Selectivity of repaglinide and glibenclamide for the pancreatic over the cardiovascular K_{ATP} channels

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Abstract

Aims/hypothesis Sulfonylureas and glinides close beta cell ATP-sensitive K^+ (K_{ATP}) channels to increase insulin release; the concomitant closure of cardiovascular K_{ATP} channels, however, leads to complications in patients with cardiac ischaemia. The insulinotrope repaglinide is successful in therapy, but has been reported to inhibit the recombinant K_{ATP} channels of beta cells, cardiocytes and non-vascular smooth muscle cells with similar potencies, suggesting that the (patho-)physiological role of the cardiovascular K_{ATP} channels may be overstated. We therefore re-examined repaglinide's potency at and affinity for the recombinant pancreatic, myocardial and vascular K_{ATP} channels in comparison with glibenclamide.

Methods K_{ATP} channel subunits (i.e. inwardly rectifying K^+ channels [Kir6.x] and sulfonylurea receptors [SURx]) were expressed in intact human embryonic kidney cells and assayed in whole-cell patch-clamp and [3H]glibenclamide binding experiments at 37°C.

Results Repaglinide and glibenclamide, respectively, were ≥ 30 and $\geq 1,000$ times more potent in closing the pancreatic than the cardiovascular channels and they did not lead to complete inhibition of the myocardial channel. Binding assays showed that the selectivity of glibenclamide was essentially based on high affinity for the pancreatic SUR, whereas binding of repaglinide to the SUR subtypes was rather non-selective. After coexpression with Kir6.x to form

the assembled channels, however, the affinity of the pancreatic channel for repaglinide was increased 130-fold, an effect much larger than with the cardiovascular channels. This selective effect of coexpression depended on the piperidino substituent in repaglinide.

Conclusions/interpretation Repaglinide and glibenclamide show higher potency and efficacy in inhibiting the pancreatic than the cardiovascular K_{ATP} channels, thus supporting their clinical use.

Keywords ATP-sensitive K^+ channel subtypes · Glibenclamide · Repaglinide · Subtype selectivity · Sulfonylurea receptors

Abbreviations

HEK	human embryonic kidney 293 cells
K_{ATP} channel	ATP-sensitive K^+ channel
Kir	inwardly rectifying K^+ channel
P1075	N-cyano-N'-(1,1-dimethylpropyl)-N''-3-pyridylguanidine
SUR	sulfonylurea receptor

Introduction

ATP-sensitive K^+ channels (K_{ATP} channels) are tetradimers composed of pore-forming subunits (inwardly rectifying K^+ channel [Kir6.x]) and sulfonylurea receptors (SURx), which act as regulatory subunits [1–3]. They have the unique property of being closed by intracellular ATP and opened by MgADP and other nucleoside diphosphates, thereby linking cell metabolism to excitability. Subtypes of the channel exist in various tissues. In the pancreatic beta cell, K_{ATP} channels of the composition Kir6.2/SUR1 couple

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insulin secretion to the plasma glucose level. Sulfonylureas and glinides, used as first-line treatment in type 2 diabetes, bind to SUR1 to induce channel closure and to promote insulin secretion. K_{ATP} channels also subserve important functions in the cardiovascular system [2]. In the coronary artery bed of many species, including man, Kir6.1/SUR2B channels play a major role in regulating vascular tone at rest [4–6] and in recruiting coronary reserve in cardiac ischaemia [7, 8]. In cardiac myocytes, Kir6.2/SUR2A channels are required for the adaptation of the heart to adrenergic stress [9], are essential mediators of cardiac preconditioning [2, 10] and, in the electrocardiogram, cause the ST segment elevation indicative of transmural myocardial infarction [11].

For these reasons, the selectivity of sulfonylureas and glinides for the pancreatic compared with the cardiovascular K_{ATP} channels has become an issue of clinical relevance [12–15]. Sulfonylureas and glinides can be divided into three groups according to their binding site(s) in the large sulfonylurea-binding pocket of SUR [15–18]. The short sulfonylureas bind to subcompartment A of the binding pocket, and a single amino acid in SUR1 (Ser 1237 which, in SUR2, is replaced by Tyr 1206) affords to these compounds a selectivity of up to 1,000-fold for the pancreatic K_{ATP} channel [18, 19]. Nateglinide also belongs to these ‘type A’ ligands [20]. The long sulfonylureas, represented by glibenclamide (see Fig. 1 for structure), occupy subcompartments A and B and form the second group; these compounds exhibit more limited selectivity (see e.g. [21]; review [18]). There has been a long-standing controversy about whether or not therapy with glibenclamide increases cardiovascular mortality (review [15]). The UK Prospective Diabetes Study did not support this suspicion [13], even in the high-risk group of patients with acute myocardial infarction [22]. A recent retrospective Canadian study showed, however, that greater exposure to glibenclamide or short sulfonylureas was associated with increased mortality among patients newly treated for type 2 diabetes [23]. The third group consists of those glinides that resemble meglitinide (Fig. 1), i.e. excluding nateglinide and mitiglinide. This subgroup of glinides binds to subcompartment B of the binding pocket (‘type B’ ligands), and these compounds do not discriminate between the K_{ATP} channel subtypes [16, 18, 19, 21]. Repaglinide (Fig. 1), the only compound of this group in clinical use, is an example in point, since it inhibits non-selectively the pancreatic, myocardial and non-vascular smooth muscle K_{ATP} channels expressed in *Xenopus* oocytes, with IC_{50} values ranging from 7 to 10 nmol/l; data on the vascular K_{ATP} channel were not provided [24]. Work with native K_{ATP} channels apparently suggests some selectivity of repaglinide for the pancreatic subtype [25, 26]. These results, however, should

