British Journal of Pharmacology (2001) 132, 1542–1548

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Effects of mitiglinide (S 21403) on Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B types of ATP-sensitive potassium channel

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1 We have investigated the mechanism of action of the novel anti-diabetic agent mitiglinide (S 21403) on Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B types of ATP-sensitive potassium (K_{ATP}) channel. These possess a common pore-forming subunit, Kir6.2, and different regulatory sulphonylurea receptor (SUR) subunits. It is believed that they correspond to native K_{ATP} channels in pancreatic β-cells, heart and non-vascular smooth muscle, respectively.

2 Kir6.2 was coexpressed with SUR1, SUR2A or SUR2B in *Xenopus* oocytes and macroscopic currents were recorded in giant inside-out membrane patches. Mitiglinide was added to the intracellular membrane surface.

3 Mitiglinide inhibited Kir6.2/SUR currents at two sites: a low-affinity site on Kir6.2 and a high-affinity site on SUR. Low-affinity inhibition was similar for all three types of K_{ATP} channel but high-affinity inhibition was greater for Kir6.2/SUR1 currents (IC_{50}, 4 nM) than for Kir6.2/SUR2A or Kir6.2/SUR2B currents (IC_{50}, 3 and 5 μM, respectively).

4 Inhibition of Kir6.2/SUR1 currents was only slowly reversible on the time scale of electrophysiological experiments.

5 Kir6.2/SUR1-S1237Y currents, which previously have been shown to lack high affinity tolbutamide inhibition, resembled Kir6.2/SUR2 currents in being unaffected by 100 nM but blocked by 10 μM mitiglinide.

6 Our results show that mitiglinide is a high-affinity drug that shows a 1000 fold greater affinity for the β-cell type than the cardiac and smooth muscle types of K_{ATP} channel, when measured in excised patches.

British Journal of Pharmacology (2001) **132,** 1542–1548

Keywords: ATP-sensitive K-channel; mitiglinide; sulphonylureas; Kir6.2; sulphonylurea receptor

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

Introduction

Mitiglinide (S 21403, KAD-1229) is a novel antidiabetic agent under development as a putative treatment for type-2 diabetes mellitus. Its chemical structure is compared to that of sulphonylureas and meglitinide in Figure 1. Mitiglinide exerts a hypoglycaemic effect with rapid onset and short duration of action, by transiently increasing insulin secretion (Ohnota *et al*., 1994). This effect results from the inhibitory effect of mitiglinide on the β-cell K_{ATP} channel (Mogami *et al*., 1994). The drug is also able to displace [^{3}H]-glibenclamide binding to HIT-15 cells microsomes with an IC_{50} of 13 nM (Ohnota *et al*., 1994).

The ATP-sensitive potassium (K_{ATP}) channel plays a major role in controlling the β-cell membrane potential. Inhibition of K_{ATP} channels by glucose, sulphonylureas or mitiglinide causes depolarization of the β-cell membrane; in turn, this triggers the opening of voltage-gated Ca^{2+} channels, eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+} which stimulates the exocytosis of insulin-containing secretory granules (Ashcroft & Rorsman, 1989). K_{ATP} channels are also found at high density in a variety of other cell types including cardiac, smooth and skeletal muscle, and some brain neurones (Ashcroft & Ashcroft, 1990). Although their roles in extra-pancreatic tissues are less well characterized, it is likely that they open in response to metabolic stress, such as that which occurs during cardiac and cerebral ischaemia (Nichols & Lederer, 1991). They are also important in the control of vascular smooth muscle tone, and therefore of blood pressure (Quayle *et al*., 1997). Thus drugs which 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 sulphonylurea-treated patients (UKPDS, 1998), less dramatic effects may be difficult to detect if they are subtle, unexpected or only evident under certain conditions (such as ischaemia). It is therefore of importance to know whether mitiglinide (S 21403) interacts with other types of K_{ATP} channel, especially that of cardiac muscle.

