). This phenomenon is the result of increased glucose metabolism in the β-cell and the generation of one or more intracellular metabolic signal(s).

The exact identity of these signals is unknown, but the current 'consensus model' involves the closure of K_{ATP} channels due to an increase in intracellular [ATP] (Cook and Hales, 1984) and/or ATP/ADP ratio (Dunne and Petersen, 1986). However, there is increasing evidence that glucose can stimulate electrical activity and insulin release by one or more mechanisms that do not involve K_{ATP} channel closure (Best et al., 1992; Gembal et al., 1993; Straub et al., 1998; Henquin, 2000; Westerlund et al., 2001; Best, 2002a). We have suggested that activation of a volume-sensitive anion channel (VSAC) by glucose could be one such mechanism. We have provided evidence that glucose activates this channel and generates an inward depolarising current (Best, 1999, 2002c; Best et al., 1997), possibly by increasing β-cell volume (Miley et al., 1997). Consistent with this suggestion, we have also shown that glucose-induced electrical activity and insulin release are sensitive to inhibitors of the VSAC, including 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS; Best, 1997) 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB; Best, 1997) and 4-hydroxytamoxifen

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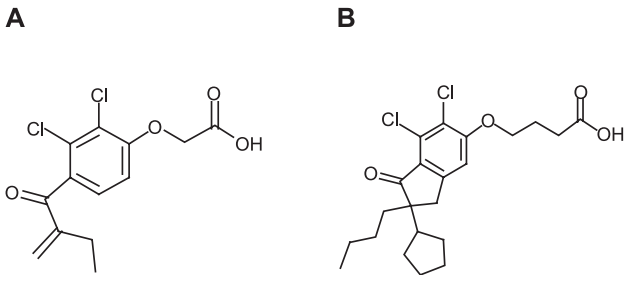

Fig. 1. Chemical structures of (A) ethacrynic acid and (B) 4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid (DCPIB).

(Best, 2002b). However, all of these drugs are nonselective and affect activity of several other ion channels and transporters, making it difficult to interpret these findings definitively.

The ethacrynic acid derivative 4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid (DCPIB) has recently been reported to be a potent and selective inhibitor of the VSAC [or volume-regulated anion channel (VRAC)] in bovine pulmonary artery endothelial cells and in guinea pig atrial myocytes (Fig. 1) (Decher et al., 2001). In the present study, we have investigated the effects of this drug on glucose-induced electrical and secretory activity in rat pancreatic β-cells.

2. Methods

Pancreatic islets were isolated from Sprague–Dawley rats by collagenase digestion (Lacy and Kostianovsky, 1967). For electrophysiological studies, islets were dispersed into single cells by a brief (~ 5 min) exposure to Ca^{2+}-free medium containing (in mM) NaCl (125), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), glucose (4), EGTA (1) and HEPES–NaOH (20; pH 7.4). Cells were suspended in HEPES-buffered Minimal Essential Medium (Sigma, Dorset, UK) and cultured in polystyrene dishes for up to 10 days. The standard medium used for all incubations contained NaCl (125), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), $CaCl_2$ (1.2), glucose (4) and HEPES–NaOH (25; pH 7.4).

Activity of the VSAC was measured using the conventional whole-cell configuration of the patch-clamp technique (Best, 2002b) and at the single channel level in cell-attached patches (Best, 1999, 2002c). In the former case, cells were subjected to 200 ms voltage pulses of ± 100 mV at 4-s intervals from a holding potential of 0 mV. A hypertonic pipette solution (330 mosM) was used to induce cell swelling and consisted of CsCl (40), ATP (2), $MgCl_2$ (3), EGTA (1), HEPES–NaOH (10; pH 7.2) and mannitol (220). For cell-attached recordings of the VSAC, the pipette solution consisted of Cs_2SO_4 (50), CsCl (40), 1 $MgCl_2$ (1) and HEPES–CsOH (10; pH 7.4), with a pipette potential of 0 mV. Diazoxide (150 μM) was included in the bath solution to clamp the cell membrane potential close to E_K (~ − 70 mV) and prevent any changes in membrane potential. Channel open probability was measured using pClamp 6 software (Axon Instruments, Foster City, CA, USA). Input conductance (G_{input}) of the cells was measured under perforated patch conditions (Best, 2000) with a pipette solution containing K_2SO_4 (60); KCl (20), NaCl (10), HEPES–NaOH, (10; pH 7.2) and amphotericin B (240 μg/ml). Cells were voltage-clamped at − 70 mV and subjected to 50 ms pulses of ± 10 mV at 4-s intervals. Net K^+ conductance was also measured under perforated patch conditions in cells voltage-clamped at − 15 mV (close to the reversal potential of the VSAC under these conditions; Best et al., 1996a) and in conventional whole-cell mode using a pipette solution containing KCl (140), $MgSO_4$ (2), ATP (0.5) and HEPES (10; pH 7.2).

