Tissue Specificity of Sulfonylureas
Studies on Cloned Cardiac and β-Cell K_{ATP} Channels

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Sulfonylureas stimulate insulin secretion from pancreatic β-cells by closing ATP-sensitive K^+ (K_{ATP}). The β-cell and cardiac muscle K_{ATP} channels have recently been cloned and shown to possess a common pore-forming subunit (Kir6.2) but different sulfonylurea receptor subunits (SUR1 and SUR2A, respectively). We examined the mechanism underlying the tissue specificity of the sulfonylureas tolbutamide and glibenclamide, and the benzamido-derivative meglitinide, using cloned β-cell (Kir6.2/SUR1) and cardiac (Kir6.2/SUR2A) K_{ATP} channels expressed in *Xenopus* oocytes. Tolbutamide inhibited Kir6.2/SUR1 (K_i ~5 μmol/l), but not Kir6.2/SUR2A, currents with high affinity. Meglitinide produced high-affinity inhibition of both Kir6.2/SUR1 and Kir6.2/SUR2A currents (K_is ~0.3 μmol/l and ~0.5 μmol/l, respectively). Glibenclamide also blocked Kir6.2/SUR1 and Kir6.2/SUR2A currents with high affinity (K_is ~4 nmol/l and ~27 nmol/l, respectively); however, only for cardiac-type K_{ATP} channels was this block reversible. Physiological concentrations of MgADP (100 μmol/l) enhanced glibenclamide inhibition of Kir6.2/SUR1 currents but reduced that of Kir6.2/SUR2A currents. The results suggest that SUR1 may possess separate high-affinity binding sites for sulfonylurea and benzamido groups. SUR2A, however, either does not possess a binding site for the sulfonylurea group or is unable to translate the binding at this site into channel inhibition. Although MgADP reduces the inhibitory effect of glibenclamide on cardiac-type K_{ATP} channels, drugs that bind to the common benzamido site have the potential to cause side effects on the heart. *Diabetes* 47:1412–1418, 1998

S ulfonylureas stimulate insulin secretion from pancreatic β-cells and are widely used in the treatment of type 2 diabetes (1). Their principal target is the ATP-sensitive K^+ (K_{ATP}) channel, which plays a major role in controlling the β-cell membrane potential. Inhibition of K_{ATP} channels by glucose or sulfonylureas 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 (2). 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 (3). Although their roles in extrapancreatic tissues are less well characterized, it is likely that they open in response to metabolic stress, such as occurs during cardiac and cerebral ischemia (4). K_{ATP} channels are also important in the control of vascular smooth muscle tone, and therefore of blood pressure (5). The question of whether sulfonylureas cause adverse cardiovascular side effects under clinical conditions has been hotly debated, and conflicting results have been reported from different studies (6,7). A detailed comparison of the mechanism of action of sulfonylureas on β-cell and cardiac K_{ATP} channels would therefore be valuable. The recent cloning of both the β-cell and the cardiac K_{ATP} channel makes it possible to investigate the molecular basis for the differences in their tissue specificity. Furthermore, by studying both types of channel in the same heterologous expression system, it is possible to identify those differences which pertain directly to the sulfonylurea receptor (SUR) subunit and to exclude any that are conferred by the different tissues in which they are normally expressed.

K_{ATP} channels are formed from two types of subunit (8,9): a pore-forming subunit, Kir6.2, and a sulfonylurea receptor, SUR, which coassemble with a 4:4 stoichiometry (10–12). Kir6.2 is strongly expressed in β-cells and cardiac muscle and is likely to form the pore of the K_{ATP} channels in both these tissues (8,9). The sulfonylurea receptor is a member of the ATP-binding cassette (ABC) transporter family, and appears to act as a regulatory subunit. Two genes, encoding the sulfonylurea receptors SUR1 and SUR2, have been cloned, of which the former, SUR1, serves as the regulatory subunit of β-cell K_{ATP} channels (13), and a splice variant of the latter, SUR2A, acts as the cardiac sulfonylurea receptor (14,15).

Metabolic regulation of K_{ATP} channel activity in response to glucose is believed to be mediated by the opposing effects of ATP (a K_{ATP} channel blocker) and MgADP (a channel activator) (2–4). K_{ATP} channels are also the target for a number of pharmacological agents, including inhibitors such as the sulfonylureas and a structurally unrelated group of K-channel openers (1,16). Studies of cloned K_{ATP} channel subunits have significantly advanced our understanding of how nucleotides and drugs bring about changes in channel activity. Thus, Kir6.2 appears intrinsically sensitive to ATP inhibition (17), whereas the sulfonylurea receptor endows the channel with sensitivity to sulfonylureas, K-channel openers, and the stimulatory effects of MgADP (14,17–19).

Native β-cell and cardiac K_{ATP} channels exhibit different sensitivities to sulfonylureas and K-channel openers. These

differences can also be demonstrated for the cloned channels; thus, coexpression of Kir6.2 with SUR1 generates K_{ATP} channels that are blocked by tolbutamide and activated by diazoxide, whereas coexpression of Kir6.2 with SUR2A results in K_{ATP} channels that are blocked by glibenclamide, activated by pinacidil, and insensitive to diazoxide (14). These properties resemble those of β-cell and cardiac K_{ATP} channels, respectively, and confirm that the pharmacological profile of the native channel is conferred by the sulfonylurea receptor subunit. Native β-cell and cardiac K_{ATP} channels also differ in the effect of MgADP on sulfonylurea inhibition: the tolbutamide block of the β-cell K_{ATP} channel is apparently enhanced in the presence of a low concentration of intracellular MgADP, while that of cardiac K_{ATP} channels is reduced (20–22).

