British Journal of Pharmacology (2002) 136, 746–752
www.nature.com/bjp

Effect of metabolic inhibition on glimepiride block of native and cloned cardiac sarcolemmal K_{ATP} channels

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1 We have investigated the effects of the sulphonylurea, glimepiride, currently used to treat type 2 diabetes, on ATP-sensitive K^+ (K_{ATP}) currents of rat cardiac myocytes and on their cloned constituents Kir6.2 and SUR2A expressed in HEK 293 cells.

2 Glimepiride blocked pinacidil-activated whole-cell K_{ATP} currents of cardiac myocytes with an IC_{50} of 6.8 nM, comparable to the potency of glibenclamide in these cells. Glimepiride blocked K_{ATP} channels formed by co-expression of Kir6.2/SUR2A subunits in HEK 293 cells in outside-out excised patches with a similar IC_{50} of 6.2 nM.

3 Glimepiride was much less effective at blocking K_{ATP} currents activated by either metabolic inhibition (MI) with CN^- and iodoacetate or by the K_{ATP} channel opener diazoxide in the presence of inhibitors of F_0/F_1-ATPase (oligomycin) and creatine kinase (DNFB). Thus 10 μM glimepiride blocked pinacidil-activated currents by >99%, MI-activated currents by 70% and diazoxide-activated currents by 82%.

4 In inside-out patches from HEK 293 cells expressing the cloned K_{ATP} channel subunits Kir6.2/SUR2A, increasing the concentration of ADP (1–100 μM), in the presence of 100 nM glimepiride, lead to significant increases in Kir6.2/SUR2A channel activity. However, over the range tested, ADP did not affect cloned K_{ATP} channel activity in the presence of 100 nM glibenclamide. These results are consistent with the suggestion that ADP reduces glimepiride block of K_{ATP} channels.

5 Our results show that glimepiride is a potent blocker of native cardiac K_{ATP} channels activated by pinacidil and blocks cloned Kir6.2/SUR2A channels activated by ATP depletion with similar potency. However, glimepiride is much less effective when K_{ATP} channels are activated by MI and this may reflect a reduction in glimepiride block by increased intracellular ADP.

British Journal of Pharmacology (2002) **136,** 746–752

Keywords: Cardiac myocytes; clones; glimepiride; K_{ATP} channel; Kir6.2/SUR2A

Abbreviations: CN^-, cyanide; DMSO, dimethylsulphoxide; DNFB, 2,4 dinitro-1-fluorobenzene; IAA, iodoacetic acid; IPC, ischaemic pre-conditioning; MI, metabolic inhibition

Introduction

Glimepiride is a relatively new sulphonylurea used in the treatment of type 2 diabetes (Klepzig *et al*., 1999; Sonnenberg *et al*., 1997; Langtry & Balfour, 1998; Riddle & Schneider, 1998; Schade *et al*., 1998). Glimepiride has become an attractive alternative to the more common anti-diabetic drug glibenclamide. Not only is glimepiride thought to be equipotent in lowering blood glucose, but it is also thought to have fewer and less potent extra-pancreatic effects than glibenclamide.

Sulphonylureas stimulate insulin secretion from pancreatic β-cells by blocking ATP-sensitive K^+-channels (K_{ATP} channels), however, sulphonylureas may also block K_{ATP} channels of other tissues. Of particular concern is the effect of these agents on cardiac function *via* their action on cardiac K_{ATP} channels. K_{ATP} channels are thought to play a key role in the cardioprotection seen with K_{ATP} channel openers and ischaemic pre-conditioning (IPC), a powerful protective mechanism endogenous to cardiac muscle (Terzic *et al*., 1995; Yellon *et al*., 1998). A number of reports have suggested that mitochondrial rather than sarcolemmal K_{ATP} channels are the mediators of this protection, particularly since protection was still observed in the absence of action potential shortening. It should be noted however, that the molecular identity of the mitochondrial channel remains unknown (Garlid *et al*., 1997; Liu *et al*., 1998). More recently it has been suggested that cardioprotection involves both mitochondrial and sarcolemmal K_{ATP} channels and that activation of either channel is independently modulated by different trigger substances (Sanada *et al*., 2001). Once activated, mitochondrial and sarcolemmal K_{ATP} channels may initiate different protective pathways, both of which may be integral to either limiting damage or recovering function (Tanno *et al*., 2001; Toyoda *et al*., 2000).

