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Glimepiride block of cloned β-cell, cardiac and smooth muscle K_{ATP} channels

[1,3]Dae-Kyu Song & *[,2]Frances M. Ashcroft

[1]Department of Physiology, Keimyung University School of Medicine, 194 Dongsan Dong, Choong Gu, Taegu, 700-712 Korea and [2]University Laboratory of Physiology, Parks Road, Oxford OX1 3PT

1 We examined the effect of the sulphonylurea glimepiride on three types of recombinant ATP-sensitive potassium (K_{ATP}) channels.

2 K_{ATP} channels share a common pore-forming subunit, Kir6.2, which associates with different sulphonylurea receptor isoforms (SUR1 in β-cells, SUR2A in heart and SUR2B in smooth muscle).

3 Kir6.2 was coexpressed with SUR1, SUR2A or SUR2B in *Xenopus* oocytes and macroscopic K_{ATP} currents were recorded from giant inside-out membrane patches. Glimepiride was added to the intracellular membrane surface.

4 Glimepiride inhibited Kir6.2/SUR currents by interaction with two sites: a low-affinity site on Kir6.2 ($IC_{50} = \sim 400\ \mu M$) and a high-affinity site on SUR ($IC_{50} = 3.0$ nM for SUR1, 5.4 nM for SUR2A and 7.3 nM for SUR2B). The potency of glimepiride at the high-affinity site is close to that observed for glibenclamide (4 nM for SUR1, 27 nM for SUR2A), which has a similar structure.

5 Glimepiride inhibition of Kir6.2/SUR2A and Kir6.2/SUR2B currents, but not Kir6.2/SUR1 currents, reversed rapidly.

6 Our results indicate that glimepiride is a high-affinity sulphonylurea that does not select between the β-cell, cardiac and smooth muscle types of recombinant K_{ATP} channel, when measured in inside-out patches. High-affinity inhibition is mediated by interaction of the drug with the sulphonylurea receptor subunit of the channel.

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

Introduction

Sulphonylureas stimulate insulin secretion from pancreatic β-cells and are widely used in the treatment of type 2 diabetes mellitus. Their principal target is the ATP-sensitive potassium (K_{ATP}) channel, which plays a major role in controlling the β-cell membrane potential. Inhibition of K_{ATP} channels by glucose or sulphonylureas causes depolarization of the β-cell membrane; in turn, this triggers the opening of voltage-gated Ca^{2+} channels, eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+} which stimulates the exocytosis of insulin-containing secretory granules (Ashcroft & Rorsman, 1989).

K_{ATP} channels are also found at high density in a variety of other cell types including cardiac, smooth and skeletal muscle, and some brain neurones (Ashcroft & Ashcroft, 1990). Although their roles in extra-pancreatic tissues are less well characterised, it is likely that they open in response to metabolic stress, such as that which occurs during cardiac and cerebral ischaemia (Nichols & Lederer, 1991). They are also important in the control of vascular smooth muscle tone, and thus of blood pressure (Quayle *et al*., 1997). It is therefore of importance to know the affinity of the different types of K_{ATP} channel for the various sulphonylureas.

*Author for correspondence;
E-mail: frances.ashcroft@physiol.ox.ac.uk
[3]Current address: University Laboratory of Physiology, Parks Road, Oxford OX1 3PT

The K_{ATP} channel is a hetero-octameric complex of two structurally distinct proteins: an inwardly-rectifying K-channel, Kir6.2, and a sulphonylurea receptor, SUR (Aguilar-Bryan *et al*., 1995; Inagaki *et al*., 1995; 1996; 1997; Isomoto *et al*., 1996; Sakura *et al*., 1995; Clement *et al*., 1997; Shyng & Nichols, 1997). The former acts as an ATP-sensitive K-channel pore while SUR is a channel regulator which endows Kir6.2 with sensitivity to drugs such as the inhibitory sulphonylureas and the K-channel openers (Tucker *et al*., 1997). K_{ATP} channels in different tissues usually share a common Kir6.2 subunit, but possess different types of SUR subunit, which accounts for their different drug sensitivities (Ashcroft & Gribble, 1998; 1999). The β-cell K_{ATP} channel is composed of Kir6.2 and SUR1, the cardiac type of Kir6.2 and SUR2A and that in some smooth muscle types, probably, of Kir6.2 and SUR2B (smooth muscle channels may also be composed of Kir6.1 and SUR2B subunits). Both Kir6.2/SUR1 and Kir6.2/SUR2B combinations are also found in the brain. Although wild-type K_{ATP} channels require both types of subunit (Kir6.2 and SUR) for functional activity, a mutant form of Kir6.2 with a C-terminal truncation of $\sim 20-40$ amino acids (Kir6.2ΔC) is capable of independent expression (Tucker *et al*., 1997). Kir6.2ΔC therefore provides a useful tool for studying the effects of drugs on the pore-forming subunit of the K_{ATP} channel.