In this study, we have characterized the effects of first and second generation sulfonylureas (represented by tolbutamide and glibenclamide) and the related compound meglitinide, on cloned β-cell (Kir6.2/SUR1) and cardiac (Kir6.2/SUR2A) K_{ATP} channels heterologously expressed in *Xenopus* oocytes. We show that both tolbutamide and glibenclamide are more effective inhibitors of β-cell than cardiac K_{ATP} channels, but that the mechanism underlying this specificity is different. Tolbutamide produces high-affinity inhibition of β-cell but not cardiac-type K_{ATP} channels. Glibenclamide, on the other hand, inhibits both β-cell and cardiac K_{ATP} channels with high affinity, but the effect on the cardiac channel is largely abolished by the presence of physiological concentrations of intracellular MgADP.

RESEARCH DESIGN AND METHODS

Molecular biology. Mouse Kir6.2 (Genbank D50581 [8,9]), rat SUR1 (Genbank L40624 [13]) and rat SUR2A (Genbank D83598 [14]) were used in this study. A 36-amino acid COOH-terminal deletion of mouse Kir6.2 (Kir6.2ΔC36) was made by introduction of a stop codon at the appropriate residue using site-directed mutagenesis (17). In vitro synthesis of mRNA was carried out as previously described (23).

Electrophysiology

Oocyte collection. Female *Xenopus laevis* were anesthetized with MS222 (2 g/l added to the water). One ovary was removed via a minilaparotomy, the incision sutured, and the animal allowed to recover. When the wound had healed, the second ovary was removed in a similar operation, and the animal was killed by decapitation under anesthesia. Immature stage V-VI *Xenopus* oocytes were incubated for ~75 min with 1.5 mg/ml collagenase (Boehringer, type A) and manually defolliculated. For coexpression experiments, ~0.04 ng Kir6.2 was coinjected with ~2 ng of SUR1 or ~2 ng of SUR2A (giving a 1:50 ratio). In some experiments, oocytes were injected with ~2 ng of mRNA encoding Kir6.2ΔC36. The final injection volume was ~50 nl/oocyte. Isolated oocytes were maintained in tissue culture and studied 1–4 days after injection (23).

Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV and at 20–24°C (23). Patch electrodes were pulled from thick-walled borosilicate glass (GC150; Clark Electromedical Instruments, Pangbourne, U.K.) and had resistances of 200–400 MΩ when filled with a pipette solution. Currents were evoked by repetitive 3-s voltage ramps from –110 to +100 mV and recorded using an EPC7 patch-clamp amplifier (List Electronik, Darmstadt, Germany). They were filtered at 0.2 kHz, digitized at 0.5 kHz using a Digidata 1200 Interface, and analyzed using pClamp software (Axon Instruments, Foster City, CA).

The pipette solution contained the following (in millimoles per liter): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, and 10 HEPES (pH 7.4 with KOH). The internal (bath) solution contained the following (in millimoles per liter): 110 KCl, 1.4 $MgCl_2$, 30 KOH, 10 EGTA, 10 HEPES (pH 7.2 with KOH), and nucleotides, as indicated. Tolbutamide was made up as a 0.1 mol/l stock solution in 0.1 mol/l KOH (or 0.14 mol/l KOH for SUR2A experiments) and diluted as required. Glibenclamide was prepared as a 100 or 1,000× stock solution in DMSO. Meglitinide was prepared as a 1,000× stock solution in DMSO or (to make the 1 mmol/l solution) as a 100 mmol/l stock solution in 0.14 mol/l KOH. Diazoxide was prepared as a 500× stock solution in 0.1 mol/l KOH and pinacidil as a 1,000× stock solution in ethanol. 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.

Data analysis. The slope conductance was measured by fitting a straight line to the current-voltage relation between –20 and –100 mV: the average of 5 consec-

utive ramps was calculated in each solution. To control for rundown, the control conductance was usually taken as the mean of that obtained in control solution before and after application of the test compound. The effect of glibenclamide on Kir6.2/SUR1 currents was not obviously reversible, so each patch was exposed to only one concentration, and only one patch was tested per oocyte. The conductance in the presence of glibenclamide was expressed relative to that in control solution before drug application for both Kir6.2/SUR1 and Kir6.2/SUR2A currents. Most currents were not leak-corrected because the leak current (that remaining in the presence of 1 mmol/l ATP) was <1% of the total current. However, as Kir6.2ΔC36 expressed smaller currents, we corrected the dose-response curves for leak by subtraction of the mean conductance measured in patches excised from water-injected oocytes.

The conductance (G) is plotted as a fraction of that obtained in the control solution (G_c). Sulfonylurea dose-response curves were fit to the following:

$$\frac{G}{G_c} = x \times y \tag{1}$$

where x is a term describing the high-affinity site and y a term describing the low-affinity site.

$$x = L + \frac{(1 - L)}{1 + ([X]/K_{i_1})^{h1}} \tag{2}$$

$$y = \frac{1}{1 + ([X]/K_{i_2})^{h2}} \tag{3}$$

where $[X]$ is the tolbutamide, meglitinide, or glibenclamide concentration (as applicable); K_{i_1} and K_{i_2} are the concentrations at which inhibition is half maximal at the high- and low-affinity sites, respectively; $h1$ and $h2$ are the Hill coefficients (slope factors) for the high- and low-affinity sites, respectively; and L is the fractional conductance remaining when all of the high-affinity inhibitory sites are occupied. When only a single binding site is present (as observed for example, for Kir6.2ΔC36 currents), Eq. 1 reduces to

$$\frac{G}{G_c} = x \tag{4}$$

ATP dose-response curves were also fit to Eq. 4.