The K_{ATP} channel is an octameric complex of two different protein subunits: an inwardly-rectifying K-channel, Kir6.x, and a sulphonylurea receptor, SUR (Aguilar-Bryan *et al*., 1995; Sakura *et al*., 1995; Inagaki *et al*., 1995; 1996; 1997; Clement *et al*., 1997; Shyng & Nichols, 1997). The former acts as an ATP-sensitive K-channel pore while SUR1 is a channel regulator which endows Kir6.x with sensitivity to

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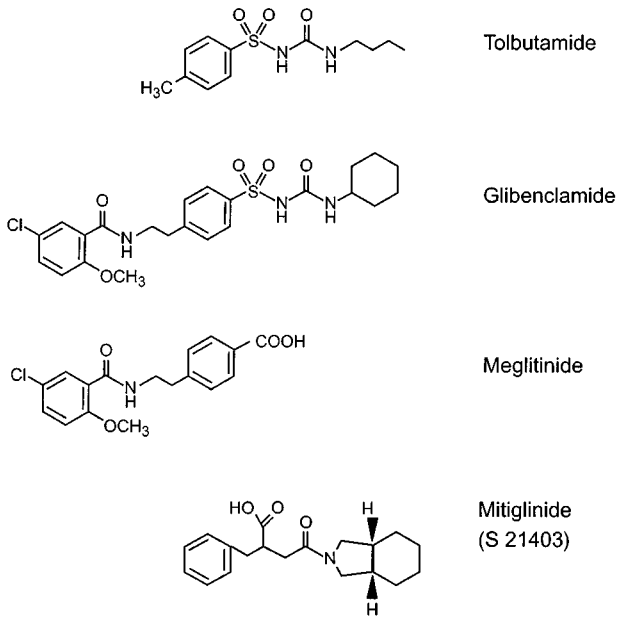

Figure 1 Molecular structures of tolbutamide, glibenclamide, meglitinide and mitiglinide (S 21403).

drugs such as the inhibitory sulphonylureas and the K-channel opener diazoxide (Tucker *et al*., 1997). K_{ATP} channels in different tissues comprise either Kir6.2 or Kir6.1 subunits, which associate with different types of SUR subunit (Ashcroft & Gribble, 1998). The β-cell K_{ATP} channel is composed of Kir6.2 and SUR1, the cardiac type of Kir6.2 and SUR2A and the smooth muscle type, probably, of SUR2B together with either Kir6.2 or Kir6.1. Both Kir6.2/SUR1 and Kir6.2/SUR2B combinations are found in the brain. Although wild-type K_{ATP} channels require both types of subunit (Kir6.2 and SUR) for functional activity, a mutant form of Kir6.2 with a C-terminal truncation of 26 or 36 amino acids (Kir6.2ΔC) is capable of independent expression (Tucker *et al*., 1997). Kir6.2ΔC therefore provides a useful tool for studying the effects of drugs on the pore-forming subunit of the K_{ATP} channel.

A number of studies suggest that the different types of K_{ATP} channel exhibit different specificities towards the various sulphonylureas. In particular, tolbutamide reversibly inhibits Kir6.2/SUR1 but not Kir6.2/SUR2A channels with high potency (Gribble *et al*., 1998). The low tolbutamide sensitivity of Kir6.2/SUR2A currents is consistent with that reported for native cardiac K_{ATP} channels (IC_{50} $\sim$1 mM; Ventakesh *et al*., 1991) and is similar to that found when Kir6.2ΔC36 is expressed in the absence of a sulphonylurea receptor (IC_{50} $\sim$2 mM) (Gribble *et al*., 1997b). Thus it appears that SUR2A, unlike SUR1, does not confer high-affinity tolbutamide inhibition on the K_{ATP} channel. Similar results are found for gliclazide (Gribble & Ashcroft, 1999).

Glibenclamide blocks both Kir6.2/SUR1 (IC_{50} = $\sim$4 nM) and Kir6.2/SUR2A (IC_{50} = $\sim$27 nM) channels with high potency (Gribble *et al*., 1998). Similar potencies are reported for native channels: the IC_{50} of the β-cell channel is 4 nM (Zünckler *et al*., 1988), that of skeletal muscle is 6 nM (Barret-Jolley & McPherson, 1998) and that of cardiac muscle is 7 nM (Findlay, 1992; Krause *et al*., 1995). However, in contrast to glibenclamide block of Kir6.2/SUR2A channels, which is readily reversible, that of Kir6.2/SUR1 channels is irreversible. In an attempt to explain this phenomenon, it has been suggested that SUR1 contains two binding sites for glibenclamide, one that is shared with SUR2A and one that is absent from SUR2A but similar to the tolbutamide binding site of SUR1. Simultaneous binding of glibenclamide to both sites of SUR1 results in an irreversible inhibition of the channel on the time scale of electrophysiological experiments. This hypothesis is strengthened by the fact that meglitinide, a benzamido compound which resembles the benzamido part of the glibenclamide molecule (rather than the tolbutamide part; Figure 1), blocks both SUR1- and SUR2A-containing channels reversibly and with a similar affinity (K_i = $\sim$0.3 μM Kir6.2/SUR1 and $\sim$0.5 μM for Kir6.2/SUR2A; Gribble *et al*., 1997b; 1998).