A number of studies suggest that the different types of K_{ATP} channel exhibit different specificities towards the various sulphonylureas. In particular, both tolbutamide and gliclazide

inhibit Kir6.2/SUR1, but not Kir6.2/SUR2A, channels by interaction with a high-affinity site located on SUR1 (Gribble *et al*., 1997b; Gribble & Ashcroft, 1999). Similar results have been reported for glibenclamide block of native β-cell and cardiac K_{ATP} channels (Trube *et al*., 1986; Ventakesh *et al*., 1991). These results suggest that only SUR1 possesses a high-affinity binding site for these sulphonylureas. In contrast, meglitinide, a benzamido derivative equivalent to the non-sulphonylurea moiety of glibenclamide, mediates high-affinity inhibition of both β-cell and cardiac types of K_{ATP} channel (native and recombinant) (Garrino *et al*., 1985; Gribble *et al*., 1997b; 1998). These results have been interpreted to indicate that both SUR1 and SUR2A may possess a benzamido-binding site. Like meglitinide, but in contrast to tolbutamide, glibenclamide blocks both native and recombinant β-cell and cardiac K_{ATP} channels with high affinity (Gribble *et al*., 1998; Zünckler *et al*., 1988a; Barret-Jolley & McPherson, 1998; Findlay, 1992). Because glibenclamide contains both the sulphonylurea and benzamido moieties, this suggests that the drug may bind simultaneously to both tolbutamide and benzamido-binding sites of SUR1, but only to the benzamido-binding site on SUR2A. This may account for the fact that inhibition of Kir6.2/SUR1 currents by glibenclamide is only poorly reversible in electrophysiological experiments, while inhibition of Kir6.2/SUR2A and Kir6.2/SUR2B currents is readily reversed (Ashcroft & Gribble, 1999).

Glimepiride (Figure 1) is a sulphonylurea that stimulates insulin secretion and has recently been introduced for the treatment of type-2 diabetes. Despite blocking K_{ATP} channels in pancreatic β-cells with high affinity (Schwanstecher *et al*., 1994) it has been suggested that the mechanism of action of glimepiride differs from that of other sulphonylureas (Kramer *et al*., 1994, 1996; Müller *et al*., 1994). In this paper we examine the effect of glimepiride on three types of recombinant K_{ATP} channels expressed in *Xenopus* oocytes: Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B. These correspond to the β-cell, cardiac and smooth muscle types of K_{ATP} channel, respectively. We conclude that the drug blocks all three types of K_{ATP} channel with similar efficacy in excised membrane patches, and that its mechanism of action is similar to that of glibenclamide.

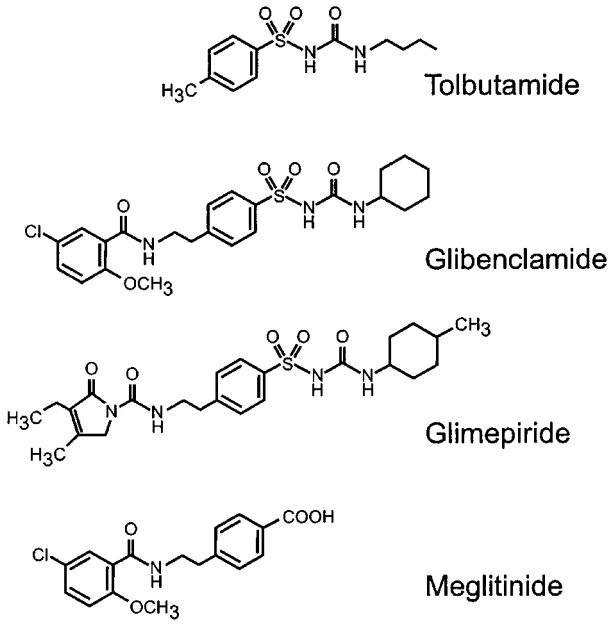

Figure 1 Molecular structures of tolbutamide, glibenclamide, glimepiride and meglitinide.

