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Mechanism of terfenadine block of ATP-sensitive K^+ channels

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1 The ATP-sensitive K^+ (K_{ATP}) channel is a complex of a pore-forming inwardly rectifying K^+ channel (Kir6.2) and a sulphonylurea receptor (SUR). The aim of the present study was to gain further insight into the mechanism of block of K_{ATP} channels by terfenadine.

2 Channel activity was recorded both from native K_{ATP} channels from the clonal insulinoma cell line RINm5F and from a C-terminal truncated form of Kir6.2 (Kir6.2Δ26), which – in contrast to Kir6.2 – expresses independently of SUR. Kir6.2Δ26 channels were expressed in COS-7 cells, and enhanced green fluorescent protein (EGFP) cDNA was used as a reporter gene. EGFP fluorescence was visualized by a laser scanning confocal microscope.

3 Terfenadine applied to the cytoplasmic side of inside-out membrane patches concentration-dependently blocked both native K_{ATP} channel and Kir6.2Δ26 channel activity, and the following values were calculated for IC_{50} (the terfenadine concentration causing half-maximal inhibition) and n (the Hill coefficient): 1.2 μM and 0.7 for native K_{ATP} channels, 3.0 μM and 1.0 for Kir6.2Δ26 channels.

4 Terfenadine had no effect on slope conductance of either native K_{ATP} channels or Kir6.2Δ26 channels. Intraburst kinetics of Kir6.2Δ26 channels were not markedly affected by terfenadine and, therefore, terfenadine acts as a slow channel blocker on Kir6.2Δ26 channels. Terfenadine-induced block of Kir6.2Δ26 channels demonstrated no marked voltage dependence, and lowering the intracellular pH to 6.5 potentiated the inhibition of Kir6.2Δ26 channels by terfenadine.

5 These observations indicate that terfenadine blocks pancreatic B-cell K_{ATP} channels *via* binding to the cytoplasmic side of the pore-forming subunit. The presence of the pancreatic SUR1 has a small, but significant enhancing effect on the potency of terfenadine.
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Abbreviations: EGFP, enhanced green fluorescent protein; HERG, human ether a-go-go related gene; I_{Kr}, cardiac rapid delayed rectifier K^+ current; K_{ATP} channels, ATP-sensitive K^+ channels; SUR, sulphonylurea receptor

Introduction

Terfenadine is a nonsedating H_1 receptor antagonist which undergoes extensive first-pass hepatic metabolism, by the cytochrome P-450 enzyme CYP3A4, to its pharmacologically active metabolite terfenadine carboxylate (fexofenadine). In patients taking either excessive dosages or using concomitant drugs that are known to inhibit cytochrome P-450 3A4 activity (such as ketoconazole or erythromycin) or in patients with impaired liver function, terfenadine may accumulate to plasma levels of about 0.2 μM. In rare cases terfenadine induces QT prolongation and life-threatening torsades de pointes cardiac arrhythmias (Woosley *et al*., 1993). A likely mechanism for QT prolongation and torsades de pointes is blockade of the rapid component of the cardiac delayed rectifier K^+ current (I_{Kr}). HERG (human ether a-go-go related gene) channels, which are likely to present I_{Kr} channels in cardiac myocytes, and I_{Kr} channels are blocked by terfenadine at nanomolar concentrations (Salata *et al*., 1995; Suessbrich *et al*., 1996; Roy *et al*., 1996).

In addition, terfenadine blocks other cardiac K^+ channels, e.g. ATP-sensitive K^+ (K_{ATP}) channels (Nishio *et al*., 1998). Under ischaemic conditions the activation of K_{ATP} channels causes shortening of the cardiac action potential and, by decreasing Ca^{2+}-entry and ATP consumption, this may protect the cell from irreversible impairment of its cellular functions. Under these conditions, blockers of K_{ATP} channels would be expected to increase myocardial damage. On the other hand, blockers of K_{ATP} channels might have anti-arrhythmic effects on re-entrant ventricular arrhythmias during early myocardial ischaemia (review in Wilde & Janse, 1994).

