The antimalarial agent mefloquine inhibits ATP-sensitive K-channels

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1 The aim of this study was to determine whether antimalarial agents inhibit ATP-sensitive potassium (K_{ATP}) channels and thereby contribute to the observed side-effects of these drugs.

2 Mefloquine (10–100 μM), but not artenusate (100 μM), stimulated insulin release from pancreatic islets *in vitro*.

3 Macroscopic K_{ATP} currents were studied in inside-out patches excised from *Xenopus* oocytes expressing cloned K_{ATP} channels.

4 Mefloquine ($IC_{50} \sim 3$ μM), quinine ($IC_{50} \sim 3$ μM), and chloroquine inhibited the pancreatic β-cell type of K_{ATP} channel Kir6.2/SUR1. Artenusate (100 μM) was without effect.

5 Mefloquine and quinine also blocked a truncated form of Kir6.2 (Kir6.2ΔC36) when expressed in the absence of SUR1. The extent of block was similar to that observed for Kir6.2/SUR1 currents.

6 Our results suggest that inhibition of the β-cell K_{ATP} channel accounts for the ability of quinoline-based antimalarial drugs to stimulate insulin secretion, and thereby produce hypoglycaemia.

7 The results also indicate that quinoline-based antimalarial agents inhibit K_{ATP} channels by interaction with the Kir6.2 subunit. This subunit is common to β-cell, neuronal, cardiac, skeletal muscle, and some smooth muscle K_{ATP} channels suggesting that K_{ATP} channel inhibition may contribute to the other side effects of these drugs, which include cardiac conduction abnormalities and neuropsychiatric disturbances.

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Introduction

The quinoline drugs, which include quinine, quinidine, chloroquine, mefloquine and halofantrine are widely used as antimalarial agents. These drugs have a variety of side-effects, of which the most clinically important are hypoglycaemia, cardiac conduction abnormalities and neuropsychiatric disturbances (Assan *et al.*, 1995; Davis, 1997; Davis *et al.*, 1997; Jaspers *et al.*, 1996; Nosten *et al.*, 1993; Bateman & Dyson, 1996; Bhatia, 1991; Bem *et al.*, 1992). When combinations of quinoline drugs are used, toxic effects may be accentuated (Nosten *et al.*, 1993). The artemisinin derivatives are antimalarial drugs that are chemically unrelated to quinolines but which are also associated with neurological and cardiac toxicity in animal experiments (Brewer *et al.*, 1994).

Quinine causes hypoglycaemia by stimulating insulin secretion, as a consequence of its ability to close ATP-sensitive potassium (K_{ATP}) channels in the plasma membrane of pancreatic β-cells (Bokvist *et al.*, 1990). This induces a membrane depolarization that triggers calcium entry, a rise in intracellular calcium and, consequently, insulin release (Ashcroft & Rorsman, 1989). Sulphonylurea compounds used in the treatment of type 2 diabetes mellitus and imidazoline drugs such as phentolamine (Proks & Ashcroft, 1997) also close K_{ATP} channels. By contrast, a diverse class of drugs known as K_{ATP}-channel openers enhance K_{ATP} channel activity (Ashcroft & Ashcroft, 1992; Edwards & Weston, 1993). The effects of drugs that close or open K_{ATP}

channels can be mediated through different types of K_{ATP} channel subunit (Ashcroft & Gribble, 1998; 1999).

Recent molecular studies have revealed that the K_{ATP} channel is an octameric complex of two types of subunit, Kir6.2 and SUR, which coassemble with 4:4 stoichiometry (Sakura *et al.*, 1995; Inagaki *et al.*, 1995; Clement *et al.*, 1997). Kir6.2 forms the pore of the K_{ATP} channel and contains the ATP-inhibitory site, while the sulphonylurea receptor, SUR, acts as a regulatory subunit that endows the channel with sensitivity to sulphonylureas and K_{ATP}-channel openers (Aguilar-Bryan *et al.*, 1995; Inagaki *et al.*, 1996; Tucker *et al.*, 1997). Although the wild-type K_{ATP} channel requires both types of subunit (Kir6.2 and SUR1) to make a functional channel, 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.

