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Effects of sulphonylureas on the volume-sensitive anion channel in rat pancreatic β-cells

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1 The volume-sensitive anion conductance in rat pancreatic β-cells was studied directly using the conventional whole-cell and perforated patch recording techniques, and indirectly by measuring ^{3}H-taurine efflux from pre-loaded, perifused islets.

2 Using the conventional whole-cell recording configuration, activation of the outwardly-rectifying, DIDS-sensitive conductance was induced by glibenclamide (10 μM) but not by tolbutamide (100 μM) nor by meglitinide (20 μM). A high concentration of glibenclamide (100 μM) caused a voltage-and time-dependent inhibition of the conductance. Tolbutamide had a modest inhibitory effect on swelling-induced inward currents.

3 In perforated patch recordings, glibenclamide, tolbutamide and meglitinide were all without effect on the conductance, although activation could be induced under these conditions by exposure to a hypotonic bath solution.

4 The rate of efflux of ^{3}H-taurine, a marker for activity of the volume-sensitive anion channel, from preloaded, perifused islets was markedly stimulated by exposure to a hypotonic solution. However, glibenclamide and tolbutamide were both without effect.

5 Electrical activity of β-cells in response to glibenclamide or tolbutamide was not inhibited by 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid (DIDS), an inhibitor of the volume-sensitive anion channel.

6 It is concluded that activity of the volume-sensitive anion conductance in rat pancreatic β-cells is not modulated by the sulphonylurea receptor. The activation of the conductance by glibenclamide in whole-cell recordings could be the result of a non-specific interaction of the drug with plasma membrane lipids.

Keywords: Anion channel; volume regulation; pancreatic β-cell; sulphonylurea

Introduction

A volume-sensitive anion channel has recently been described in insulin-secreting cells (Best *et al*., 1995; 1996b; Kinard & Satin, 1995). This channel is activated by cell swelling, inhibited by the anion channel blockers 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and is ATP-dependent. Activation of the channel in intact β-cells leads to the generation of an inward depolarizing current and electrical activity (Best *et al*., 1996a). This inward current is presumably due to Cl^- efflux since intracellular [Cl^-] has been reported to be maintained above its electrochemical equilibrium ($E_{Cl} = -18$ to -9 mV; Sehlin, 1978).

It has also been reported that the volume-sensitive anion channel is activated by the sulphonylurea glibenclamide (Glyburide; Kinard & Satin, 1995). In general, sulphonylureas are thought to stimulate β-cell function by inhibiting K_{ATP} channels (Ashcroft & Rorsman, 1989), of which the sulphonylurea receptor is a functional subunit (Aguilar-Bryan *et al*., 1995). The finding that the volume-sensitive anion channel is activated by glibenclamide led Kinard & Satin (1995) to suggest that the sulphonylurea receptor might also interact with this channel, and that such an interaction could contribute towards depolarization, electrical and secretory activity in the β-cell. Such a mechanism would have important implications both for the regulation of β-cell function and for the design of potential antidiabetic drugs. The present study has therefore further investigated the effects of sulphonylureas and a related compound meglitinide on activity of the volume-sensitive anion channel in rat pancreatic β-cells using four distinct experimental approaches.

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Methods

Rat pancreatic β-cells were prepared as described previously (Best *et al*., 1996b). Cells were superfused with a solution containing (mM) NaCl (135), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), $CaCl_2$ (1.2), glucose (4) and HEPES-NaOH (10; pH 7.4, 285mOsM). In experiments where cells were exposed to a hypotonic solution, 100 mM mannitol was substituted for 50 mM NaCl and a 33% hypotonic solution (190mOsM) made by omission of mannitol.

