Diabetologia (1999) 42: 845–848

Diabetologia

Rapid communication

Differential sensitivity of beta-cell and extrapancreatic K_{ATP} channels to gliclazide

F. M. Gribble, F. M. Ashcroft

University Laboratory of Physiology, Oxford, UK

Abstract

Aims/hypothesis. To investigate the tissue specificity of gliclazide for cloned beta-cell, cardiac and smooth muscle ATP-sensitive K-channels (K_{ATP} channels). These channels share a common pore-forming subunit, Kir6.2, which associates with different sulphonylurea receptor isoforms (SUR1 in beta-cells, SUR2A in heart, SUR2B in smooth muscle).
Methods. Kir6.2 was coexpressed with SUR1, SUR2A or SUR2B in *Xenopus* oocytes, and channel activity was measured by recording macroscopic currents in giant inside-out membrane patches. Gliclazide was added to the intracellular membrane surface.
Results. We reported previously that Kir6.2-SUR1 currents are blocked at two sites by tolbutamide: a high-affinity site on SUR1 and a low-affinity site on Kir6.2. We now show that gliclazide also inhibits beta-cell K_{ATP} channels at two sites: a high-affinity site, which is half-maximally blocked (K_i) at 50 ± 7 nmol/l ($n = 8$) and a low-affinity site with a K_i of 3.0 ± 0.6 mmol/l ($n = 4$). The high-affinity site on SUR1 was thus about 40-fold more sensitive to gliclazide than to tolbutamide ($K_i \sim 2$ μmol/l). Cloned cardiac and smooth muscle K_{ATP} channels did not show high-affinity block by gliclazide. Kir6.2-SUR2A currents exhibited a single low-affinity site with a K_i of 0.8 ± 0.1 mmol/l ($n = 5$), which is likely to reside on the Kir6.2 subunit.
Conclusion/interpretation. Our results show that gliclazide is a sulphonylurea with high affinity and strong selectivity for the beta-cell type of K_{ATP} channel. [Diabetologia (1999) 42: 845–848]

Keywords ATP-sensitive K-channel, gliclazide, sulphonylureas, Kir6.2, sulphonylurea receptor.

Sulphonylureas, which are widely used in the treatment of Type II (non-insulin-dependent) diabetes mellitus, stimulate insulin secretion from pancreatic beta-cells by inhibiting ATP-sensitive K-channels (K_{ATP} channels). Members of the K_{ATP} channel family are found in a variety of other tissues, including cardiac, skeletal and smooth muscle and neurones, where they couple the metabolic state of a cell to its electrical activity. In beta-cells, the activity of K_{ATP} channels is modulated by cell metabolism and regulates the membrane potential. Closure of the K_{ATP} channels in response to glucose results in membrane depolarisation. This opens voltage-gated Ca^{2+} channels, allowing calcium influx and thereby triggering insulin release [1].

The K_{ATP} channel is an octameric (4 + 4) complex of two types of subunit: a pore-forming Kir6.2 subunit and a regulatory sulphonylurea receptor (SUR), which endows the channel with high-affinity sulphonylurea sensitivity [2–4]. Two *SUR* genes have been identified, one of which encodes the beta-cell isoform (SUR1), and the other the cardiac muscle (SUR2A) and smooth muscle (SUR2B) isoforms of the sulphonylurea receptor [2, 5, 6]. These account for the differential tissue selectivity to sulphonylureas. Tolbutamide, for example, blocks beta-cell K_{ATP} channels

Received: 20 January 1999 and in final revised form: 4 March 1999

Corresponding author: Professor F. M. Ashcroft, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK
Abbreviations: K_{ATP} channel, ATP-sensitive K-channel; SUR, sulphonylurea receptor.

with high affinity but has relatively little effect on cardiac channels. Glibenclamide, however, is less tissue specific, blocking both beta-cell and cardiac K_{ATP} channels [7, 8]. In this paper we have investigated the selectivity of gliclazide, a widely used second generation sulphonylurea, for beta-cell, cardiac and smooth muscle K_{ATP} channels.

Materials and methods

Molecular biology. Mouse *Kir6.2* (Genbank D50581; [3]), rat *SUR1* (Genbank L40624; [2]), *SUR2A* (Genbank D83598; [5]) and *SUR2B* (Genbank D86038; [6]) cDNAs were cloned in 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 [4]. Capped mRNA was prepared using the mMessage mMachine large scale in vitro transcription kit (Ambion, Austin, Tex., USA).

