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Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells

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Abstract. The influence of the antidiabetic sulphonylurea tolbutamide on K^+ channels of mouse pancreatic β-cells was investigated using different configurations of the patch clamp technique. The dominant channel in resting cells is a K^+ channel with a single-channel conductance of 60 pS that is inhibited by intracellular ATP or, in intact cells, by stimulation with glucose. In isolated patches of β-cells membrane, this channel was blocked by tolbutamide (0.1 mM) when applied to either the intracellular or extracellular side of the membrane. The dose-dependence of the tolbutamide-induced block was obtained from whole-cell experiments and revealed that 50% inhibition was attained at approximately 7μM. In cell-attached patches low concentrations of glucose augmented the action of tolbutamide. Thus, the simultaneous presence of 5 mM glucose and 0.1 mM tolbutamide abolished channel activity and induced action potentials. These were not produced when either of these substances was added alone at these concentrations. The inhibitory action of tolbutamide or glucose on the K^+ channel was counteracted by the hyperglycaemic sulphonamide diazoxide (0.4 mM). Tolbutamide (1 mM) did not affect Ca^{2+}-dependent K^+ channels. It is concluded that the hypo- and hyperglycaemic properties of tolbutamide and diazoxide reflect their ability to induce the closure or opening, respectively, of ATP-regulated K^+ channels.

Key words: Pancreatic β-cell – Patch clamp – ATP-dependent K^+ channel – Tolbutamide – Diazoxide

Introduction

Glucose has recently been shown to depolarize pancreatic β-cells by inducing the closure of a K^+ channel which is regulated by intracellular ATP (Cook and Hales 1984; Ashcroft et al. 1984; Rorsman and Trube 1985: Findlay et al. 1985b). The antidiabetic sulphonylurea tolbutamide reduces the outflow of ^{86}Rb, used as a tracer for K^+, in a way resembling the inhibitory effect of glucose (Henquin 1980). Like glucose the sulphonylureas induce electrical activity in pancreatic islets (Henquin and Meissner 1982) leading to an increase of cytosolic $[Ca^{2+}]$ (Abrahamsson et al. 1985) and to insulin release (see review by Gylfe et al. 1984). Circumstantial evidence thus indicates that glucose and sulphonylureas act at a common site in the stimulus-secretion coupling of the β-cell. Preliminary patch clamp studies on rat islet (Gillis et al. 1986) and insulinoma β-cells (Sturgess et al. 1985) suggest that the two pathways of glucose- and sulphonylurea-induced insulin secretion converge at the level of the ATP-regulated K^+ conductance. We now describe the action of tolbutamide on K^+ channels in greater detail. The effects are compared with those of diazoxide, another sulphonamide, which despite being chemically related to the sulphonylureas has hyperglycaemic properties and abolishes glucose-stimulated insulin release (Frerichs et al. 1966; Henquin et al. 1982), increases K^+ efflux and inhibits glucose- and tolbutamide-induced electrical activity in the β-cell (Henquin and Meissner 1982). It will be shown that the reported hypo- and hyperglycaemic effects of tolbutamide and diazoxide are correlated with their respective abilities to open or close the ATP-regulated K^+ channels.

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Methods

Preparation of β-cells. β-Cells were prepared from the pancreases of NMRI-mice and cultured as previously described (Rorsman and Trube 1985). Whereas single cells were used for whole-cell measurements, small clusters were preferentially used for cell-attached recordings.

Recording and analysis. The different recording modes of the patch-clamp technique were employed: the whole-cell, cell-attached patch, inside-out patch and outside-out patch configurations (see Hamill et al. 1981). The cell-attached patch configuration is the only recording mode not affecting the composition of the cytoplasm and the cell metabolism. When using other configurations, the intracellular milieu is replaced by the medium in the bath (inside-out patches) or by the solution in the recording pipette (outside-out patches and whole-cell experiments). Pipettes were pulled from aluminosilicate glass (Hilgenberg, Malsfeld, FRG) and had resistances between 3 and 5 MΩ. A patch-clamp amplifier (EPC7, List Electronic, Darmstadt, FRG) was used. During the experiment the current and voltage signals were recorded on magnetic tape. Signals were displayed on a storage oscilloscope (5100, Tektronix, Beaverton, ON, USA) or by using a chart recorder (2200, Gould, Cleveland, OH, USA). Traces in the figures were photographed directly from the oscilloscope screen with exception of the records in Figs. 3 and 4, which were plotted by the chart recorder. Records were filtered at 0.1 kHz (−3 dB point) unless otherwise indicated. The degree of channel block was estimated in some experiments by time-averaging single-channel currents

before and after drug application. Current records of at least 1 min duration were filtered (0.2 kHz), digitized (1 kHz) and stored in a computer (PDP 11/34, DEC, Maynard, MA, USA). The level of the baseline was set visually to zero and the average current calculated by integration. Data in the text and in the graphs are presented as mean values ± SEM.