Activity of the volume-sensitive anion channel was measured in single isolated cells by the conventional whole-cell and perforated-patch configurations of the patch-clamp technique using a List EPC-7 amplifier. In the former case, the pipette solution consisted of CsCl (40), ATP (2), $MgCl_2$ (3), EGTA (1), HEPES-NaOH (10) and mannitol (180). This solution was slightly hypotonic (275mOsM) in order to prevent cell swelling and activation of the channel. In experiments where channel activation was required, the mannitol concentration in the pipette solution was raised to 220 mM. For perforated patch recordings, the pipette solution consisted of Cs_2SO_4 (60), CsCl (20), NaCl (10), HEPES-NaOH (10; pH 7.2) and amphotericin B (240 μg/ml). All current amplitudes shown represent the maximum values, usually achieved within 2–4 min, for each cell under set experimental conditions. Current amplitudes were measured 20 ms after the start of each voltage pulse. Membrane potential was recorded using the perforated

patch technique in current-clamp mode, though in this case, K^+ was substituted for Cs^+ in the pipette solution.

^{3}H-taurine efflux, reported to provide an assessment of activity of the volume-sensitive anion conductance (Manolopoulos *et al.*, 1997), was measured from intact rat islets. Groups of 100 islets were preincubated in 0.5 ml incubation medium containing 4 μCi ^{3}H-taurine (sp. act. 24 Ci mmol^{-1}) for 2 h at 37°C. The islets were then placed in small (200 μl) chambers and perifused at a rate of 1 ml min^{-1}. After a 15 min 'washout' period, samples of effluent were collected at 1 min intervals and counted for radioactivity content. Results are expressed as fractional outflow rates. Patch-clamp experiments were performed at 30–32°C and ^{3}H-taurine efflux at 37°C. The chemical structures of the drugs used are shown in Figure 1; the concentrations used are known to cause depolarization and stimulate insulin release in pancreatic β-cells.

Meglitinide was a kind gift from Dr J.C. Henquin, University of Louvain, Belgium. All other drugs and chemicals were obtained from the Sigma Chemical Co., Poole, U.K. ^{3}H-taurine was supplied by the Radiochemical Centre, Amersham, U.K.

Results

In the first series of experiments, the volume-sensitive anion conductance was studied under conventional whole-cell recording conditions. In preliminary experiments, it was found that spontaneous activation of the conductance was common when using an isotonic intracellular (pipette) solution. This could be prevented by using a slightly hypotonic intracellular solution. Under such conditions, addition of 10 μM glibenclamide to the bath solution activated the outwardly-rectifying conductance (Figures 2 and 3), as previously reported by Kinard & Satin (1995). This activation was not reversed upon withdrawal of the sulphonylurea (not shown), but was inhibited by 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid (DIDS, 100 μM; Figure 3), an inhibitor of the volume-sensitive anion channel (Kinard & Satin, 1995; Best *et al.*, 1996b). This observation, together with the outward-rectification and the composition of the solutions, suggests that this conductance is identical to the volume-sensitive anion conductance previously described in these cells (Kinard & Satin, 1995; Best *et al.*, 1996b). When a higher concentration of the sulphonylurea (100 μM) was subsequently applied, a voltage- and time-dependent inactivation of this current was apparent at strongly depolarizing potentials (Figure 2c). As previously reported by Kinard & Satin (1995), this high concentration of glibenclamide also inhibited swelling-induced channel activity (not shown).

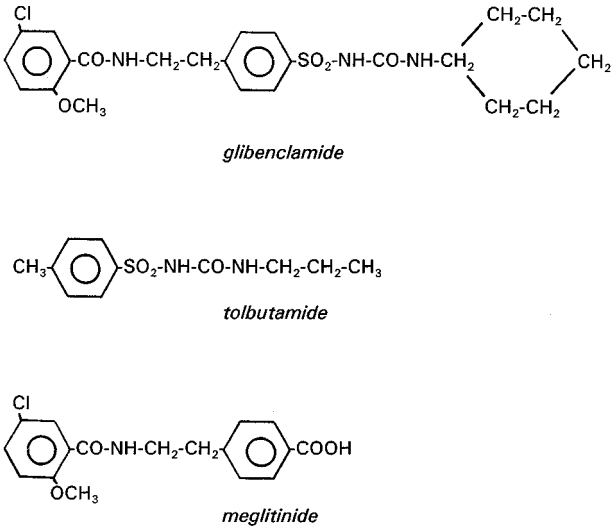

Figure 1 Chemical structures of the three compounds used in this study.