Fig. 1 Structures of glibenclamide and the glinides tested or discussed in this study. Compounds are aligned to the negative charge of the sulfonylurea or benzoic acid group

be considered with caution, as in one study the pancreatic and cardiovascular K_{ATP} channels were opened using different openers at different concentrations [25], and in the other repaglinide was applied for only 15 min [26].

The case of repaglinide is intriguing. If the compound does not discriminate between K_{ATP} channel subtypes [24], how can it have been successful in therapy since 1999 without reports of increased cardiovascular mortality? Is the importance of the functional role of cardiovascular K_{ATP} channels in human (patho-)physiology overstated? In view of this question, we reassessed the potency of repaglinide in closing the major recombinant K_{ATP} channels, including the vascular channel; glibenclamide was used in comparison. The channels were expressed in human embryonic kidney (HEK) cells and their inhibition by repaglinide and glibenclamide was assayed using the whole-cell patch-clamp technique in the presence of nucleotides and Mg^{2+} . In order to assess the affinity of repaglinide and glibenclamide for the different SUR subtypes, radioligand binding assays were performed in intact cells expressing SUR alone and together with Kir6.x. This was done because coexpression with Kir6.2 increases the affinity of SUR1 for repaglinide much more than for glibenclamide [27, 28], and more than observed with the other K_{ATP} channel subtypes [29, 30]. Hence, these differential effects of coexpression with Kir6.x on the affinity of SURx may contribute to the observed differences in selectivity.

Materials and methods

Materials

[3H]Glibenclamide (specific activity 1.85 TBq/mmol) was purchased from PerkinElmer Life Sciences (Bad Homburg, Germany). The reagents and media used for cell culture and transfection were from Invitrogen (Karlsruhe, Germany). Repaglinide and its optical antipode, (–)repaglinide, were a gift from Novo Nordisk (Bagsvaerd, Denmark); in addition, repaglinide was purchased from Toronto Research Chemicals (Toronto, ON, Canada). (–)AZ-DF 265, AG-EE 319 and AG-DD 1461 (Fig. 1) were a gift from Boehringer Ingelheim (Germany) and P1075 (*N*-cyano-*N'*-(1,1-dimethylpropyl)-*N''*-3-pyridylguanidine) from Leo Pharmaceuticals (Ballerup, Denmark). Glibenclamide was purchased from Sigma (Deisenhofen, Germany). The K_{ATP} channel modulators were dissolved in dimethyl sulfoxide/ethanol (50/50, v/v) and further diluted with the same solvent or with incubation buffer (final solvent concentration in the assays <1%).

Cell culture and transfection

HEK 293 cells were cultured in Minimum Essential Medium containing glutamine and supplemented with 10% fetal bovine serum and 20 μ g/ml gentamicin as described [29]. Cells were transfected with rat SUR1 (GenBank X97279) or the murine clones of SUR2A (D86037), SUR2B (D86038), Kir6.1 (D88159) or Kir6.2 (D50581) using the pcDNA 3.1 vector Lipofectamine 2000 and Opti-MEM (Invitrogen) according to the manufacturer's instructions [31]. In order to improve the signal-to-noise ratio in the assays of [3H]glibenclamide binding to Kir6.1/SUR2B channels, a HEK cell line permanently expressing the channel was generated as described by Giblin et al. [32].

Patch-clamp experiments

Patch-clamp experiments were generally performed in the whole-cell configuration at 37°C as described by Russ et al. [33]. The bath was filled with (mmol/l): NaCl, 142; KCl, 2.8; $MgCl_2$, 1; $CaCl_2$, 1; D(+)-glucose, 11; HEPES, 10; pH 7.4. Patch pipettes were filled with (mmol/l) K-glutamate, 132; NaCl, 10; $MgCl_2$, 2; HEPES, 10; EGTA (ethylene glycol-bis-(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid), 1; Na_2ATP , 1; and $MgGDP$, 0.27 at pH 7.2; resistance was 3–5 M Ω . Cells were clamped at –60 mV and subjected to test pulses from –110 to 10 mV (Fig. 2).

Some experiments on Kir6.2/SUR1 were performed in the inside-out configuration at 22°C. The bath and pipette were filled with a high- K^+ Ringer solution containing (mmol/l) KCl, 142; NaCl, 2.8; $MgCl_2$, 1; $CaCl_2$, 1; D(+)-glucose, 11; HEPES, 10, titrated to pH 7.4 with NaOH. After filling with buffer, pipettes had a resistance of 1.0–1.5 M Ω . After patch excision, the pipette was moved in front of a pipe filled with a high- K^+ EGTA-buffered solution containing (in mmol/l) KCl, 143; $MgCl_2$, 1.1; $CaCl_2$, 1; D(+)-glucose, 11; Na_2ATP , 0.03; Na_2UDP , 0.3; EGTA, 5; HEPES, 10, titrated to pH 7.2 with NaOH at 22°C. Patches were clamped at –50 mV.