Cardioprotection, derived from either K_{ATP} channel openers or IPC, can be abolished by glibenclamide which blocks both sarcolemmal and mitochondrial K_{ATP} channels. In contrast to glibenclamide, however, glimepiride does not appear to abolish IPC (Klepzig *et al*., 1999; Mocanu *et al*., 2001). It has been postulated therefore, that glimepiride has selective effects between different K_{ATP} channels (Ladriene *et al*., 1997; Olbrich *et al*., 1999).

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Here, we have investigated the effects of glimepiride on native sarcolemmal K_{ATP} channels of adult rat cardiac myocytes, and recombinant cardiac sarcolemmal K_{ATP} channels (Kir6.2/SUR2A) stably expressed in human embryonic kidney (HEK) cells 293. We report that glimepiride is an effective inhibitor of native and cloned cardiac sarcolemmal K_{ATP} channels under normal conditions with a concentration that produces half-maximal inhibition (IC_{50}) similar to that which we have measured previously for glibenclamide (Lawrence *et al*., 2001). However, its blocking effectiveness is reduced when K_{ATP} channels are activated by metabolic inhibition. Parallel experiments on cloned K_{ATP} channels suggest that this is due to an interaction with ADP. We suggest that block of the cardiac sarcolemmal K_{ATP} channel by glimepiride diminishes in a metabolically compromised environment such as that which occurs during myocardial ischaemia, but under physiological conditions where ADP concentration is not raised glimepiride is equipotent to glibenclamide.

Methods

Isolation of cardiac myocytes

Adult male Wistar rats (300–400 g) were killed by cervical dislocation. The care and sacrifice of animals conformed to the requirements of the U.K. Animals (Scientific Procedures) Act 1986. The heart was rapidly removed and perfused using the Langendorff technique with collagenase (type I, Sigma) and protease (type XV, Sigma) solution as described previously (Lawrence & Rodrigo, 1999). Myocytes were then mechanically dispersed and washed twice in normal Tyrode. Typically, there was a 70–90% yield of quiescent, rod-shaped cells. Cells were stored at 10°C in Tyrode for a maximum of 24 h.

HEK 293 cells stably expressing Kir6.2/SUR2A subunits

Cloned Kir6.2/SUR2A channel subunits stably expressed in human embryonic kidney HEK 293 cells were kindly provided by Dr Andrew Tinker (Centre for Clinical Pharmacology, Department of Medicine, University College London). Cells were cultured in MEM with Earl's salts also containing 10% FCS and 10 mM L-glutamine. Zeocin (364 $\mu g\ ml^{-1}$) and G418 (727 $\mu g\ ml^{-1}$) were included in the media for selection purposes. Cells were used between passages 18–26.

Solutions

Isolated ventricular myocytes were superfused with normal Tyrode containing (in mM): NaCl 135, KCl 6, NaH_2PO_4 0.33, Na-pyruvate 5, glucose 10, $MgCl_2$ 1, $CaCl_2$ 2, and N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid] (HEPES) 10, titrated to pH 7.4 with NaOH. Extracellular solution for experiments using HEK 293 cells contained (in mM): KCl 70, NaCl 70, $MgCl_2$ 2, $CaCl_2$ 2, and HEPES 10, titrated to pH 7.4 with NaOH. Other reagents were added to these solutions as described in the text.

Electrophysiology

Conventional patch pipettes were used in the whole-cell configuration to record from cardiac myocytes, and in excised outside-out and inside-out patch configurations from HEK 293 cells. Voltage was controlled and membrane currents recorded using an Axopatch 200B amplifier (Axon Instruments). Currents were filtered at 2 or 5 kHz and analogue signals were collected and digitised using a DigiData 1200 Series interface. Records were acquired and analysed using either pClamp 8 (Axon) or custom software, Excel 2000 (Microsoft) and SigmaPlot 5.0 (Jandel Scientific). To allow for variation in cell size whole-cell currents were normalized to cell capacitance and expressed as pA pF^{-1}.

Patch pipettes used to record from cardiac myocytes were made from thin-walled borosilicate glass, filled with a solution containing (in mM): KCl 140, $MgCl_2$ 1, ATP 2, ADP 0.1, GTP 0.1, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 5, HEPES 10, pH 7.2 and had resistances of 4–6 MΩ when filled. Experiments were carried out at 32±2°C. Patch pipettes used to record from HEK 293 cells were made from thick-walled borosilicate glass, fire-polished and coated with Sylgard (Dow Corning). In the outside-out patch configuration, pipettes were filled with a (intracellular) solution containing (in mM): KCl 140, ATP 0.01, EDTA 10, HEPES 10, pH 7.2 and had resistances of 14–18 MΩ when filled. In the inside-out patch configuration, pipettes were filled with extracellular solution (as described above) and the superfusing (intracellular) solution was supplemented as described in the text. Experiments on HEK 293 cells were carried out at room temperature, 22±2°C.