The structure of mitiglinide (S21403) is quite different from that of either tolbutamide or meglitinide (Figure 1). It is therefore unclear whether S21403 interacts with the sulphonylurea or benzamido sites of SUR, or with yet another site on SUR1, or if it mediates its effect primarily by interaction with the Kir6.2 subunit. In this study, we explore the mechanism of block of the K_{ATP} channel by S21403 in more detail.

Methods

Molecular biology

Mouse Kir6.2 (GenBank D50581; Inagaki *et al*., 1995; Sakura *et al*., 1995), rat SUR1 (GenBank L40624; Aguilar-Bryan *et al*., 1995), rat SUR2A (Genbank D83598; Inagaki *et al*., 1996) and mouse SUR2B (GenBank D86038; Isomoto *et al*., 1996) cDNAs were cloned in the pBF vector. A mutant form of SUR1 (SUR1-S1237Y) and 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, were prepared as described previously (Ashfield *et al*., 1999; Tucker *et al*., 1997). Capped mRNA was prepared using the mMESSAGE mMACHINE large scale *in vitro* transcription kit (Ambion, Austin, TX, U.S.A.), as previously described (Gribble *et al*., 1997a).

Oocyte collection

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

Electrophysiology

Patch pipettes were pulled from 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 (Gribble *et al*., 1997a). 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 0.4 kHz using a Digidata 1200 Interface and analysed using pClamp software (Axon Instruments, Burlingame, U.S.A.).

The pipette (external) solution contained (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The intracellular (bath) solution contained (mM): 110 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH; final $[K^+]$ ~140 mM). The sodium salt of mitiglinide (S 21403-2 or 2S-2-benzyl-3-(cis-hexahydro-2-isoindolinyl-carbonyl) proprionate sodium, monohydrate) was supplied by Technologie Servier, Orléans, France. All other chemicals were from Sigma. Mitiglinide was prepared as a 0.1 M stock solution in ethanol. The stock solution was kept at 4°C and experimental solutions were prepared freshly each day. The drug concentration is expressed as that of the free acid. The maximum concentration of ethanol used (1%) was without effect on all types of cloned K_{ATP} channel.

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 order of increasing concentrations (because of the slow reversibility of the drug in the time frame of the experiment). Patches were exposed to 1 mM 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 and −100 mV: the average of five consecutive ramps was calculated in each solution. 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 mitiglinide as a fraction of the mean of the conductances measured in control solution before and after addition of the drug.

Concentration-response curves for Kir6.2/SUR1 currents were fit to the following equation (Gribble *et al*., 1997b):

$$\frac{G}{G_C} = x * y \quad (1)$$

where G is the conductance in the presence of mitiglinide, 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.

$$x = L + \frac{(1-L)}{1+\frac{[S]}{IC_{50(i1)}{}^{h1}}} \quad (2)$$

$$y = \frac{1}{1+\frac{[S]}{IC_{50(i2)}{}^{(h2)}}} \quad (3)$$

where [S] is the concentration of mitiglinide, $IC_{50(i1)}$, and $IC_{50(i2)}$ are the mitiglinide concentrations at which inhibition is half maximal at the high- and low-affinity sites, respectively; h1, h2 are the Hill coefficients (slope factors) for the high- and low-affinity sites, respectively; and L is the fractional conductance remaining when the high-affinity sites are maximally occupied. Concentration-response curves for Kir6.2/SUR2A or Kir6.2/SUR2B currents were also fit to equation 1, but in this case the Hill coefficient was assumed to be unity for both the high- and low-affinity sites (i.e. h1 = h2 = 1).

Data were fit using Microcal Origin software. Data are presented as mean ± 1 standard error of the mean.

Results

Macroscopic currents were recorded in inside-out membrane patches from *Xenopus* oocytes expressing Kir6.2ΔC36, or coexpressing Kir6.2 and either SUR1, SUR2A, SUR2B. In all cases, the currents were small in the cell-attached configuration but increased markedly when the patch was excised into nucleotide-free solution. This is consistent with the idea that the K_{ATP} channel is blocked in the intact oocyte by cytoplasmic nucleotides such as ATP.

Figure 2 shows that application of 100 nM mitiglinide to the intracellular membrane surface blocked Kir6.2/SUR1 currents by 43.8 ± 6.7% (n = 6), but had little effect on either Kir6.2/SUR2A currents (1.4 ± 2.2% block, n = 5) or Kir6.2/SUR2B currents (1.8 ± 2.9% block, n = 5). The effect of the drug on Kir6.2/SUR1 currents was not fully reversible on the time scale of our experiments.