Changes in β-cell membrane potential were recorded by means of the perforated patch technique under current-clamp conditions as described previously (Best, 1997, 2000). Recordings were made only from cells that showed a depolarisation in response to a stimulatory glucose concentration. Insulin release was measured in groups of 10 islets incubated for 60 min in 1 ml medium containing 1 mg/ml bovine serum albumin. The insulin content of the medium was measured by radioimmunoassay. The patch-clamp experiments were performed at 30–32 °C and secretion measurements at 37 °C.

[^{125}I]-insulin was obtained from the Radiochemical Centre, Amersham, UK, collagenase (type 4) from Worthington Biochemicals (Freehold, NJ, USA) and all chemicals from the Sigma (Poole, UK). DCPIB was obtained from Aventis Pharma, Frankfurt am Main, Germany, and was diluted into the incubation medium at the required concentration from a 10 mM stock solution in dimethylsulphoxide (DMSO) at a final concentration of 0.1% (v/v) or less. DMSO at this concentration did not affect control responses. Statistical significance was assessed using Student's unpaired or paired *t*-test.

3. Results

The first series of experiments examined the effects of DCPIB on activity of the VSAC in rat pancreatic β-cells. Fig. 2 shows the effects of the drug on VSAC activity under conventional whole-cell recording conditions. The channel was activated by the use of a hypertonic pipette solution, generating a characteristic outwardly rectifying current in response to voltage pulses of ± 100 mV. Application of 10 μM DCPIB inhibited both outward and inward components of the current, the effect being virtually complete within 2–3 min (Fig. 2, upper panel). Inhibition of the channel by DCPIB was very slowly reversible, and required 15 min or more following withdrawal of the drug before channel activity reappeared. The inhibition of VSAC activity by DCPIB was concentration dependent, with IC_{50} values for the inhibition of outward and inward currents of 2.2 and 1.7 μM respectively (Fig. 2, lower panel).