Oocyte collection. Oocytes were collected by mini-laparotomy from female *Xenopus laevis* anaesthetised with MS222 (2 g/l added to the water). Immature stage V-VI oocytes were incubated for 60 min with 1.0 mg/ml collagenase (Sigma, type V, Poole, UK) and manually defolliculated. Oocytes were either injected with approximately 1 ng Kir6.2ΔC36 mRNA or coinjected with approximately 0.1 ng Kir6.2 mRNA and approximately 2 ng of mRNA encoding one of the types of SUR. The final injection volume was 50 nl/oocyte. Isolated oocytes were maintained in Barth's solution and studied 1–4 days after injection [9].

Electrophysiology. Patch pipettes were pulled from thick-walled 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 [9]. 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 0.2 kHz, digitised at 0.4 kHz using a Digidata 1200 Interface and analysed using pClamp software (Axon Instruments, Burlingame, USA). Records were stored on video tape and resampled at 20 Hz for presentation in the figures.

The pipette (external) solution contained (mmol/l): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The intracellular (bath) solution contained (mmol/l): 110 KCl, 1.4 $MgCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH; final $[K^+]$ ~ 140 mmol/l). Gliclazide (a gift from Servier) was prepared as a 0.1 mol/l stock solution in 0.1 mol/l KOH, and the pH of the bath solution was readjusted after drug addition. The final $[K^+]$ was approximately 140 mmol/l after addition of gliclazide. 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 mmol/l MgATP at intervals throughout the experiment, to reverse channel rundown.

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 means ± 1 SEM.

Dose-response curves were fit to the following equation (eq.) [9]:

$$\frac{G}{G_c} = x^*y \qquad \text{eq. 1}$$

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

$$x = L + \frac{(1-L)}{(1+([Glic]/K_{i1})^{h1})} \qquad \text{eq. 2}$$

$$y = \frac{1}{1+([Glic]/K_{i2})^{h2}} \qquad \text{eq 3}$$

where [Glic] is the gliclazide concentration, K_{i1}, K_{i2} are the gliclazide concentrations at which inhibition is half maximum at 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 fully occupied. When only a single site is present, the equation reduces to $G/G_c = x$ (eq. 4). To control for the rundown of channel activity that occurs in excised patches, dose-response curves were constructed by expressing the conductance in the presence of gliclazide as a fraction of the mean of the conductances measured in control solution before and after addition of the drug.

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 considerably when the patch was excised into nucleotide-free solution. This is consistent with the idea that the K_{ATP} channel is blocked in the intact oocyte by cytoplasmic nucleotides such as ATP. The application of 10 μmol/l gliclazide to the intracellular membrane surface reversibly blocked Kir6.2-SUR1 currents by 61 ± 4 % (n = 8), but had little effect on either Kir6.2-SUR2A currents (6 ± 3 % block, n = 6) or Kir6.2-SUR2B currents (8 ± 2 % block, n = 5) (Fig. 1A).

We reported previously that tolbutamide inhibits beta-cell K_{ATP} channels at two sites: a high-affinity site, conferred by SUR1 and a low-affinity site on Kir6.2 [9]. The high-affinity site has a K_i of approximately 2 μmol/l and, when fully saturated by 100 μmol/l tolbutamide, the current amplitude is reduced by about 50 %. Kir6.2-SUR2A currents show only low-affinity tolbutamide inhibition [8]. We therefore measured the relation between the gliclazide concentration and the K_{ATP} current for both Kir6.2-SUR1 and Kir6.2-SUR2A channels (Fig. 1Bi). As with tolbutamide, the inhibition of Kir6.2-SUR1 currents by gliclazide showed both high- and low-affinity sites with K_is of 50 ± 7 nmol/l (n = 8) and 3.0 ± 0.6 mmol/l (n = 4), respectively. Kir6.2-SUR2A