In most cases, data are given as means ± 1 SEM, and the symbols in the figures indicate the mean and the vertical bars 1 SEM (where this is larger than the symbol). Statistical significance was tested using unpaired Student's *t* test.

RESULTS

We first compared the basic properties of Kir6.2/SUR1 and Kir6.2/SUR2A currents, when expressed in *Xenopus* oocytes. The mean current amplitudes at –100 mV after patch excision were –3.0 ± 0.3 nA (n = 50) for Kir6.2/SUR1 currents and –6.2 ± 0.7 nA (n = 47) for Kir6.2/SUR2A currents. This difference may reflect a greater expression of Kir6.2/SUR2A channels and/or a higher channel open probability. The latter has previously been reported for single-channel recordings (14).

In contrast to earlier reports using a mammalian cell expression system (14), we did not observe a significant difference in ATP sensitivity between Kir6.2/SUR1 and Kir6.2/SUR2A currents (Fig. 1*A*). The mean K_i for ATP inhibition was 21 ± 2 μmol/l (n = 7) for Kir6.2/SUR1 currents, compared with 29 ± 4 μmol/l (n = 12) for Kir6.2/SUR2A currents (not significant by *t* test). The Hill coefficients (h) were 1.0 ± 0.1 and 1.4 ± 0.1 for Kir6.2/SUR1 and Kir6.2/SUR2A currents, respectively. The values of K_i and h we observe are similar to those found for native β-cell and cardiac K_{ATP} channels (2–4,24).

Figure 1*B* compares the effects of various stimulators of K_{ATP} channel activity, which mediate their effects by interaction with the sulfonylurea receptor. MgADP (100 μmol/l) activated both β-cell and cardiac types of K_{ATP} channel to a similar extent (~140%). By contrast, the efficacies of K-channel openers were markedly different. Diazoxide (340 μmol/l) induced marked activation of Kir6.2/SUR1 but was without effect on Kir6.2/SUR2A currents, while the reverse was true
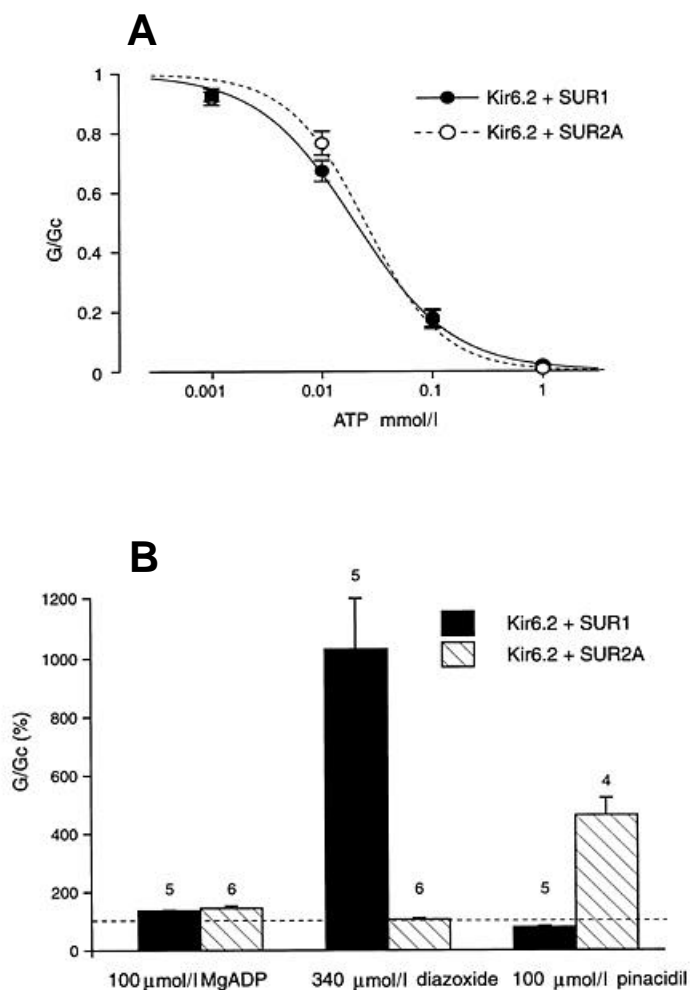
FIG. 1. Sensitivity of Kir6.2/SUR1 and Kir6.2/SUR2A currents to ATP and K-channel openers. *A*: Mean ATP dose-response relationships for Kir6.2/SUR1 currents (*n* = 7) and Kir6.2/SUR2A currents (*n* = 6). The slope conductance (*G*) is expressed relative to that (*G*$_c$) obtained in control solution. The lines are the best fit of the data to the Hill equation (Eq. 4) using the mean values for *K*$_i$ and *h* given in the text. *B*: Mean amplitude of Kir6.2/SUR1 or Kir6.2/SUR2A currents recorded in response to the agents indicated, expressed as a percentage of the current amplitude in the absence of test compound. The dashed line indicates the level of activity in the absence of the test compound. Diazoxide and pinacidil were tested in the presence of 100 µmol/l MgATP: the current recorded in the presence of both activator and ATP is expressed relative to that recorded in the ATP-containing solution. The number of patches is given above each bar.

for 100 µmol/l pinacidil (Fig. 1*B*). Both drugs were tested in the presence of 100 µmol/l intracellular ATP because their effect on native K$_{ATP}$ channels is known to be dependent on the presence of hydrolysable nucleotide (25,26). These results resemble those previously reported for cloned K$_{ATP}$ channels (14,18). They also confirm that Kir6.2/SUR2A channels expressed in *Xenopus* oocytes display properties similar to those of native cardiac K$_{ATP}$ channels (4,27).