The relations between mitiglinide concentration and the K_{ATP} current for the β-cell (Kir6.2/SUR1), cardiac (Kir6.2/

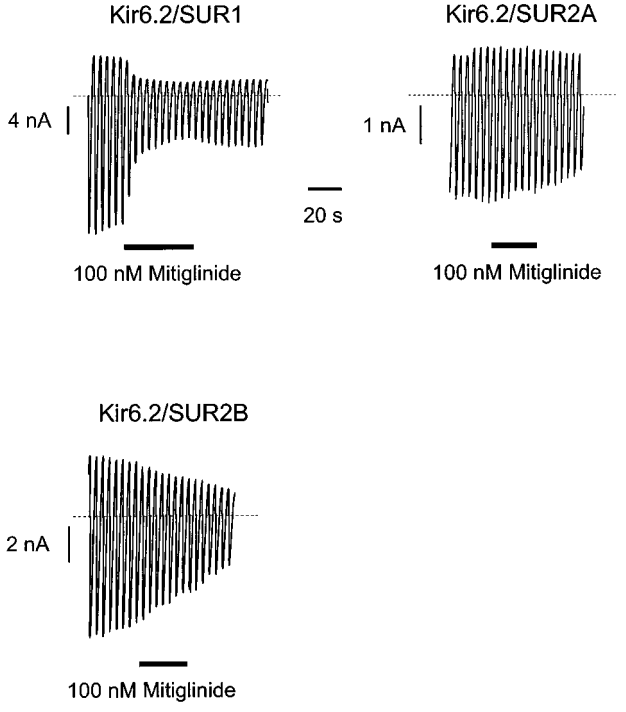

Figure 2 Inhibition of K_{ATP} currents by mitiglinide. Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1 or SUR2A or SUR2B. Mitiglinide (100 nM) was added as indicated by the bars.

SUR2A) and smooth muscle (Kir6.2/SUR2B) types of K_{ATP} channel are shown in Figure 3. In all cases, the concentration-response curve is best described by a two-site model, in which mitiglinide interacts with both a high-affinity and a low-affinity site.

The IC_{50} for mitiglinide inhibition of Kir6.2/SUR1 channels at the high-affinity site was 3.8 ± 1.6 nM ($n=5$ oocytes): the Hill coefficient was 0.7 ± 0.2 and the percentage of block mediated by interaction with the high-affinity site was $45 \pm 3\%$. The IC_{50} for the low-affinity site was >1 mM but could not be measured accurately because it was not possible to dissolve higher concentrations of the drug. In contrast to Kir6.2/SUR1 channels, Kir6.2/SUR2A currents were less sensitive to mitiglinide, the IC_{50} being 3.2 ± 1.2 μM ($n=5$) at the high-affinity site as compared with 3.8 nM for Kir6.2/SUR1. It was not possible to measure the low-affinity block but, as in the case of Kir6.2/SUR1 currents, the IC_{50} was greater than 1 mM. The percentage of block mediated by interaction with the high-affinity site was $67 \pm 1\%$ ($n=5$), compared with 45% for Kir6.2/SUR1 currents. The sensitivity of the high-affinity site on Kir6.2/SUR2B currents to mitiglinide most closely resembled that of Kir6.2/SUR2A currents, the IC_{50} being 4.6 ± 1.6 μM ($n=5$), as compared with 3.2 μM for Kir6.2/SUR2A and 3.8 nM for Kir6.2/SUR1. As is the case for both Kir6.2/SUR2A and Kir6.2/SUR2B currents, the IC_{50} for the low-affinity site was >1 mM. The percentage of block mediated by interaction with the high-affinity site was $56 \pm 5\%$ ($n=5$). These results demonstrate that the IC_{50} of the high-affinity site for mitiglinide is around 1000 fold lower for Kir6.2/SUR1 channels than for Kir6.2/SUR2A or Kir6.2/SUR2B channels, whereas that of the low-affinity site may be similar for all three types of channel.