K_{ATP} channels are also found in a wide variety of other tissues including cardiac, smooth and skeletal muscle and some neurones of the central nervous system (Ashcroft & Ashcroft, 1992; Ashcroft & Gribble, 1999). There is considerable variation in the sensitivity of K_{ATP} channels in different tissues to both sulphonylureas and K-channel openers. Both of these drugs bind to the SUR subunit and the observed variability results from the presence of different types of SUR subunit in different tissues (Inagaki *et al.*, 1995; 1996; Isomoto *et al.*, 1996). In contrast, phentolamine does

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not interact with the sulphonylurea receptor, and it is equally effective at blocking Kir6.2ΔC26 channels as Kir6.2/SUR1 channels (Proks & Ashcroft, 1997). The aim of this study was to determine whether the antimalarial drugs mefloquine and artenusate functionally interact with one or other type of K$_{ATP}$ channel subunit, and thereby contribute to the observed side-effects of these drugs.

Methods

Molecular biology

Mouse Kir6.2 (GenBank D50581) and rat SUR1 (Genbank L40624) were used in this study. A 36 amino acid C-terminal deletion of mouse Kir6.2 (Kir6.2ΔC36) was made by introduction of a stop codon at the appropriate residue using site-directed mutagenesis (Tucker *et al.*, 1997). Constructs were subcloned into the pBF expression vector and synthesis of capped mRNA was carried out using the mMessage mMachine large-scale *in vitro* transcription kit (Ambion, Austin, TX, U.S.A.).

Electrophysiology

Female *Xenopus laevis* were anaesthetized with MS222 (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. Once the wound had completely healed, the second ovary was removed in a similar operation and the animal was then killed by decapitation whilst under anaesthesia. Immature stage V-VI *Xenopus* oocytes were manually defolliculated, injected with ∼2 ng of mRNA encoding Kir6.2ΔC36 or coinjected with mRNAs encoding Kir6.2 (∼0.05 ng) and SUR1 (∼2 ng), and studied 1–4 days after injection (Gribble *et al.*, 1997).

Macroscopic currents were recorded from giant inside-out patches using an EPC7 patch-clamp amplifier (List Electronik, Darmstadt, Germany) at 20–24°C (Gribble *et al.*, 1997). The holding potential was 0 mV and currents were evoked by repetitive 3 s voltage ramps from −110 mV to +100 mV. Currents were filtered at 0.2 kHz, digitized at 0.5 kHz using a Digidata 1200 Interface and analysed using pClamp software (Axon Instruments, Burlingame, U.S.A.). The pipette solution contained (mM): KCl 140, MgCl$_2$ 1.2, CaCl$_2$ 2.6, and HEPES 10 (pH 7.4 with KOH). The internal (bath) solution contained (mM): KCl 110, MgCl$_2$ 1.4, KOH 30, EGTA 10, and HEPES 10 (pH 7.2 with KOH). Drugs were prepared as 1000× stock solutions in DMSO (mefloquine; Larium®, Roche, Basel, Switzerland), ethanol (artesunate; Guilin no.2 Pharmaceutical Factory, Giangxi, China) or water (quinine and chloroquine; Sigma) and added to the internal solution as required.

Insulin secretion

Islets were isolated from mouse pancreas by collagenase digestion (type 11, Sigma) and hand-picking. They were cultured overnight in RPMI medium containing 8.3 mM glucose (1 ml per dish) at 37°C in a humidified atmosphere of 95% air/5% CO$_2$. Next day, the medium was replaced with fresh RPMI medium with or without (for control) the test compound and islets were cultured for up to 6 days. Aliquots of the medium were collected at 48, 96 and 140 h for insulin assay, and replaced with fresh media. Insulin was assayed using a Pharmacia Insulin RIA 100 kit, which shows 100% cross-reactivity with rodent insulin, and was quantitated as pmol of insulin release per islet per 48 h. Secretion was compared with that of control islets isolated from the same batch, incubated in RPMI containing 8.3 mM glucose.