Effects of sulfonylureas in the absence of MgADP. We next compared the effects of tolbutamide, glibenclamide, and meglitinide on cardiac- and β-cell–type K$_{ATP}$ channels. The structure of these drugs is given in Fig. 2*A*. As Fig. 2 also shows, the effects of tolbutamide and glibenclamide on Kir6.2/SUR1 and Kir6.2/SUR2A currents were quite different.
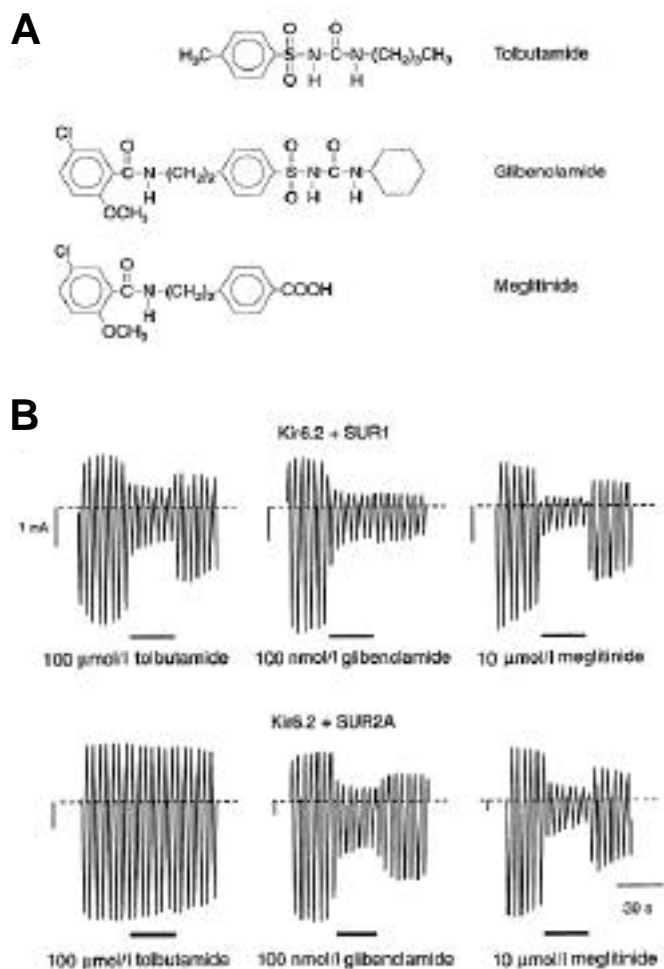

FIG. 2. Effects of sulfonylureas on Kir6.2/SUR1 and Kir6.2/SUR2A currents. *A*: Chemical structure of tolbutamide, glibenclamide, and meglitinide. *B*: Oocytes were coinjected with mRNAs encoding Kir6.2 and either SUR1 (top) or SUR2A (bottom) and macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from –110 to +100 mV. Tolbutamide (100 µmol/l), glibenclamide (100 nmol/l), or meglitinide (10 µmol/l) were added to the internal solution as indicated.

Tolbutamide (100 µmol/l) inhibited Kir6.2/SUR1 currents by 58 ± 2% (*n* = 16) but had little effect on Kir6.2/SUR2A currents (5 ± 2% inhibition, *n* = 13). By contrast, 100 nmol/l glibenclamide blocked both types of channel to a similar extent, inhibiting Kir6.2/SUR1 currents by 71 ± 1% (*n* = 4) and Kir6.2/SUR2A currents by 68 ± 4% (*n* = 6). Meglitinide (10 µmol/l) also blocked β-cell– and cardiac-type K$_{ATP}$ channels with a similar potency; by 76 ± 1% (*n* = 6) and 69 ± 1% (*n* = 5), respectively. Inhibition of K$_{ATP}$ currents by tolbutamide (in the case of Kir6.2/SUR1) and by meglitinide was rapid in onset and easily reversible. This was also the case for the block of cardiac-type K$_{ATP}$ channels by glibenclamide. In contrast, glibenclamide inhibition of Kir6.2/SUR1 currents was essentially irreversible and, at low drug concentrations, the onset of the block was very slow.