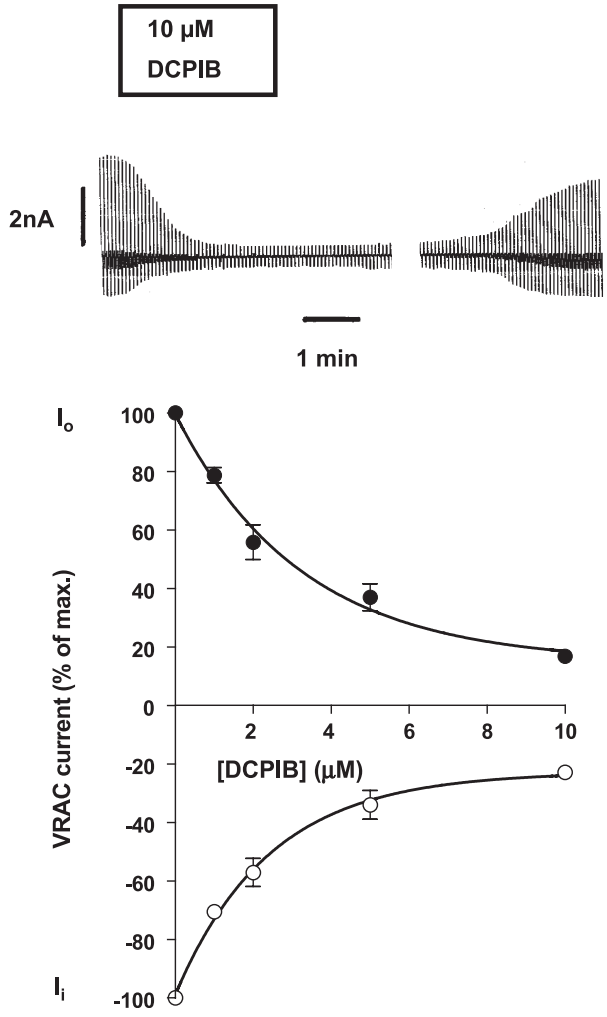

Fig. 2. Effect of DCPIB on activity of the VSAC in rat pancreatic β-cells under conventional whole-cell recording conditions. Cells were held at 0 mV and subjected to 200 ms voltage pulses of ±100 mV. Channel activation was achieved by the use of a hypertonic pipette solution. Outward and inward currents are shown as upward and downward deflections respectively. The break in the upper trace represents a period of 10 min following washout of the drug. The lower panel shows data for maximal inhibition of the channel by various concentrations of DCPIB from 4–6 similar experiments (mean ± S.E.M.). All concentrations of the drug shown significantly ($P<0.01$) inhibited both outward and inward currents.

The above experiments demonstrate that DCPIB inhibits the VSAC under conventional whole-cell recording conditions, where nonliving cells are exposed to a large osmotic gradient, causing near-maximal activation of the conductance. We therefore investigated the effects of DCPIB on glucose-stimulated VSAC activity in intact β-cells. As previously reported, single channel activity can be recorded under such conditions in cell-attached patches (Best, 1999, 2002c). Two such recordings are shown in Fig. 3 from cells where the channel was activated by 16 mM glucose. Under such conditions, channel open probability was 0.59 ± 0.19 ($n=5$). Within approximately 1–2 min exposure of the cell to 10 μM DCPIB, channel activity was completely inhibited (open probability zero) in all cases (Fig. 3A). The inhibitory effect of this high concentration was irreversible for at least 20 min following removal of the drug, probably due to the slow rate of washout of the hydrophobic drug from the cell interior. A lower concentration of the drug (2 μM) also inhibited channel activity (Fig. 3B). In this case, however, inhibition required longer exposure to the drug and residual channel activity could still be detected consisting of occasional small amplitude currents (see trace b in Fig. 3B). Thus, channel inhibition by DCPIB appears to be due to a progressive reduction in both open probability and current amplitude. With the lower concentration of the drug, a degree of reversibility could be seen following withdrawal, although channel activity after exposure to the drug consisted of a somewhat 'noisy' pattern of current (see trace c in Fig. 3B). It was consistently observed that, before the inhibitory effect of DCPIB on channel activity became apparent, open probability of the channel showed a transient increase. A similar phenomenon has recently been reported with the VSAC inhibitors DIDS and NPPB (Best, 2002c) and has been attributed to the initial inhibition of channels outside the patch from which the recording is being made,

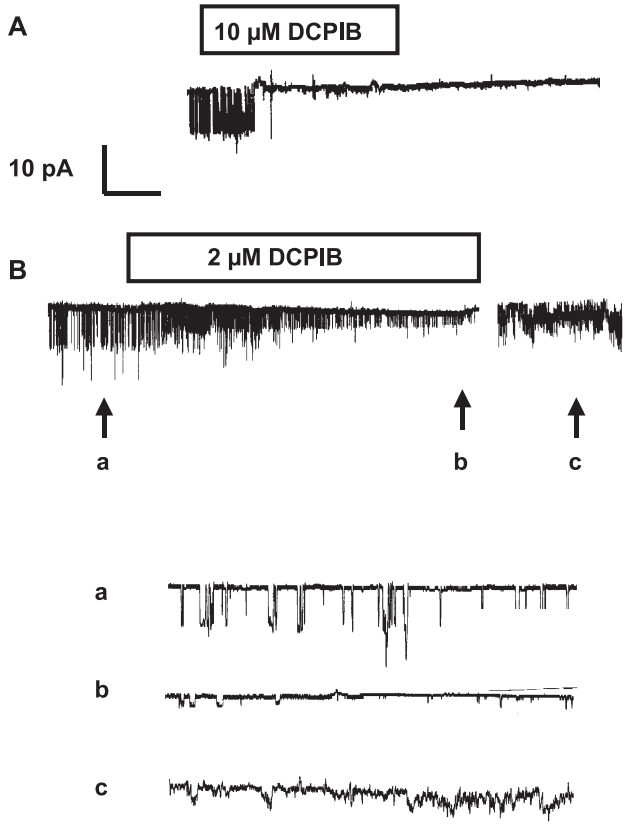

Fig. 3. Cell-attached recordings of the VSAC in rat pancreatic β-cells in the presence of 16 mM glucose: inhibition of channel activity by (A) 10 μM or (B) 2 μM DCPIB. Traces a, b and c are segments from trace B as shown on an expanded time scale. Horizontal calibration bar: 1 min (traces A and B); 2 s (traces a, b and c). The pipette potential was 0 mV in all cases. The gap in trace B is approximately 5 min following drug 'washout'. The recordings are representative of those from a total of five experiments showing similar results.

resulting in raised intracellular [Cl^-] and thereby increasing current flow via the channel in the patch, possibly accentuated by increased cell volume. The subsequent inhibition of the recorded channel presumably occurs when the drug has permeated the cell membrane and reached a sufficient intracellular concentration. Clarification of the kinetics of VSAC inhibition by DCPIB will require studies of channel activity in excised patches.

