Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole

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Abstract The widely used histamine receptor antagonists terfenadine and astemizole were shown to prolong the QT interval in electrocardiographic recordings in cases of overdose or inappropriate co-medications, indicating a possible interaction with cardiac K^+ channels. Here, terfenadine and astemizole both inhibited the *h*uman *e*ther-a-go-go *r*elated *g*ene (HERG) encoded channels expressed in *Xenopus* oocytes at nanomolar concentrations in a use- and voltage-dependent fashion. In contrast, inhibition of other delayed rectifier (Kv1.1 and I_{sK}) or inward rectifier K^+ channels (IRK1) was much weaker and occurred only at high micromolar concentrations. These results suggest that blockade of HERG channels by terfenadine and astemizole might contribute to the cardiac side effects of these compounds.

Key words: Histamine receptor antagonist; HERG; K^+ channel; Torsades de pointes

1. Introduction

Terfenadine and astemizole are widely used non-sedating histamine receptor antagonists. They were shown to produce in some patients prolongation of the QT interval in electrocardiographic recordings (ECG) resulting in torsades de pointes, a life-threatening form of polymorphic ventricular tachycardia [1-3]. These observations pointed to effects of terfenadine and astemizole on repolarizing conductances in ventricular myocytes. Recently, several groups demonstrated terfenadine blockage of distinct cardiac K^+ conductances [4,5], but the molecular identity of the blocked K^+ channels remains unclear. On their molecular basis, three general classes of K^+ channels can be distinguished [6]: slowly activating I_{sK} channels [7], inwardly rectifying K^+ channel (for example IRK1 channels first described by Kubo et al. [8]) and delayed rectifying K^+ channels (see Ruppersberg et al. [9]). The aim of this study was to test representatives of distinct classes of K^+ channels expressed in *Xenopus* oocytes for their sensitivity to terfenadine, astemizole and other histamine receptor antagonists in order to identify the molecular basis for their action. Special attention was paid to HERG channels, which are likely to represent in cardiac myocytes the K^+ conductance I_{Kr} [10]. I_{Kr} contributes significantly to the action potential repolarizing currents, and inhibition of I_{Kr} prolongs action potential duration [11]. HERG channels expressed in *Xenopus* oocytes show identical biophysical properties as the cardiac K^+ conductance I_{Kr} [11,12]. Recently, Salata et al. [5] demonstrated terfenadine- and astemizole-mediated I_{Kr} inhibition in guinea pig cardiac myocytes. In this study we therefore tested the effects of terfenadine and astemizole on HERG expressed in *Xenopus* oocytes and compared them with their effects on other K^+ conductances.

2. Materials and methods

Handling and injection of *Xenopus* oocytes and synthesis of cRNA have been described previously in detail [13,14]. The two-microelectrode voltage or current clamp configuration was used to record currents or cell membrane potential changes from *Xenopus laevis* oocytes. In several sets of experiments oocytes were individually injected with cRNA encoding for the K^+ channels HERG [10], rat and human I_{sK} [7,15], rat Kv1.1 [13] or rat IRK1 [16]. Recordings were performed at 22°C using a Geneclamp amplifier (Axon Instruments, Foster City, USA) and MacLab D/A converter and software for data acquisition and analysis (ADInstruments, Castle Hill, Australia). Outward currents through HERG channels were in general evoked with 0.5 s depolarizing pulses from a holding potential of −80 mV to 40 mV at variable intervals as described later (filtered at 0.5 kHz). Tail currents were analyzed at −85 mV. For some experiments longer depolarizing pulses to distinct voltages were performed as described later. Outward currents through I_{sK} or Kv1.1 channels were evoked with 15 s or 0.5 s depolarizing pulses to −10 mV from a holding potential of −80 mV, and filtered at 10 Hz and 1 kHz, respectively. K^+ inward currents through inwardly rectifying IRK1 channels were evoked by polarizing the cells for 0.5 s to −120 mV from a holding potential of −40 mV (filtered at 1 kHz). The amplitudes of the recorded currents were measured at the end of the test voltage steps; HERG currents were measured as the amplitude of the tail currents. The control solution contained (mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (titrated with NaOH to pH 7.4). The microelectrodes were filled with 3 M KCl solution and had resistances between 0.5 and 0.9 MΩ. Astemizole was a generous gift from Janssen Pharmaceuticals (Beerse, Belgium). Terfenadine, diphenhydramine and chlorpheniramine were purchased from Sigma. All histamine receptor antagonists were solubilized in DMSO as stock solutions. Data are presented as means with standard errors (S.E.M.), where n represents the number of experiments performed. Concentration-blockage relationships were calculated with the Hill equation. A Student's t-test was used to test for statistical significance, which was obtained for $P < 0.05$.