The relationship between tolbutamide concentration and the amplitude of Kir6.2/SUR1, or Kir6.2/SUR2A, currents is shown in Fig. 3*A*. As previously described, the dose-response
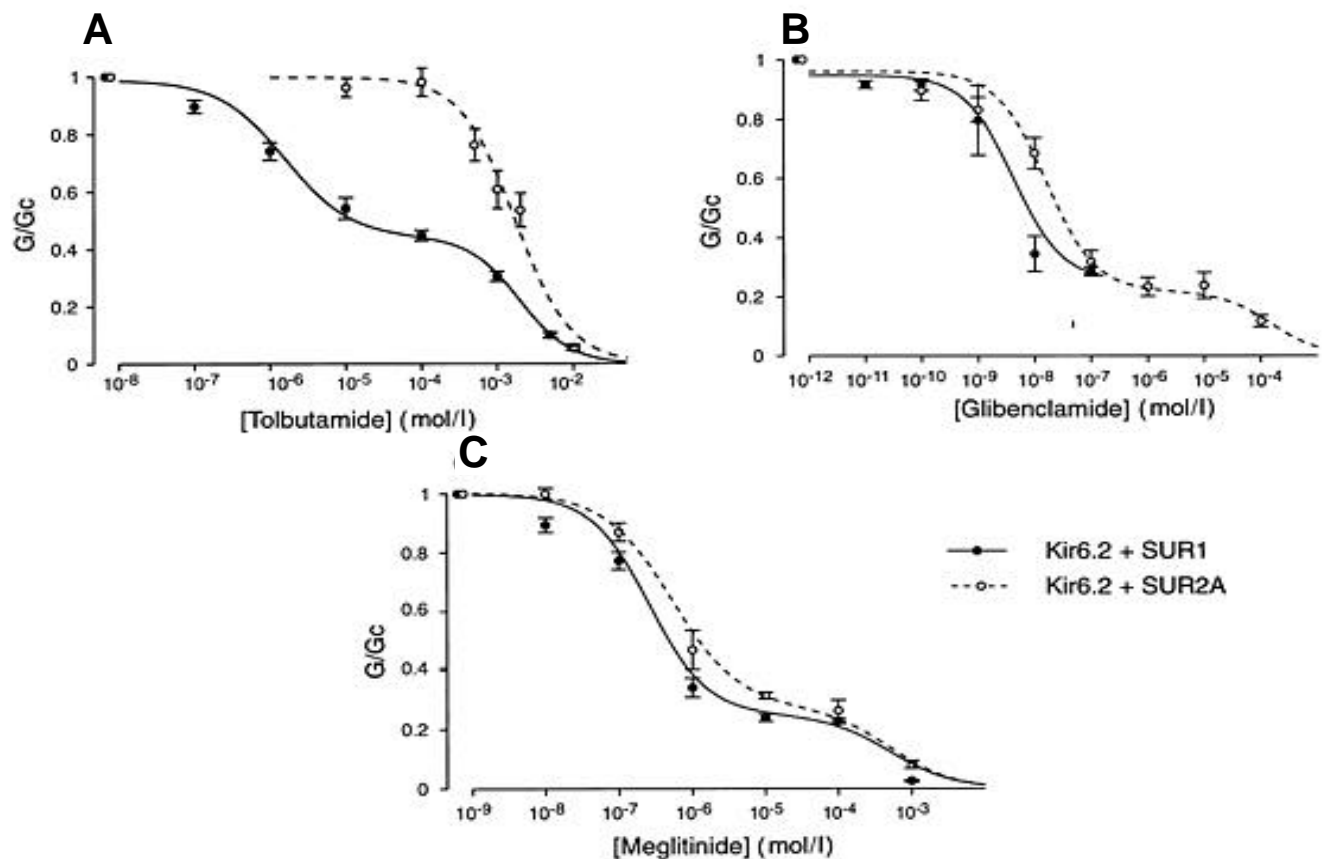
A

B

C

FIG. 3. Dose-response curves for sulfonylurea inhibition of Kir6.2/SUR1 currents and Kir6.2/SUR2A currents. *A*: Relationship between tolbutamide concentration and the macroscopic K_{ATP} conductance, expressed as a fraction of its amplitude in the absence of the drug. Kir6.2/SUR1 currents (n = 11) were fit with Eq. 1: K_{i1} = 5 μmol/l, *h1* = 1.0, K_{i2} = 2 mmol/l, *h2* = 1.3, *L* = 0.46. Kir6.2/SUR2A currents (n = 6) were fit with Eq. 4: K_i = 1.7 mmol/l, *h* = 1.2, *L* = 0. *B*: Relationship between glibenclamide concentration and the macroscopic K_{ATP} conductance, expressed as a fraction of its amplitude in the absence of the drug. Kir6.2/SUR1 currents (n = 4) were fit with Eq. 1: K_{i1} = 4.2 nmol/l, *h1* = 1, *L* = 0.23. Kir6.2/SUR2A currents (n = 6) were fit with Eq. 4: K_{i1} = 27 nmol/l, *h1* = 1.0, K_{i2} = 110 μmol/l, *h2* = 1, *L* = 0.25. *C*: Relationship between meglitinide concentration and the macroscopic K_{ATP} conductance, expressed as a fraction of its amplitude in the absence of the drug. The lines are the best fit of the data to Eq. 1 of the text. Kir6.2/SUR1 currents (n = 6): K_{i1} = 0.26 μmol/l, *h1* = 1.1, K_{i2} = 380 nmol/l, *h2* = 1, *L* = 0.26. Kir6.2/SUR2A currents (n = 6): K_{i1} = 0.48 μmol/l, *h1* = 1.2, K_{i2} = 380 nmol/l, *h2* = 1, *L* = 0.3.

curve for tolbutamide block of Kir6.2/SUR1 currents was best fit by assuming there are two drug binding sites on the K_{ATP} channel (28,29). The high-affinity binding site had a mean K_i of 5.4 ± 3.4 μmol/l (n = 11) and the low-affinity site had a mean K_i of 2.0 ± 0.3 mmol/l (n = 11). The Hill coefficients were 1.0 ± 0.1 and 1.3 ± 0.1, respectively. In contrast, Kir6.2/SUR2A currents did not show high-affinity inhibition by tolbutamide (Fig. 3*A*). Instead, the block was best described by a single low-affinity site with a mean K_i of 1.7 ± 0.2 mmol/l and a Hill coefficient of 1.2 ± 0.1 (n = 6). We have shown elsewhere that the low-affinity site of Kir6.2/SUR1 does not lie on SUR1 but is associated either with Kir6.2 or, possibly, with a regulatory protein endogenous to the *Xenopus* oocyte (28). This result therefore suggests that SUR2A either does not possess a high-affinity binding site for tolbutamide or that binding of the drug does not result in channel inhibition.