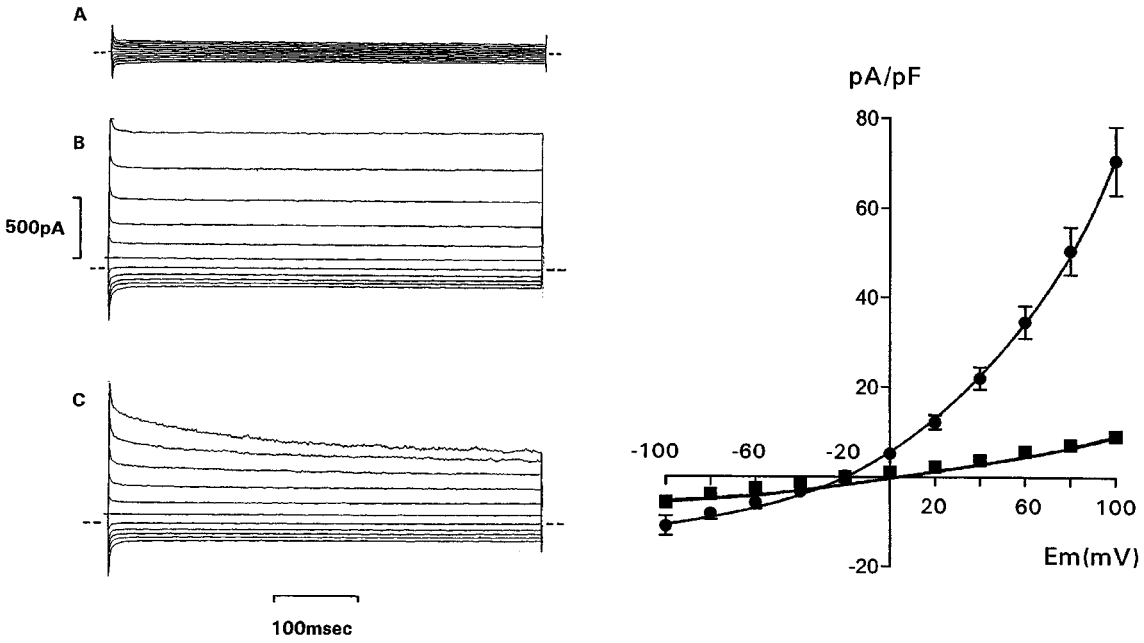

Figure 2 Conventional whole-cell recordings of volume-sensitive anion channel activity in single rat pancreatic β-cells. Left panel: Currents obtained from a typical cell under control conditions (A), 4 min after the application of 10 μM glibenclamide (B) and 2 min after raising the concentration of glibenclamide to 100 μM (C). The traces are typical of those from a total of four to six similar experiments. Right panel: Current-voltage relationships showing means$\pm$s.e.mean values for 4 cells; ■ control, ● 10 μM glibenclamide. Error bars, where not shown, are smaller than the symbols.

In order to assess whether activation of the conductance by glibenclamide could also be evoked by other sulphonylureas, the effect of tolbutamide was investigated. As shown in Figure 3, addition of 100 μM tolbutamide had no significant effect on channel activity in the absence of cell swelling; no effect of the drug was apparent over the concentration range 10–500 μM.