Solutions. At the beginning of the experiments, the cells were immersed in a medium (solution A) containing 140 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$ and 10 mM HEPES-NaOH (pH = 7.4). Glucose, diazoxide and tolbutamide were included at the concentrations stated in the text or figures. Tolbutamide and diazoxide were added as 20 mM stock solutions prepared from NaOH and KOH (0.15 M). The pH of the final solutions was readjusted if necessary. In the cell-attached and inside-out patch experiments, the pipette solution (i.e. the solution adjacent to the extracellular side of the plasma membrane) was composed of 146 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$ and 10 mM HEPES-KOH (pH = 7.4; solution B). The same medium was also used as the bath solution for outside-out patches when a high extracellular concentration of K^+ was desired. In experiments on outside-out patches and in whole-cell recordings, the intracellular side of the membrane is exposed to the pipette solution. Pipettes were therefore filled with a medium mimicking the composition of the cytoplasm and containing 125 mM KCl, 30 mM KOH, 1 mM $MgCl_2$, 6 mM NaCl, 2 mM $CaCl_2$, 10 mM EGTA and 5 mM HEPES (pH = 7.15, $[Ca^{2+}] = 0.06$ μM; solution C). This "intracellular" solution was also used for filling the bath during measurements from inside-out patches. For the activation of Ca^{2+}-dependent K^+ channels the EGTA in solution C was replaced by equimolar HEDTA. The concentrations of $MgCl_2$ and $CaCl_2$ were increased to 8 mM and 5 mM to give free concentrations of 1.2 mM and 50 μM of Mg^{2+} and Ca^{2+}, respectively (solution D). Na_2ATP at concentrations of 0.3 – 3 mM was included, when necessary, in the intracellular media with concomitant changes of NaCl and $MgCl_2$ to keep the free concentrations of these cations constant. The binding constants given by Martell and Smith (1974) were used to calculate $[Ca^{2+}]$ and $[Mg^{2+}]$. The bath could be perifused to exchange solutions within 10 – 40 s. However, the speed of solution exchange was quite variable from experiment to experiment and in different parts of the bath. The analysis of time-dependent effects during solution changes was therefore not attempted. All experiments were performed at room temperature (20 – 22° C).

Results

Effects of tolbutamide and $[K^+]_0$ on ATP-dependent channels in outside-out patches

Figure 1 A shows a record from an inside-out patch to illustrate the ATP-dependence of a channel previously characterized in the β-cell membrane (Cook and Hales 1984; Ashcroft et al. 1984; Rorsman and Trube 1985). This channel is permanently closed when the intracellular side of the membrane is exposed to a bath solution containing 1 mM ATP. After removal of the ATP, openings of the channel are seen as deflections of the current trace. Two of these openings are shown at better time resolution in Fig. 1 C. At potentials more negative than −50 mV, the openings are typically interrupted by frequent brief closures which are seen as vertical lines in Fig. 1 C.