Methods

Molecular biology

Mouse Kir6.2 (Genbank D50581; Inagaki *et al*., 1995; Sakura *et al*., 1995), rat SUR1 (Genbank L40624; Aguilar-Bryan *et al*., 1995), SUR2A (Genbank D83598; Inagaki *et al*., 1996) and SUR2B (Genbank D86038; Isomoto *et al*., 1996) cDNAs were cloned into the pBF vector. A truncated form of Kir6.2 (Kir6.2ΔC36), which lacks the C-terminal 36 amino acids and forms functional channels in the absence of SUR, was prepared as described previously (Tucker *et al*., 1997). Capped mRNA was prepared using the mMESSAGE mMACHINE large scale *in vitro* transcription kit (Ambion, Austin, TX, U.S.A.), as previously described (Gribble *et al*., 1997a).

Oocyte collection

Female *Xenopus laevis* were anaesthetized with MS222 (2 g l^{-1} added to the water). One ovary was removed *via* a mini-laparotomy, the incision sutured and the animal allowed to recover. Immature stage V–VI oocytes were incubated for 60 min with 1.0 mg ml^{-1} collagenase (Sigma, type V) and manually defolliculated. Oocytes were either injected with $\sim$1 ng Kir6.2ΔC36 mRNA or coinjected with $\sim$0.1 ng Kir6.2 mRNA and $\sim$2 ng of mRNA encoding either SUR1, SUR2A or SUR2B. The final injection volume was 50 nl per oocyte. Isolated oocytes were maintained in Barth's solution and studied 1–4 days after injection (Gribble *et al*., 1997a).

Electrophysiology

Patch pipettes were pulled from borosilicate glass and had resistances of 250–500 kΩ when filled with pipette solution. Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV and at 20–24°C (Gribble *et al*., 1997a). Currents were evoked by repetitive 3 s voltage ramps from $-$110 mV to $+$100 mV and recorded using an EPC7 patch-clamp amplifier (List Electronik, Darmstadt, Germany). They were filtered at 10 kHz, digitized at 0.4 kHz using a Digidata 1200 Interface and analysed using pClamp 8 software (Axon Instruments, Foster City, U.S.A.). Records were stored on videotape and resampled at 20 Hz for presentation in the figures.

The pipette (external) solution contained (mM): KCl 140, $MgCl_2$ 1.2, $CaCl_2$ 2.6, HEPES 10 (pH 7.4 with KOH). The intracellular (bath) solution contained (mM): KCl 107, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 10, HEPES 10 (pH 7.2 with KOH; final $[K^+]$ $\sim$140 mM). Glimepiride (supplied by IRIS) was prepared as a 50 mM stock solution in DMSO, and the pH of the bath solution was readjusted after drug addition. The final $[K^+]$ was about 140 mM after addition of glimepiride.

Rapid exchange of solutions was achieved by positioning the patch in the mouth of one of a series of adjacent inflow

pipes placed in the bath. Test solutions were applied in random order, and patches were exposed to 1 mM MgATP at intervals throughout the experiment, to reverse channel rundown.

In a previous study of glimepiride on native K_{ATP} currents (Schwanstecher *et al*., 1994), pancreatic β-cells were preincubated with the drug and the K_{ATP} current subsequently measured as the whole-cell current activated on washout of cellular ATP. The current amplitudes were then compared with those of β-cells that had not been exposed to the drug. This strategy relies on two assumptions: (i) that all cells express approximately the same amount of K_{ATP} current (because it is not possible to record the control current, in the absence of the drug, in the same β-cell); and (ii), that the drug does not accumulate inside the cell to a concentration higher than that in the external solution. Neither of these assumptions is valid for K_{ATP} channels heterologously expressed in *Xenopus* oocytes. The level of expression may vary from oocyte to oocyte; furthermore, the lipid-soluble sulphonylureas appear to accumulate within the oocyte (which has a high lipid content) because drugs like tolbutamide, which are readily reversible in excised patches, are not reversible on intact oocytes. We therefore added glimepiride to the intracellular surface of excised inside-out membrane patches.

Data analysis

The slope conductance was measured by fitting a straight line to the current-voltage relation between -20 mV and -100 mV: the average of five consecutive ramps was calculated in each solution. Data are presented as mean$\pm$1 s.e.mean.

