Diabetologia (2001) 44: 1019–1025

Diabetologia

Gliclazide produces high-affinity block of K_{ATP} channels in mouse isolated pancreatic beta cells but not rat heart or arterial smooth muscle cells

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Abstract

Aims/hypothesis. Sulphonylureas stimulate insulin secretion by closing ATP-sensitive potassium (K_{ATP}) channels in the pancreatic beta-cell membrane. K_{ATP} channels are also found in other tissues, including heart and smooth muscle, where they link cellular metabolism to electrical activity. The sulphonylurea gliclazide blocks recombinant beta-cell K_{ATP} channels (Kir6.2/SUR1) but not heart (Kir6.2/SUR2A) or smooth muscle (Kir6.2/SUR2B) K_{ATP} channels with high potency. In this study, we examined the specificity of gliclazide for the native (as opposed to recombinant) K_{ATP} channels in beta cells, heart and smooth muscle.
Methods. The action of the drug was studied by whole-cell current recordings of native K_{ATP} channels in isolated pancreatic beta-cells and myocytes from heart and smooth muscle.
Results. Gliclazide blocked whole-cell beta-cell K_{ATP} currents with an IC_{50} of 184 ± 30 nmol/l (n = 6–10) but was much less effective in cardiac and smooth muscle (IC_{50}s of 19.5 ± 5.4 μmol/l (n = 6–12) and 37.9 ± 1.0 μmol/l (n = 5–10), respectively). In all three tissues, the action of the drug on whole-cell K_{ATP} currents was rapidly reversible. In inside-out patches on beta-cells, gliclazide (1 μmol/l) produced a maximum of 66 ± 13 % inhibition (n = 5), compared with more than 98 % block in the whole-cell configuration.
Conclusion/interpretation. Gliclazide is a high-potency sulphonylurea which shows specificity for the pancreatic beta-cell K_{ATP} channel over heart and smooth muscle. In this respect, it differs from glibenclamide. The difference in the maximal block observed in the excised patch and whole-cell recordings from beta-cells, may be due to the absence of intracellular Mg-nucleotides in the excised patch experiments. [Diabetologia (2001) 44: 1019–1025]

Keywords Gliclazide, ATP-sensitive K^+-channel, sulphonylurea receptor, heart, smooth muscle.

The sulphonylurea gliclazide is widely used in the treatment of Type II (non-insulin-dependent) diabetes mellitus because of its ability to stimulate insulin secretion from pancreatic beta-cells. Like other sulphonylureas, its principal target is the ATP-sensitive potassium (K_{ATP}) channel. This channel plays a major role in controlling the beta-cell membrane potential and thereby insulin release. At low plasma glucose concentrations, K_{ATP} channels are open and the resulting K^+ efflux holds the beta-cell membrane at a hyperpolarized potential. Closure of K_{ATP} channels by glucose or sulphonylureas causes depolarization of the beta-cell membrane, leading to opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx and a rise in intracellular Ca^{2+} which stimulates the exocytosis of insulin-containing secretory granules [1].

K_{ATP} channels are also found in a variety of other cell types including cardiac, smooth and skeletal muscle, and some brain neurones [2]. Although their roles in extrapancreatic tissues are less well characterised, it is likely that they open in response to metabolic stress, such as during cardiac and cerebral is-

Received: 21 March 2001 and in revised form: 30 April 2001

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Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; SUR, sulphonylurea receptor

chaemia [3]. In the heart, it is believed that they play a key role in the intrinsic mechanisms that protect cardiac muscle during ischaemia [4]. In arterial smooth muscle, K_{ATP} channels are important in the control of contractile tone and therefore of blood pressure and blood flow [5]. Cardiovascular disease is a major cause of death in patients with diabetes and it has been argued that this could, at least in part, be related to the effects of sulphonylureas on K_{ATP} channels in cardiac and vascular smooth muscles [6–8]. In view of the clinical use of gliclazide in Type II diabetes, it is important to know to what extent the different types of K_{ATP} channel are blocked by this sulphonylurea.