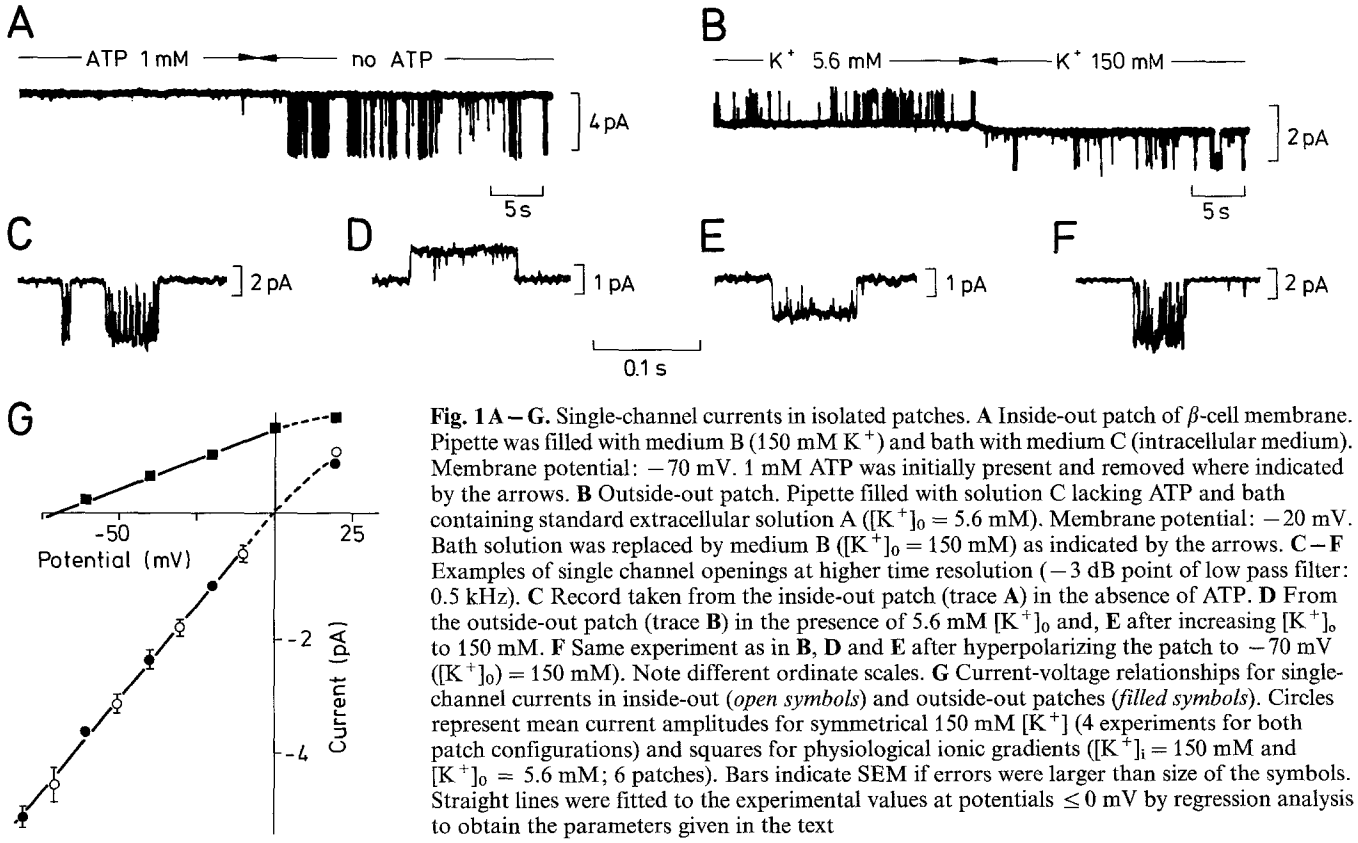

Fig. 1 A – G. Single-channel currents in isolated patches. **A** Inside-out patch of β-cell membrane. Pipette was filled with medium B (150 mM K^+) and bath with medium C (intracellular medium). Membrane potential: −70 mV. 1 mM ATP was initially present and removed where indicated by the arrows. **B** Outside-out patch. Pipette filled with solution C lacking ATP and bath containing standard extracellular solution A ($[K^+]_0 = 5.6$ mM). Membrane potential: −20 mV. Bath solution was replaced by medium B ($[K^+]_0 = 150$ mM) as indicated by the arrows. **C – F** Examples of single channel openings at higher time resolution (−3 dB point of low pass filter: 0.5 kHz). **C** Record taken from the inside-out patch (trace **A**) in the absence of ATP. **D** From the outside-out patch (trace **B**) in the presence of 5.6 mM $[K^+]_0$ and, **E** after increasing $[K^+]_0$ to 150 mM. **F** Same experiment as in **B**, **D** and **E** after hyperpolarizing the patch to −70 mV ($[K^+]_0$) = 150 mM). Note different ordinate scales. **G** Current-voltage relationships for single-channel currents in inside-out (*open symbols*) and outside-out patches (*filled symbols*). Circles represent mean current amplitudes for symmetrical 150 mM $[K^+]$ (4 experiments for both patch configurations) and squares for physiological ionic gradients ($[K^+]_i = 150$ mM and $[K^+]_0 = 5.6$ mM; 6 patches). Bars indicate SEM if errors were larger than size of the symbols. Straight lines were fitted to the experimental values at potentials ≤ 0 mV by regression analysis to obtain the parameters given in the text

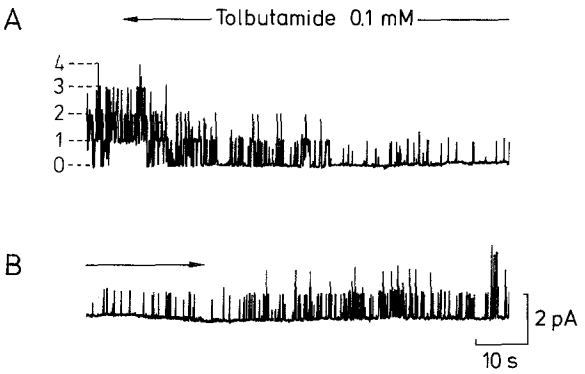

Fig. 2 A, B. Effects of tolbutamide on channel activity in an outside-out patch. Pipette containing solution C without ATP and bath filled with solution A. 0.1 mM tolbutamide was applied during the period indicated by the arrows. Membrane potential: −20 mV. Figures 0–4 at beginning of trace **A** denote numbers of simultaneous channel openings. 20 s of recording between traces **A** and **B** is not shown

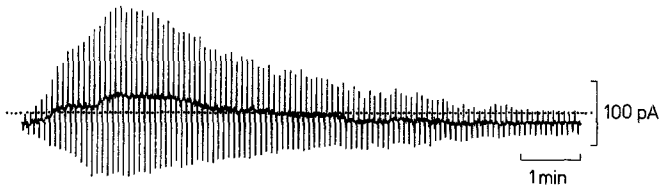

Fig. 3. Whole-cell currents from a single isolated β-cell. Bath filled with solution A and pipette with solution C lacking ATP. The cell membrane potential was clamped at −70 mV and hyper- and depolarizing voltage pulses of 10 mV were applied alternatively. Pulse duration: 200 ms. Interval: 2 s. Current responses are seen as vertical lines due to the compressed time scale. The dotted horizontal line indicates the zero current level. No drugs were added in this experiment