Dose-response curves were fit to the following equation (Gribble *et al*., 1997b):

$$\frac{G}{G_c} = x^* y \qquad (1)$$

where G is the conductance in the presence of glimepiride, G_c is the conductance in control solution, x is a term describing the high-affinity site and y is a term describing the low-affinity site.

$$x = \mathrm{L} + \frac{(1-\mathrm{L})}{(1+([\mathrm{Glim}]/IC_{50(1)})^{\mathrm{h1}})} \qquad (2)$$

$$y = \mathrm{L} + \frac{1}{(1+([\mathrm{Glim}]/IC_{50(2)})^{\mathrm{h2}})} \qquad (3)$$

where [Glim] is the glimepiride concentration, $IC_{50(1)}$, $IC_{50(2)}$ are the glimepiride concentrations at which inhibition is half maximal at the high and low-affinity sites, respectively; *h1*, *h2* are the Hill coefficients (slope factors) for the high and low-affinity sites, respectively; and L is the fractional conductance remaining when the high-affinity sites are maximally occupied. When only a single site is present, the equation reduces to $G/G_c = x$ (eqn 4). Data were fit using Microcal Origin software.

To control for the rundown of channel activity that occurs in excised patches, dose-response curves for Kir6.2/SUR1 currents were constructed by expressing the conductance in the presence of glimepiride as a fraction of the conductance measured in control solution before addition of the drug. Because the drug was essentially irreversible on the time scale of our experiments, it was not possible to calculate the mean conductance in control solution before and after drug addition. The lack of reversibility also meant that a drug concentration could only be applied to a given patch once. Thus each data point represents a different oocyte.

Results

Macroscopic currents were recorded in inside-out membrane patches from *Xenopus* oocytes coexpressing Kir6.2 and either SUR1, SUR2A or SUR2B. In all cases, the currents were small in the cell-attached configuration but increased markedly when the patch was excised into nucleotide-free solution, consistent with the idea that the K_{ATP} channel is blocked in the intact oocyte by cytoplasmic nucleotides such as ATP.

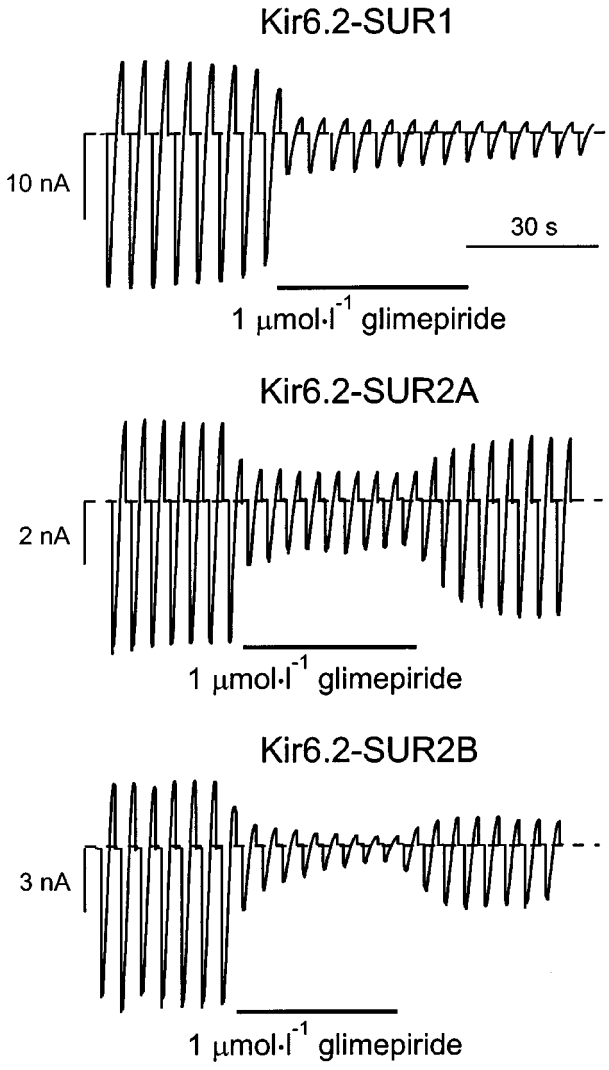

Figure 2 Inhibition of K_{ATP} currents by glimepiride. Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from -110 mV to $+100$ mV from oocytes coexpressing Kir6.2 and either SUR1, SUR2A or SUR2B. Glimepiride (1 μM) was added as indicated by the bars. The dashed line indicates the zero current level.