Low-affinity inhibition of both Kir6.2/SUR1 and Kir6.2/SUR2A channels by sulphonylureas such as tolbutamide and glibenclamide is mediated by the Kir6.2 subunit (Gribble *et al.*, 1997a; 1998). To investigate whether mitiglinide also interacts with Kir6.2, we tested the effect of the drug on Kir6.2ΔC36 (a truncated form of Kir6.2) expressed in the absence of the sulphonylurea receptor. Figure 4 shows that mitiglinide blocked Kir6.2ΔC36 currents with very low affinity, 1 mM mitiglinide producing less than 20% inhibition (it was not possible to test the effect of higher concentrations

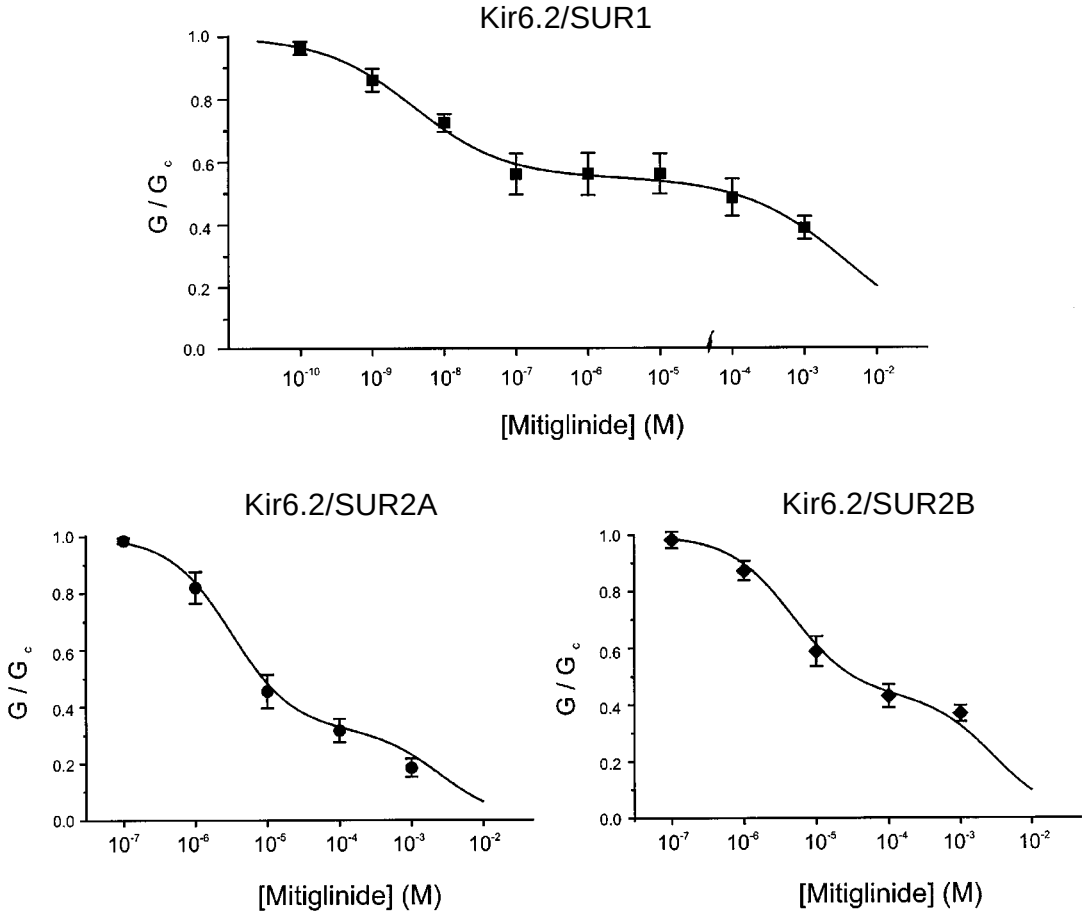

Figure 3 Concentration-response relationships for Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B currents. Mitiglinide concentration-response relationships measured for Kir6.2/SUR1, Kir6.2/SUR2A or Kir6.2/SUR2B currents. The macroscopic conductance in the presence of mitiglinide (G) is expressed as a fraction of its mean amplitude in the absence of the drug (G_c). The symbols represent the mean, and the vertical bars indicate 1 s.e.mean. Kir6.2/SUR1 data ($n=5$) were fit with a two-site model (eqn 1): $IC_{50(1)}=3.8$ nM, h1 = 0.7, $IC_{50(2)}=4.1$ mM, h2 = 0.6, L = 0.55. Kir6.2/SUR2A and Kir6.2/SUR2B data were fit with the same two-site model (eqn. 1), assuming that both Hill coefficients were unity. For Kir6.2/SUR2A ($n=5$), $IC_{50(1)}=3.2$ nM, $IC_{50(2)}=2.5$ mM, L = 0.32. For Kir6.2/SUR2B data ($n=5$), $IC_{50(2)}=4.6$ μM, $IC_{50(1)}=2.9$ mM, L = 0.44. The lines were drawn according to the mean fits for the individual patches.