Inside-out patches are appropriate for studying the action of intracellular regulators like ATP. However, sulphonylureas are known to be effective from the outside (Gylfe et al. 1984). Outside-out patches are therefore better suited for experiments on tolbutamide because they allow easy exchange of the solution at the extracellular side of the membrane. It was also convenient to maintain the extracellular K^+ concentration ($[K^+]_o$) at the normal value of 5.6 mM, whereas previously the channel has been mainly studied in 150 mM $[K^+]_o$. With 5.6 mM $[K^+]_o$ we found a channel with a conductance of 19 ± 1 pS ($n = 6$) and a zero-current potential of −70 ± 3 mV provided ATP was omitted from the intracellular medium (I-V relation in Fig. 1 G, squares). The latter value agrees with that reported for ATP-dependent whole-cell currents (Rorsman and Trube 1985) and is close to the K^+ equilibrium potential (−83 mV). To characterize this channel, $[K^+]_o$ was increased to 150 mM (Fig. 1 B). As expected, single-channel currents recorded at −20 mV reversed direction during the exchange of the bath solution. Fig. 1 D, E show single openings for both concentrations of extracellular K^+ at an expanded time scale. If the membrane potential was increased to −70 mV in 150 mM $[K^+]_o$, single channel currents in outside-out patches (Fig. 1 F) appeared similar to those in inside-out patches (compare Fig. 1 C). The current-voltage relations for both patch configurations (Fig. 1 G, circles) superimposed. The values of the single-channel conductances were 60 ± 3 pS ($n = 4$) in outside-out patches and 60 ± 4 pS ($n = 4$) in inside-out patches. Hence, it is concluded that the channel with a conductance of 19 pS in 5.6 mM $[K^+]_o$ is the ATP-regulated K^+-channel seen before in the inside-out patches (see also Findlay et al. 1985 b).

Figure 2 shows a record from another experiment in which 0.1 mM tolbutamide was applied to the extracellular side of the membrane. At least four channels were initially active in this patch as indicated by superpositions of four unit current levels. When the drug was added to the bath, the activity of the channels decreased until only rare openings remained. The slowness of the response probably reflects the slow exchange of the solutions in the bath rather than a slow onset of the drug action. By averaging the current flowing through the channels before the addition of tolbutamide and after reaching a steady state in the presence of the drug, it could be calculated that tolbutamide reduced the open state probability by 95%. The activity of the channels did not recover completely after washout of tolbutamide (Fig. 2 B). However, since control experiments without any additions demonstrated a progressive run-down of the frequency and duration of the channel openings (not shown here, but see Findlay et al. 1985 b; Trube and Hescheler 1984), this does not necessarily mean that the blocking action of tolbutamide is only partially reversible. Several experiments with 0.1 mM tolbutamide suggested that this concentration was 80–95% effective in blocking the channel. The efficiency of lower concentrations could not be determined unequivocally due to the problem with run-down. We were therefore obliged to seek an alternative experimental protocol to obtain the concentration dependence of the tolbutamide block.

Whole-cell measurements

An alternative way to assess the inhibitory action of tolbutamide is to apply the drug in whole-cell experiments. In this configuration changes of the ATP-dependent conductance can be visualized if the cell interior is dialyzed with a pipette medium low in ATP.

Figure 3 shows current responses from a whole-cell experiment where small voltage pulses (± 10 mV) were applied from a holding potential of −70 mV. The record starts 1 min after disrupting the membrane in the pipette tip. The progressive increase of the current responses, which are seen as vertical lines, reflects the washout of ATP resulting in the activation of the ATP-regulated K^+ conductance (Rorsman and Trube 1985). Since the holding potential was slightly more positive than the true K^+ equilibrium potential, the holding current became more outward as the K^+ conductance increased. However, approximately 3 min after starting the experiment, the K^+ conductance began to decline as indicated both by the decrease in the amplitude of the current responses and the change of holding current. Thus the problem with run-down persisted in the whole-cell configuration (cf. Kakei et al. 1985). It was later discovered that the rate of run-down could be reduced by including 0.3 mM ATP in the pipette solution and that experiments could last for more than 30 min under this condition. Apparently ATP not only inhibits the activity of the channel but is also necessary to maintain its integrity. Although 0.3 mM blocks the channel by 96% in inside-out patches (Cook and Hales 1984; own unpublished observations), there was still

a marked increase of the K^+ conductance in most cells after reaching the whole-cell recording mode (Fig. 4A). A discrepancy in the ATP-sensitivity between inside-out patches and whole-cell recordings has also been reported for heart cells (Kakei et al. 1985; Noma and Shibasaki 1985). Once the conductance induced by the low ATP concentration was maximal (end of trace A of Fig. 4), different concentrations of tolbutamide were applied. As can be seen from the examples in Fig. 4B, C, 3 μM slightly and 30 μM markedly reduced the magnitude of the K^+ current. After washout of the drug, the current returned to the expected level as indicated by the dotted lines. Fig. 4D demonstrates that adding 0.4 mM diazoxide reverses the block induced by 30 μM tolbutamide.