Unlike tolbutamide, glibenclamide inhibited both β-cell– and cardiac-type K_{ATP} currents with high affinity: the K_i was 4.2 nmol/l (n = 4) for Kir6.2-SUR1 channels and 27 ± 2 nmol/l (n = 6) for Kir6.2-SUR2A currents (Fig. 3*B*). A second low-affinity site was also apparent from the glibenclamide dose-response curve for Kir6.2-SUR2A currents. The K_i for this site was not measured, but appeared to be ~100 μmol/l (Fig. 3*B*). Both β-cell– and cardiac-type K_{ATP} channels were blocked by

meglitinide, with dose-response curves suggesting the presence of both high- and low-affinity sites (Fig. 3*C*). The mean K_i for the high-affinity site was 0.26 ± 0.06 μmol/l (n = 6) for Kir6.2/SUR1 currents and 0.53 ± 0.11 μmol/l (n = 5) for Kir6.2/SUR2A currents.

We next examined the effects of sulfonylureas on a truncated form of Kir6.2 (Kir6.2ΔC36), which lacks the COOH-terminal 36 amino acids and does not require a sulfonylurea receptor for functional expression (17). Kir6.2ΔC36 currents were also inhibited by sulfonylureas, but only at very high concentrations (Fig. 4) (28). The dose-response curves were described by a single binding site with a K_i of 42 ± 5 μmol/l (n = 5) for glibenclamide and of 1.7 ± 0.1 mmol/l (n = 8) for tolbutamide and Hill coefficients of 0.9 ± 0.1 and 1.2 ± 0.1, respectively. These values are similar to those found for the low-affinity sites of Kir6.2/SUR1 and Kir6.2/SUR2A currents. Meglitinide also inhibited Kir6.2ΔC36 currents with low affinity (37 ± 3% inhibition by 1 mmol/l meglitinide [n = 5]), but a full dose-response curve could not be obtained because the drug was insoluble at higher concentrations. The data argue that, like tolbutamide (28), glibenclamide and meglitinide bind to a low-affinity site on Kir6.2. This binding is unlikely to be of clinical significance, however, as the concentrations involved are suprapharmacological.
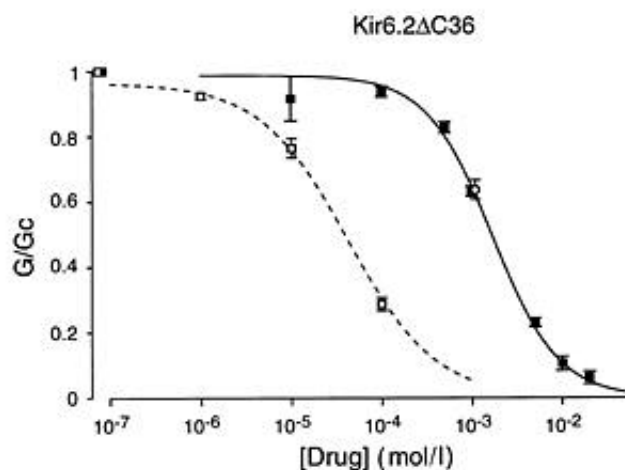
FIG. 4. Effects of sulfonylureas on Kir6.2ΔC36 currents. Mean relationship between sulfonylurea concentration and the macroscopic Kir6.2ΔC36 conductance, expressed as a fraction of its amplitude in the absence of the drug. The lines are drawn to Eq. 4 of the text. □,

Effects of sulfonylureas in the presence of intracellular MgADP. We have shown previously that tolbutamide inhibition of Kir6.2/SUR1 currents is enhanced in the presence of intracellular MgADP (28). Figure 5A shows that this was also the case for glibenclamide. In contrast, cardiac-type K$_{ATP}$ channels showed a very different behavior; glibenclamide appeared to block Kir6.2/SUR2A currents less effectively in the presence of MgADP (Fig. 5B). Figure 5C shows that although 100 nmol/l glibenclamide blocked both Kir6.2/SUR1 and Kir6.2/SUR2A currents to a similar degree (~70% inhibition) in the absence of nucleotide, the same concentration applied in the presence of 100 μmol/l MgADP inhibited Kir6.2/SUR1 currents by ~90%, but Kir6.2/SUR2A currents by only ~10%. It therefore appears that MgADP enhances the block of Kir6.2/SUR1 currents by glibenclamide, but reduces its effect on Kir6.2/SUR2A currents. Similar results have been reported for native β-cell and cardiac K$_{ATP}$ channels (20–22).

DISCUSSION

Our results suggest that under physiological conditions, both tolbutamide and glibenclamide will be more effective on β-cells than on cardiac muscle, but that different mechanisms underlie this tissue specificity.