The K_{ATP} channel is an octameric complex of two different protein subunits: an inwardly rectifying potassium channel, Kir6.2 or Kir6.1, and a sulphonylurea receptor, SUR [9–15]. The former acts as an ATP-sensitive K^+ channel pore while SUR is a channel regulator that endows Kir6.2 with sensitivity to drugs such as the inhibitory sulphonylureas and to K^+-channel openers like diazoxide [16]. K_{ATP} channels in different tissues are composed of different Kir and SUR subunits. Based on northern blotting, *in situ* hybridization, immunocytochemistry and functional studies of cloned K_{ATP} channels expressed in several expression systems, it seems that Kir6.2 is used as the pore-forming subunit in most tissues, except for various vascular smooth muscles where Kir6.1 serves the same function [10–12]. The type of SUR is far more variable between tissues. The beta-cell K_{ATP} channel is composed of SUR1, cardiac and skeletal muscle K_{ATP} channels of SUR2A and smooth muscle K_{ATP} channels of SUR2B [9,13–15].

The different types of SUR subunit endow K_{ATP} channels with different sensitivities to various drugs [17]. In particular, gliclazide and tolbutamide produce high-affinity inhibition of Kir6.2/SUR1 (beta-cell type) channels but not Kir6.2/SUR2A (cardiac) or Kir6.2/SUR2B (smooth muscle) types of K_{ATP} channels, when expressed in *Xenopus* oocytes [18,19]. Glimepiride, glibenclamide and meglitinide, in contrast, inhibit both Kir6.2/SUR1 and Kir6.2/SUR2A channels with high potency [19]. It has therefore been proposed that SUR1 possesses high-affinity binding sites for both gliclazide and glibenclamide while SUR2A has only a single high-affinity binding site for glibenclamide [18–19].

We have investigated whether gliclazide interacts with different types of native K_{ATP} channels with a similar potency and specificity to that found for their cloned counterparts by studying the effect of the drug on the native channels of pancreatic beta-cells, cardiac muscle and vascular smooth muscle. Our results indicate that, unlike glibenclamide, gliclazide produces high-affinity inhibition of the pancreatic beta-cell K_{ATP} channel but is much less effective on K_{ATP} channels of cardiac and arterial smooth muscle.

Materials and methods

Cell isolation: Mouse pancreatic islets and β-cells. Mice were killed by cervical dislocation. The pancreas was removed, cut into pieces and placed in Hank's solution containing collagenase (in mmol/l): 137 NaCl, 1.2 $NaHPO_4$, 5.6 KCl, 1.2 $CaCl_2$, 10 HEPES (pH 7.4 with NaOH), 4.2 $NaHCO_3$, 2.8 glucose plus 5 mg/ml bovine serum albumin and 2.5 mg/ml collagenase. The pancreas was digested by hand shaking in this solution for 8 min and islets were then removed by hand. Isolated islets were dispersed into single cells by trituration at 37 °C in a solution containing (mmol/l): 137 NaCl, 1.2 $NaHPO_4$, 5.6 KCl, 1 EGTA, 10 HEPES (pH 7.4 with NaOH), 4.2 $NaHCO_3$, and 2.8 glucose. Beta cells were maintained in RPMI tissue culture medium containing 11 mmol/l glucose supplemented with 10 % fetal calf serum at 37 °C in a humidified atmosphere of 5 % CO_2 in air. Cells were used 1 to 4 days after the isolation.

Cell isolation: Rat cardiac ventricular and mesenteric arterial myocytes. Male adult Wistar rats were killed by cervical dislocation, and ventricular myocytes isolated from adult rat hearts by enzymatic dissociation with collagenase and protease in a Langendorff apparatus [20]. Cells were stored in plastic petri dishes at 10 to 12 °C and used within 2 days. Mesenteric arteries were removed and cleaned of connective tissue. Second and third order branches of the arteries were dissected and treated with papain, collagenase and hyaluronidase [21]. Single smooth muscle cells were obtained by trituration with a wide-bore pipette. Cells were stored at 4 to 6 °C and used on the day of preparation.