Because β-cell membrane potential can be influenced by changes in K^+ channel activity, notably that of K_{ATP} channels, experiments were carried out to determine whether these channels were affected by DCPIB. Fig. 4A shows the effect of DCPIB on β-cell input conductance (G_{input}), to which K_{ATP} channel activity makes a major contribution (Ashcroft and Rorsman, 1989). In the presence of 10 mM glucose, application of 10 μM DCPIB caused a gradual increase in G_{input} from a value of 1.73 ± 0.23 nS to a maximum of 8.50 ± 0.65 nS ($n=4$; $P<0.001$). In cells voltage-clamped at −15 mV (the approximate reversal potential of the VSAC under these conditions), 10 μM DCPIB evoked a gradual outward current with an EC_{50} value of approximately 1.5 μM (Fig. 4B). This current was completely and reversibly inhibited by 100 μM tolbutamide (Fig. 4B), suggesting that it resulted from K_{ATP} channel activation. In contrast to the perforated patch experiments, DCPIB had no effect on net K^+ current under conventional whole-cell conditions, where the cell interior was perfused with a K^+-rich solution containing 0.5 mM ATP (not shown).

The next series of experiments investigated the effects of DCPIB on glucose-induced electrical activity in β-cells.

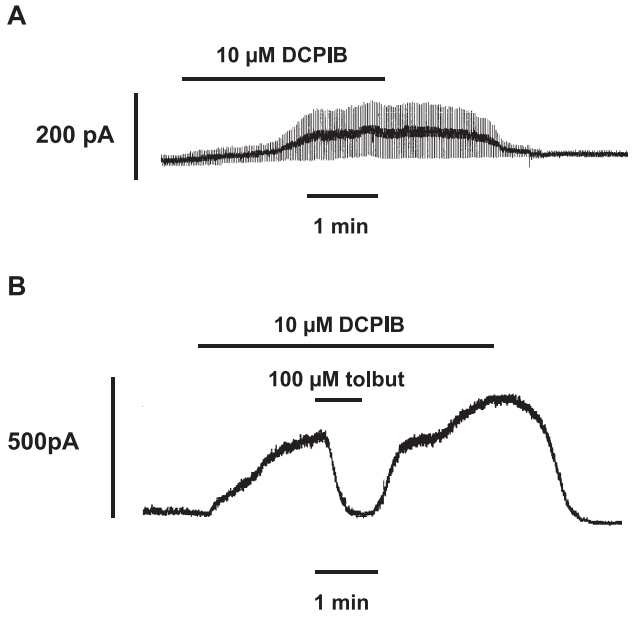

Fig. 4. (A) Effect of 10 μM DCPIB on β-cell input conductance in the presence of 10 mM glucose under perforated patch conditions. The cells were held at −70 mV and subjected to 50 ms pulses of ±10 mV. (B) Inhibition by tolbutamide of the outward current evoked by DCPIB. The cell was held at −15 mV in the presence of 10 mM glucose. Both are perforated patch recordings and are representative of 4–5 similar experiments.