[3H]Glibenclamide competition experiments

Binding experiments were performed in intact cells as described by Hambrock et al. [29] using an incubation buffer containing (mmol/l): NaCl, 129; KCl, 5; $MgCl_2$, 1.2; $CaCl_2$, 1.25; D(+)-glucose, 11; $NaHCO_3$, 5; HEPES, 10, at pH 7.4. [3H]Glibenclamide was ~2 nmol/l (SUR1) or ~4 nmol/l (SUR2). At higher concentrations (e.g. glibenclamide >100 nmol/l), the inhibitors interfered with [3H]glibenclamide binding to binding sites in HEK cells other than SUR and non-specific binding was therefore determined in the presence of 100 nmol/l glibenclamide (SUR1)

Fig. 2 Whole-cell recordings showing the inhibition of (a) pancreatic (Kir6.2/SUR1), (b) myocardial (Kir6.2/SUR2A) and (c) vascular (Kir6.1/SUR2B) K_{ATP} channels by repaglinide and glibenclamide. Dialysis of the cells with MgATP (1 mmol/l) and MgGDP (0.27 mmol/l) generated a current ($I_{K_{ATP}}$) which was totally or partially inhibited by repaglinide or glibenclamide at the concentrations indicated. The dotted lines indicate the zero current level. Recordings were made at physiological K^+ concentrations at 37°C and the holding

potential was -60 mV. The *inset* (a) shows the currents at test potentials from -110 to 10 mV taken at the times indicated by the numbers 1 and 2. The *broken curves* (a) show the fit of an exponential function to the data. Both curves extrapolated to the zero current level and gave half-times of 2 and 8 min for the inhibition by repaglinide (10 nmol/l) and glibenclamide (1 nmol/l), respectively; mean half times were 3 and 7 min ($n=4$). $BaCl_2$ (b) was 10 mmol/l

or 100 μ M P1075 (SUR2) [29]; it was between 10% (SUR1) and 40–60% (SUR2) of total binding.

Data analysis and statistics

Channel inhibition curves were analysed according to the equation

$$y = 100 - A / \left(1 + 10^{[px - pIC_{50}]} \right) \quad (1)$$

as described [29], where y denotes the current, A the maximum inhibition (amplitude), and x the inhibitor concentration, with $px = -\log x$ and $pIC_{50} = -\log IC_{50}$. The IC_{50} values with their 95% confidence interval are given in the text. If, at concentration x , the channel is inhibited to

$\alpha\%$, an estimate of the IC_{50} value can be obtained using the non-logarithmic form of Eq. 1 with $A=100$:

$$IC_{50} = x(100 - \alpha) / \alpha. \quad (2)$$

Individual binding inhibition curves were evaluated with $A=100$ and IC_{50} values were corrected for the presence of the radioligand according to the Cheng–Prusoff equation [34], giving the inhibition constant K_i :

$$IC_{50} = K_i(1 + L/K_D) \quad (3)$$

where L denotes the concentration and K_D the equilibrium dissociation constant of the radioligand. The affinity of

glibenclamide for SUR2 expressed alone is insufficient to allow precise binding experiments in intact cells. Binding assays were therefore conducted using high-affinity mutants of SUR2 carrying the Y1206S mutation [29, 30]. Assuming that the affinity shift due to the mutation SUR2(YS) was the same for SUR2 expressed alone or together with Kir6, the pK_i values were calculated according to the equation

$$pK_i(\text{SUR2}) = pK_i(\text{SUR2(YS)}) - [pK_i(\text{Kir6/SUR2(YS)}) - pK_i(\text{Kir6/SUR2})] \quad (4)$$

using the appropriate values published previously [29, 30].

K_i/IC_{50} values are log normally distributed and statistical analysis was performed at the level of the corresponding pK_i/pIC_{50} values. Groups were compared using ANOVA or Student's *t*-test after the data had passed the normality and equal variance tests.

Results

Inhibition of K_{ATP} channel subtypes

Figure 2 illustrates the inhibition of the (recombinant) pancreatic, myocardial and vascular K_{ATP} channels by repaglinide and glibenclamide in whole-cell recordings at 37°C. Cells were dialysed with ATP (1 mmol/l), GDP (0.27 mmol/l) and free Mg^{2+} (~1 mmol/l) since these nucleotide Mg^{2+} salts (which are present in the cell) affect the inhibition of K_{ATP} channels by sulfonylureas in a subtype-specific manner. MgADP in particular enhances the inhibition of Kir6.2/SUR1 channels by sulfonylureas but leaves that of Kir6.2/SUR2 channels unchanged [24, 35] or even weakens the inhibition of Kir6.2/SUR2A channels [36]. In the presence of nucleotides, repaglinide (10 nmol/l) induced a slow inhibition of the pancreatic channel, which extrapolated to completion (Fig. 2) and which was maintained upon washout (not illustrated). In contrast, inhibition of the cardiovascular channels by repaglinide (30 nmol/l and 1 $\mu\text{mol/l}$) was incomplete and readily reversed by washout (Fig. 2). Similarly, glibenclamide (1 nmol/l) induced complete inhibition of the pancreatic channel; however, higher concentrations (100 nmol/l or 1 $\mu\text{mol/l}$) produced only partial and reversible inhibition of the cardiovascular channels (Fig. 2).