Electrophysiology: beta-cells. Whole-cell K_{ATP} currents were recorded using the conventional whole-cell configuration of the patch-clamp technique and single-channel currents were recorded from inside-out membrane patches. Patch pipettes were pulled from borosilicate glass capillaries coated with Sylgard close to their tips and fire-polished immediately before use. They had resistances of 2 to 5 MΩ when filled with pipette solution. Membrane potentials and whole-cell currents were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Union City, Calif., USA) and stored on digital audio tape for later analysis. The zero current potential of the pipette was adjusted with the pipette in the bath before the seal was established and no corrections have been made for liquid junction potentials. Whole-cell currents flowing through K_{ATP} channels were monitored using alternate ± 20 mV pulses of 200 ms duration which were applied at a frequency of 0.5 Hz from a holding potential of –70 mV. Single-channel currents were recorded at –60 mV. Currents were filtered at 2 kHz, digitized at 5 kHz using a Digidata 1200 Interface and subsequently analysed using pCLAMP 7 (Axon Instruments) and in-house software.

After formation of the whole-cell configuration, whole-cell K_{ATP} currents gradually increase in size, as ATP dialyses out of the cell into the pipette solution [22]. Drugs were therefore applied only once the whole-cell K_{ATP} current had reached a steady state amplitude. The standard extracellular (bath) solution contained (mmol/l): 137 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with NaOH). The intracellular (pipette) solution contained (mmol/l): 107 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.15 with KOH). ATP (0.3 mmol/l) was added to the pipette to prevent rundown of K_{ATP} channel activity. For inside-out patch recordings, the extracellular (pipette) solution contained (mmol/l); 140 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 10 K-HEPES (pH 7.4). The bath (intracellular) solution contained (mmol/l): 107 KCl, 1 $MgCl_2$, 1

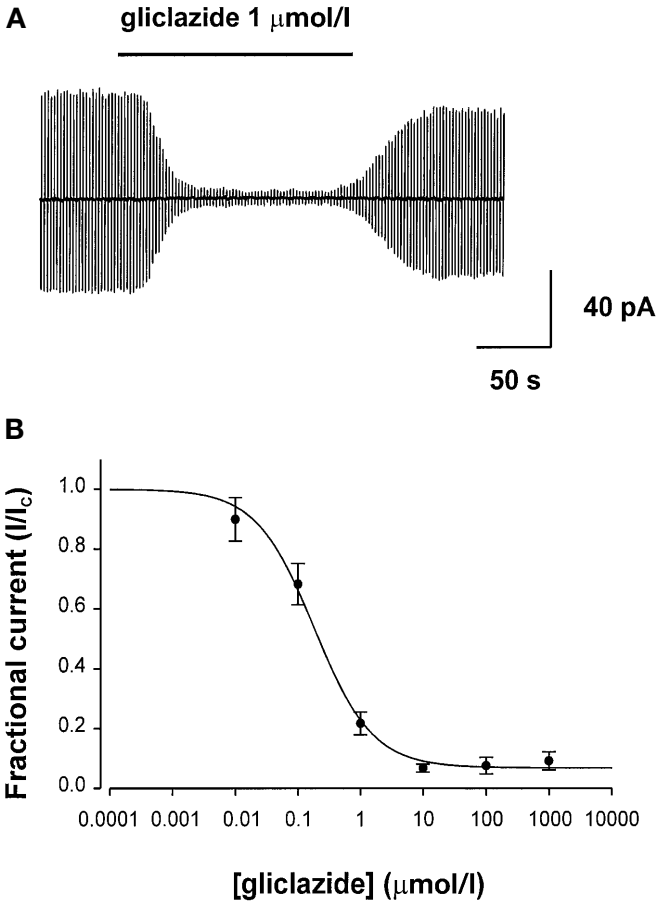

Fig. 1 A, B. Inhibition of beta-cell K_{ATP} currents by gliclazide. (**A**) Whole-cell K_{ATP} currents recorded from a single beta-cell in response to a series of depolarizing and hyperpolarizing 20 mV pulses from a holding potential of –70 mV. Gliclazide (1 μmol/l) was added as indicated by the *bars*. (**B**) Gliclazide concentration-response relation for beta-cell K_{ATP} currents. The current evoked by a 20 mV voltage step in the presence of gliclazide (G) is expressed as a fraction of its mean amplitude in the absence of the drug (G_c). (The *lines* are fit to equation 1 of the text) with IC_{50} = 184 nmol/l, n = 0.93, a = 0.07