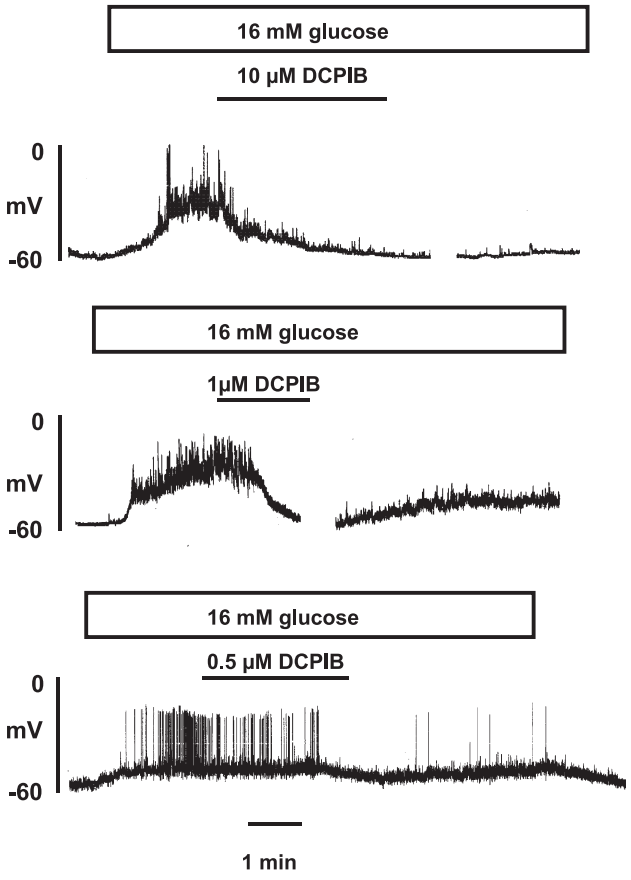

Fig. 5. Perforated patch recordings of β-cell membrane potential. Effects of DCPIB on electrical activity evoked by raising the glucose concentration from 4 to 16 mM. The drug was added at the concentrations shown for the period indicated by the horizontal bars. The gaps in the upper and centre traces represent a period of approximately 10 min following washout of the drug. Each recording is representative of at least five similar experiments.

Electrical activity was routinely evoked by raising the glucose concentration of the superfusate from 4 to 16 mM. The subsequent application of DCPIB (10, 1 or 0.5 μM) gradually repolarised the cell and terminated electrical activity (Fig. 5). With lower concentrations of the drug, longer exposure was required to inhibit electrical activity. Again, complete reversibility of the drug's inhibitory action was difficult to achieve. Thus, inhibition by 10 μM DCPIB was effectively irreversible, at least within a period of approximately 15 min 'washout' of the drug (Fig. 5, upper panel). At lower concentrations of DCPIB, the cells gradually repolarised following drug withdrawal, but this was rarely sufficient to permit full glucose-induced electrical activity (Fig. 5, centre and lower panels). In cells exposed to 10 μM DCPIB, a rise in glucose concentration failed to cause any depolarisation (Fig. 6, upper panel). At a substimulatory concentration of glucose (4 mM), application of 10 μM DCPIB was found to cause a gradual hyperpolarisation of the cell membrane potential from −56 ± 3 to −65 ± 4 mV ($n=4$, $P<0.05$ by paired t-test; Fig. 6, lower panel). Again, following a subsequent 15 min washout period, raising the concentration of glucose to 16 mM caused

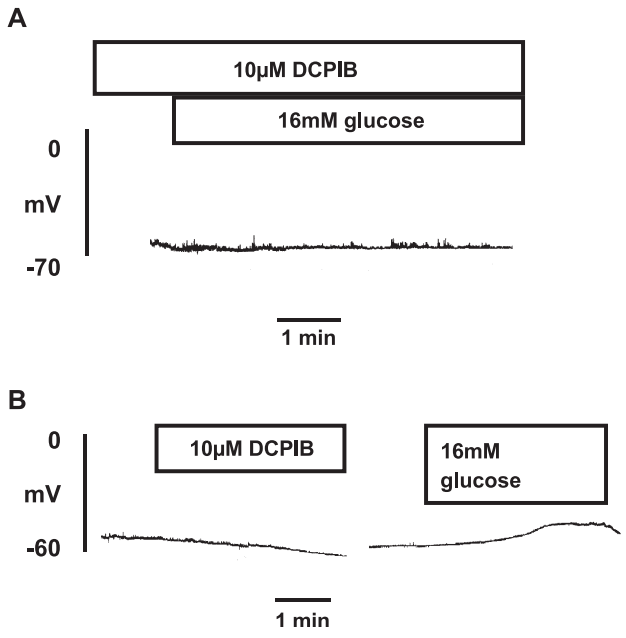

Fig. 6. Perforated patch recordings of β-cell membrane potential. (A) Effect of 16 mM glucose on cells preexposed to DCPIB and of (B) DCPIB on resting membrane potential in the presence of 4 mM glucose. The gap in the lower trace represents a period of approximately 12 min following washout of the drug, prior to raising the glucose concentration.