Results

We first tested the effects of the quinoline-based antimalarial drugs quinine and mefloquine, and the structurally unrelated artemisinin derivative artenusate, on insulin secretion. The basal rate of insulin secretion from islets incubated in control solution was 2.3±1.2 pmol islet^{-1} 24 h^{-1} ($n=18$). In the presence of 10 μM mefloquine, insulin secretion was approximately double that of control islet cultures. Islets treated with 100 μM mefloquine, however, showed a ∼5 fold increase in insulin secretion ($P=0.002$; Figure 1). This degree of stimulation was similar to that produced by 0.1 mM IBMX plus 20 mM glucose, which generally induces near-maximal insulin secretion. Artesunate (100 μM) did not affect insulin secretion.

To investigate whether the stimulatory effect of antimalarial drugs is mediated *via* inhibition of K$_{ATP}$ channel activity, we tested their effect on the cloned β-cell K$_{ATP}$ channel (Kir6.2/SUR1) expressed in *Xenopus* oocytes. These channels show properties typical of native β-cell K$_{ATP}$ channels (Gribble *et al.*, 1997). Macroscopic currents were recorded in excised membrane patches, and test solutions were applied to the intracellular membrane surface. Figure 2 shows that Kir6.2/SUR1 currents were blocked by quinine, mefloquine, chloroquine and quinidine, but were only slightly affected by the structurally unrelated compound artesunate, consistent with the effects of the drugs on insulin secretion from islets *in vitro*. Half-maximal channel inhibition was produced by ∼3 μM quinine or mefloquine, and <100 μM chloroquine. The block was voltage independent and was slowly reversible. The ability of quinoline drugs to inhibit the β-cell K$_{ATP}$ channel may explain their ability to stimulate insulin secretion.

There is currently controversy about whether it is safe to use combinations of quinolines, including mefloquine and quinine, in a clinical setting, because of the possibility of drug interactions (Nosten *et al.*, 1993; Palmer *et al.*, 1993; World
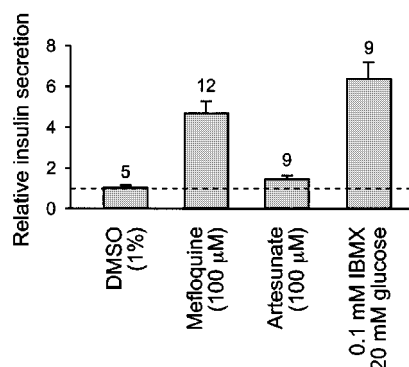

Figure 1 Effect of antimalarial drugs on insulin secretion. Batches of islets were incubated for 6 days in the presence of the test solution, and the culture medium was collected every 48 h for insulin assay. The rate of insulin secretion is expressed relative to that of control islets from the same batch. Data collected over the 6 day period is pooled, and the number of insulin assays is given above each bar. The dashed line indicates the rate of insulin secretion from control islets exposed to 8.3 mM glucose but no drug. $P=0.002$ vs DMSO, for mefloquine; and $P=0.1$ vs DMSO for artenusate.

Health Organization, 1996). We therefore tested the effect of this drug combination on K$_{ATP}$ channel activity. As shown in Figure 2, when quinine and mefloquine were applied together, the extent of block was consistent with a simple model in which both drugs compete for the same site.

To determine whether the site at which quinoline-related drugs interact lies on Kir6.2 or on SUR1, we tested the drugs on a truncated form of Kir6.2 (Kir6.2ΔC36) which expresses functional channels in the absence of SUR1. Figure 3 shows that Kir6.2ΔC36 currents were inhibited by quinine and mefloquine in a similar manner to Kir6.2/SUR1 currents. This result indicates that the inhibitory effect of these drugs is not mediated by SUR1, and further suggests that the quinoline antimalarial agents interact either with Kir6.2ΔC36 or (possibly) with a third protein, endogenously expressed in *Xenopus* oocytes, that modulates the activity of Kir6.2ΔC36. In this respect, the quinoline antimalarial agents resemble imidazoline drugs such as phentolamine (Proks & Ashcroft, 1997).