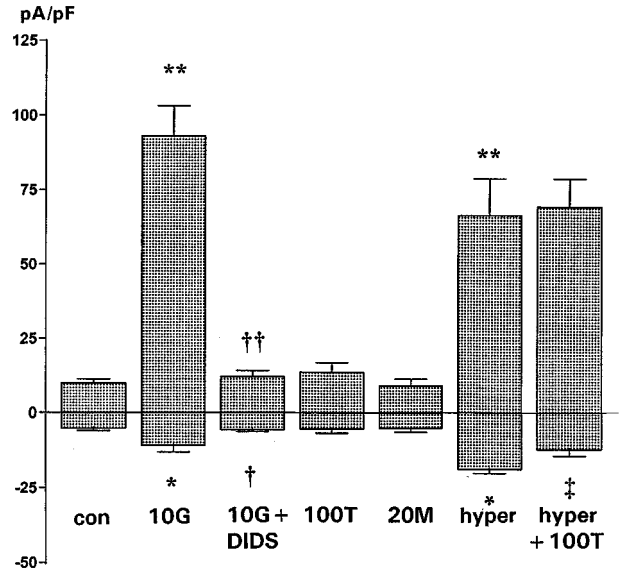

Figure 3 Current amplitudes evoked by voltage pulses of +100 mV (positive currents) and −100 mV (negative currents) in single rat β-cells under conventional whole-cell recording conditions. Effects of glibenclamide (G; 10 μM) in the absence and presence of DIDS (100 μM), tolbutamide (T; 100 μM), meglitinide (M; 20 μM); hyper: channel activated by hypertonic pipette solution. $^{**}P<0.001$, $^{*}P<0.05$ compared to control; $\dagger\dagger P<0.001$, $\dagger P<0.05$ compared to 10G; $\ddagger P<0.05$ compared to hyper. Data represent the means ± s.e.mean of at least four different cells.

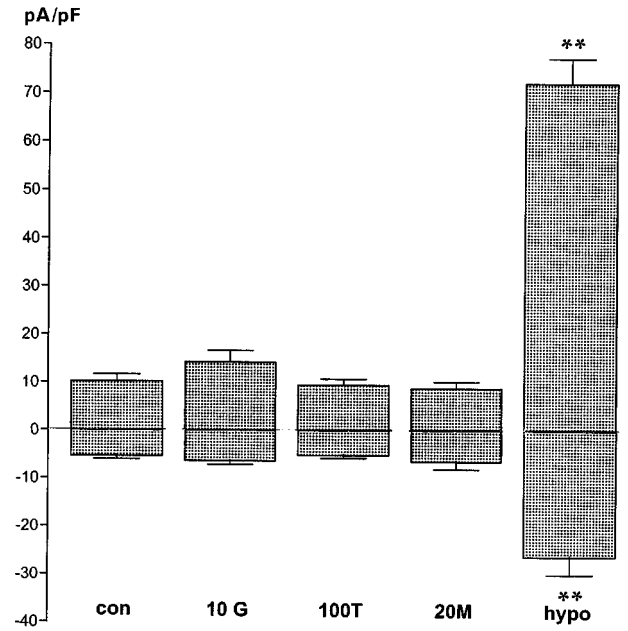

Figure 4 Current amplitudes evoked by voltage pulses of +100 mV (positive currents) and −100 mV (negative currents) in intact single rat β-cells under perforated patch conditions. Effects of glibenclamide (G; 10 μM), tolbutamide (T; 100 μM), meglitinide (M; 20 μM) and a hypotonic bath solution (hypo). $^{**}P<0.001$, compared to control. Data represent the means ± s.e.mean of four different cells.

However, a small though statistically significant inhibition of the swelling-activated inward current was observed in the presence of 100 μM tolbutamide (Figure 3), although there was no significant effect on outward current.

Glibenclamide, unlike tolbutamide, consists of sulphonylurea and non-sulphonylurea moieties (Figure 1). In view of the contrasting effects on the anion conductance of the two drugs, the actions of meglitinide, which represents the non-sulphonylurea moiety of glibenclamide, were investigated. This compound has been reported to cause β-cell electrical and secretory activity, and like sulphonylureas has been shown to inhibit K_{ATP} channel activity (Henquin, 1990). However, as shown in Figure 3, this drug also failed to activate the volume-sensitive anion channel in the pancreatic β-cell.

The second series of experiments utilized the perforated patch technique to study the effects of the above compounds on activity of the volume-sensitive anion channel in intact rat β-cells. As shown in Figure 4, glibenclamide, tolbutamide and meglitinide were all ineffective in causing significant activation of the anion channel. Activation of the conductance could, however, be induced under these conditions by application of a hypotonic bath solution (Figure 4).