3. Results

Expression of I_{sK}, IRK1 and Kv1.1 channels in *Xenopus* oocytes resulted in the induction of K^+ conductances with described activation and rectification properties [13,14,16]. I_{sK} and Kv1.1 induced slow and fast activating voltage-gated K^+ conductances, respectively, while IRK1 induced an inward rectifier K^+ conductance in *Xenopus* oocytes. Terfenadine at 3 μM produced only weak effects on the above channels (Fig. 1A). The blockade was 4.2 ± 0.5% (n = 7), 1.8 ± 0.7% (n = 7) and 5.5 ± 0.6% (n = 7), for I_{sK}, IRK1 and Kv1.1 channels, respectively. In full concentration-response experiments, terfenadine produced half-maximal block of I_{sK}, IRK1 and Kv1.1 channels at 29.1 ± 0.8 μM (n = 7), 23.6 ± 2.2 μM (n = 7) and 34.1 ± 2.9 μM (n = 7), respectively. At concentrations of 3–10

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μM astemizole had no significant effects on these channels (data not shown). At the maximal soluble concentration of 60 μM astemizole inhibited I_{sK}, IRK1 and Kv1.1 by 26.0 ± 1.4% (n = 5), 12.2 ± 1.3% (n = 5) and 14.3 ± 1.9% (n = 5), respectively.

Expression of HERG channels resulted in a K^+ conductance with similar properties as previously described by Sanguinetti et al. [12]. Depolarizations to potentials more positive than 0 mV caused a negative rectification because of a fast C-type inactivation [12]. Because of its negative rectification outward currents at 40 mV are small, but the tail currents at repolarizing steps to −85 mV are maximal after such depolarizing pulses (Fig. 1B). In contrast to the K^+ conductances described above, terfenadine (3 μM) and astemizole (1 μM) almost completely abolished HERG channel tail currents (Fig. 1B). Terfenadine and astemizole blocked both small outward currents at 40 mV and the large outward tail currents at −85 mV; only the blockade of tail currents was subsequently analyzed. The inhibition of HERG channels by terfenadine (3 μM) and astemizole (1 μM) was 96.6 ± 0.7% (n = 6) and 92.5 ± 1.5% (n = 4). Subsequently complete concentration-blockade relationships were analyzed for HERG channels by terfenadine and astemizole. Fig. 2A shows an original trace: both terfenadine and astemizole blocked HERG channels at submicromolar concentrations. Analysis of the data with the Hill equation gave IC_{50} values of 246.1 ± 28.9 nM (n = 6) and 48.4 ± 3.8 nM (n = 6) and Hill coefficients of 1.09 ± 0.07 and 1.38 ± 0.08 for terfenadine and astemizole, respectively (Fig. 2B). The effects of the tested histamine receptor antagonists on HERG channels were very slowly but completely reversible upon washout (washout period > 30 min). The reasons for the slow recovery of HERG channels from astemizole and terfenadine block are unclear, but both blockers are hydrophobic compounds which may accumulate within the membrane.

The non-cardiotoxic histamine receptor antagonists chlorpheniramine and diphenhydramine blocked HERG channels with a much weaker potency with IC_{50} values of 20.9 ± 2.1 μM (n = 5) and 27.1 ± 1.5 μM (n = 5; Fig. 2B).

Blockade of HERG channels by terfenadine and astemizole was strongly use-dependent. To analyze this use dependence in detail, HERG channels were activated with 0.5 s depolarizing steps to 40 mV at intervals of 3 s, 12 s or 36 s in the presence of 0.3 μM terfenadine and 0.1 μM astemizole. It is obvious from Fig. 3 that the time course in which HERG channel blockade occurs is dramatically dependent on the activation frequency, but not on the time of drug superfusion. HERG blockade by terfenadine and astemizole occurred much faster at higher activation frequencies. Because the effects of terfenadine and astemizole were only very slowly reversible, the use dependence of washout could not be studied.