Discussion

Our results demonstrate that the quinoline-based drug mefloquine inhibits the β-cell K$_{ATP}$ channel and stimulates islet insulin secretion, and confirm previous findings that this is also the case for quinine (Bokvist *et al.*, 1990). They further show that inhibition is mediated by interaction of the drugs with the Kir6.2 subunit of the K$_{ATP}$ channel, rather than
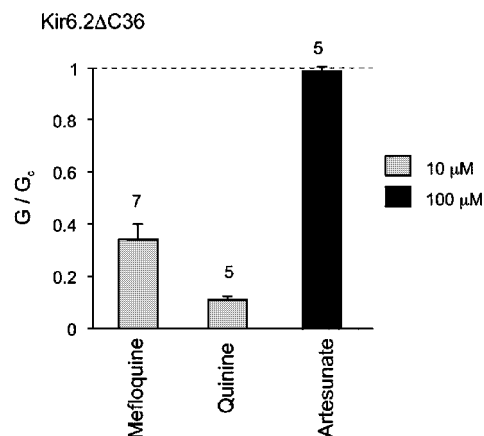

Figure 3 The mean effect of antimalarial drugs on Kir6.2ΔC36 currents. The conductance in the presence of the drug (G) is expressed as a fraction of the mean conductance in control solution before and after exposure to the drug (Gc). The number of patches is given above the bars. The dashed line indicates the control conductance in the absence of drug.
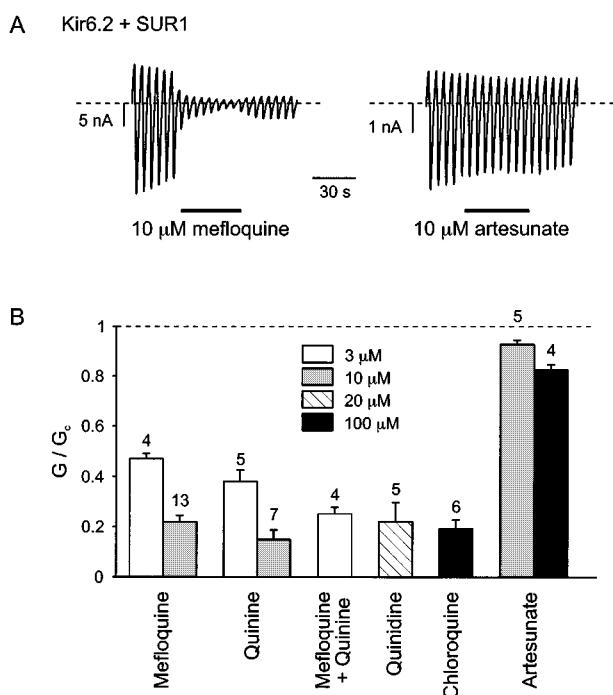
with the regulatory SUR subunit. Thus, quinoline drugs resemble imidazolines, rather than sulphonylureas, in their mode of action. The structurally unrelated compound artesunate was without effect on K$_{ATP}$ channel activity.

The raised serum insulin levels found in human subjects given quinoline antimalarial agents may, therefore, be due to the inhibition of β-cell K$_{ATP}$ channels. Serum concentrations of mefloquine achieved during treatment are 2–6 μM and the range for quinine is 30–60 μM. Mefloquine is 98% or more protein bound (free serum concentration ≈0.1 μM), while 88–93% of quinine is bound to plasma proteins (free serum concentration ≈5 μM). Thus the free serum concentrations of mefloquine, but not those of quinine, are low compared to the media concentrations that produce half-maximal K$_{ATP}$ channel inhibition in our *in vitro* experiments (∼3 μM). In addition, the media mefloquine concentrations needed to stimulate measurable insulin secretion by islet cell cultures were greater than the predicted free drug concentrations *in vivo*.

Although the effects of quinoline antimalarial agents are considered to reflect the free drug concentrations, other factors also have to be considered. In conventional pharmacodynamic analysis, the rate constant for elimination of drug from the 'effect' compartment is the prime determinant of the response (Sheiner *et al.*, 1979). We found that K$_{ATP}$ channel block was only slowly reversible on the time scale of our electrophysiological experiments, which may contribute to an enhanced efficacy *in vivo*. The biological activity of mefloquine may also be influenced by its association with lipoproteins (Foley & Tilley, 1997). Detailed analysis of β-cell function and free serum concentrations of quinine, its diastereoisomer quinidine, and mefloquine given in conventional doses to healthy volunteers suggests a drug-specific concentration-effect relationship (Davis, 1997). At therapeutic doses, quinine has the most potent stimulatory effect on β-cell function but mefloquine has the greatest hyperinsulinaemic effect for a given free serum concentration. Thus, direct comparison between quinoline drugs, and of our *in vitro* data to the *in vivo* situation, must be made with caution.