$CaCl_2$, 10 EGTA, 10 HEPES (pH 7.15 with KOH). Gliclazide (supplied by Institut de Recherches Internationales Servier, Paris, France) was prepared as a 50 mmol/l stock solution in DMSO and the pH of the bath solution was readjusted after the drug was added. Test solutions were applied in random order. The perfusion system allowed the bath to be exchanged within 1 min. Experiments were carried out at room temperature (20–24 °C).

Electrophysiology: Cardiac myocytes. The conventional whole-cell configuration of the patch-clamp technique was used to record glibenclamide-sensitive K_{ATP} currents at a holding potential of 0 mV. The extracellular solution contained (mmol/l): 135 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 0.33 NaH_2PO_4, 5 Na pyruvate, 10 HEPES (pH 7.4). The intracellular (pipette) solution contained (mmol/l): 140 KCl, 1 $MgCl_2$, 5 EGTA, 2 ATP, 0.1 ADP, 0.1 GTP, 10 HEPES (pH 7.2). Currents were recorded, and voltage controlled, using an Axopatch 200B patch-clamp amplifier (Axon Instruments), and analysed using pCLAMP 8 software. Sulphonylureas were added to the extracellular solution, and concentration-response curves were constructed as described below. The perfusion system allowed the bath solution to be exchanged within 1 min. Experiments were done at 30 °C.

Electrophysiology: Mesenteric arterial myocytes. Glibenclamide-sensitive K_{ATP} currents were recorded at –60 mV using the conventional whole-cell patch-clamp and an Axopatch 200B patch-clamp amplifier. The extracellular solution contained (mmol/l): 140 KCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 HEPES (pH 7.4), 10 glucose. The intracellular solution contained (mmol/l): 110 KCl, 30 KOH, 10 HEPES (adjusted to pH 7.2), 10 EGTA, 1 $MgCl_2$, 1 $CaCl_2$, 0.1 Na_2ATP, 0.1 ADP, 0.5 GTP. Sulphonylureas were added to the extracellular solution and concentration-response curves constructed as described below. The perfusion system allowed the bath to be exchanged within 2 min. Experiments were done at room temperature (20–24 °C).

Electrophysiology: Data analysis. For beta-cell experiments, concentration-response relations for gliclazide inhibition of K_{ATP} currents were obtained by alternating test solutions with control (gliclazide-free) solution. The control conductance was taken as the mean of that obtained in control solution before and after application of the test gliclazide concentration. In the case of cardiac and smooth muscle experiments, the control current amplitude was measured as described in the Results.

The K_{ATP} current in gliclazide (I) is plotted as a fraction of that obtained in the control solution (I_c). Data were fit with the Hill equation

$$I/I_c = a + \frac{1-a}{1+([G]/IC_{50})^n} \quad (1)$$

where [G] is the gliclazide concentration, IC_{50} is the gliclazide concentration that produces half-maximal inhibition, n is the slope factor (Hill coefficient) and a is the fraction of remaining current at maximal gliclazide concentration. In heart and smooth muscle, a = 0. Data are given as means ± 1 SEM. The symbols in the figures indicate the mean and the vertical bars indicate ± one SEM (where this is larger than the symbol).

Results

K_{ATP} channels in pancreatic beta-cells. K_{ATP} currents recorded from a single beta-cell using the standard whole-cell configuration of the patch clamp technique are shown in Figure 1 A. In this configuration, the cell cytoplasm is dialysed with the pipette solution. Consequently, at low ATP concentrations, the K_{ATP} currents increase with time after the whole-cell mode is established, as cytosolic ATP washes out of the cell [22]. In general, the maximum washout current is observed with a pipette concentration of 0.3 mmol/l ATP, as at lower ATP concentrations the currents 'rundown' rapidly with time [22]. We therefore used an intracellular concentration of 0.3 mmol/l ATP in all whole-cell recordings and we only tested the effect of gliclazide after the current had reached a steady-state. This was normally achieved about 3 min after the whole-cell configuration was established.