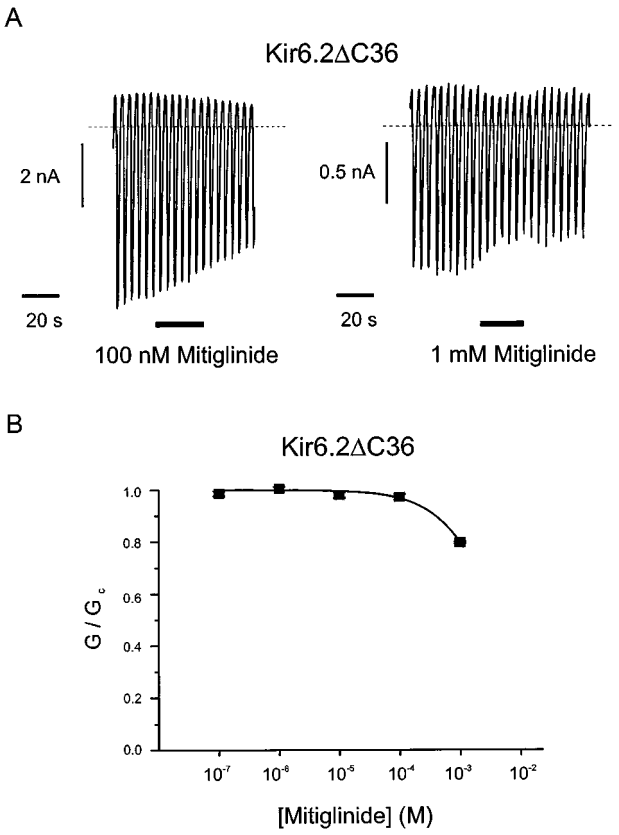

Figure 4 Inhibition of Kir6.2ΔC36 currents by mitiglinide. (A) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes expressing Kir6.2ΔC36. Mitiglinide (0.1 μM or 1 mM) was added as indicated by the bars. (B) Mitiglinide concentration-response relationships measured for Kir6.2ΔC36 currents. The macroscopic conductance in the presence of mitiglinide (G) is expressed as a fraction of its mean amplitude in the absence of the drug (G_c). The symbols represent the mean, and the vertical bars indicate 1 s.e.mean. The line is drawn through the points by eye.

because of difficulty in dissolving the compound). This result would be consistent with low-affinity inhibition of Kir6.2/SUR1 channels being associated with the Kir6.2 subunit and the high-affinity site being located on SUR1. Likewise, the high-affinity site on Kir6.2/SUR2 variant channels lies on SUR2 and low-affinity inhibition is mediated by Kir6.2.

We next investigated whether mitiglinide binds to the same (or an overlapping) site on SUR1 as tolbutamide, by examining the effect of mitiglinide on channels containing a mutant form of SUR1, SUR1-S1237Y. This mutation has previously been shown to abolish high-affinity tolbutamide inhibition of K_{ATP} currents, *via* disruption of sulphonylurea binding (Ashfield *et al*., 1999). As shown in Figure 5, Kir6.2/SUR1-S1237Y currents were unaffected by 100 nM mitiglinide but were blocked by 65.5±3.0% ($n=9$) in the presence of 10 μM of the drug. There was no significant difference in the extent of inhibition of Kir6.2/SUR1, Kir6.2/SUR1-S1237Y or KIR6.2/SUR2A currents produced by 10 μM mitiglinide. However, the block of Kir6.2/SUR1-S1237Y currents was readily reversible, which is in contrast to the block of Kir6.2/SUR1 currents but similar that of Kir6.2/SUR2-type currents.

Discussion

Our results demonstrate that S 21403 (mitiglinide) blocks Kir6.2/SUR1 currents with high affinity (IC_{50}, 4 nM), by interacting with a site located on SUR1. The IC_{50} value is in good agreement with the K_i determined for the high-affinity binding site for the drug by displacement of [^{3}H]-glibenclamide to hamster insulinoma (HIT) cell microsomes (13 nM, Ohnota *et al*., 1994). This is consistent with the fact that native K_{ATP} channels in pancreatic β-cells comprise Kir6.2 and SUR1 subunits. High-affinity block of Kir6.2/SUR1 currents by mitiglinide is similar to that found for glibenclamide ($IC_{50}=\sim4$ nM; Gribble *et al*., 1998), but more potent than that observed for tolbutamide ($IC_{50}=2$ μM; Gribble *et al*., 1998). The displacement studies further suggest that the binding sites for mitiglinide and glibenclamide must either overlap or be able to interact allosterically.