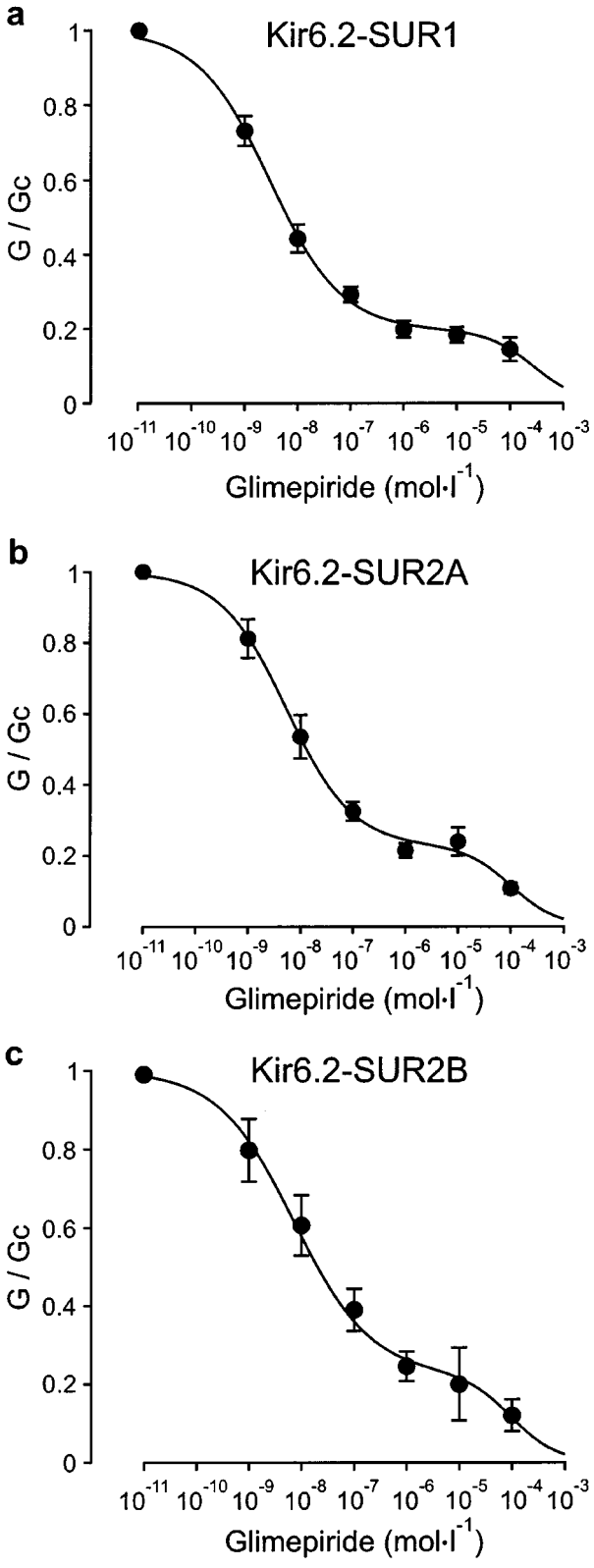

Figure 3 Dose-inhibition relationships for block of K_{ATP} currents by glimepiride. Glimepiride dose-response relationships for (a) Kir6.2/SUR1, (b) Kir6.2/SUR2A and (c) Kir6.2/SUR2B currents. The macroscopic conductance in the presence of glimepiride (G) is expressed as a fraction of its mean amplitude in the absence of the drug (G_c). The symbols represent the mean and the vertical bars indicate 1 s.e.mean. The lines are fit to equation 1 of the text using the following values. Kir6.2/SUR1 channels: $IC_{50(1)}=3.0$ nM, $h_1=0.64$, $IC_{50(2)}=234$ μM, $h_2=1$, L = 0.20. Kir6.2/SUR2A channels: $IC_{50(1)}=5.4$ nM, $h_1=0.69$, $IC_{50(2)}=104$ μM, $h_2=1$, L = 0.23. Kir6.2/SUR2B channels: $IC_{50(1)}=7.3$ nM, $h_1=0.6$, $IC_{50(2)}=99$ μM, $h_2=1$, L = 0.23.