To construct a dose-inhibition curve, the amplitudes of current responses during exposure to tolbutamide (I) were compared with those observed under control conditions (I_c). The mean amplitude before each application of the drug and after recovery was used as the control. The ratios I/I_c for 11 different cells are plotted against the respective tolbutamide concentration in Fig. 4E. The values are fitted by the function

$$I/I_c = 1/(1 + 10^{n \cdot \lg[\text{tolbutamide}]-\lg K}) \quad (1)$$

which is derived from the chemical equilibrium

$$\text{channel} + n \cdot \text{tolbutamide} \overset{K}{\rightleftharpoons} \text{channel} \cdot \text{tolbutamide}_n. \quad (2)$$

The best values of n and lgK as defined in Eq. (2) are 1.02 ± 0.14 and -5.23 ± 0.06 respectively, i.e. 50% inhibition is attained at 7 μM and one molecule of tolbutamide is bound per channel. Several concentrations of tolbutamide were applied in most experiments but similar parameters are obtained when only the values for the first exposure in each experiment are used for the fit. Experiments withouth ATP in the pipette solution also indicate that 50% inhibition is reached between 1 and 10 μM suggesting that the blocking action of tolbutamide is not influenced by intracellular ATP levels.

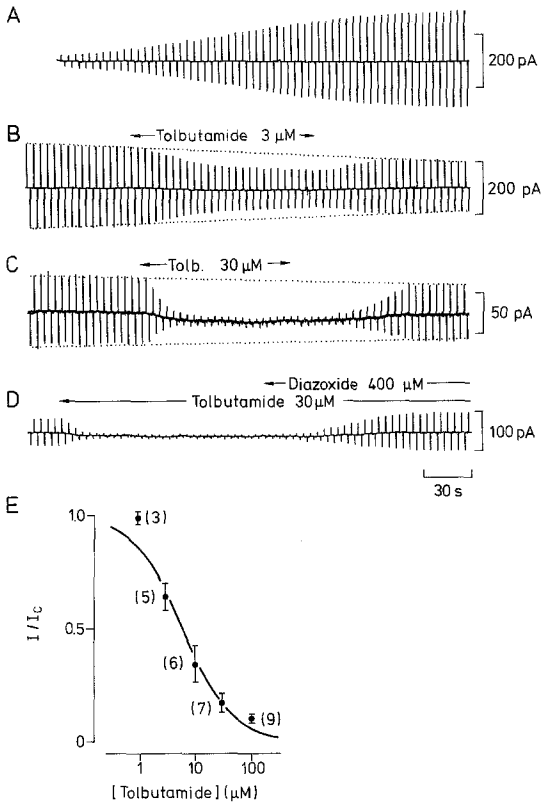

Fig. 4A–E. Effects of tolbutamide and diazoxide on whole-cell K^+-currents. Same experimental protocol as in Fig. 3 except that pipette solution C contained 0.3 mM ATP. **A** Increase of current responses after disrupting the membrane in the pipette tip. **B, C** Effects of 3 and 30 μM tolbutamide. Drug was applied during periods indicated by arrows. Dotted lines represent interpolations of the current responses before and after drug exposures. Cell was exposed to tolbutamide in the order of 1, 3, 10, 30, 100 μM (other sequences tried in other experiments). Each exposure lasted approximately 2 min and current was allowed to recover for 2–3 min between each application of the drug. **D** Effect of 400 μM diazoxide added in the presence of 30 μM tolbutamide. Record obtained 7 min after trace **C** and subsequent to the exposure to 100 μM tolbutamide. All traces from same experiment. Total duration of experiment: 25 min. Note different ordinate scales. **E** Dose-inhibition curve for tolbutamide from 11 similar experiments. Values are given as fractions of control currents and are means ± SEM of indicated number of experiments. Curve was drawn by fitting the function of Eq. (1) to the observed values using a non-linear least-squares routine

Effects of tolbutamide on inside-out and cell-attached patches

Tolbutamide is believed to stimulate insulin release after binding to the surface of the β-cell (Gylfe et al. 1984). It was therefore unexpected that tolbutamide also blocks the ATP-regulated K^+ channel when applied to the intracellular face of the membrane. As can seen in Fig. 5A, B, 0.1 mM tolbutamide is at least as potent under this condition as when applied extracellularly. The inhibitory action amounted to 80–100% in five experiments. The observation that tolbutamide inhibits the channel from both sides of the membrane suggested that it might be taken up into the membrane before reaching the inhibitory site. In order to test this notion, tolbutamide was applied from the bath while recording currents from cell-attached patches with pipettes not containing the drug (Fig. 6). In these experiments spontaneous channel openings were observed in the absence of glucose (Fig. 6A) and the conductance and kinetics of these channels were identical to those of the ATP-regulated K^+ channel (Rorsman and Trube 1985). After the basal activity of the channel was reduced by increasing glucose to 5 mM (trace B), the subsequent addition of 0.1 mM tolbutamide completely suppressed channel activity. Three minutes after adding tolbutamide the cell started giving action potentials, seen as a black blur in trace C. The action potentials appear as biphasic current deflections in recordings from cell-attached patches for reasons previously explained (Fig. 6D; Rorsman and Trube 1986). In other experiments 0.1 mM tolbutamide was applied in the absence of glucose. Although channel activity was similarly blocked, action potentials were not elicited. They were, however, evoked by a tenfold higher concentration of tolbutamide or by 1 μM glibenclamide. The effects of glibenclamide differed from those of tolbutamide in not being reversible. Whereas channel activity resumed in cell-attached patches within a few minutes after removal of tolbutamide, the effects of glibenclamide persisted and action potentials were observable for at least 15 min (not shown). Figure 6E shows that the combined action of tolbutamide and glucose was