Drugs

Pinacidil, glibenclamide, diazoxide, sodium cyanide, iodoacetic acid (IAA), and oligomycin (a mixture of oligomycins A, B and C), were obtained from Sigma. 2,4 dinitro-1-fluorobenzene (DNFB) was obtained from Fluka. Drugs were dissolved in dimethylsulfoxide (DMSO) (Sigma) as stock solutions and diluted in Tyrode. DMSO, at the maximum concentration used of 0.1%, did not have any measurable effect on the parameters studied.

Data analysis

The K_{ATP} current in glimepiride (I) was plotted as a fraction of the K_{ATP} current in its absence (I_A) and data were fit with the Hill equation:

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

where [G] is the glimepiride concentration, IC_{50} is the glimepiride concentration that produces half-maximal inhibition and n is the slope factor (Hill coefficient). Data are presented as mean±s.e.mean and statistical significance was tested using Student's paired or unpaired t-tests as appropriate, or a one-way ANOVA followed by a Dunnet's Test for multiple comparisons. A value of $P<0.05$ was considered significant.

Results

Effect of glimepiride on pinacidil-activated whole-cell K_{ATP} currents in cardiac myocytes

Whole-cell cardiac K_{ATP} currents are rapidly activated by the K_{ATP} channel opener pinacidil, and such pinacidil-activated currents have been used to study block by the sulphonylurea glibenclamide (Fosset *et al*., 1988; Wilde & Janse, 1994). We used a similar strategy to investigate the blocking effect of glimepiride on K_{ATP} currents of rat cardiac myocytes. In the whole-cell configuration, myocytes were superfused with normal Tyrode, voltage-clamped initially at −65 mV and then depolarized to a holding potential of 0 mV for the duration of the experiment. Under these conditions, with the pipette solution containing 2 mM ATP, K_{ATP} channels are mainly inhibited. Addition of pinacidil (200 μM) to the extracellular solution activated substantial K_{ATP} current (4.2 ± 1.7 pA pF^{-1}, $n=7$ cells) and we measured glimepiride block of this pinacidil-activated current (Figure 1a). Glimepiride was added cumulatively, and no more than three concentrations of glimepiride were applied to a single cell (Figure 1a). At the conclusion of each experiment glibenclamide (10 μM) was added to the superfusate to block all K_{ATP} current and the K_{ATP} current amplitude was taken as the difference between the steady-state pinacidil-activated current and that in the presence of glibenclamide. In these experiments significant channel rundown did not occur. The effects of glimepiride and glibenclamide were not substantially reversible over the time scale of our experiments, and so no attempt was made to wash them off. Figure 1b shows the mean concentration–inhibition curve for glimepiride, plotted as a fraction of the maximal K_{ATP} current, which is well fit by equation (1) giving an IC_{50} of 6.8 ± 0.1 nM and a Hill coefficient of 1.4 ± 0.2 ($n=6$). These results suggest that glimepiride is a very potent inhibitor of rat cardiac K_{ATP} currents activated by pinacidil.