Figure 2 shows that application of 1 μM glimepiride to the intracellular membrane surface blocked all three types of K_{ATP} channel, to a similar extent. The mean block of Kir6.2/SUR1 currents was $80\pm2\%$ ($n=7$), of Kir6.2/SUR2A currents was $78\pm2\%$ ($n=12$), and of Kir6.2/SUR2B currents was $80\pm3\%$ ($n=8$). The very slow decline in current observed in the presence of glimepiride in some patches is probably due to rundown of channel activity, as a similar decrease is also observed in control solution (Tucker *et al*., 1997). It is believed to result from a gradual fall in the membrane concentration of the phospholipid PIP_2 (Baukrowitz *et al*., 1998; Shyng & Nichols, 1998).

The block of Kir6.2/SUR1 currents was not reversible on the time scale of our experiments for any drug concentration (Figure 2). In contrast, inhibition of Kir6.2/SUR2A and Kir6.2/SUR2B currents by glimepiride concentrations of <100 μM was partially reversed on return to control solution; at higher drug concentrations, however, the block was also irreversible.

The relationship between the glimepiride concentration and the K_{ATP} current for Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B channels is shown in Figure 3. As with other sulphonylureas, inhibition of Kir6.2/SUR1 currents by glimepiride (Figure 3a) was best fit by assuming that the drug interacted with both high and low-affinity sites. The IC_{50}s of these sites were 3.0 ± 0.5 nM and 234 ± 139 μM ($n=5-13$), respectively. Likewise, glimepiride blocked Kir6.2/SUR2A (Figure 3b) and Kir6.2/SUR2B (Figure 3c). currents by interaction with both a high-affinity and a low-affinity site. The IC_{50}s were 5.4 ± 0.1 nM and 104 ± 56 μM ($n=6-12$), respectively, for Kir6.2/SUR2A currents and 7.3 ± 0.2 nM and 98.9 ± 75 μM ($n=5-13$), respectively, for Kir6.2/SUR2B currents.

The Hill coefficients for the high-affinity site were 0.64 ± 0.06 ($n=5-13$) and 0.69 ± 0.09 ($n=6-12$) for channels containing SUR1 and SUR2A, respectively. The fact that the Hill coefficient is less than unity may indicate that there is more than one binding site for the drug and that there is negative cooperativity between these sites. This may reflect the fact that the channel is a tetramer (Clement *et al*., 1997; Shyng & Nichols, 1997). In the case of glibenclamide, it appears all four subunits bind the drug although occupation of a single site is sufficient to induce channel closure, (Dörschner *et al*., 1999).

Low-affinity inhibition of Kir6.2/SUR1 and Kir6.2/SUR2A channels by other sulphonylureas is not conferred by the sulphonylurea receptor, because it is observed when truncated Kir6.2 subunits (Kir6.2ΔC36) are expressed in the absence of SUR (Gribble *et al*., 1997b; 1998). To investigate whether the low-affinity site for glimepiride inhibition is also independent of SUR, we tested the effect of the drug on Kir6.2ΔC36 channels. Figure 4 shows that glimepiride blocked Kir6.2ΔC36 currents at low affinity, with an IC_{50} of around 0.4 mM, suggesting that the low-affinity site for glimepiride inhibition lies on Kir6.2 itself. It was not possible to dissolve the drug at concentrations greater than 0.5 mM, so that the