Fig. 2. Effect of gliclazide on single K_{ATP} channel currents in beta-cells. Single-channel currents recorded from an inside-out patch at –60 mV in the presence and absence of 10 μmol/l gliclazide, as indicated

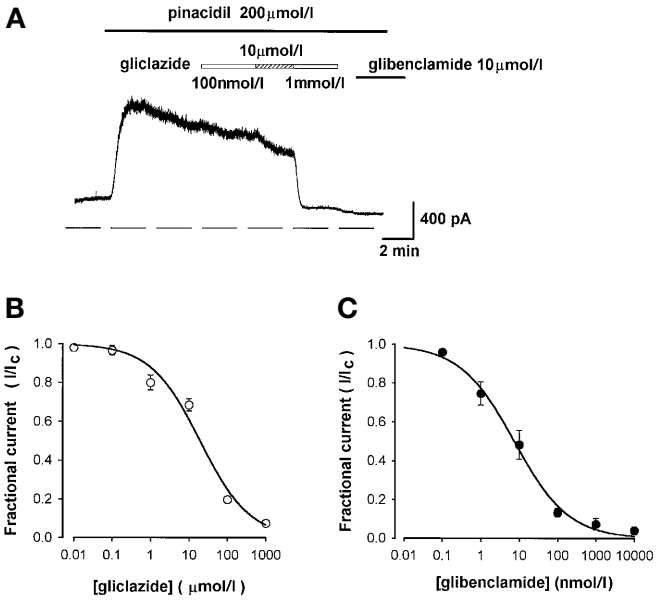

Fig. 3 A–C. Effects of gliclazide and glibenclamide on K_{ATP} currents of cardiac muscle. (**A**) Whole-cell membrane currents recorded from a cardiac myocyte held at 0 mV. Pinacidil was applied as shown to activate the K_{ATP} current. Gliclazide was applied at three concentrations and glibenclamide was applied at the end of the experiment to give complete block of K_{ATP} current. The broken line shows the zero current level. (**B**) Concentration-response curve for the effect of gliclazide. The number of cells at each concentration was 7, 7, 6, 12, 8 and 6 (in order of increasing drug concentration). The curve is drawn to equation (1) with IC_{50} = 19.5 μmol/l and n = 0.67. (**C**) Concentration-response curve for glibenclamide The number of cells at each concentration was 9, 14, 16, 18, 13 and 10 (in order of increasing drug concentration). The curve is drawn to equation (1) with IC_{50} = 7.9 nmol/l and n = 0.59

At a concentration of 1 μmol/l, gliclazide blocked the beta-cell K_{ATP} current almost completely (Fig. 1A). The onset of block was fast and inhibition was largely reversible on removal of the drug. However, at the highest drug concentrations tested (10 mmol/l, a suprapharmacological concentration), the current did not always fully reverse once the drug was removed.

The relation between gliclazide concentration and the inhibition of the K_{ATP} current is given in Figure 1B. The data are consistent with the idea that gliclazide blocks native K_{ATP} currents by interaction with a single site and the concentration-inhibition curve is well fit by the Hill equation (1). Half-maximal inhibition (IC_{50}) was observed at 184 ± 30 nmol/l gliclazide (n = 6–10) and the current was almost completely blocked by 10 μmol/l gliclazide. The Hill coefficient (slope factor) for gliclazide block was close to unity (0.93 ± 0.12; n = 6–10). This result suggests that the binding of a single molecule of gliclazide is sufficient to block the K_{ATP} channel.