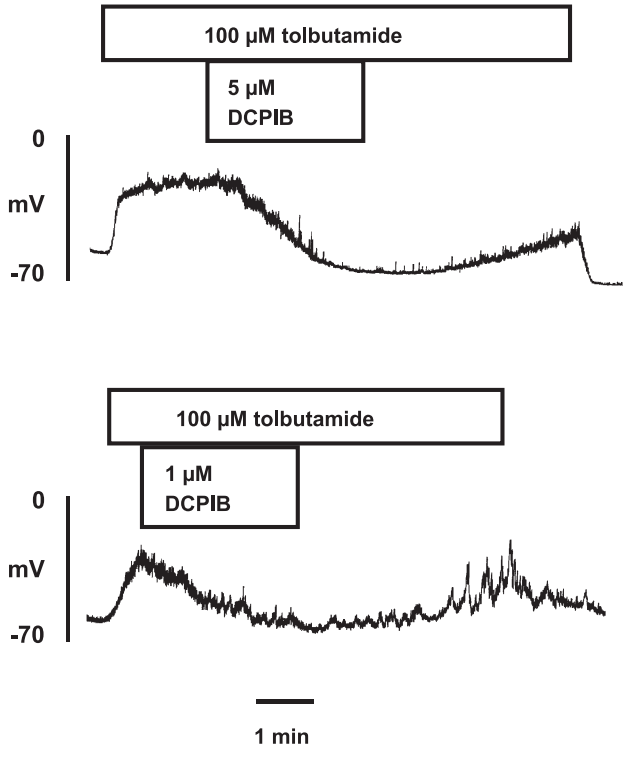

Fig. 7. Perforated patch recordings of β-cell membrane potential. Effects of DCPIB on electrical activity evoked by tolbutamide (100 μM). The traces are representative of 3–4 similar experiments.

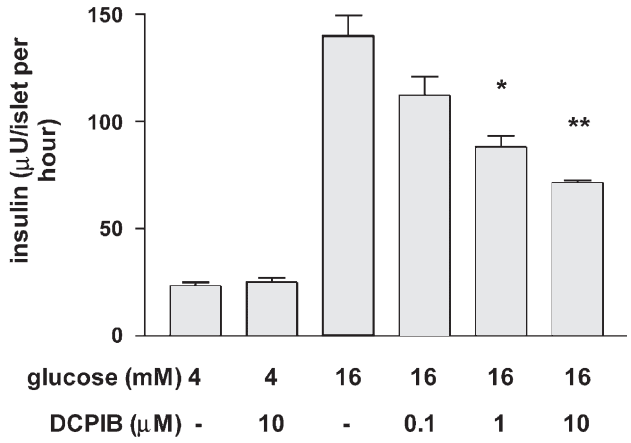

Fig. 8. Effect of DCPIB on basal and glucose-induced insulin release from intact rat islets. Groups of 10 islets were preincubated for 5 min either with or without DCPB then incubated for a further 60 min with the glucose concentration shown. Data are mean ± S.E.M. of six incubations. *$P<0.01$; **$P<0.001$ compared to column 3.

only a slow, modest depolarisation insufficient to generate electrical activity. DCPIB was also found to inhibit electrical activity in β-cells stimulated by the sulphonylurea tolbutamide (Fig. 7). In this case, the inhibitory effect of the drug was found to be partially reversible.

The final series of experiments examined the effects of DCPIB on insulin release from intact islets (Fig. 8). Basal release of insulin in the presence of 4 mM glucose (23.4 ± 1.6 μU/islet in 60 min) was not significantly affected by DCPIB (10 μM; 25.0 ± 2.1), despite the fact that the drug hyperpolarised the cells under these conditions. This is almost certainly because basal insulin release does not involve electrical activity. In contrast, insulin release evoked by 16 mM glucose (140.0 ± 9.5) was inhibited in a concentration-dependent manner by DCPIB (71.6 ± 0.92, $P<0.001$ at 10 μM DCPIB).