At low inhibitor concentrations, the inhibition kinetics of the pancreatic channel was slow, and concentration–inhibition curves could not be established. A potency estimate, however, was obtained by considering that, at the end of the long traces, determination of the zero current level (i.e. total inhibition) was within $\leq 5\%$ error. Assuming that repaglinide (10 nmol/l) and glibenclamide (1 nmol/l)

inhibited the pancreatic channel to $\geq 95\%$ gave IC_{50} values ≤ 0.5 and ≤ 0.05 nmol/l for repaglinide and glibenclamide, respectively (Eq. 2). These estimates may considerably underestimate the true potency of the drugs, and this may hold particularly for repaglinide, which showed approximately four-fold slower inhibition kinetics than glibenclamide (Fig. 2). Therefore, the pancreatic channel was also assayed in the inside-out configuration. To account for the higher inhibitory potency of MgATP in this configuration, MgATP was reduced to 0.03 mmol/l and MgUDP (0.3 mmol/l) was added to activate SUR without inhibiting Kir6.2 [37]. The inhibition kinetics was now approximately seven-fold faster (not illustrated), allowing inhibition curves to be measured (Fig. 3). Maximum channel inhibition was ~86% and IC_{50} values of 0.79 and 0.48 nmol/l were obtained for repaglinide and glibenclamide, respectively (Table 1). This showed that the compounds were more potent in the whole-cell than in the inside-out configuration of the patch-clamp technique.

The concentration–inhibition curves of the cardiovascular channels were again recorded in the whole-cell

Fig. 3 Concentration-dependent inhibition of recombinant K_{ATP} channel subtypes by (a) repaglinide and (b) glibenclamide. Squares, pancreatic channel (open squares, inside-out; closed squares, whole-cell configuration); triangles, myocardial channel (whole-cell configuration); circles, vascular channel (whole-cell configuration). Data are mean $\pm$ SEM from four to ten cells or patches per point. The fit of Eq. 1 in Materials and methods to the data is shown; it gave the parameters listed in Table 1. Conditions for whole-cell recordings were as outlined in Fig. 2; inside-out experiments were performed at 22°C in symmetrical high K^+ solution with MgATP (0.03 mmol/l) and MgUDP (0.3 mmol/l) in the pipette solution

configuration. IC_{50} values ranged from 15 to 56 nmol/l; in addition, there were differences in the efficacy of inhibition (Fig. 3, Table 1). At the myocardial channel, both inhibitors were unable to produce complete inhibition; this was particularly significant with glibenclamide (55% maximum inhibition; $n=10$; Fig. 3 and Table 1). There was also greater variability in the response of the myocardial channel to the two inhibitors than observed with the other channels. The inhibition curves for the vascular channel reached (glibenclamide) or almost reached (repaglinide) the 100% level and were slightly shifted rightwards from the curves for the myocardial channel.

Binding to K_{ATP} channel and SUR subtypes

In order to assess the affinity of repaglinide and glibenclamide binding to the three channels, [3H]glibenclamide competition assays were performed in intact cells at 37°C, i.e. under conditions close to those used in the whole-cell clamp experiments. The competition curves are shown in Fig. 4 and the K_i values derived from these curves are listed in Table 2. From these data, affinity ratios for the binding of repaglinide to the pancreatic compared with the myocardial and the vascular channels were calculated to be 2 and 20, and those for glibenclamide were 14 and 71, respectively.

In further experiments, the affinity of each SUR subtype expressed alone for repaglinide, glibenclamide and (–)AZ-DF 265 (another piperidino-glinide, Fig. 1) was compared with that after coexpression with Kir6.x. The K_i values compiled in Table 2 show that coexpression of SUR1 with Kir6.2 (which gives the pancreatic channel) increased the affinity of SUR1 for the piperidino-containing glinides ≥ 130 -fold, but that for glibenclamide only three-fold. Upon coexpression of SUR2A with Kir6.2 (myocardial channel), the increase in affinity for the piperidino-glinides was smaller (60- and 30-fold for repaglinide and AZ-DF 265,

respectively), whereas that for glibenclamide was increased six-fold. In the case of SUR2B, coexpression with Kir6.1 (vascular channel) increased the affinity for the three compounds only weakly (≤ 3 -fold). Table 2 also shows that coexpression with Kir6.1 increased the affinity of SURx for the inhibitors less than Kir6.2.