In previous studies of gliclazide inhibition of the cloned beta-cell K_{ATP} channel, Kir6.2/SUR1, heterologously expressed in *Xenopus* oocytes, we observed that high-affinity gliclazide block was not complete when the drug was applied to the intracellular surface of an excised patch [18]. To determine if this is also the case for the native channel, we examined the inhibitory effect of 10 μmol/l gliclazide in inside-out patches excised from isolated beta-cells. Gliclazide was also effective when applied to the intracellular membrane surface (Fig. 2). At a concentration of 10 μmol/l, gliclazide blocked the beta-cell K_{ATP} channel by 66 ± 3 % (n = 5). This is much less than in the intact cell (~ 95 %, Fig. 1), but is similar to the extent of the block observed under the same conditions for Kir6.2/SUR1 channels expressed in *Xenopus* oocytes (61 ± 4 %, n = 8; [18]).

Rat cardiac K_{ATP} currents. Ventricular myocytes were superfused with physiological solution containing 6 mmol/l K^+. Cells were initially voltage clamped at –70 mV and were then depolarised to 0 mV for the duration of the experiment. Unlike beta-cell K_{ATP} currents, those of cardiac myocytes are largely blocked, even when the cell is dialysed with intracellular solutions containing low ATP [3]. K_{ATP} currents of ventricular myocytes were therefore activated by applying the K_{ATP} channel opener pinacidil (200 μmol/l) to the extracellular solution (Fig. 3) and concentration-inhibition curves for gliclazide were measured in the presence of pinacidil. Because pinacidil activation was followed by a slow decline in the current amplitude (Fig. 3), gliclazide was applied after the K_{ATP} current had reached a steady state. A maximum of three concentrations were applied to any one cell, in increasing concentrations. Glibenclamide (10 μmol/l) was added at the end of the experiment, and the control K_{ATP} current was taken as the difference between the steady-state current after the addition of pinacidil and that in the additional presence of glibenclamide. We show the mean concentration-response curve for gliclazide inhibition (Fig. 3B). Fitting equation (1) to the data gave an IC_{50} of

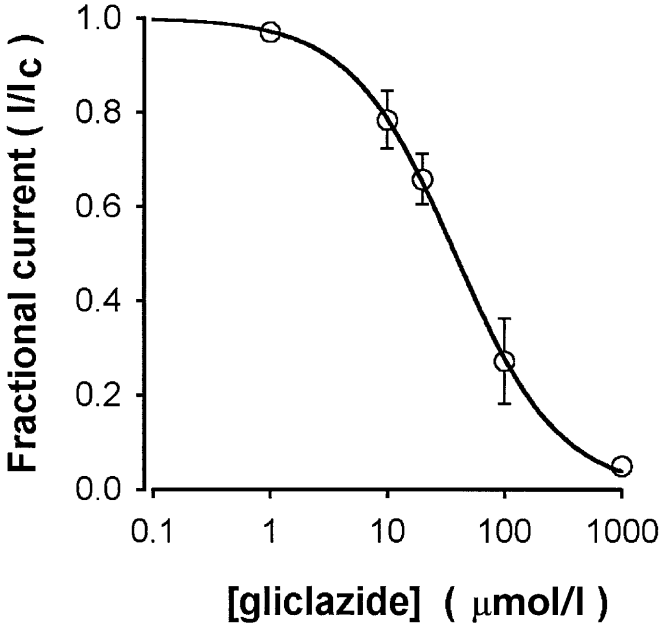

Fig. 4. Concentration-response curve for block of arterial K_{ATP} currents by gliclazide. The points show means ± SEM from the following number of cells at each concentration: 10, 6, 6, 5 and 5. The curve is drawn to equation (1) with IC_{50} = 37.9 μmol/l and n = 0.98

19.5 ± 5.4 μmol/l (n = 6–12) and a Hill coefficient of 0.67 ± 0.11.

For comparison, we also measured the concentration-response curve for the inhibition of the cardiac K_{ATP} current by glibenclamide. In these experiments, 100 μmol/l glibenclamide was added at the end of each experiment, and the control K_{ATP} current was taken as the difference between the steady-state current in pinacidil and that in 100 μmol/l glibenclamide plus pinacidil. The concentration-inhibition curve for glibenclamide (Fig. 3C) is well fit by the Hill equation with an IC_{50} of 7.9 ± 1.1 nmol/l and a Hill coefficient of 0.59 ± 0.05 (n = 9–18), consistent with glibenclamide being over 2500 times more potent than gliclazide at blocking cardiac K_{ATP} channels.