Inhibition by sulfonylureas. The finding that Kir6.2/SUR1 channels are inhibited by tolbutamide with high affinity (K_i ~5 μmol/l), whereas Kir6.2/SUR2A channels are only blocked with low affinity (K_i ~2 mmol/l), provides additional support for the idea that tolbutamide inhibition is mediated through its interaction with the sulfonylurea receptor. The low tolbutamide sensitivity of Kir6.2/SUR2A currents is consistent with that reported for native cardiac K$_{ATP}$ channels (K_i ~1 mmol/l [22]) and similar to that found when Kir6.2ΔC36 was expressed in the absence of a sulfonylurea receptor (K_i ~2 mmol/l [28]). There may be several explanations why tolbutamide does not inhibit SUR2A currents with high affinity. First, SUR2A may not possess a high-affinity tolbutamide binding site, or this site may have a markedly lower affinity. Second, tolbutamide may bind to SUR2A with high-affinity,

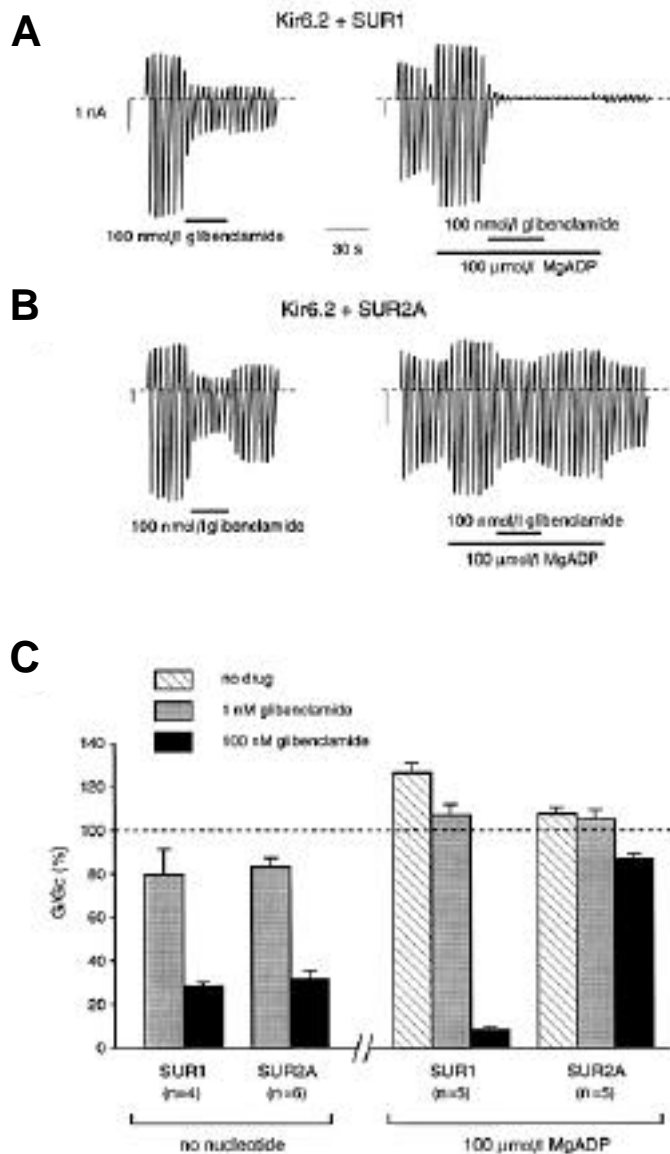**A** Kir6.2 + SUR1

B Kir6.2 + SUR2A

C

FIG. 5. Effect of MgADP on glibenclamide block of Kir6.2/SUR1 and Kir6.2/SUR2A currents. Oocytes were coinjected with mRNAs encoding Kir6.2 and either SUR1 (*A*) or SUR2A (*B*). Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from –110 to +100 mV. Glibenclamide (100 nmol/l) or MgADP (100 μmol/l) were added to the internal solution as indicated. *C*: Mean amplitude of Kir6.2/SUR1 or Kir6.2/SUR2A currents, recorded in the presence of glibenclamide (1 nmol/l or 100 nmol/l, as indicated) in the absence (left) or presence (right) of 100 μmol/l MgADP, expressed as a percentage of current in control solution (no additions). Hatched bars represent the current recorded in the presence of 100 μmol/l MgADP relative to control. The dashed line indicates the control level (100%). The number of oocytes is given below the bars.

but this binding may not result in channel closure. The latter possibility is supported by the observation that tolbutamide displaces [³H]glibenclamide binding to cardiac membranes with a K_i of 1–5 μmol/l (30,31).

In contrast to tolbutamide, meglitinide mediates reversible high-affinity inhibition of both Kir6.2/SUR1 and Kir6.2/SUR2A currents, with a similar affinity and time course. Our data confirm the previous finding that meglitinide blocks native β-cell K$_{ATP}$ currents (32) (and thereby stimulates insulin secre-

tion [33]) and demonstrate that this compound also inhibits cardiac K_{ATP} channels. Meglitinide is not a sulfonylurea but is a benzamido derivative, equivalent to the nonsulfonylurea moiety of glibenclamide (Fig. 2). Thus our results suggest that the sulfonylurea receptor may have a separate benzamido binding site that is common to SUR1 and SUR2A.