In contrast to glibenclamide, which blocks cloned K_{ATP} channels with similar potency (Gribble *et al*., 1998), the potency of mitiglinide on Kir6.2/SUR2A (cardiac type) and Kir6.2/SUR2B (smooth muscle type) K_{ATP} channels was about 1000 fold lower than on Kir6.2/SUR1 (β-cell type) channels, the IC_{50} being 3 μM, 5 μM and 4 nM respectively. This suggests that some part of SUR that differs between SUR1 and SUR2 is responsible for the difference in binding affinities. The similarity in the affinities of SUR2A and SUR2B indicates that the last 42 amino acids (the only region of sequence difference in these two isoforms) are unlikely to be important for selectivity.

SUR1 containing the mutation S1237Y lacks both high-affinity tolbutamide block and [^{3}H]-glibenclamide binding (Ashfield *et al*., 1999). We found that Kir6.2/SUR1-S1237Y channels were blocked by less than 10% by 100 nM mitiglinide, a concentration that saturates the high-affinity site on SUR1. These data suggest that SUR1 possesses a high-affinity site for mitiglinide that is either identical to, or overlaps with, the tolbutamide binding site of SUR1. In contrast, 10 μM mitiglinide blocked Kir6.2/SUR1-S1237Y channels as much as Kir6.2/SUR1 and Kir6.2/SUR2A channels, but Kir6.2ΔC channels were not affected by this drug concentration. This further suggests that mitiglinide binds with intermediate affinity to a site that is common to SUR1-S1237Y and SUR2.

As is the case for glibenclamide, high-affinity block of Kir6.2/SUR1 currents by mitiglinide reversed only slowly under our experimental conditions. In contrast, the intermediate-affinity block of Kir6.2/SUR2A, Kir6.2/SUR2B and Kir6.2/SUR1-S1237Y currents was readily reversible. By analogy with the mechanism proposed for glibenclamide block of SUR1 (Gribble *et al*., 1998), we suggest that mitiglinide binds to SUR1 simultaneously at the tolbutamide binding site and at another site, and that this accounts for the slow off-rate of the drug on Kir6.2/SUR1 currents. Our data do not allow us to conclude whether or not this second site is the same as the intermediate-affinity site of SUR2, although this is clearly a possibility.

The benzoic acid derivative meglitinide also exhibits high-affinity inhibition of Kir6.2/SUR1 (IC_{50} ~0.5 μM; Gribble *et al*., 1998). Its inhibitory effect on K_{ATP} currents differs in three aspects from that observed with mitiglinide. First, the block of Kir6.2/SUR1 currents by meglitinide is rapidly reversible, while that of mitiglinide is only poorly reversible