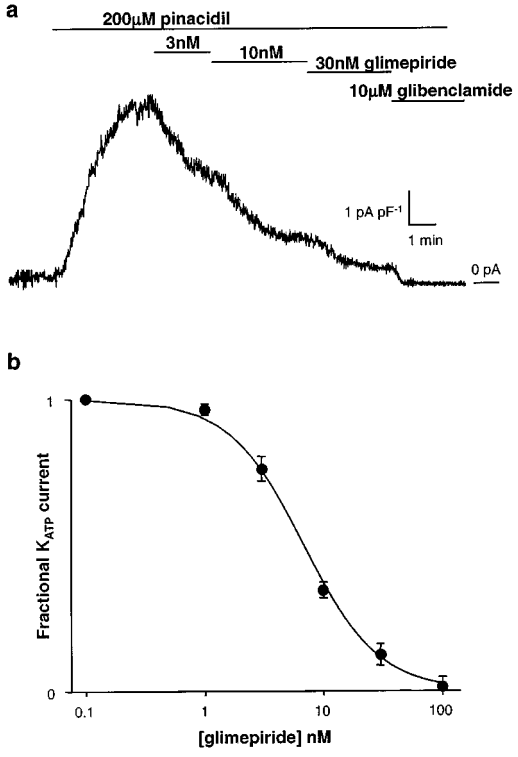

Figure 1 Glimepiride inhibition of pinacidil-activated whole-cell current from a rat cardiac myocyte. (a) Whole-cell K_{ATP} current recorded from a single rat cardiac myocyte voltage-clamped at 0 mV. 200 μM pinacidil activated a normalized whole-cell current of 4.78 pA pF^{-1}. 3, 10 and 30 nM glimepiride reduced the whole-cell current in a concentration-dependent manner and 10 μM glibenclamide blocked any remaining K_{ATP} current. The superfusion protocol is shown above the recording. (b) Glimepiride concentration–inhibition curve from native cardiac myocytes patched in the whole-cell configuration and voltage-clamped at 0 mV. Currents were evoked by application of 200 μM pinacidil ($n=6$). The K_{ATP} current in glimepiride is expressed as a fraction of the steady-state K_{ATP} current (see Methods). The line is fit to equation 1 of the text with $IC_{50}=6.8$ nM and a Hill coefficient of 1.4. Data are mean ± s.e.mean.

Effect of glimepiride on cloned K_{ATP} channels (Kir6.2/SUR2A) expressed in HEK 293 cells

To investigate the effect of glimepiride on the K_{ATP} channel without effects from other intracellular factors we recorded K_{ATP} currents from cloned K_{ATP} channel subunits. The cardiac-type sarcolemmal K_{ATP} channel is a heteromultimer composed of Kir6.2/SUR2A subunits (Isomoto *et al*., 1996; Gribble *et al*., 1998; Giblin *et al*., 1999). We recorded K_{ATP} currents from HEK 293 cells stably expressing these subunits. Membrane patches were excised in the outside-out configuration and the pipette solution contained 10 μM MgATP to maintain channel activity. This ATP concentration was chosen because the mean K_i for ATP, which we determined previously for cloned cardiac K_{ATP} currents (Kir6.2/SUR2A) in HEK 293 cells was 19.0 ± 0.04 μM ($n=4$), thus a pipette solution containing 10 μM ATP allows substantial activation of the current. Furthermore, we have observed that in the absence of ATP glibenclamide has no effect on channel activity, as has also been reported by Gribble *et al*. (1998). The K_{ATP} current dominates endogenous currents in HEK 293 cells stably expressing the subunits, Kir6.2/SUR2A, and so the current recorded immediately after patch excision, when K_{ATP} channels are still inhibited by residual intracellular ATP, was defined as the zero-current baseline. The K_{ATP} current develops spontaneously as the internal face of the membrane patch is dialyzed with 10 μM ATP and is fully activated in less than 1 min.

Figure 2a shows several current-voltage relationships recorded from a single outside-out patch in the absence and presence of glimepiride (3, 30, 100 and 300 nM). Patches were held at −20 mV, close to E_K, and pulsed from −80 to +80 mV in 10 mV steps for 100 ms at 1 Hz. In this patch block of K_{ATP} current appeared to be near-maximal at 30 nM glimepiride since the I/V relationships of 30, 100 and 300 nM glimepiride are superimposed. A concentration–inhibition curve was constructed from the average current amplitude of 10 pulses stepped to −80 mV from a holding potential of −20 mV for each concentration (Figure 2b). Each patch was exposed to a maximum of five concentrations of glimepiride applied in increasing concentrations. The IC_{50} (equation 1) for glimepiride on cloned K_{ATP} channel currents in HEK 293 cells was 6.2 ± 0.1 nM with a Hill coefficient of 1.2 ± 0.1 ($n=4$). This IC_{50} for glimepiride on cloned K_{ATP} channels is strikingly similar to that for native K_{ATP} currents of rat cardiac myocytes (6.8 nM) even though native cardiac K_{ATP}