The K_{ATP} channel is an octameric 4:4 complex of a pore-forming inwardly rectifying K^+ channel (Kir6.2) subunit and a regulatory subunit, the sulphonylurea receptor (SUR). Two different sulphonylurea receptor genes have been cloned, SUR1 and SUR2, that show different tissue expression and encode proteins with different pharmacological sensitivity. K_{ATP} channels in pancreatic B-cells comprise Kir6.2 and SUR1 subunits, while those of cardiac muscle have been suggested to be composed of Kir6.2 and a splice variant of SUR2, SUR2A (review in Aguilar-Bryan *et al*., 1998). SURs have been shown to represent the receptors for potassium channel openers and sulphonylureas. There are, however, pharmacological agents that block K_{ATP} channels by interaction with the pore-forming subunit: the α-adrenoceptor antagonist phentolamine (Proks & Ashcroft, 1997) and the class Ia antiarrhythmic agents cibenzoline (Mukai *et al*., 1998) and disopyramide (Zünkler *et al*., 2000). The Kir6.2 channel subunit forms only very few functional K^+ channels when expressed in the absence of SUR; however, truncation of the carboxy terminus of Kir6.2 by either the last 26 (Kir6.2Δ26) or 36 amino acids (Kir6.2Δ36) produces high K_{ATP} channel activity in the absence of SUR (Tucker *et al*., 1997).

The aim of the present study was to further investigate the mechanism of action of terfenadine on K_{ATP} channels. Therefore, the effects of terfenadine on native K_{ATP} channels from the clonal insulinoma cell line RINm5F and on

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Kir6.2Δ26 channels were determined in the present study, and the results were compared to those obtained previously on native cardiac K_{ATP} channels (Nishio *et al*., 1998).

Methods

Culture of RINm5F cells

Cells from the clonal insulinoma cell line RINm5F were grown in RPMI 1640 medium (10 mM glucose), supplemented with 10% foetal calf serum, penicillin (100 u ml^{-1}), streptomycin (100 μg ml^{-1}) and 2 mM glutamine at 37°C in a humidified atmosphere of 5% CO_2. Cells were passaged at 7 day intervals, plated onto 35 mm dishes and used 1–4 days after plating.

Culture and transfection of COS-7 cells

Culture and transfection of African green monkey kidney COS-7 cells were performed as described previously (Zünkler *et al*., 2000) with the following modifications. COS-7 cells were plated at a density of 2×10^6 cells per dish (85 mm diameter) and cultured (at 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) with 1 g l^{-1} glucose supplemented with 10% foetal calf serum, penicillin (100 u ml^{-1}), streptomycin (100 μg ml^{-1}) and 2 mM glutamine. COS-7 cells were transiently transfected with the pcDNA3 vector containing the coding sequence of Kir6.2Δ26 (generously provided by Dr F. Ashcroft, Oxford University) and of EGFP (enhanced green fluorescent protein). The plasmid concentrations were as follows (μg per 85 mm dish containing 4 ml of medium): 3 Kir6.2Δ26, 3 EGFP. The transfections were carried out using 20 μl GenePORTER and Opti-MEM (Gibco-BRL). Single channel currents were studied 48–96 h after transfection.

Electrophysiological recording

To aid in visualizing transfected cells, EGFP cDNA was used as a reporter gene and inside-out membrane patches were obtained only from cells expressing EGFP. An inverted Zeiss LSM-510 laser-scanning confocal imaging system with an argon laser (excitation wavelength: 488 nm) was employed and EGFP-induced fluorescence (>515 nm) was detected using a photomultiplier tube.

A standard patch-clamp technique was used in the inside-out configuration (Hamill *et al*., 1981). Unless otherwise stated, the pipette potential was held constant at +60 mV (membrane potential: −60 mV). Pipette resistances ranged between 3 and 7 MΩ when filled with solution B. Inward currents flowing from the pipette to the bath solution are indicated by downward deflections. Experiments were performed at room temperature (20–22°C).

Data analysis

Current signals were filtered at 2 kHz with the help of a Bessel filter (902, Frequency Devices, Haverhill, Massachusetts, U.S.A.) and sampled at 10 kHz using a microcomputer equipped with A–D and D–A converters (Digidata 1200 Interface, Axon Instruments, Foster City, CA., U.S.A.) and pCLAMP 6.0.2 software (Axon Instruments). Channel activity ($N\cdot P_O$) was calculated as:

$$N \cdot P_O = 1/T\cdot\Sigma\ n_i \cdot t_i \qquad (1)$$

where N was the number of available channels in the patch (estimated as the maximum number of open channels), P_O was the open probability of a single channel, t_i was the time spent at each current level n_i, and the total recording time (T) was usually 20–30 s.

For analysis of intraburst channel behaviour of Kir6.2Δ26 channels, intraburst open and closed times were each fitted with a single exponential function and closed times that exceeded 2.5 ms were omitted.