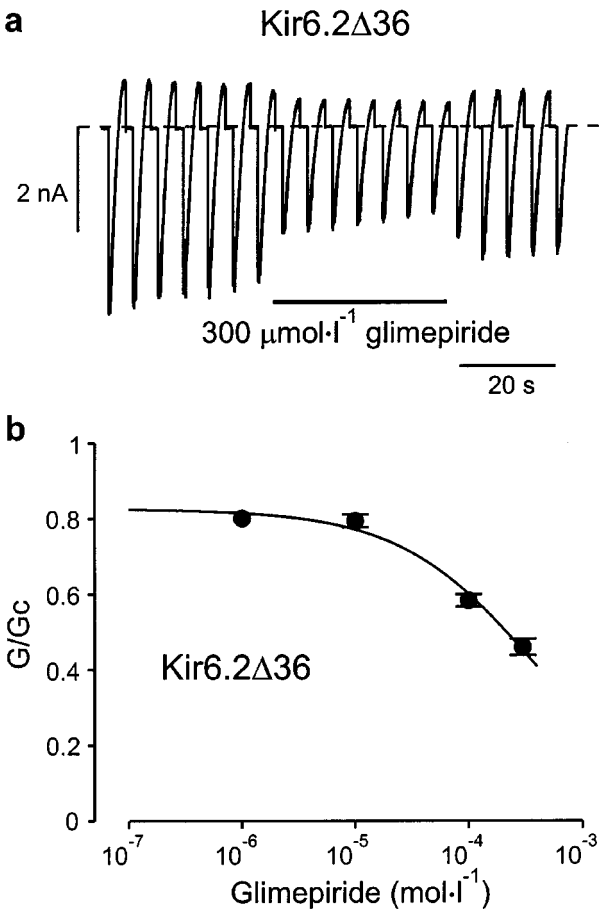

Figure 4 Block of Kir6.2ΔC36 currents by glimepiride. (a) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 mV to +100 mV from oocytes coexpressing Kir6.2ΔC36. Glimepiride (300 μM) was added as indicated by the bar. (b) Glimepiride dose-response relationships for Kir6.2ΔC36 currents. The macroscopic conductance in the presence of glimepiride (G) is expressed as a fraction of its mean amplitude in the absence of the drug (G_c). The symbols represent the mean, and the vertical bars indicate 1 s.e.mean. The line is fit to equation 4 of the text using $IC_{50}=388$ μM, $h=0.72$.

IC_{50} could not be accurately determined. Fitting of equation 4 to the data, however, gave an estimated IC_{50} of 388 ± 95 μM ($n=2-12$).

Discussion

Our results demonstrate that glimepiride blocks Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B channels by interaction with both a high-affinity and a low-affinity site. The high-affinity sulphonylurea site is located on the SUR subunit, while the low-affinity site is likely to reside on the Kir6.2 subunit, because it was also observed for Kir6.2ΔC36 currents.

The IC_{50} for high-affinity inhibition of Kir6.2/SUR1 currents by glimepiride was 3 nM. This value is in good agreement with the K_i for binding of [^{3}H]-glimepiride to intact β-cells or β-cell membranes (0.7 to 6.8 nM: Müller *et al*., 1994; Schwanstecher *et al*., 1994). It is, however, about 10 fold higher than that observed for inhibition of whole-cell K_{ATP} currents in isolated β-cells (IC_{50} of 0.3 nM, Schwanstecher *et al*., 1994), This difference probably reflects differences in the experimental protocols. Our experiments were conducted on inside-out patches, rather than intact cells, to avoid the complicating effects of intracellular nucleotides on sulphonylurea inhibition. In contrast, Schwanstecher *et al*. (1994) preincubated β-cells with glimepiride and measured the whole-cell current activated by washout of ATP from the cell. This may be advantageous when the on-rate of the drug is slow, but may give a higher affinity if glimepiride accumulates inside the β-cell to a concentration higher than that in the extracellular solution. In contrast to the experiments reported here for K_{ATP} channels in excised patches, high-affinity block by glimepiride was complete in whole-cell recordings from β-cells, and thus low-affinity inhibition was not observed (Schwanstecher *et al*., 1994). Similar findings have also been reported for tolbutamide and glibenclamide, and have been attributed to the presence of intracellular Mg-nucleotides (Trube *et al*., 1986; Zünckler *et al*., 1988b; Gribble *et al*., 1997b).

Direct photoaffinity labelling of β-cell membrane proteins with radiolabelled sulphonylureas revealed that glibenclamide binds to a 140 kDa membrane protein, the sulphonylurea receptor (Aguilar-Bryan *et al*., 1995; Kramer *et al*., 1994). In contrast, glimepiride selectively binds to a 65 kDa protein (Kramer *et al*., 1994). Both sulphonylureas are able to displace binding of the other ligand to their respective receptors. These results have been interpreted by Kramer *et al*. (1994, 1996) to indicate that the K_{ATP} channel complex contains both 140 kDa and 65 kDa subunits, which interact allosterically; and that glimepiride binds to 65 kDa subunit and glibenclamide to the 140 kDa subunit. Our results support a different interpretation. We observed that glimepiride blocks Kir6.2/SUR currents but not Kir6.2ΔC36 currents with high affinity, indicating that high-affinity inhibition requires the presence of a sulphonylurea receptor subunit. It therefore seems probable that glimepiride, like glibenclamide, binds to the 140 kDa sulphonylurea receptor subunit. Recent studies have shown that mild trypsinization of SUR1 produces a 65 kDa fragment (Matsuo *et al*., 1999). A possible explanation of the photoaffinity labelling studies, therefore, is that the SUR subunit is less susceptible to proteolysis when glibenclamide (but not glimepiride) is bound.