In addition, the effect of Kir6.2 on the affinity of SUR1 for other repaglinide-related piperidino-glinides was determined (AG-EE 319 and AG-DD 1461; Fig. 1). Table 3 shows that both exhibited a strong effect of coexpression. Finally, the impact of the sterical configuration at the optical centre of repaglinide (asterisk in Fig. 1) on the effect of coexpression was assessed. Table 3 shows that the affinity of SUR1 and Kir6.2/SUR1 for the distomer of repaglinide, (–)repaglinide, was ~ 150 -fold weaker than that for the eutomer, repaglinide ((+)repaglinide).

Discussion

Potency and efficacy

This study has shown that repaglinide and glibenclamide are more potent and, in part, also more efficient in inhibiting the pancreatic than the cardiovascular K_{ATP} channels.

Regarding the pancreatic channel, the slow inhibition kinetics in the whole-cell configuration allowed only an estimate of upper limits for the IC_{50} values of repaglinide and glibenclamide (≤ 0.5 and ≤ 0.05 nmol/l, respectively). These estimates agree well with the values reported by Gromada et al. [39] on the inhibition of the native channel in rat pancreatic beta cells in culture ($IC_{50}=0.089$ and 0.047 nmol/l, respectively). The upper limit for repaglinide is, however, much lower than the value determined in metabolically poisoned *Xenopus* oocytes [24].

Table 1 Inhibition of (recombinant) K_{ATP} channel subtypes by repaglinide and glibenclamide

Channel	Repaglinide			Glibenclamide		
	IC_{50} (nmol/l)	A (% $I_{K_{ATP}}$)	SR	IC_{50} (nmol/l)	A (% $I_{K_{ATP}}$)	SR
Pancreatic, wc	≤ 0.5	100		≤ 0.05	100	
Pancreatic, i/o	0.79 (0.68, 0.91)	86 $\pm$ 1		0.48 (0.47, 0.49)	86 $\pm$ 1	
Cardiac, wc	15 (11, 20)	86 $\pm$ 2 ^a	≥ 30	51 (32, 81)	56 $\pm$ 3 ^{a, b}	$\geq 1,000$
Vascular, wc	29 (23, 36)	94 $\pm$ 2	≥ 100	56 (52, 58)	100	$\geq 1,000$

Parameters were determined by analysis of the concentration–response curves in Fig. 2. IC_{50} values are followed by the 95% confidence interval in parentheses

wc Whole-cell configuration of the patch clamp technique, i/o inside-out configuration of the patch clamp technique, A maximum inhibition at saturation as percentage of current blocked by Ba^{2+} ($I_{K_{ATP}}$), SR selectivity ratio calculated as

$IC_{50}(\text{Kir6.2/SUR1; wc})/IC_{50}(\text{Kir6.x/SUR2x; wc})$

^aAmplitudes differ significantly ($p<0.05$)

^bFrom ten cells assayed, two were strong responders, blocking up to 88% of $I_{K_{ATP}}$

Fig. 4 Inhibition of [^3H]glibenclamide (GBC) binding to the pancreatic (circles), cardiac (open squares) and vascular (triangles) K_{ATP} channels by repaglinide (a) and glibenclamide (b). Specific binding is shown and data are means from three to five independent experiments. The fit of Eq. 1 in Materials and methods with $A=100$ to the data gave IC_{50} values which, after correction for the presence of the radioligand (Eq. 3), gave the K_i values listed in Table 2. The broken curves show the theoretical competition curves for the pancreatic channel after correction ([^3H]glibenclamide concentration ~ 2 nmol/l, correction factor 5). For the cardiac and vascular channels, [^3H]glibenclamide was 3.5 and 5 nmol/l and the correction factors were 1.6 and 1.2, respectively; for clarity, the corrected curves are omitted. Non-specific binding ranged from 10 to 50–60% of total binding for the pancreatic and cardiovascular channels, respectively

Regarding the myocardial channel, the inhibition constants of repaglinide and glibenclamide in Table 1 are well within the range of those reported in other studies ([21, 24]; review [18]). The vascular channel (Kir6.1/SUR2B) is studied only rarely, since it is not readily accessible in the inside-out mode [40]. The IC_{50} value for repaglinide in Table 1 (29 nmol/l) is similar to that obtained for the non-vascular smooth muscle channel, Kir6.2/SUR2B, in oocytes (10 nmol/l, [24]); that for glibenclamide agrees with our earlier result [33]. Accepting the values in Table 1, one calculates that repaglinide is ≥ 30 -fold selective for the pancreatic compared with the myocardial and >100 -fold compared with the vascular K_{ATP} channel; for glibenclamide, selectivity is $\geq 1,000$.

Subtype-specific differences were also observed in the efficacy of channel inhibition, since repaglinide and gliben-

clamide, respectively, were unable to close completely the vascular and myocardial K_{ATP} channels in whole cells (Table 1, Fig. 3). The latter effect was particularly marked and was also observed with the native channel in metabolically poisoned or hypoxic cardiocytes ([41–43], but see [24]).