The concentration-response relationship for inhibition of channel activity by terfenadine was calculated according to the Hill equation:

$$I/I_C = 1 - A^n/(A^n + IC_{50}{}^n) \qquad (2)$$

where A = concentration of terfenadine, n = slope parameter (Hill coefficient), IC_{50} = half-maximally inhibitory concentration of terfenadine. I_C was the channel activity during the control periods before and after application of terfenadine and I was the channel activity in the presence of terfenadine. Equation (2) was fitted using the program Sigma Plot Windows 1.0 (Jandel Scientific).

The results are expressed as mean ± s.e.mean. Significances were calculated by the two-tailed non-paired *t*-test and $P<0.05$ was considered to be significant.

Solutions and chemicals

The bath (solution A) contained (in mM): KCl 140, $MgCl_2$ 1, EGTA 10, $CaCl_2$ 2 and HEPES 5 titrated to pH = 7.15 with KOH (free $[Ca^{2+}]$ = 50 nM; free $[Mg^{2+}]$ = 0.7 mM). In some experiments, 5 mM PIPES (piperazine-N,N′-bis-(2-ethanesulphonic acid)) buffer was used instead of HEPES buffer for solution A having a pH of 6.5. The pipette (solution B) contained (in mM): KCl 146, $CaCl_2$ 2.6, $MgCl_2$ 1.2 and HEPES 10 titrated to pH = 7.40 with KOH.

Drugs

Terfenadine was purchased from Sigma (St. Louis, MO, U.S.A.). Stock solution of 30 mM terfenadine was prepared in DMSO and applied to solution A to give the final concentrations.

Results

K_{ATP} channels from RINm5F cells and Kir6.2Δ26 channels had similar current amplitudes (5.26 ± 0.03 pA (n = 40) and 5.24 ± 0.05 pA (n = 25), respectively, at a membrane potential of −60 mV).

Terfenadine applied to the cytoplasmic side of inside-out membrane patches concentration-dependently blocked both native K_{ATP} channel and Kir6.2Δ26 channel activity, and this block was reversible after washout of terfenadine (Figure 1). According to equation (2) the following values were calculated for IC_{50} (the terfenadine concentration causing half-maximal inhibition) and n (Hill coefficient; mean values ± s.e.mean): 1.2 ± 0.2 μM and 0.7 ± 0.1 for native K_{ATP} channels, 3.0 ± 0.5 μM and 1.0 ± 0.2 for Kir6.2Δ26 channels (Figure 2). The difference of the IC_{50} values between the two types of channel was statistically significant ($P<0.05$).

Terfenadine had no effect on slope conductance of either native K_{ATP} channels or Kir6.2Δ26 channels, indicating that it does not act as a very fast open channel blocker. Intraburst open and closed times of Kir6.2Δ26 channels were not markedly affected by