K_{ATP} channels of arterial smooth muscle. To record K_{ATP} currents from smooth muscle cells isolated from mesenteric arteries, cells were held at –60 mV in 140 mmol/l extracellular [K^+]. These experimental conditions minimise activation of other K^+ channels [21, 23]. K_{ATP} currents were activated by applying 10 μmol/l pinacidil to the extracellular solution, and under these conditions the pinacidil-activated current was blocked completely by 10 μmol/l glibenclamide. We measured the reduction in the pinacidil-activated current produced by subsequent application of gliclazide, a maximum of three concentrations being applied to any one cell. Glibenclamide (10 μmol/l) was applied at the end of each experiment to completely inhibit the K_{ATP} current. The control K_{ATP} current was measured as the difference between the current in the presence of pinacidil and that in the presence of pinacidil plus glibenclamide. The concentration-response curve is shown in Figure 4 and fitting of equation (1) gave an IC_{50} of 37.9 ± 1.0 μmol/l (n = 5–10) and a Hill coefficient of 0.98 ± 0.02. Thus gliclazide seems slightly less potent at blocking K_{ATP} channels of arterial smooth muscle than those of cardiac muscle.

Discussion

Extent of block. Our results show that gliclazide blocks K_{ATP} channels in pancreatic beta-cells by interaction with a single high-affinity site. Half-maximal inhibition (IC_{50}) was produced by 184 nmol/l gliclazide. This is only slightly less potent than the IC_{50} measured for the high-affinity site of Kir6.2/SUR1 channels expressed in *Xenopus* oocytes (50 ± 7 nmol/l; n = 8 [18]) and is consistent with the view that the beta-cell K_{ATP} channel is composed of Kir6.2 and SUR1 subunits.

Gliclazide was much less effective at blocking K_{ATP} currents in cardiac myocytes and arterial smooth muscle, the IC_{50}s being 19.5 ± 5.4 μmol/l (n = 6–12) and 37.9 ± 1.0 μmol/l (n = 5–10), respectively. These values are more than 100-fold and more than 200-fold, respectively, higher than that observed for the pancreatic beta-cell K_{ATP} channel under similar conditions. Thus, gliclazide is a high-affinity sulphonylurea that has specificity for the pancreatic beta-cell K_{ATP} channel.

In contrast, the sulphonylurea glibenclamide seems to show much less tissue selectivity, as it blocks K_{ATP} channels in beta-cells and cardiac muscle with IC_{50} s in the low nanomolar range. The IC_{50} of 7.9 nmol/l we obtained for inhibition of cardiac K_{ATP} channels by glibenclamide is consistent with previous measurements [24, 25] and suggests that glibenclamide is more than 2500-fold more potent in inhibiting cardiac K_{ATP} channels than gliclazide. The potency of glibenclamide in blocking beta-cell K_{ATP} channels (IC_{50} ~ 4 nmol/l [26]) is greater than that found for gliclazide (50 nmol/l), and similar to glibenclamide block of cardiac K_{ATP} channels (8 nmol/l).

Cardiac K_{ATP} channels are thought to be composed of Kir6.2 and SUR2A subunits [13]. Consistent with this idea, glibenclamide inhibited Kir6.2-SUR2A expressed in *Xenopus* oocytes with an IC_{50} similar (27 nmol/l [19]) to our present results on the native channel. The IC_{50} of 800 μmol/l for gliclazide inhibition of Kir6.2/SUR2A in *Xenopus* oocytes [18] was higher than that measured in native cardiac myocytes. There are several possible reasons for this difference. Firstly, it might relate to the different cell types (oocyte vs cardiac myocyte). Secondly, in the *Xenopus* experiments, gliclazide was added to the in-

tracellular face of excised macropatches, while it was applied in the extracellular solution bathing intact cardiac myocytes. Thirdly, K_{ATP} currents in cardiac muscle were activated by the K_{ATP} channel opener pinacidil, whereas the drug was not present in the oocyte experiments. Finally, intracellular nucleotides were not present in the *Xenopus* inside-out patch experiments but might be expected to be present in the whole-cell recordings from cardiac myocytes.