Several studies have demonstrated that the volume-sensitive anion channel is permeable to a number of organic osmolytes, including taurine. Consequently, the rate of efflux of ^{3}H-taurine has been used as an index of activity of the channel (Manolopoulos *et al.*, 1997). Consistent with this suggestion, exposure of ^{3}H-taurine-loaded intact islets to a hypotonic solution resulted in a DIDS-sensitive stimulation of ^{3}H-taurine efflux. The mean fractional outflow rate of ^{3}H-taurine during the 10 min pre-stimulatory period was $6.4 \pm 0.9 \times 10^{-3}$ min^{-1} ($n=5$). Three minutes after exposure to a 33% hypotonic solution, a peak rate of $59.8 \pm 7.6 \times 10^{-3}$ min^{-1} was attained ($P<0.001$). In the presence of 100 μM DIDS, the corresponding peak value was $34.3 \pm 2.4 \times 10^{-3}$ min^{-1} ($P<0.01$; $n=4$). In contrast to the effect of exposure to a hypotonic solution, application of neither glibenclamide (10 μM) nor tolbutamide (100 μM) had any significant effect on ^{3}H-taurine fractional outflow rate; the respective peak values achieved were $8.0 \pm 1.6 \times 10^{-3}$ min^{-1} ($n=4$) and $7.5 \pm 0.8 \times 10^{-3}$ min^{-1} ($n=3$).

The final series of experiments investigated the effect of DIDS, an inhibitor of the volume-sensitive anion channel, on β-cell electrical activity induced by sulphonylureas. As shown in Figure 5, 10 μM glibenclamide evoked a rapid, marked depolarization of the membrane potential leading to spiking electrical activity. The subsequent addition of 100 μM DIDS failed to inhibit this electrical activity. The effect of tolbutamide (100 μM) on β-cell membrane potential was also unaffected by DIDS (Figure 5).

Discussion

The results of the present study confirm the previous report of Kinard & Satin (1995) that the volume-sensitive anion channel in insulin-secreting cells, when studied with the conventional whole-cell recording technique, can be activated by glibenclamide. However, our findings do not support those authors' conclusions that the channel could be regulated by interaction with the sulphonylurea receptor. Four separate lines of evidence suggest that this is not the case. First, activity of the channel was not stimulated by tolbutamide, a sulphonylurea whose structure, unlike glibenclamide, does not contain a benzoic acid derivative. Initially, this finding raised the possibility that anion channel activation might be evoked

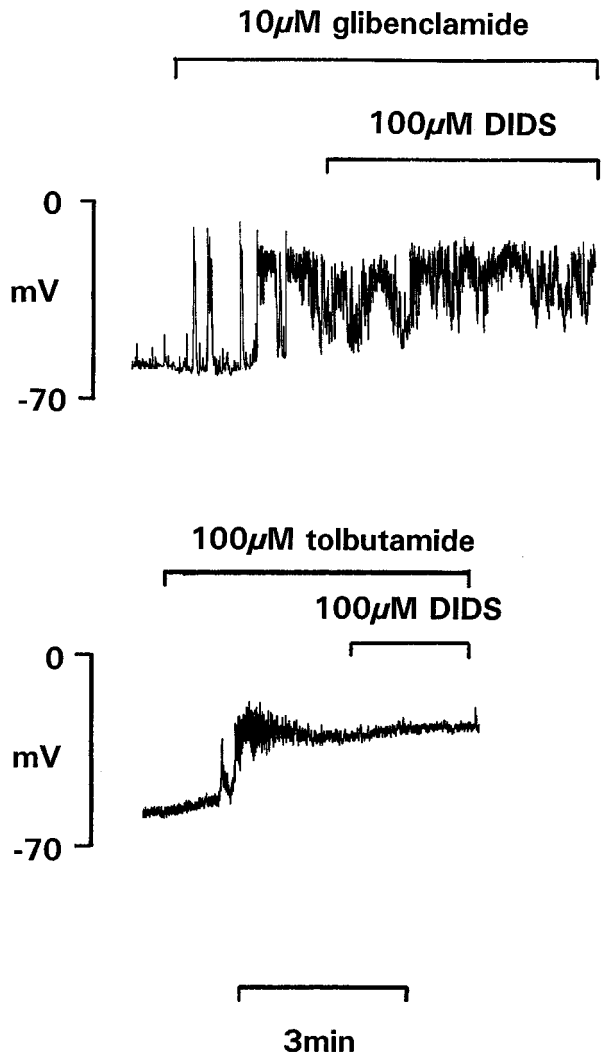

Figure 5 Electrical activity in single rat β-cells induced by glibenclamide (10 μM) and tolbutamide (100 μM): effect of DIDS (100 μM). Recordings were made using the perforated patch technique in current-clamp mode. Each trace is representative of three to four similar experiments.