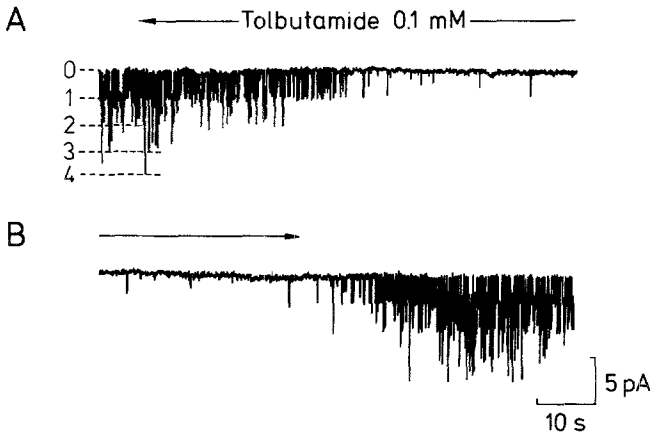

Fig. 5A, B. Effects of tolbutamide on single-channel currents in an inside-out patch. Pipette and bath filled with solutions B and C respectively. Membrane potential: −70 mV. Patch was exposed to 0.1 mM tolbutamide during the period indicated by arrow. Figures 0–4 indicate number of simultaneous channel openings. Trace **B** is the immediate continuation of trace **A**

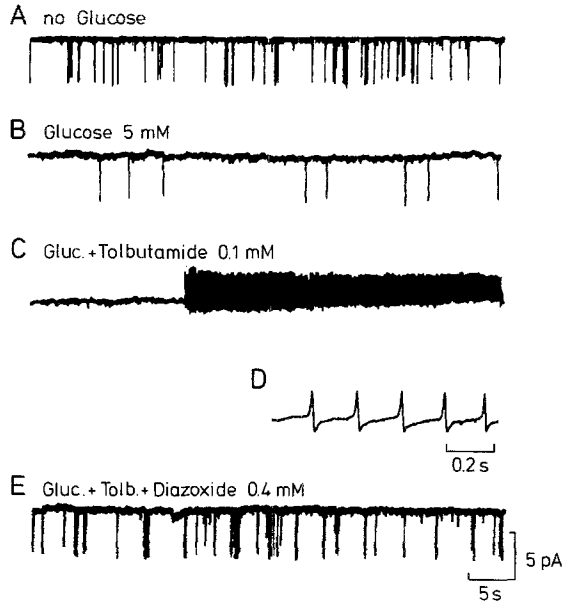

Fig. 6A–E. Effects of tolbutamide, glucose and diazoxide in a cell-attached patch. Bath filled with solution A and pipette with solution B. Currents were recorded from a β-cell in a cluster at the cell's membrane potential. **A** Channel openings under basal conditions (0 mM glucose). **B** 5 min after adding 5 mM glucose to bath. **C** Same patch after adding 0.1 mM tolbutamide. Channel activity is fully inhibited and cell starts to give action potentials. **D** Examples of the biphasic current deflections due to action potentials displayed at an expanded time scale. **E** Conditions as in **C** but 0.4 mM diazoxide included in the bath solution 5 min before

counteracted by 0.4 mM diazoxide as would be expected from the whole-cell measurements (cf. Fig. 4D). The cell stopped given action potentials and openings of the channel could be observed again within 2 min. After 6 min channel activity was as intense as during control conditions (compare Fig. 6A and E).

Diazoxide activation of ATP-regulated K^+ channels

The action of diazoxide was tested under conditions where the channel activity had been completely blocked by a high

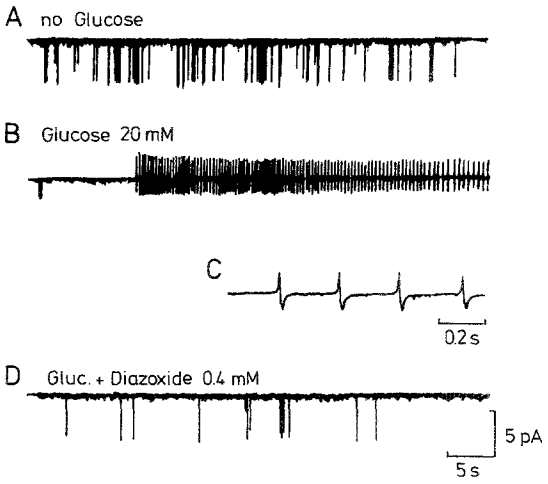

Fig. 7A–D. Effects of diazoxide and glucose in a cell-attached patch. Solutions and recording conditions as in Fig. 6. **A** Basal conditions. **B** 6 min after adding 20 mM glucose. Cell starts giving action potentials. **C** Action potentials at expanded time scale. **D** 6 min after adding 0.4 mM diazoxide

concentration of glucose (Fig. 7). In the absence of glucose there were numerous openings of the channel and addition of 20 mM glucose abolished channel activity within 3–4 min. In three out of six cells, this inhibitory action was preceded by a transient stimulation of the channels during the first 2 min. Measurements with conventional intracellular electrodes have shown a transient increase of the input conductance of pancreatic islets shortly after addition of glucose (Atwater et al. 1978). These observations can be explained by a temporary reduction of the intracellular ATP content (Malaisse et al. 1979) produced by the consumption of ATP in the phosphorylation of glucose.