Glibenclamide blocked both β-cell– and cardiac-type K_{ATP} currents, but the mechanism of this block appears to be different, since inhibition could not be reversed for Kir6.2/SUR1 currents but was readily reversible in the case of Kir6.2/SUR2A currents. Since glibenclamide comprises both tolbutamide and meglitinide moieties, our data could be explained if the drug binds to SUR1 at two sites (i.e., a tolbutamide and a benzamido site), but only at a single (benzamido) site on SUR2A. If this were the case, the drug would only dissociate from SUR1 when both halves of the molecule unbound from the receptor simultaneously. This could account for the irreversible inhibition of Kir6.2/SUR1 currents by glibenclamide, since simultaneous unbinding is likely to occur with a low probability. It might also contribute to the long washout time observed when glibenclamide therapy is discontinued in patients. By contrast, unbinding from SUR2A would occur more rapidly, since the drug need only dissociate from a single site. Although this is an attractive explanation of our results, it remains speculative since we cannot distinguish whether the loss of high-affinity inhibition of Kir6.2/SUR2A currents by tolbutamide is due to a loss of the high-affinity binding site for this drug, or to a failure to transduce binding into channel inhibition.

Although a previous study was unable to demonstrate high-affinity binding of ^{125}I-labeled iodoglibenclamide to SUR2A (14), our results are consistent with earlier electrophysiological and binding studies showing interaction of glibenclamide with native cardiac membranes (K_i 0.3–3 nmol/l) (30). One possible explanation for the different findings in these studies is that, because the glibenclamide block of Kir6.2/SUR2A currents is rapidly reversible, washing of the membranes (which occurs in binding studies) might dislodge the drug from SUR2A under some conditions.

We reported previously that Kir6.2/SUR1 currents exhibit both high- and low-affinity block by tolbutamide (28). We show here that low-affinity inhibition is also observed for meglitinide and glibenclamide. In both cases, this inhibition was independent of the type of sulfonylurea receptor and was also observed for Kir6.2ΔC36 currents (expressed in the absence of SUR). This indicates that the low-affinity block is not mediated through SUR and suggests it may be a property of Kir6.2 itself (or possibly an additional subunit endogenously expressed in *Xenopus* oocytes). Although therapeutic concentrations of sulfonylureas are unlikely to reach levels that would interact with this low-affinity site, it is important to recognize its existence because it influences the interpretation of sulfonylurea dose-response curves.

Interaction between sulfonylureas and MgADP. It has been known for several years that nucleotides such as MgADP enhance the inhibitory effect of tolbutamide and meglitinide on the β-cell K_{ATP} channel (20,21). Recently, we proposed that this is because tolbutamide abolishes the stimulatory action of MgADP, unmasking an inhibitory effect of the nucleotide (28). The present results demonstrate that MgADP is also able to enhance the inhibitory effects of glibenclamide on Kir6.2/SUR1 currents. In con-

trast, MgADP did not enhance the inhibitory effect of glibenclamide on Kir6.2/SUR2A currents, which is consistent with earlier reports from native cardiac K_{ATP} channels (22,34). As the stimulatory effect of MgADP is mediated by the nucleotide-binding domains (NBDs) of SUR1 (17–19), these data suggest that the sulfonylurea binding site(s) and the NBDs of SUR1 may interact with each other. A difference in either, or both, of these sites might underlie the different effect of MgADP on the glibenclamide block of β-cell and cardiac type K_{ATP} channels.

In intact cells, glibenclamide appears to inhibit Kir6.2/SUR2A channels less effectively than Kir6.2/SUR1 channels, when measured by Rb^+ efflux (14). Our data suggest that this may be because intracellular MgADP enhances glibenclamide inhibition of Kir6.2/SUR1 channels but alleviates inhibition of Kir6.2/SUR2A channels. They also argue that a similar effect would be observed for native K_{ATP} currents in vivo.

ATP sensitivity. Although previous authors have suggested that Kir6.2/SUR2A currents are less sensitive to ATP inhibition than Kir6.2/SUR1 currents (14), we do not find this to be the case. The sensitivity we report is, however, similar to that measured for K_{ATP} channels from rat ventricular myocytes (~25 µmol/l) (24). The difference between our results and those of previous studies may be related either to the precise experimental protocol or to the different expression system (*Xenopus* oocytes as compared with mammalian cells).

Clinical relevance. Since K_{ATP} channels are found in a wide variety of extrapancreatic tissues (cardiac, skeletal, and smooth muscle, and some brain neurones), drugs that cross-react with different members of the K_{ATP} channel family have the potential to cause undesired side effects. Although no major side effects have been demonstrated in sulfonylurea-treated patients, less dramatic effects may be difficult to detect if they are subtle, unexpected, or only evident under certain conditions (such as ischemia) (6,7). Clearly, the ideal sulfonylurea for treatment of type 2 diabetes would be one which interacts only with the β-cell K_{ATP} channel.

Our results indicate that separate mechanisms may underlie the apparent lack of effect of tolbutamide and glibenclamide on cardiac muscle when used at therapeutic concentrations in humans. Tolbutamide is without effect either because the SUR2A subunit of the cardiac K_{ATP} channel lacks a high-affinity site for the drug that mediates channel inhibition or because SUR2A fails to translate tolbutamide binding into closure of the pore. By contrast, glibenclamide does inhibit cardiac-type K_{ATP} channels with high-affinity, but may be ineffective in vivo because this inhibition is markedly reduced in the presence of physiological concentrations of MgADP. We cannot exclude the possibility that other cytosolic substances might likewise influence the response of cardiac K_{ATP} channels to glibenclamide. Thus glibenclamide and related drugs might interact with cardiac K_{ATP} channels under some conditions encountered in vivo. Based on this data, a drug which interacts only with SUR1, but which has higher affinity than that of tolbutamide, would appear to be the best sulfonylurea for treatment of type 2 diabetes. Further studies are required, however, to ascertain the interaction of first and second generation sulfonylureas with the cloned K_{ATP} channel from vascular smooth muscle.

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