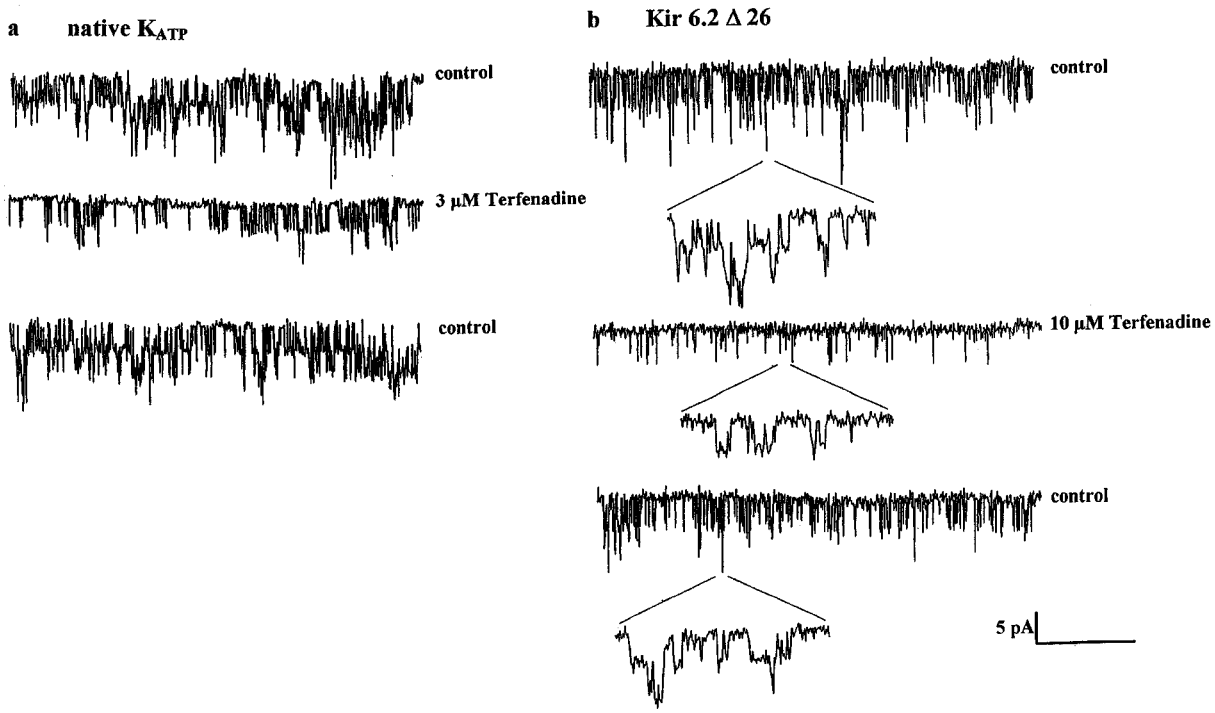

Figure 1 Effects of terfenadine on activity of K_{ATP} channels from a RINm5F cell and of Kir6.2Δ26 channels expressed in a COS-7 cell. The inside-out configuration was employed at a membrane potential of −60 mV and the pH value of the bath solution was 7.15. The traces are continuous recordings (from top to bottom) from native K_{ATP} channels (a) and from Kir6.2Δ26 channels (b), respectively (current recordings during exchange of the bath solution have been omitted). (a) Up to 4 K_{ATP} channels are simultaneously open in a patch from a RINm5F cell. Values for N * P_O were: 1.192 (control); 0.316 (3 μM terfenadine); 0.962 (control). Horizontal scale bar corresponds to 5 s. (b) Up to 3 Kir6.2Δ26 channels are simultaneously open in the patch. Values for N * P_O were: 0.115 (control); 0.042 (10 μM terfenadine); 0.089 (control). Segments of channel activities from the continuous traces are shown below each trace on an expanded time scale to illustrate the short open times of Kir6.2Δ26 channels. Horizontal scale bar corresponds to 2 s for the continuous traces and 20 ms for the expanded segments. Note the faster kinetics of Kir6.2Δ26 channels compared to native K_{ATP} channels.

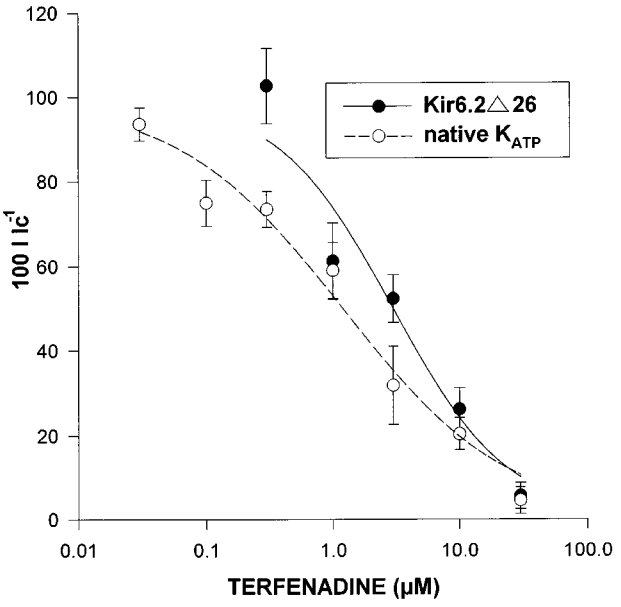

Figure 2 Concentration-response relationship for the inhibition of K_{ATP} channel activity from RINm5F cells and of Kir6.2Δ26 channel activity by terfenadine. The ordinate represents channel activity in the presence of terfenadine (I) in per cent of the channel activity in the absence of terfenadine (control; I_C). The abscissa indicates the terfenadine concentration (logarithmic scale). Points represent means and the vertical lines the s.e.m. Numbers of observations were 3–13 for each terfenadine concentration. The lines are fits to equation (2).

terfenadine: mean open and closed times were 0.61 ± 0.03 ms and 0.26 ± 0.02 ms, respectively, under control conditions, and 0.59 ± 0.03 ms and 0.26 ± 0.01 ms, respectively, in the presence of 3 μM terfenadine (data were obtained from seven experiments). Therefore, terfenadine seems to reduce the open probability of Kir6.2Δ26 channels either by reducing burst duration or by prolonging interburst interval and thus acts as a slow channel blocker. An analysis of K_{ATP} channel kinetics from RINm5F cells was not possible due to the large number of channels active in inside-out membrane patches.