After 6–10 min in 20 mM glucose, 50% of the tested cells gave spontaneous action potentials (Fig. 7B and C). Addition of 0.4 mM diazoxide terminated spiking activity and lead to a gradual return of channel openings (Fig. 7D). Diazoxide also induced openings of the channel in outside-out patches where channel activity was first reduced by the inclusion of 0.3 mM ATP in the pipette solution (Fig. 8). No effect could be detected, however, when the pipette contained 3 mM ATP, a finding presently not understood.

Specificity of tolbutamide action

In many earlier investigations tolbutamide has been shown to reduce the resting K^+ permeability of β-cells (e.g. Henquin and Meissner 1982). As this conductance has previously been proposed to be controlled by $[Ca^{2+}]_i$ (Atwater et al. 1983), it was important to explore whether tolbutamide also affects the Ca^{2+}-activated K^+ channels recently described in the β-cell membrane (Cook et al. 1984; Findlay et al. 1985a). This channel was studied in five patches with an "intracellular" Ca^{2+} concentration of 50 μM. The single-channel conductance in symmetrical 150 mM K^+ solutions was 195 ± 5 pS. Figure 9A shows that even 1 mM tolbutamide failed to affect the currents recorded from an inside-out patch. The same negative observation was made in four outside-out patches. The Ca^{2+}-dependence of the channel could be directly demonstrated in inside-out patches by reducing the free Ca^{2+} concentration from 50 to 0.06 μM (Fig. 9B). The delayed rectifying K^+ current previously de-

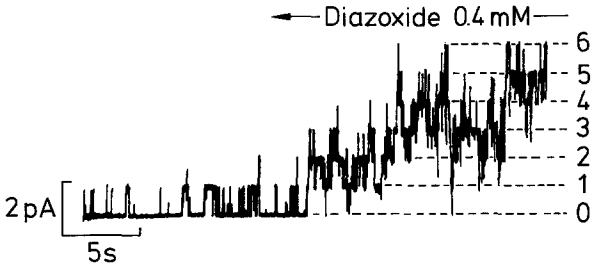

Fig. 8. Action of diazoxide on channels in an outside-out patch. Bath filled with solution A, pipette with solution C containing 0.3 mM ATP. Membrane potential: 0 mV. Diazoxide was introduced during period indicated by arrow. Figures 0–6 indicate numbers of simultaneous channel openings

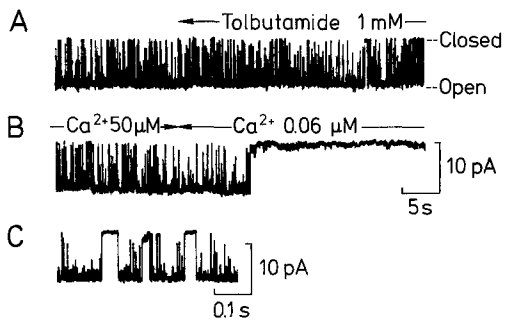

Fig. 9 A–C. Lack of influence of tolbutamide on Ca^{2+}-activated K^+ channel in an inside-out patch. Pipette was filled with solution B and bath contained solution D with 3 mM ATP to suppress activity of ATP-dependent K^+ channel. **A** 1 mM tolbutamide was added to the bath solution during period indicated by arrow. **B** Free Ca^{2+} concentration was lowered from 50 to 0.06 μM by replacing solution D with solution C. **C** Channel activity shown with expanded time scale. Filter: 1 kHz in C

scribed in β-cells is also unaffected by tolbutamide and glibenclamide (Rorsman and Trube 1986). Openings of smaller channels, which may be identical to the channel named K_{SIR} by Findlay et al. (1985b) were sometimes observed in our experiments, but these, too, remained active also in the presence of the sulphonylureas (not shown).