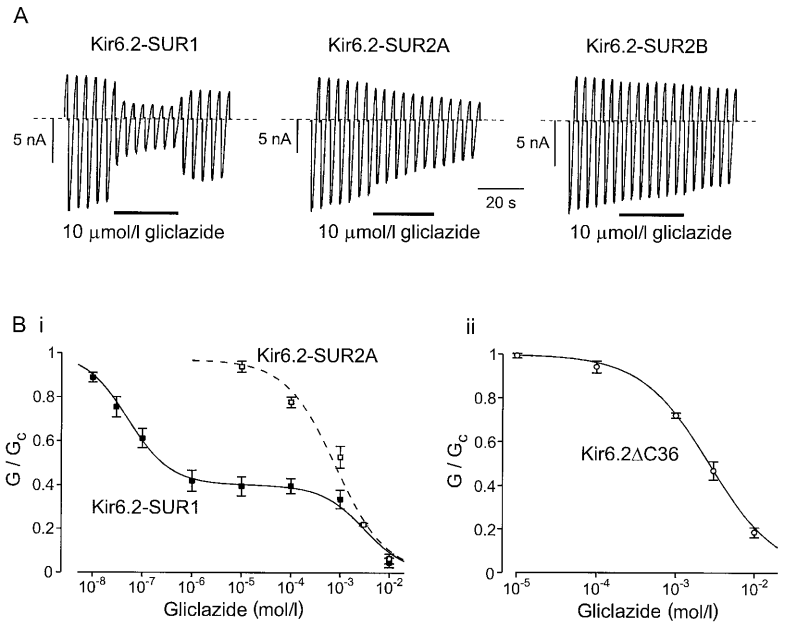

Fig. 1 A, B. Inhibition of K_{ATP} currents by gliclazide. **A** Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from –110 mV to + 100 mV from oocytes coexpressing Kir6.2 and either SUR1, SUR2A or SUR2B. Gliclazide (10 μmol/l) was added as indicated by the bars. **B** Gliclazide dose-response relation for (i) Kir6.2-SUR1 and Kir6.2-SUR2A or (ii) Kir6.2ΔC36 currents. The macroscopic conductance in the presence of gliclazide (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 SEM. Kir6.2-SUR1 data (n = 4 to 8) were fit with a two-site model (eq. 1): K_{i1} = 51 nmol/l, $h1$ = 1.0, K_{i2} = 3.1 mmol/l, $h2$ = 1, L = 0.40. Kir6.2-SUR2A data (n = 5) were fit with a single-site model (eq. 4): K_{i1} = 0.8 mmol/l, $h1$ = 0.9, L = 0. Kir6.2ΔC36 data (n = 5) were also fit with eq. 4: K_{i1} = 2.7 mmol/l, $h1$ = 1.0, L = 0

currents, in contrast, showed only low-affinity inhibition by gliclazide, with a K_i of 0.8 ± 0.1 mmol/l (n = 5).

Low-affinity inhibition of Kir6.2-SUR1 and Kir6.2-SUR2A channels by sulphonylureas has also been reported for tolbutamide. This site is not conferred by the sulphonylurea receptor, since it is also observed when truncated Kir6.2 subunits (Kir6.2ΔC36) are expressed in the absence of SUR [8, 9]. To investigate whether the low-affinity site for gliclazide is also independent of SUR, we tested the effect of the drug on Kir6.2ΔC36 channels. Gliclazide blocked Kir6.2ΔC36 currents at low affinity, with a K_i of 2.7 ± 0.3 mmol/l (n = 5), suggesting that the low-affinity gliclazide site lies on Kir6.2 itself (Fig. 1Bii).

Discussion

Our results show that gliclazide blocks K_{ATP} channels from beta-cells but not those from cardiac or smooth muscle, with high affinity. This high-affinity sulphonylurea site, located on SUR1, is about 40-fold more sensitive to gliclazide than to tolbutamide. A low-affinity site for gliclazide was observed for Kir6.2-SUR1, Kir6.2-SUR2A and Kir6.2ΔC36 currents and is likely to reside on the Kir6.2 subunit.

The responses of beta-cell and cardiac K_{ATP} currents to gliclazide are qualitatively similar to those previously observed with tolbutamide, which blocked Kir6.2-SUR1 currents at high-affinity and low-affinity sites (K_is of ~ 2 μmol/l and ~ 2 mmol/l, respectively) and Kir6.2-SUR2A currents at a single low-affinity site (K_i ~ 2 mmol/l; [8]). The principal difference between the two drugs, therefore, is the greater sensitivity of the high-affinity site on SUR1 to gliclazide (K_i ~ 50 nmol/l) than to tolbutamide (K_i ~ 2 μmol/l). Since the only structural difference between these drugs is the substitution of an azobicyclo ring in the gliclazide molecule (Fig. 2), this group is likely to be involved in high-affinity binding to SUR1.