Binding and effect

The ability of the cardiovascular channels to open despite full occupancy of SUR by the inhibitor shows that the transduction from inhibitor binding into channel closure is weaker in the cardiovascular than in the pancreatic channels. Subtype-specific differences in signal transduction are also evident when the binding affinities of the inhibitors for the K_{ATP} channel subtypes are compared with their potencies in channel inhibition. For both inhibitors, the differences in affinity were considerably smaller than the differences in potency (Tables 1 and 2). For the pancreatic channel, the inhibition curves for repaglinide and glibenclamide were to the left of the respective binding curves by factors of ≥ 2.4 and ≥ 9 ; for the vascular channel, they were slightly (<2 -fold) to the right, and for the myocardial channel this rightward shift was eight- to ten-fold. These results show that transduction of inhibitor binding into channel closure is easiest for the pancreatic and most difficult for the myocardial channel, the vascular channel being intermediate; this resembles the rank order in efficacy.

Mechanism of selectivity

The radioligand binding studies provided some insight into the mechanism underlying the selectivity of the inhibitors for the pancreatic channel. Hansen et al. have shown that coexpression with Kir6.2 greatly enhanced the affinity of repaglinide binding to SUR1, whereas the effect was small for glibenclamide [27]. Hence, the effect of coexpression could contribute to the observed selectivity of repaglinide, provided that it was subtype-specific. Here we have shown this to be the case, and also included a further piperidino-glinide (AZ-DF 265) in the analysis. Table 2 shows that the (limited) selectivity of these two piperidino-glinides for binding to the pancreatic channel arises essentially from the large effect of Kir6.2 on the affinity of SUR1. In contrast, the selectivity of glibenclamide binding relies on differences in affinity for the SUR subtypes, which is only slightly modulated by coexpression with Kir6.x.

Further evidence suggests that the large effect of Kir6.2 on the affinity of SUR1 for glinides depends on the piperidino substituent in the ortho position of the left phenyl ring: first, the large effect seen with repaglinide and (–)AZ-DF 265 was also found with two additional piperidino-substituted glinides (Table 3). Secondly, unpub-

Table 2 Binding of repaglinide, glibenclamide and (–)AZ-DF 265 to combinations of Kir6.x/SURx and to SURx expressed alone

Receptor	Repaglinide K_i (nmol/l)	f	Glibenclamide K_i (nmol/l)	f	(–)AZ-DF 265 K_i (nmol/l)	f
Kir6.2/SUR1	0.72 (0.60, 0.87)	130	0.45 (0.31, 0.65)	3.2	3.7 (2.8, 4.9)	370
Kir6.1/SUR1	1.7 (1.6, 1.8)	54	0.96 (0.59, 1.5)	1.6	18 (16, 21)	77
SUR1	91 (83, 100)	–	1.5 (1.4, 1.6)	–	1,400 (1,200, 1,600)	–
Kir6.2/SUR2A	1.5 (1.4, 1.7)	56	6.2 (5.6, 6.8)	~6	25 (23, 28)	~30
SUR2A ^a	~90	–	~40	–	~700	–
Kir6.1/SUR2B	15 (13, 18)	~3	32 (27, 39)	~1	280 (220, 350)	~3
Kir6.2/SUR2B	2.0 (1.9, 2.1)	~25	16 (13, 19)	~2	56 (48, 63)	~10
SUR2B ^a	~50	–	~30 ^b	–	~600	–

The recombinant pancreatic, cardiac and vascular K_{ATP} channels have the composition Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.1/SUR2B, respectively; Kir6.2/SUR2B is the K_{ATP} channel in non-vascular smooth muscle

K_i values (95% confidence intervals) were derived from experiments shown in Fig. 4 ($n=3-5$ per condition)

f Factor by which coexpression with Kir6.x enhances the affinity of SUR for the inhibitor; $f=K_i(\text{SUR})/K_i(\text{Kir6.x/SUR})$

^aCalculated according to Eq. 4 in **Materials and methods**

^bMembranes + MgATP (1 mmol/l) [38]

lished observations show that the small effect seen with glibenclamide occurs with many other glinides and sulfonylureas, all lacking the piperidino group. Thirdly, repaglinide analogues lacking the piperidino ring or having it in another position are devoid of hypoglycaemic activity [44]. Conformational analysis suggests that the piperidine of repaglinide protrudes from the common pharmacophore structure with glibenclamide [20]. Hence, the piperidino group may make contact with parts of the channel not reached by non-piperidino compounds, and it is interesting that the effect of coexpression on the affinity of SUR1 is lost if the first 14 amino acids of Kir6.2 are deleted [27].

Clinical implications

This study has shown that repaglinide and glibenclamide are more potent and, in part, more efficacious in inhibiting the (recombinant) pancreatic than the cardiovascular K_{ATP} channels. In view of the large selectivity ratios for glibenclamide (see also [18, 21]), the fact that the UK Prospective Diabetes Study showed no excess mortality among diabetic patients with acute myocardial infarction treated with glibenclamide in comparison with insulin [22]

seems plausible. A recent Canadian study [23], however, again casts doubt. Regarding repaglinide, the potency ratios were more limited. On the other hand, repaglinide was unable to induce complete closure of the cardiovascular channels, and, during therapy, exposure of the body to repaglinide is less than to glibenclamide (despite more frequent daily dosing) since the duration of action of repaglinide in man is shorter than that of glibenclamide (2 vs 8–12 h [45]). Collectively, these arguments help our understanding of the apparent therapeutic safety of repaglinide; however, a prospective clinical study addressing this issue would be desirable.