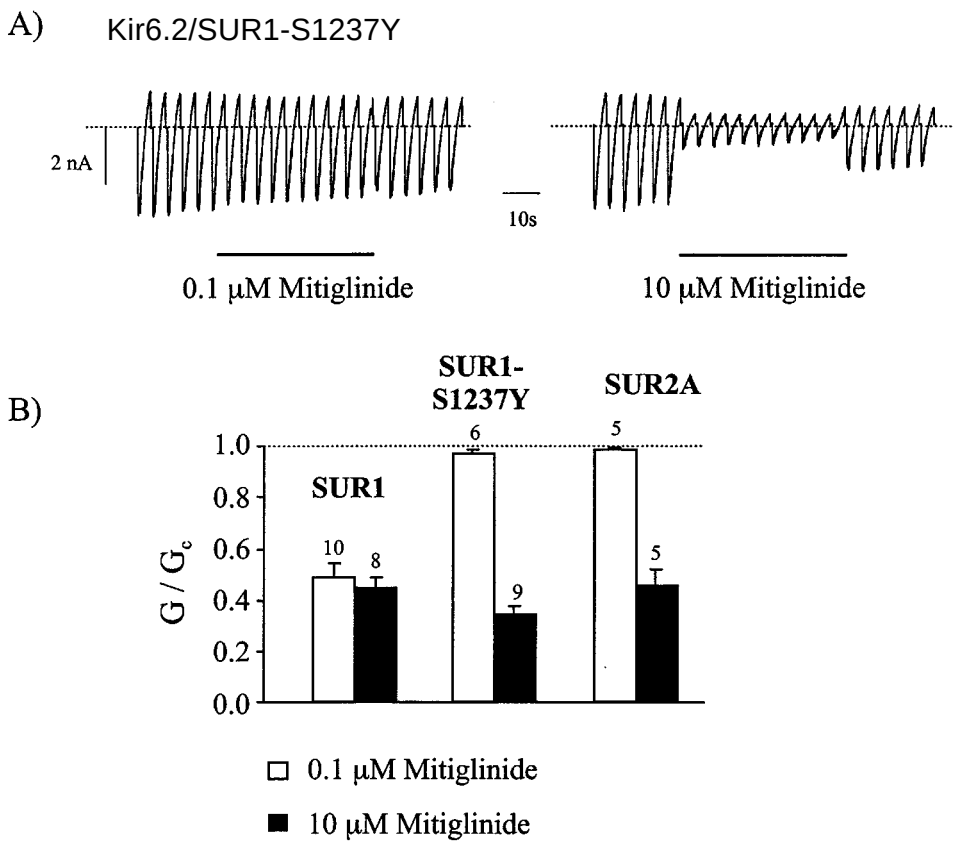

Figure 5 Inhibition of Kir6.2/SUR1-S1237Y currents by S21403. (A) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes expressing Kir6.2 and SUR1-S1237Y. Mitiglinide (0.1 or 10 μM) was added as indicated by the bar. (B) Mean conductance recorded in the presence of either 0.1 or 10 μM mitiglinide, expressed relative to the mean of that recorded before and after exposure to the drug, for the channels indicated. The number of patches is given above each bar.

on the time scale of our electrophysiological experiments. Secondly, the affinity of SUR1 for mitiglinide is much greater than that of meglitinide, a finding which may account for the differences in reversibility. Thirdly, meglitinide, but not mitiglinide, blocks both Kir6.2/SUR1 and Kir6.2/SUR2A channels with similar potency. In contrast to the wild-type channel, Kir6.2/SUR1-S1237Y channels are blocked to a similar extent by 10 μM meglitinide and 10 μM mitiglinide (~65%; this study and Ashfield *et al*., 1999). Although the structures of the two drugs are rather different, they both contain a hydrophobic substituted carbon acidamide (Figure 1). It is therefore possible that they bind to overlapping sites but that, in addition, mitiglinide binds to the tolbutamide-binding site. This may account for the higher affinity of mitiglinide than meglitinide and its slower reversibility in electrophysiological experiments.

Some very low-affinity inhibition by mitiglinide was observed for Kir6.2/SUR1, Kir6.2/SUR2A, Kir6.2/SUR2B and Kir6.2ΔC36 currents. Because of limited solubility, it was not possible to investigate the effect of mitiglinide at concentrations greater than 1 mM, and it was therefore not possible to study the low-affinity block in any detail. Given that it was also observed for Kir6.2ΔC36 currents, however, it seems likely to be a property of the Kir6.2 subunit (although we cannot exclude a small additional effect on SUR). It is unlikely to be of clinical relevance because the therapeutic concentration of the drug in diabetic patients is much lower.

In the excised patch recordings reported here, interaction of mitiglinide with the high-affinity site produces a maximum of ~45% block of Kir6.2/SUR1 currents. Similar findings have been reported for other sulphonylureas for both native β-cell K_{ATP} channels (Zünckler *et al*., 1988) and for cloned Kir6.2/SUR1 channels (Gribble *et al*., 1998; 1999). By contrast, high-affinity inhibition by sulphonylureas is complete when measured in the intact cell (Trube *et al*., 1986; Gribble *et al*., 1997a). In the case of tolbutamide, this anomaly has been shown to result from the presence of intracellular MgADP in the intact cell (Zünckler *et al*., 1988; Gribble *et al*., 1997b). It seems likely that, by analogy, high-affinity inhibition of Kir6.2/SUR1 currents by mitiglinide will also be complete when measured in intact cells.

In conclusion, our results suggest that concentrations of mitiglinide below 100 nM should block the β-cell type of K_{ATP} channel (which comprises Kir6.2/SUR1), but produce little cross-reactivity with either the cardiac (Kir6.2/SUR2A) or smooth muscle (Kir6.2/SUR2B) types of K_{ATP} channel. This conclusion is based on experiments in which the drug was applied to the intracellular surface of excised membrane patches and therefore should not be extrapolated directly to the whole-cell condition, because cytosolic substances may modify the drug properties (e.g. Ventakesh *et al*., 1991;

Gribble *et al*., 1997a,b). Nevertheless, the data indicate that mitiglinide shows some specificity for the β-cell type of K_{ATP} channel in excised membrane patches and provide an encouraging basis for further experiment and drug development.

This work was supported by the Institut de Recherches Internationales Servier. We thank Dr Denis Ravel for advice and helpful criticism.

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(Received August 8, 2000
Revised December 11, 2000
Accepted January 19, 2001)