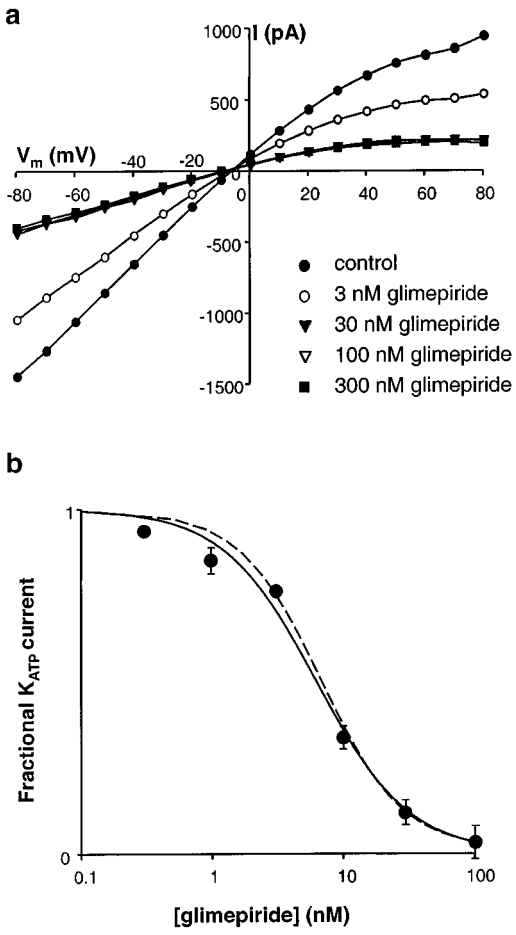

Figure 2 Glimepiride inhibition of cloned K_{ATP} channel subunits Kir6.2/SUR2A evoked by ATP depletion. (a) Current-voltage relationships obtained from an excised outside-out patch from HEK 293 cells stably expressing Kir6.2/SUR2A subunits in the absence of glimepiride (control) and at 3, 30, 100 and 300 nM glimepiride. Patches were voltage-clamped at −20 mV and pulsed from −80 to +80 mV in 10 mV steps for 100 ms at 1 Hz. (b) Mean (±s.e.mean) concentration-inhibition curve for glimepiride at −80 mV from outside-out patches from HEK 293 cells stably expressing the cloned cardiac K_{ATP} channel subunits, Kir6.2/SUR2A. Currents were evoked by decreasing ATP_i to 10 μM ($n=4$). The K_{ATP} current in glimepiride is expressed as a fraction of the K_{ATP} current (see Methods). The line is fit to equation 1 of the text with $IC_{50}=6.2$ nM and a Hill coefficient of 1.2. The glimepiride concentration–inhibition curve for native cardiac myocytes taken from Figure 1b is shown as a dashed line for comparison.

channels were activated by pinacidil and cloned cardiac K_{ATP} channels were activated by ATP depletion.

Glimepiride inhibition of native K_{ATP} currents activated by metabolic inhibition and diazoxide

The open probability of K_{ATP} channels is increased under conditions of metabolic stress, such as myocardial ischaemia, and the activation of these channels during ischaemia has been suggested to protect cells against reperfusion injury. Thus it is important to evaluate K_{ATP} channel block by sulphonylureas when metabolism is compromised. Interestingly, Findlay (1993) has shown that K_{ATP} channel block by glibenclamide is significantly less effective during periods of metabolic stress. We therefore investigated whether block by glimepiride is similarly affected. To induce metabolic inhibition (MI) we used substrate-free Tyrode (without pyruvate or glucose) with 0.5 mM IAA to inhibit glycolysis and 1 mM CN^- to uncouple mitochondrial respiration. MI activated a maximal outward current of 40.7 ± 6.1 pA pF^{-1} ($n=7$) as shown in Figure 3b.

We compared the effect of glimepiride at 10 μM on K_{ATP} currents induced by pinacidil and by MI (Figure 3). As expected from the concentration–inhibition curve in Figure