4. Discussion

The recent characterisation of DCPIB as a selective blocker of the VSAC presented the opportunity to assess the role of this channel in the regulation of pancreatic β-cell function. It should be noted that, although sharing several characteristics with the VSAC in other cell types, the β-cell channel appears to be distinct, at least on the basis of its halide selectivity sequence Br>Cl>I (Kinard and Satin, 1995; Best et al., 1996b) and relatively large single channel conductance (Best, 1999, 2002c). It was therefore initially confirmed that DCPIB inhibited VSAC activity in the β-cell. Under conventional whole-cell recording conditions, half-maximal inhibition of both the outward and inward current was observed at approximately 2 μM DCPIB. This value compares with that of 4.1 μM previously reported for bovine pulmonary artery endothelial cells (Decher et al., 2001). Channel inhibition was voltage-independent (i.e., a

similar degree of inhibition of inward and outward currents), again as previously reported in other cell types (Decher et al., 2001) and in marked contrast to other VSAC blockers such as DIDS and NPPB (see, e.g., Best et al., 1996b). Inhibition of VSAC activity could also be demonstrated at the single channel level in intact cells stimulated by glucose. However, it should be noted that the experimental conditions used for recording whole-cell VSAC currents, namely near-maximal activation by the use of a hypertonic pipette solution, are not strictly representative of normal physiological conditions with intact living cells, which are required to study channel activation by glucose. This could explain, for example, why 2 μM DCPIB reduced whole-cell currents by less than 50% but almost totally inhibited single channel currents.

Previous studies of the ion channel pharmacology of DCPIB revealed no apparent action on a number of K^+ channels implicated in repolarisation of heart muscle, including I_{Kr}, I_{Ks} and I_{K1}, or on Ca^{2+} currents (Decher et al., 2001). We confirmed that the drug had no direct effect on net K^+ current under similar conventional whole-cell recording conditions. However, in perforated patch recordings from intact, living β-cells, there was evidence for activation of K_{ATP} channel activity via an indirect mechanism, possibly involving an effect of the drug on cellular metabolism. The kinetics of K_{ATP} channel activation in intact cells broadly resembled those of VSAC inhibition, that is a gradual onset and slow reversibility after withdrawal of the drug over the concentration range 0.5–10 μM. It is likely that the delayed onset and reversibility seen with DCPIB reflect the strong hydrophobicity of the drug, which was also a complicating factor in obtaining a precise assessment of the concentration dependence of channel (in)activation. In general, the effects of the drug on channel activity increased with longer periods of exposure, both at whole-cell and single channel levels. This phenomenon could reflect the binding and accumulation of the hydrophobic drug in cellular lipid membranes. The finding that DCPIB produced a net increase in β-cell input conductance suggests that K_{ATP} channel activation by the drug was of a relatively greater magnitude than VSAC inhibition, presumably reflecting the dominating influence of the K_{ATP} channel on β-cell membrane conductance (Ashcroft and Rorsman, 1989).

Inhibition of VSAC and activation of K_{ATP} channel activities by DCPIB was associated with a predictable inhibition of glucose-induced electrical activity. Because electrical activity in isolated rat β-cells does not involve a regular 'bursting' pattern, it is difficult to quantify the inhibitory effect of DCPIB in terms of an IC_{50} value. In fact, the drug was able to suppress electrical activity over the concentration range 0.5–10 μM, although this effect required longer exposure with lower concentrations of the drug. However, the above range of concentrations is broadly similar to that effective in inhibiting VSAC and activating K_{ATP} activities. These actions would thus account for the marked hyperpolarisation of the β-cell membrane potential observed, even in the presence of nonstimulatory glucose concentrations. It was also notable that DCPIB reversed the depolarisation of β-cells induced by tolbutamide, despite the fact that the sulphonylurea was able to inhibit K_{ATP} channel activation by DCPIB in intact cells. It is possible that this could reflect the dual action of DCPIB, namely K_{ATP} channel activation and VSAC inhibition) in hyperpolarising the β-cell.

It was evident that, whilst electrical activity induced by 16 mM glucose was virtually abolished by 10 μM DCPIB, glucose-induced insulin release was not completely inhibited in the presence of the same concentration of the drug. It should be noted that different experimental systems (isolated β-cells vs. intact islets) are used for these studies, with longer incubations necessary for the secretion measurements. However, it is possible that the DCPIB-resistant component of glucose-induced insulin release represents the secretory pathway reported to be independent of electrical activity (see Henquin, 2000 for review of this topic).

In conclusion, the ethacrynic acid derivative DCPIB was found to be a potent inhibitor of the VSAC in rat pancreatic β-cells whilst activating K_{ATP} channel activity in an equipotent manner. This reciprocal effect caused a marked hyperpolarisation of the β-cell membrane potential and hence inhibited glucose-induced electrical activity and insulin release.

Acknowledgements

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