It is possible that mefloquine has other effects on glucose handling, in addition to inhibition of β-cell K$_{ATP}$ channel activity. Thus, mefloquine, like quinine (Bokvist *et al.*, 1990),

Figure 2 Effect of antimalarial drugs on Kir6.2-SUR1 currents. (A) Macroscopic currents recorded in inside-out patches from oocytes coinjected with Kir6.2 and SUR1 mRNAs. Drugs were applied as indicated by the bars. The voltage was ramped repetitively from −110 to +100 mV from a holding potential of 0 mV. The dashed line indicates the zero current level. (B) Mean effect of antimalarial agents, at the concentrations indicated, on Kir6.2/SUR1 currents. The conductance in the presence of the drug (G) is expressed as a fraction of the mean conductance in control solution before and after exposure to the drug (G$_c$). The number of patches is given above the bars. The dashed line indicates the control conductance level in the absence of drug.

might block other types of K$^+$ channels in the β-cell, thereby enhancing insulin secretion. Furthermore, mefloquine is metabolized to quinoline-carboxylic acid, which is structurally related to quinolinic acid, a well-known inhibitor of gluconeogenesis (Blackshear *et al.*, 1975). The hypothesis that this mefloquine metabolite inhibits gluconeogenesis *in vivo* was not assessed in the present study.

We found no evidence for a synergistic interaction between mefloquine and quinine on Kir6.2/SUR1 currents. When both drugs were present, the extent of block was simply the sum of that expected for the two compounds individually, consistent with competition for the same binding site on the K$_{ATP}$ channel. This finding adds weight to the hypothesis that the hyperinsulinaemic effect of mefloquine is mediated by K$_{ATP}$ channel inhibition, in common with other quinoline drugs.

Kir6.2 serves as a common pore-forming subunit for K$_{ATP}$ channels in β-cells, cardiac and skeletal muscle and neurones of the hippocampus, cerebellum and substantia nigra (Ashcroft & Gribble, 1999; Inagaki *et al.*, 1996; Karschin *et al.*, 1998; Liss *et al.*, 1999). Our results therefore suggest that K$_{ATP}$ channels in all these tissues may also be affected by therapeutic concentrations of quinoline antimalarial drugs. Studies on native tissues are now required to confirm our findings. In cardiac muscle, K$_{ATP}$ channel opening during ischaemia reduces the QT interval of the electrocardiogram (Nichols & Lederer, 1991). It is uncertain whether cardiac K$_{ATP}$ channels also play a role in determining the QT interval

under physiological conditions, but it is possible that their closure by quinoline drugs, especially quinine (Davis *et al.*, 1990), prolongs the QT interval during treatment of acute malaria. Likewise, although the roles of K$_{ATP}$ channels in the hippocampus and other brain regions are uncertain, our results suggest the possibility that quinoline drugs, most notably mefloquine (Bem *et al.*, 1992), might cause neurotoxicity through a direct interaction with neuronal K$_{ATP}$ channels.

The artemisinin-associated neurological and cardiac toxicity in animal experiments (Brewer *et al.*, 1994) has not been found in humans despite widespread and increasing use of this relatively safe class of drugs in human malaria (Hien & White, 1993). Our data confirm that concentrations of artesunate that are in excess of therapeutic levels (peak plasma concentrations after intravenous injection $\sim 30\ \mu$M; Batty *et al.*, 1998) have no effect on K$_{ATP}$ channel activity *in vitro*. The QT prolongation seen in animals given high doses of artemisinin drugs for long periods is, therefore, unlikely to be due to K$_{ATP}$ channel closure. However, our identification of Kir6.2 as a molecular target for commonly-used quinoline drugs may provide a specific mechanism for a range of clinically significant side-effects in humans.

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