Time course of block. Gliclazide blocked beta-cell K_{ATP} currents rapidly when applied to the intracellular surface of the excised patch and when applied to the outside of the cell. The slight difference in time course that we observe may reflect differences in the rate of drug application rather than in the rate of binding. However, the washout of the drug was distinctly slower in the whole-cell configuration. This is in accordance with the idea that the drug binding site may lie on the intracellular side of the membrane; in this case, the slower time course of unblock observed in intact cells would reflect the rate at which the drug can be lost from the beta-cell. There is accumulating evidence that the site at which tolbutamide binds is located on the intracellular side of the membrane [27–29].

Comparison of whole-cell and inside-out patch recordings. There is an interesting discrepancy between the results of our experiments on beta-cells obtained using whole-cell recordings and those in excised patches. Thus in whole-cell recordings, 10 μmol/l gliclazide causes almost complete block of the K_{ATP} currents, whereas inhibition only amounts to 66 % in excised patches. It is of interest that the maximal extent of high-affinity block of Kir6.2/SUR1 currents measured in excised patches was also 60 %. Our results show that this difference is the result of the patch configuration rather than any difference between the cloned channel and its native counterpart.

A similar anomaly is found for tolbutamide inhibition of beta-cell K_{ATP} channels, where it has been shown to result from modulation by MgADP [30–31]. It is well established that the inhibitory effects of tolbutamide on native beta-cell K_{ATP} channels and on Kir6.2/SUR1 channels are enhanced in the presence of intracellular MgADP [30–32]. The enhanced block does not reflect an increased binding affinity for sulphonylureas when MgADP is present. Instead, it results because sulphonylureas abolish the stimulatory action of MgADP mediated via SUR1, whilst leaving the inhibitory effect of the nucleotide on Kir6.2 intact [32]. Inhibition is thus caused by the combined actions of MgADP and tolbutamide. Because MgADP is always present in the intact cell but not (unless deliberately added) in excised patches, this effect can account for the increased block observed in intact cells. Our results suggest that a similar effect may operate for gliclazide. Indeed, we have observed that this is the case for Kir6.2/SUR1 channels (data not shown).

Possible therapeutic relevance. The extent to which sulphonylurea therapy contributes to the increased risk from cardiovascular disease in diabetes has been debated for several years [6–8]. Furthermore, blockade of vascular K_{ATP} channels can affect blood flow in coronary and other circulations [5]. Thus, it seems likely that high selectivity for pancreatic beta-cell K_{ATP} channels over those of cardiovascular tissues is a desirable property for sulphonylureas to be used therapeutically in Type II diabetes.

Like tolbutamide, but in contrast to glibenclamide and glimepiride, gliclazide is able to produce a selective block of K_{ATP} channels in beta cells. At a concentration of 1 μmol/l, the block is 83 %, 12 % and 3 % for beta-cell, cardiac and smooth muscle K_{ATP} channels, respectively. The total concentration of gliclazide (free plus bound) in the plasma of patients treated with the drug is between 1 and 25 μmol. Because of drug binding to plasma proteins, however, only about 5 % of the drug is free giving an active concentration of less than 1 μmol/l. This suggests that cross-reactivity of gliclazide with K_{ATP} channels in the surface membranes of heart and vascular smooth muscle is likely to be very small throughout the therapeutic range. Recent experiments suggest that K_{ATP} channels in the inner mitochondrial membrane are involved in cardiac ischemic preconditioning [4] and further studies are needed to determine the specificity of different sulphonylureas with regard to these channels.

Acknowledgements. Supported by the Wellcome Trust, the British Heart Foundation and the Institut de Recherches Internationales Servier. We thank Dr D. Ravel for helpful criticism of the manuscript.

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