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Duality of interest The authors declare that they have no conflict of interest.

Table 3 Effect of coexpression with Kir6.2 on the affinity of SUR1 for some piperidino-glinides

Compound	SUR1 K_i (μmol/l)	Kir6.2/SUR1 K_i (μmol/l)	f
AG-EE 319	1.4 (1.2, 1.6)	0.0033 (0.0028, 0.0040)	410
AG-DD 1461	13 (10, 17)	0.12 (0.11, 0.13)	100
(–)Repaglinide	14 (14, 14)	0.14 (0.13, 0.16)	100

K_i values (95% confidence intervals) were derived from experiments as illustrated in Fig. 4 ($n=3$)

$f=K_i(\text{SUR1})/K_i(\text{Kir6.2/SUR1})$

References

- Ashcroft FM, Gribble FM (1998) Correlating structure and function in ATP-sensitive K^+ channels. Trends Neurosci 21:288–294
- Seino S, Miki T (2003) Physiological and pathophysiological roles of ATP-sensitive K^+ channels. Prog Biophys Mol Biol 81:133–176
- Bryan J, Vila-Carriles WH, Zhao G, Babenko AP, Aguilar-Bryan L (2004) Toward linking structure with function in ATP-sensitive K^+ channels. Diabetes 53(Suppl 3):S104–S112