Discussion

The reversibility of the action of tolbutamide as well as the limited distribution volume have been taken as arguments against an intracellular uptake of the drug and infer that this drug acts by binding extracellularly (Hellman and Täljedal 1975; Gylfe et al. 1984). Because the gigaohm seal between the patch electrode and the plasma membrane forms an extracellular diffusion barrier (Sakmann and Neher 1984), it was surprising that tolbutamide blocked the ATP-regulated K^+ channels when applied both outside the pipette in cell-attached patch recordings and to the intracellular face of the plasma membrane in inside-out patch experiments (see also Gillis et al. 1986). These findings suggest that the action of tolbutamide can spread within the plasma membrane and that tolbutamide can reach the inhibitory site from both sides of the membrane. It has been proposed that sulphonylureas are inserted into the phospholipid domain of the β-cell membrane since they bind similarly to artificial lipid bilayers (Deleers and Malaisse 1984) and to biological membranes from brain or tumoural β-cells (Geisen et al. 1985). If the sulphonylurea "receptor" is embedded in the lipid phase, the potency of a sulphonylurea would be expected to be determined in part by its lipophilicity. This may explain why tolbutamide, which at physiological pH is dissociated to 99% ($pK_a = 5.3$; Häussler and Pechtold 1971), is a less potent insulin secretagogue than other sulphonylureas with higher pK_a-values or large hydrophobic end groups such as glibenclamide. If only relatively small amounts of tolbutamide are dissolved in the membrane, a failure to detect much intracellular uptake of the drug (Gylfe et al. 1984) is not surprising.

The model outlined above seems adequate to explain our experimental findings and is also consistent with what is known about sulphonylurea interactions with the β-cell membrane. However, it is still premature to exclude other possibilities such as the participation of sulphydryl groups within the membrane (see Gylfe et al. 1984) or lateral mobility of channel subunits. Further patch clamp studies using sulphonylureas with different pK_a-values may prove useful in elucidating the nature of sulphonylurea-membrane interactions.

The effects of diazoxide can be explained by a mechanism similar to that suggested above for tolbutamide. Uptake of this drug into the β-cell membrane seems likely from the oil-water partition coefficient (0.13 at pH = 7.4; Pruitt et al. 1973). The relative lipophilicity of diazoxide is probably enhanced by the fact that more than 90% of this compound exists in the undissociated form at physiological pH (pK_a = 8.5; Pruitt et al. 1973). An appreciable uptake of diazoxide into the membrane is consistent with our observation that its effects were less rapidly reversed than those of tolbutamide.

Different sulphonylureas bind competitively to artificial (Deleers and Malaisse 1984) and tumoural β-cell membranes (Geisen et al. 1985). Measurements of the displacement of bound ^{3}H-glibenclamide by other sulphonylureas indicate that half-maximal binding of tolbutamide occurs at 6–10 μM. This concentration is close to that where 50% of the ATP-dependent K^+ current was blocked in our experiments (Fig. 4). The dose-response curve shows that 0.1 mM tolbutamide decreases the ATP-dependent conductance by 90–95%. The remaining 5–10% are probably enough to keep the β-cell sufficiently hyperpolarized not to produce action potentials. Action potentials were observed when the β-cell was exposed to 1 mM tolbutamide, a concentration which from the dose-inhibition curve can be estimated to reduce channel activity by 99%. It therefore seems that the ATP-dependent K^+ conductance must be almost completely blocked in order to stimulate electrical and secretory activity. This also provides a basis for the potentiating action of low glucose concentrations (< 10 mM) on tolbutamide responses. In the presence of 5 mM glucose channel activity is already reduced (see Fig. 5A, B) by 85% as indicated from tracer flux measurements (Malaisse and Herchuelz 1982). The subsequent addition of 0.1 mM tolbutamide blocks about 90–95% of the remaining conductance, assuming that the inhibitions by tolbutamide and glucose are independent, leading to further depolarization with resultant initiation of spiking activity.

Henquin and Meissner (1982) have reported that tolbutamide paradoxically is more effective at a low (74 μM) than at a high (370 μM) concentration in reducing the efflux of ^{86}Rb (K^+ analogue). We believe that this behaviour reflects the existence of several types of K^+ channels. The tolbutamide-induced reduction of the basal ATP-regulated conductance is nearly complete (80–90%) at the lower con-

centration. Increasing the concentration of tolbutamide leads to a further fall in this K^+ conductance and more pronounced depolarization of the β-cell. Once the cells start giving action potentials, other pathways of K^+ efflux come into operation. Voltage-clamp experiments on β-cells have disclosed the existence of a delayed rectifying K^+ current (Rorsman and Trube 1986) which is activated during each action potential and hence contributes to the observed efflux rate of K^+ (cf. Matthews and Shotton 1984). In addition, the increase of cytosolic Ca^{2+} observed during exposure to tolbutamide (Abrahamsson et al. 1985) may open Ca^{2+}-dependent K^+ channels, which would be consistent with the described Ca^{2+}-dependence of the "paradox".

The observation that tolbutamide does not block other types of K^+ channels in the β-cell (Fig. 9; Rorsman and Trube 1986) suggests that the sulphonylureas are selective for the ATP-dependent K^+ channel. The absence of this channel in pituitary and adrenal chromaffin cells (Sakmann and Neher, personal communications) can explain why tolbutamide does not depolarize these cells (Gylfe and Hellman 1982). The ATP-regulated K^+ channel exists in rat (Cook and Hales 1984), mouse, (Rorsman and Trube 1985) and guinea-pig (own unpublished observations) β-cells. It is therefore tempting to speculate that the actions of tolbutamide and diazoxide on mouse β-cells here described can be extended to explain the therapeutic effects of these compounds in man.

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