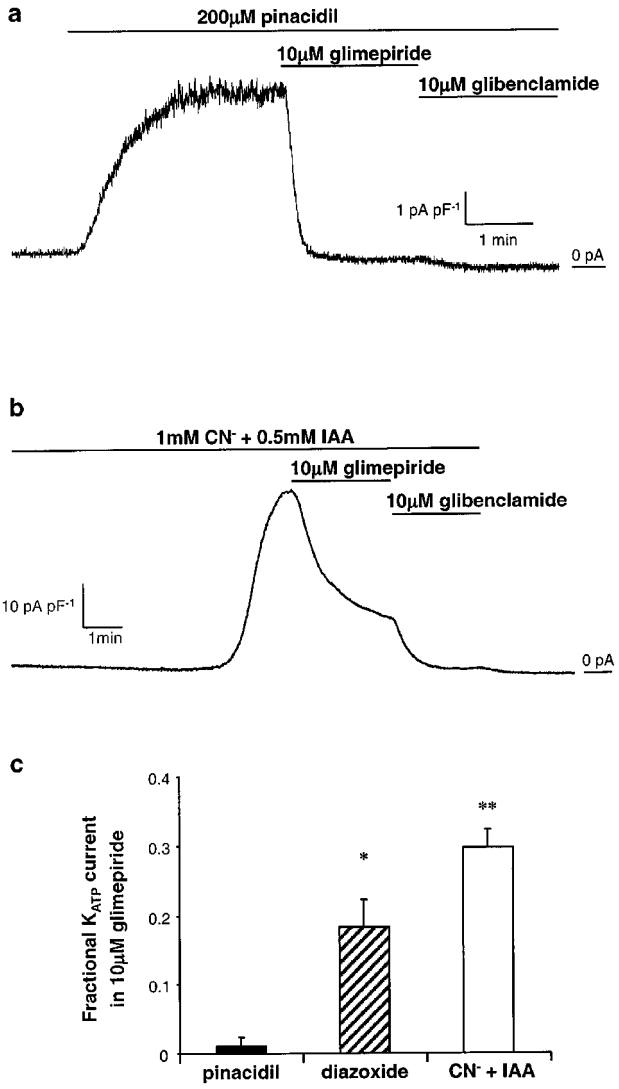

Figure 3 Glimepiride inhibition of native K_{ATP} currents activated by pinacidil, metabolic inhibition or diazoxide. (a) Effect of glimepiride on K_{ATP} current activated by pinacidil. The trace shows whole-cell current recorded from a single rat cardiac myocyte voltage-clamped at 0 mV. Pinacidil, glimepiride and glibenclamide were added as indicated. (b) Effect of glimepiride on K_{ATP} current activated by metabolic inhibition (MI). Metabolic inhibition was induced by superfusion with CN^- (1 mM) + IAA (0.5 mM) and glimepiride and glibenclamide were added as indicated. (c) Mean + -s.e.mean fractional K_{ATP} currents in 10 μM glimepiride activated by pinacidil (200 μM; $n=7$), diazoxide (300 μM; $n=6$) or MI (CN^-: 1 mM + IAA: 0.5 mM; $n=8$) $^*P<0.005$, $^{**}P<0.001$.

1b, 10 μM glimepiride induced near-complete block, 99%, of pinacidil-activated K_{ATP} current though a small additional component of current comprising about 1% of the total was inhibited by the addition of 10 μM glibenclamide (Figure 3a). The fractional pinacidil-activated current in 10 μM glimepiride was 0.01 ± 0.01 ($n=7$, Figure 3c). Figure 3b, c show that glimepiride was much less effective in blocking K_{ATP} currents induced by MI. The fractional MI-induced K_{ATP} current in 10 μM glimepiride was 0.30 ± 0.03 ($n=8$; $P<0.001$ compared to block of the pinacidil-activated current) corresponding to 70% block of the current (Figure 3c).

In view of these differences, we investigated glimepiride block of K_{ATP} currents activated by a third procedure. The K_{ATP} channel opener diazoxide has been proposed to show selectivity for cardiac mitochondrial over sarcolemmal K_{ATP} channels (Garlid *et al*., 1997; Liu *et al*., 1998). However, under certain conditions, such as those that might be expected to develop during myocardial ischaemia, diazoxide can also activate sarcolemmal K_{ATP} channels (D'Hahan *et al*., 1999). A dominant aspect of ischaemia or a metabolically-compromised environment is an increase in intracellular ADP (ADP_i) together with a decrease in ATP_i. (Jennings & Reimer, 1991). Intracellular ADP antagonizes the ATP-induced inhibition of K_{ATP} channels (Terzic *et al*., 1995) and has been suggested to serve as an essential cofactor for diazoxide activation of sarcolemmal K_{ATP} channels (D'Hahan *et al*., 1999). The intracellular concentration of ADP can be raised by applying 2,4 dinitro-1-fluorobenzene (DNFB), a creatine kinase inhibitor which prevents the phosphorylation of 90% of cellular ADP, together with oligomycin, the mitochondrial ATP-synthase inhibitor.

We therefore used the combination of DNFB, oligomycin and diazoxide to investigate K_{ATP} channel activation and its subsequent block by glimepiride. Cells were incubated for 5 min with oligomycin (5 μg ml^{-1}), then voltage-clamped at 0 mV and superfused for 5 min with DNFB (100 μM). Generally, 5-min superfusion with DNFB alone did not cause an increase in baseline current at 0 mV, however in some cells a modest increase in current was observed. Subsequent application of diazoxide (300 μM) activated an outward current of 39.1 ± 9.2 pA pF^{-1} ($n=6$). This was reduced by glimepiride (10 μM) to a fractional current of 0.18 ± 0.04 ($n=6$; $P<0.005$ compared to pinacidil-activated current, Figure 3c), corresponding to 82% block by glimepiride. Thus, glimepiride was a less effective blocker when the K_{ATP} current was activated by either MI (70% block) or partial MI + diazoxide (82% block) compared to pinacidil (99% block). As both MI and the combination of inhibitors together with diazoxide lead to a rise in ADP_i (D'Hahan *et al*., 1999), it may be that elevated ADP_i interferes with the blocking effect of glimepiride on the K_{ATP} channel (Findlay, 1993).