4. Miki T, Suzuki M, Shibasaki T et al (2002) Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nature Med* 8:466–472
5. Chutkow WA, Pu J, Wheeler MT et al (2002) Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K_{ATP} channels. *J Clin Invest* 110:203–208
6. Farouque HMO, Worthley SG, Meredith IT, Skyrme-Jones RAP, Zhang MJ (2002) Effect of ATP-sensitive potassium channel inhibition on resting coronary vascular responses in humans. *Circ Res* 90:231–236
7. Brayden JE (2002) Functional roles of K_{ATP} channels in vascular smooth muscle. *Clin Exp Pharmacol Physiol* 29:312–316
8. Miura H, Wachtel RE, Loberiza FR et al (2003) Diabetes mellitus impairs vasodilation to hypoxia in human coronary arterioles—reduced activity of ATP-sensitive potassium channels. *Circ Res* 92:151–158
9. Zingman LV, Hodgson DM, Bast PH et al (2002) Kir6.2 is required for adaptation to stress. *Proc Natl Acad Sci USA* 99:13278–13283
10. Gross GJ, Peart JN (2003) K_{ATP} channels and myocardial preconditioning: an update. *Am J Physiol (Heart Circ Physiol)* 285:H921–H930
11. Huizar JF, Gonzalez LA, Alderman J, Smith HS (2003) Sulfonylureas attenuate electrocardiographic ST-segment elevation during an acute myocardial infarction in diabetics. *J Am Coll Cardiol* 42:1017–1021
12. Meinert CL, Knatterud GL, Prout TE, Klimt CR (1970) A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. II. Mortality results. *Diabetes* 19:789–830
13. UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853
14. Wascher TC, Boes U (2003) Ischemia in type 2 diabetes: tissue selectivity of sulfonylureas and clinical implications. *Metabolism* 52:3–5
15. Quast U, Stephan D, Bieger S, Russ U (2004) The impact of ATP-sensitive K^+ channel subtype selectivity of insulin secretagogues for the coronary vasculature and the myocardium. *Diabetes* 53: S156–S164
16. Gribble FM, Reimann F (2003) Sulphonylurea action revisited: the post-cloning era. *Diabetologia* 46:875–891
17. Bryan J, Crane A, Vila-Carriles WH, Babenko AP, Aguilar-Bryan L (2005) Insulin secretagogues, sulfonylurea receptors and K_{ATP} channels. *Curr Pharm Design* 11:2699–2716
18. Proks P, Reimann F, Green N, Gribble F, Ashcroft F (2002) Sulfonylurea stimulation of insulin secretion. *Diabetes* 51 (Suppl 3):S368–S376
19. Ashfield R, Gribble FM, Ashcroft SJH, Ashcroft FM (1999) Identification of the high-affinity tolbutamide site on the SUR1 subunit of the K_{ATP} channel. *Diabetes* 48:1341–1347
20. Hansen AMK, Christensen IT, Hansen JB, Carr RD, Ashcroft FM, Wahl P (2002) Differential interactions of nateglinide and repaglinide on the human β -cell sulphonylurea receptor 1. *Diabetes* 51:2789–2795
21. Dörschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M (1999) Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. *Mol Pharmacol* 55:1060–1066
22. Stevens RJ, Coleman RL, Adler AI, Stratton IM, Matthews DR, Holman RR (2004) Risk factors for myocardial infarction case fatality and stroke case fatality in type 2 diabetes: UKPDS 66. *Diabetes Care* 27:201–207
23. Simpson SH, Majumdar SR, Tsuyuki RT, Eurich DT, Johnson JA (2006) Dose-response relation between sulfonylurea drugs and mortality in type 2 diabetes mellitus: a population-based cohort study. *CMAJ* 174:169–174
24. Dabrowski M, Wahl P, Holmes WE, Ashcroft FM (2001) Effect of repaglinide on cloned beta cell, cardiac and smooth muscle types of ATP-sensitive potassium channels. *Diabetologia* 44:747–756
25. Hu S, Wang S, Dunning BE (1999) Tissue selectivity of antidiabetic agent nateglinide: study on cardiovascular and β -cell K_{ATP} channels. *J Pharmacol Exp Ther* 291:1372–1379
26. Weyermann A, Vollert H, Busch AE, Bleich M, Gögelein H (2004) Inhibitors of ATP-sensitive potassium channels in guinea pig isolated ischemic hearts. *Naunyn-Schmiedeberg Arch Pharmacol* 369:374–381
27. Hansen AMK, Hansen JB, Carr RD, Ashcroft FM, Wahl P (2005) Kir6.2-dependent high-affinity repaglinide binding to β -cell K_{ATP} channels. *Br J Pharmacol* 144:551–557
28. Stephan D, Winkler M, Russ U, Bryan J, Quast U (2005) The impact of the Kir6.2 N-terminus for inhibitor binding to the ATP-sensitive potassium channel. *Naunyn-Schmiedeberg Arch Pharmacol* 371:R58
29. Hambrock A, Löffler-Walz C, Russ U, Lange U, Quast U (2001) Characterization of a mutant sulfonylurea receptor SUR2B with high affinity for sulfonylureas and openers: differences in the coupling to Kir6.x subtypes. *Mol Pharmacol* 60:190–199
30. Stephan D, Stauss E, Lange U et al (2005) The mutation Y1206S increases the affinity of the sulphonylurea receptor SUR2A for glibenclamide and enhances the effects of coexpression with Kir6.2. *Br J Pharmacol* 144:1078–1088
31. Hambrock A, Löffler-Walz C, Kurachi Y, Quast U (1998) Mg^{2+} and ATP dependence of K_{ATP} channel modulator binding to the recombinant sulphonylurea receptor, SUR2B. *Br J Pharmacol* 125:577–583
32. Giblin JP, Leaney JL, Tinker A (1999) The molecular assembly of ATP-sensitive potassium channels—determinants on the pore forming subunit. *J Biol Chem* 274:22652–22659
33. Russ U, Hambrock A, Artunc F et al (1999) Coexpression with the inward rectifier K^+ channel Kir6.1 increases the affinity of the vascular sulfonylurea receptor SUR2B for glibenclamide. *Mol Pharmacol* 56:955–961
34. Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem Pharmacol* 22:3099–3108
35. Gribble FM, Tucker SJ, Seino S, Ashcroft FM (1998) Tissue specificity of sulfonylureas: studies on cloned cardiac and β -cell K_{ATP} channels. *Diabetes* 47:1412–1418
36. Reimann F, Dabrowski M, Jones P, Gribble FM, Ashcroft FM (2003) Analysis of the differential modulation of sulphonylurea block of beta-cell and cardiac ATP-sensitive K^+ (K_{ATP}) channels by Mg-nucleotides. *J Physiol (Lond)* 547:159–168
37. Okuyama Y, Yamada M, Kondo C et al (1998) The effects of nucleotides and potassium channel openers on the SUR2A/Kir6.2 complex K^+ channel expressed in a mammalian cell line, HEK293T cells. *Pflügers Arch Eur J Physiol* 435:595–603
38. Löffler-Walz C, Hambrock A, Quast U (2002) Interaction of K_{ATP} channel modulators with sulfonylurea receptor SUR2B: implication for tetramer formation and allosteric coupling of subunits. *Mol Pharmacol* 61:407–414
39. Gromada J, Dissing S, Kofod H, Frøkjær-Jensen J (1995) Effects of the hypoglycaemic drugs repaglinide and glibenclamide on ATP-sensitive potassium-channels and cytosolic calcium levels in β TC3 cells and rat pancreatic beta cells. *Diabetologia* 38:1025–1032
40. Satoh E, Yamada M, Kondo C et al (1998) Intracellular nucleotide-mediated gating of SUR/Kir6.0 complex potassium channels expressed in a mammalian cell line and its modification by pinacidil. *J Physiol (Lond)* 511:663–674

41. Findlay I (1993) Sulphonylurea drugs no longer inhibit ATP-sensitive K^+ channels during metabolic stress in cardiac muscle. *J Pharmacol Exp Ther* 266:456–467
42. Krause E, Englert H, Gögelein H (1995) Adenosine triphosphate-dependent K currents activated by metabolic inhibition in rat ventricular myocytes differ from those elicited by the channel opener rilmakalim. *Pflügers Arch Eur J Physiol* 429:625–635
43. Venkatesh N, Lamp ST, Weiss JN (1991) Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ Res* 69:623–637
44. Grell W, Hurnaus R, Griss G et al. (1998) Repaglinide and related hypoglycemic benzoic acid derivatives. *J Med Chem* 41:5219–5246
45. Hardman JG, Limbird LE, Gilman AG (2001) Goodman and Gilman's the pharmacological basis of therapeutics, 10th edn. McGraw-Hill Medical Division, New York