by the non-sulphonylurea component of glibenclamide. However, the lack of effect of meglitinide, which represents this component, does not support this possibility. Evidence against modulation of the volume-sensitive anion channel by the sulphonylurea receptor also arises from the lack of effect of either glibenclamide or tolbutamide on channel activity in perforated patch recordings from intact β-cells. Furthermore, neither sulphonylurea was able to stimulate ^{3}H-taurine efflux from intact islets. Whilst it remains to be established that taurine efflux in the β-cell occurs *via* the volume-sensitive anion channel, the finding that this process was markedly stimulated by exposure to a hypotonic solution and inhibited by DIDS is consistent with this mechanism. The final piece of evidence against anion channel activation playing a role in β-cell stimulation by sulphonylureas emerges from the lack of effect of the anion channel blocker DIDS on sulphonylurea-induced electrical activity. This contrasts markedly with the inhibition by DIDS of electrical activity evoked by hypotonically-induced β-cell swelling (Best *et al*., 1996a).

A question raised by the present study is why glibenclamide caused activation of the volume-sensitive anion channel activity under conventional whole-cell recording techniques, whereas the compounds which represent the two pharmacologically active components of this drug, namely tolbutamide and meglitinide, were ineffective. Whilst we have no definitive answer to this question, the most likely explanation is that glibenclamide causes channel activation *via* an indirect biophysical interaction with the lipid components of the plasma membrane. Indeed, it has been documented that the volume-sensitive anion channel in cardiac cells is susceptible to activation by both anionic and cationic amphipathic compounds (Tseng, 1992), presumably due to their ability to alter the conformation of lipid membranes. It is possible that the volume-sensitive anion channel is more susceptible to activation by such amphipathic compounds under conventional whole-cell recording conditions, where the cell interior is dialyzed with the pipette solution with the possible removal or disruption of intracellular regulatory elements. This could also account for the lack of activation of the channel by glibenclamide in perforated patch recordings made from intact cells. The investigation of the effects of a range of amphipathic compounds on activity of the volume-sensitive anion channel in different recording configurations could provide further insight into this subject. Incidentally, the irreversible nature of β-cell stimulation by glibenclamide, as noted in the present study, has also been ascribed to the high lipid solubility of the drug (Carpentier *et al*., 1986).

In contrast to the stimulatory effects on the volume-sensitive anion channel of low concentrations of glibenclamide (1 – 10 μM), Kinard & Satin (1995) demonstrated that a higher concentration of the drug (100 μM) produced an inhibition of swelling-induced channel activity. Whilst we were able to confirm this finding, an inhibitory component of glibenclamide was also apparent when its concentration was raised from 10 to 100 μM. Under such circumstances, a voltage- and time-dependent inhibition of outward currents evoked by strongly depolarizing potentials was apparent. A similar pattern of inhibition of this type of channel has been documented in the presence of a number of organic anion channel blockers (Okada, 1997). A different pattern of inhibition of the swelling-induced conductance was observed with tolbutamide, where only the amplitude of the inward current was reduced. High concentrations of both glibenclamide and tolbutamide have been reported to inhibit a number of anion channels (Wangemann *et al*., 1986; Doroshenko *et al*., 1991), including CFTR (Sheppard & Welsh, 1992), though whether these effects are mediated *via* the sulphonylurea receptor or represent direct effects of the drugs on the anion channel is at present unknown.

In conclusion, the results of the present study indicate that the activation by glibenclamide of the volume-sensitive anion channel in insulin-secreting cells occurs only under whole-cell recording conditions and is unlikely to be of pharmacological or therapeutic significance. Channel activation under such conditions could be the result of interaction of the drug with lipid components of the plasma membrane.

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