Voltage dependence of block of Kir6.2Δ26 channels by terfenadine was tested in experiments where activity of Kir6.2Δ26 channels was recorded at both −60 mV and +60 mV membrane potential. Kir6.2Δ26 channels showed slight inward rectification (current amplitudes were 3.69 ± 0.09 pA (n = 7) at a membrane potential of +60 mV). No voltage dependence of 3 μM terfenadine-induced block was found: channel activity was reduced by 44.3 ± 6.2% at −60 mV and by 44.1 ± 9.8% at +60 mV membrane potential, respectively (n = 7).

Lowering the pH to 6.5 enhanced the inhibition of Kir6.2Δ26 channel activity by terfenadine, since at this pH 3 μM terfenadine blocked channel activity by 69.7 ± 4.7% at 3 μM (n = 10). This value was significantly different from the extent of terfenadine-induced block at pH 7.15 (52.3 ± 5.7% at 3 μM, Figure 2).

Discussion

It has been demonstrated in the present study that terfenadine blocks both K_{ATP} channels from RINm5F cells and Kir6.2Δ26 channels. The half-maximally inhibitory terfenadine concentrations were 1.2 μM and 3.0 μM, respectively. Therefore, block of pancreatic K_{ATP} channels by terfenadine seems to be mediated by the pore-forming subunit. The presence of the pancreatic SUR1 has a small, but significant enhancing effect on the potency of terfenadine.

The terfenadine concentrations which are necessary to block pancreatic K_{ATP} channels and the pore-forming subunit are much higher than free plasma concentrations obtained under conditions of terfenadine accumulation (<10 nM, taking 97% plasma protein binding into account (Slater *et al*., 1999)).

Inhibition of Kir6.2Δ26 channel activity by terfenadine was more intense at pH 6.5 when compared to pH 7.15. This pH dependence cannot be explained by different proportions of protonated terfenadine at the pH values studied. Terfenadine is a base with a pK_a value of 8.6 and, therefore, most of the terfenadine molecules in the internal solution are present in the positively charged form at pH 6.5 and 7.15. The pH dependence must be attributed to Kir6.2Δ26 channels, which are transformed into a state of higher affinity for terfenadine at lower pH values. It has previously been shown that lowering the pH value in the intracellular solution from 7.15 to 6.5 reduces both the amplitude and the open probability of single Kir6.2Δ26 channels (Zünkler *et al*., 2000). Therefore, it is possible that the terfenadine sensitivity of Kir6.2Δ26 channels is enhanced under acidic conditions as a secondary consequence of pH-dependent changes in gating of the channels.

The binding site for terfenadine both on native cardiac K_{ATP} channels (Nishio *et al*., 1998) and on HERG channels (Roy *et al*., 1996; Taglialatela *et al*., 1998) seems to be located on the cytoplasmic side of the channels. It has been assumed that terfenadine accesses its binding site on the K_{ATP} channel *via* the hydrophilic pathway as a charged form (Nishio *et al*., 1998). Alternatively, the non-protonated form of terfenadine might slowly diffuse through the lipid biomembrane and reaches its binding site near the internal membrane surface.

When compared to pancreatic K_{ATP} channels and Kir6.2Δ26 channels, K_{ATP} channels from rabbit ventricular myocytes demonstrated a higher sensitivity towards terfenadine-induced block (the half-maximally inhibitory concentration was 0.19 μM in the inside-out configuration; Nishio *et al*., 1998). Furthermore, in contrast to the results obtained in the present study, block of K_{ATP} channels from rabbit ventricular myocytes by terfenadine was voltage dependent and was accompanied by flickering of the channels, suggesting that terfenadine blocks cardiac K_{ATP} channels in the open state (Nishio *et al*., 1998). A possible explanation for the different effects of terfenadine on pancreatic K_{ATP} channels and Kir6.2Δ26 channels on the one hand and on cardiac K_{ATP} channels on the other hand is that the presence of SUR affects the blocking action of terfenadine on Kir6.2, the cardiac isoform SUR2A being much more effective in this regard than the pancreatic isoform SUR1.

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