The effect of intracellular ADP on glimepiride and glibenclamide block of cloned K_{ATP} channels

To investigate the possibility that an increase in ADP_i reduces the effectiveness of glimepiride block on K_{ATP} channels we used inside-out patches excised from HEK 293 cells expressing the K_{ATP} channel. Inside-out rather than outside-out patches were used so that we could apply different concentrations of ADP to the intracellular face of the same patch. ADP (0, 1, 10 or 100 μM) was applied in decreasing concentrations, together with either glimepiride or glibenclamide (100 nM), while cytoplasmic ATP was kept constant at 10 μM. Currents were recorded for 100 ms during steps to -80 mV as described above. Figure 4 shows that increasing intracellular ADP substantially and progressively reduced glimepiride block of K_{ATP} current. As ADP increased, the fractional K_{ATP} current in 100 nM glimepiride increased significantly from 0.21 ± 0.02 in the absence of ADP to 0.71 ± 0.06 at 100 μM ADP ($n=6$; $P<0.05$ *vs* control). In these experiments using inside-out patches the fractional K_{ATP} current in 100 nM glimepiride at 0.21 ± 0.02 was greater than that measured previously from outside-out patches (0.04 ± 0.04) and shown in Figure 2b. This apparent difference in the extent of sulphonylurea block of K_{ATP} channels has been noted previously (Lawrence *et al*., 2001) and may reflect a decrease in the rate at which sulphonylureas can access their receptor from the inside. These results show that glimepiride becomes increasingly less effective at blocking cloned K_{ATP} channels as intracellular ADP increases. In contrast, the presence of ADP had little impact on the inhibitory effect of glibenclamide on K_{ATP} current ($n=6$ patches). The fractional K_{ATP} current in glibenclamide in the absence of ADP was not significantly different from that in 1, 10 or 100 μM ADP.

Discussion

In this study we have investigated the effects of glimepiride on native and recombinant (Kir6.2/SUR2A) K_{ATP} channels in rat ventricular myocytes and HEK 293 cells. Our data show that glimepiride is a very effective blocker of native and cloned cardiac sarcolemmal K_{ATP} channels. Our results from experiments on both native and cloned cells suggest that glimepiride block of K_{ATP} channels is increasingly overcome as intracellular ADP rises.

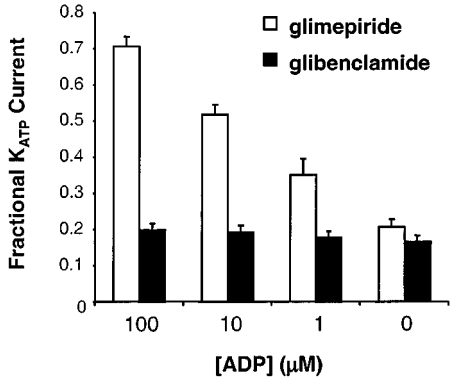

Figure 4 Effect of intracellular ADP on glimepiride or glibenclamide inhibition of the fractional K_{ATP} current from cloned Kir6.2/SUR2A subunits expressed in HEK 293 cells. Mean + s.e.mean fractional K_{ATP} from inside-out patches of cloned K_{ATP} channels in the presence of 10 μM ATP and either glimepiride (100 nM) or glibenclamide (100 nM) ($n=6$) and in the absence or presence of ADP at 100, 10, or 1 μM. Patches were voltage-clamped at -20 mV, currents were recorded at -80 mV for 100 ms and the average current amplitude was calculated.

Glimepiride block of K_{ATP} channels under physiological conditions

We find that glimepiride blocks both native and cloned cardiac K_{ATP} channels with high affinity (IC_{50}s of 6.8 and 6.2 nM, respectively). These results are consistent with those of Song & Ashcroft (2001) who reported an IC_{50} of 5.4 nM for glimepiride in *Xenopus* oocytes expressing Kir6.2/SUR2A subunits. The similarity of IC_{50}s between native and cloned channels suggest that the effect of glimepiride in native cells is solely derived from its effect on the K_{ATP} channel. The IC_{50} for glimepiride on pinacidil-activated cardiac K_{ATP} channels is also similar to that for glibenclamide (7.9 nM) measured in the same way (Lawrence *et al*., 2001). Thus, glimepiride appears to be equipotent to glibenclamide as a blocker of native K_{ATP} channels in cardiac myocytes and cloned K_{ATP} channels in HEK 293 cells (shown here) or in oocytes (Gribble *et al*., 1998).