Glibenclamide (Fig. 2) also exhibits high-affinity binding to SUR1 (K_i ~ 4 nmol/l; [2, 8]), but its inhibitory effect on K_{ATP} currents differs in two important respects from that observed with gliclazide. Firstly, the block of Kir6.2-SUR1 currents by glibenclamide is effectively irreversible, whereas that of gliclazide reverses rapidly. Secondly, glibenclamide, but not gliclazide, blocks the cardiac type of K_{ATP} channel with high affinity. We suggested previously that the non-sulphonylurea moiety of glibenclamide is responsible both for its effectiveness on cardiac K_{ATP} channels and for the irreversibility of its action on the beta-cell K_{ATP} channel. The data presented here

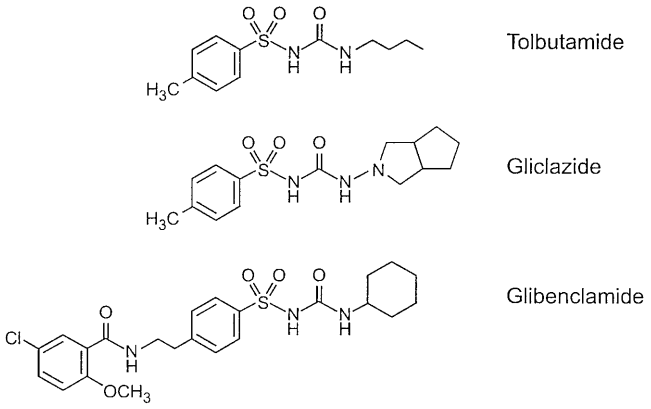

Fig. 2. Molecular structures of tolbutamide, gliclazide and glibenclamide

suggest that the ring on the sulphonylurea tail of glibenclamide may contribute to the high affinity of the drug.

Gliclazide and tolbutamide blocked Kir6.2ΔC36 currents with similar K_is (~ 2 mmol/l and ~ 3 mmol/l, respectively), suggesting that the low-affinity site on Kir6.2 is not sensitive to the structural differences between the two drugs.

Our results suggest that therapeutic concentrations of gliclazide (1–25 μmol/l; [10]) should selectively block beta-cell K_{ATP} channels and produce little cross-reactivity with cardiac and smooth muscle K_{ATP} channels. The clinical importance of the extrapancreatic actions of sulphonylureas remains controversial, since both beneficial and harmful side effects have been reported in the literature. It is clear that the sulphonylureas should not be considered as a homogeneous group when looking for possible side effects, since only some of the drugs interact with K_{ATP} channels in extrapancreatic tissues. Understanding the tissue specificity of individual sulphonylureas is therefore a crucial step in planning further studies in this area.

Acknowledgements. We thank the Wellcome Trust and the British Diabetic Association for support. We thank Dr. S. Seino (Chiba University, Japan) for providing SUR2A, Dr. H. Sakura for cloning SUR2B and Dr. D. Ravel of Servier for the gift of gliclazide and for helpful discussion.

References

1. Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54: 87–143
2. Aguilar-Bryan L, Nichols CG, Wechsler SW et al. (1995) Cloning of the β-cell high-affinity sulphonylurea receptor: a regulator of insulin secretion. Science 268: 423–425
3. Sakura H, Ämmälä C, Smith PA, Gribble FM, Ashcroft FM (1995) Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel expressed in pancreatic β-cells, brain, heart and skeletal muscle. FEBS Lett 377: 338–344
4. Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM (1997) Truncation of Kir6.2 produces ATP-sensitive K-channels in the absence of the sulphonylurea receptor. Nature 387: 179–183
5. Inagaki N, Gonoi T, Clement JP et al. (1996) A family of sulfonylurea receptors determines the properties of ATP-sensitive K^+ channels. Neuron 16: 1011–1017
6. Isomoto S, Kondo C, Yamada M et al. (1996) A novel sulphonylurea receptor forms with BIR (Kir6.2) a smooth muscle type of ATP-sensitive K^+ channel. J Biol Chem 271: 24321–24325
7. 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
8. Gribble FM, Tucker SJ, Seino S, Ashcroft FM (1998) Tissue specificity of sulphonylureas: studies on cloned cardiac and β-cell K_{ATP} channels. Diabetes 47: 1412–1418
9. Gribble FM, Tucker SJ, Ashcroft FM (1997) The interaction of nucleotides with the tolbutamide block of K-ATP currents: a reinterpretation. J Physiol 504.1: 35–45
10. Palmer KJ, Brodgen R (1993) Gliclazide: an update of its pharmacological properties and therapeutic efficacy in Non Insulin Dependent Diabetes Mellitus. Drugs 46: 92–125