The responses of both Kir6.2/SUR1 and Kir6.2/SUR2A currents to glimepiride are qualitatively similar to those previously observed with glibenclamide, which also blocked both Kir6.2/SUR1 and Kir6.2/SUR2A currents at high- and low-affinity sites. The sensitivity of the high-affinity site on SUR1 to glibenclamide is similar to that for glimepiride, with IC_{50}s of 4.2 nM (Gribble *et al*., 1998) and 3.0 nM, respectively. Likewise, glimepiride is roughly as potent as glibenclamide at blocking Kir6.2/SUR2A currents, the IC_{50}s being $\sim$5 nM and $\sim$27 nM (Gribble *et al*., 1997b; 1998), for glimepiride and glibenclamide respectively. Similar values were reported for whole-cell native cardiac K_{ATP} currents activated by rimakalim: an IC_{50} of 32 nM was obtained for glimepiride block and one of 7 nM for glibenclamide block (Geisen *et al*., 1996). This is perhaps not surprising, given the structural similarity of the two drugs. Thus it appears that both SUR1 and SUR2 possess a high-affinity site for glimepiride.

The block of Kir6.2/SUR1 currents by glimepiride, like that of glibenclamide, is effectively irreversible in electrophysiological experiments, while block of Kir6.2/SUR2A and Kir6.2/SUR2B currents is partially reversible at the lower drug concentrations. These data are consistent with the idea that SUR2 does not possess a high-affinity site for the sulphonylurea moiety of glibenclamide and glimepiride, and that these drugs block Kir6.2/SUR2 currents primarily through interaction with the benzamido site.

Glimepiride and glibenclamide blocked Kir6.2ΔC36 currents with IC_{50}s of $\sim$400 μM and 42 μM, respectively, suggesting that the low-affinity site on Kir6.2 is not very sensitive to the structural differences between the two drugs. When a therapeutic dose of glimepiride is administered orally, the reported free plasma drug concentration is less than 10 μM (Lehr & Damm, 1990; Schwanstecher *et al*., 1994). Thus the inhibitory effect of glimepiride on Kir6.2 is unlikely to be of clinical relevance and the effect of the drug on insulin secretion is mediated entirely *via* the high-affinity site.

Our results demonstrate that, like glibenclamide, glimepiride blocks all three types of recombinant K_{ATP} channel with similar affinity in excised patches; and that the affinity for both drugs is similar. Yet a number of studies in the literature claim that glimepiride has less effect on the electrical properties of the heart *in vivo* than glibenclamide and has led to the suggestion that glibenclamide, but not glimepiride, decreases 'ischemic preconditioning' by blocking K_{ATP} channel activation (Geisen *et al*., 1996; Klepzig *et al*., 1999). The reason for the differences between glibenclamide and glimepiride observed *in vivo* are unclear. One possibility is that the effects of glimepiride and glibenclamide on native cardiac K_{ATP} channels may not be identical in the intact cell. It is also worth pointing out that inhibition of whole-cell K_{ATP} currents by glimepiride may vary between different tissues, despite being identical in excised patches. In particular, intracellular Mg-nucleotides enhance the inhibitory effect of sulphonylureas in β-cells, but reduce inhibition in cardiac muscle (Zünckler *et al*., 1988b; Ventakesh *et al*., 1991; Gribble *et al*., 1998). This finding, together with the fact that cardiac K_{ATP} channels are thought to be closed under physiological conditions and open only in response to ischaemic stress (Nichols & Lederer, 1991), may help explain why sulphonylureas have relatively few side effects on the heart and why cardiac mortality is not different for diabetic patients on insulin or sulphonylurea therapy (UKPDS, 1998).

In conclusion, our results suggest that therapeutic concentrations of glimepiride (10 μM; Lehr & Damm, 1990) block three types of recombinant K_{ATP} channel – Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B (corresponding to the β-cell, cardiac and smooth muscle types of K_{ATP} channel) with similar affinity in excised patches. They also indicate that this inhibition is produced by interaction of the drug with the SUR subunit of the channel.

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