Glimepiride block of K_{ATP} channels is reduced in the presence of elevated intracellular ADP

While K_{ATP} currents activated by pinacidil were almost completely blocked (99%) by 10 μM glimepiride, the degree of block was considerably less (70%) when currents were activated by metabolic inhibition. These results are in agreement with suggestions that nucleoside diphosphates antagonize ATP_i and sulphonylurea-induced K_{ATP} channel inhibition, or may even increase channel activity by increasing the time spent in intraburst, ligand-insensitive states (Terzic *et al*., 1995; Nichols *et al*., 1996; Alekseev *et al*., 1998; Gribble *et al*., 1998). We would expect ADP_i to be increased under the conditions used in this study to induce metabolic inhibition. In HEK 293 cells expressing cardiac K_{ATP} channel subunits we found that increasing ADP_i in the presence of glimepiride caused an increase in K_{ATP} channel activity. These results are consistent with ADP_i relieving block by glimepiride and suggest that the sulphonylurea binding site and the nucleotide-binding domains of SUR2A interact (Nichols *et al*., 1996). Interestingly, we did not observe marked relief of glibenclamide block by metabolic inhibition in cardiac myocytes (Figure 3), though Findlay (1993) has reported such relief induced by long-term treatment with 2,4 dinitrophenol. Ripoll *et al*. (1993) have reported that ADP, in the presence of 2 mM Mg^{2+}, antagonizes the blocking action of glibenclamide, however, in the absence of Mg^{2+} glibenclamide block of K_{ATP} current is maintained. As our experiments were conducted in the absence of intracellular Mg^{2+}, our findings are in close agreement with those of Ripoll *et al*. (1993). It has also been suggested that K_{ATP} channel sensitivity to sulphonylureas, such as glibenclamide, is dependent on the gating state of the channel. In the presence of dinucleotides, the ligand-insensitive state (Alekseev *et al*., 1998) may be prolonged, therefore decreasing the inhibitory effect of glibenclamide. It is likely that channel block by glimepiride is similarly affected by the raised ADP levels in our experiments and is more sensitive than glibenclamide. Thus block by glimepiride may be more readily relieved by ADP_i than that by glibenclamide. Consistent with this, block of cloned channels by glibenclamide was unaffected by changing ADP_i over the range 0–100 μM. This contrasts with the work of Gribble *et al*. (1998), a difference that may relate to our use of a mammalian rather than a *Xenopus* expression system.

Possible therapeutic significance

Several studies have suggested that glimepiride, used for the treatment of type 2 diabetes, may give better glycaemic control and affect cardiovascular variables less than does glibenclamide (Sonnenberg *et al*., 1997; Langtry & Balfour, 1998; Riddle & Schneider, 1998; Schade *et al*., 1998; El-Reyani *et al*., 1999). It has also been shown recently that glimepiride, unlike glibenclamide, does not abolish the cardioprotective effects of IPC (Klepzig *et al*., 1999; Mocanu *et al*., 2001). A possible explanation for these findings is that the observed protection is conferred by mitochondrial, rather than sarcolemmal, K_{ATP} channels (Grover & Garlid, 2000) and that these channels are unaffected by glimepiride. However recent work suggests that sarcolemmal K_{ATP} channels also play an important role in the cardioprotection elicited by IPC (Toyoda *et al*., 2000; Sanada *et al*., 2001; Tanno *et al*., 2001). Our results are consistent with such a suggestion. During myocardial ischaemia, ADP_i rises (Weiss *et al*., 1992) and we show that glimepiride becomes a less effective blocker of sarcolemmal K_{ATP} channels under such conditions.

We conclude that glimepiride is equipotent to glibenclamide as a blocker of both native and cloned cardiac sarcolemmal K_{ATP} channels under normal physiological conditions. However, glimepiride block is reduced under conditions of metabolic inhibition in cardiac myocytes and when ADP_i is elevated at the cytoplasmic face of cloned channels. A reduction in the blocking effect of glimepiride on sarcolemmal K_{ATP} channels under ischaemic conditions may contribute to its observed failure to block the cardioprotection induced by ischaemic preconditioning.

We thank Dr Andrew Tinker for providing Kir6.2/SUR2A subunits and the British Heart Foundation and Wellcome Trust for support.

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(Received January 31, 2002
Revised April 18, 2002
Accepted April 22, 2002)