HERG blockade by terfenadine and astemizole was also analyzed for its voltage dependence. We tested the steady-state effects of terfenadine (0.3 μM) and astemizole (0.1 μM) on HERG channel tail currents after 3 s depolarizing prepulses to −20 mV and 20 mV. Terfenadine blocked HERG channel tail currents by 47.0 ± 9.5% (n = 6) after depolarizing steps to −20 mV and by 66.2 ± 7.6% (n = 6) at 20 mV (Fig. 4A,B). 50 nM astemizole blocked HERG tail currents by 54.5 ± 2.9% (n = 6) and 84.5 ± 1.5% (n = 6), after prior depolar-

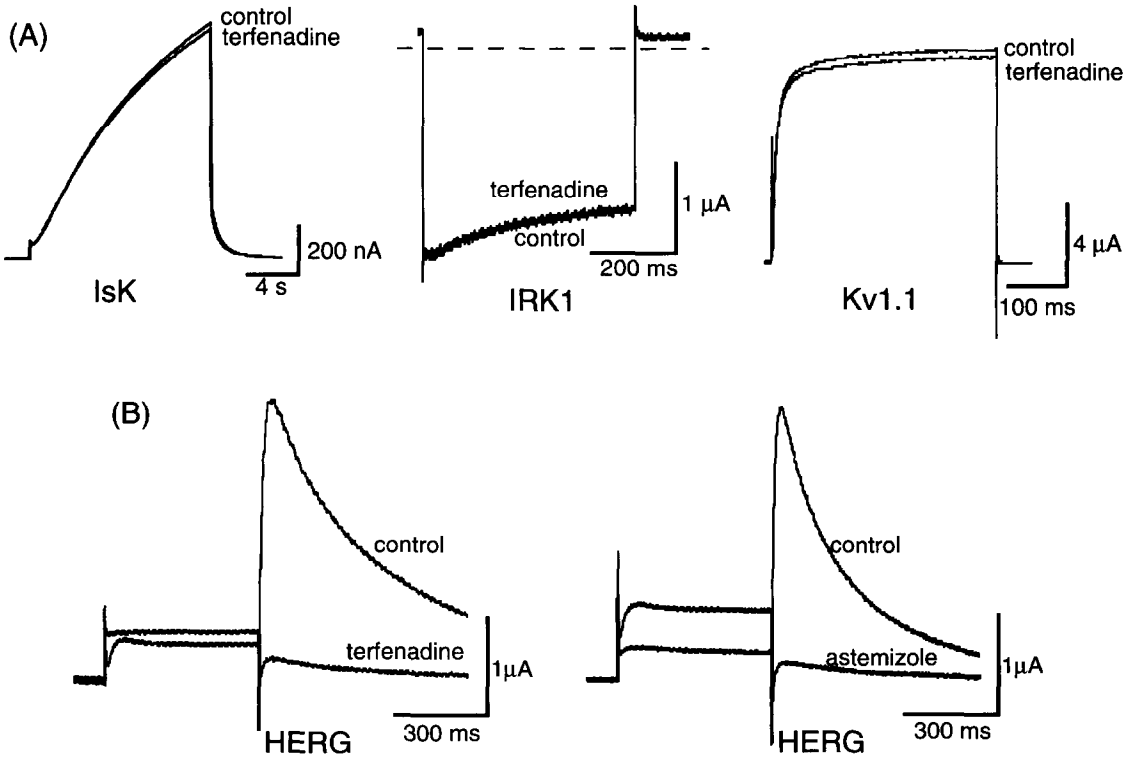

Fig. 1. (A) Effects of 3 μM terfenadine on I_{sK}, IRK1 and Kv1.1 channels expressed in *Xenopus* oocytes. I_{sK} or Kv1.1 were evoked with 15 s or 0.5 s voltage steps to −10 mV from a holding potential of −80 mV every 45 s or 3 s, respectively. IRK1 channels were evoked with 0.5 s polarizing pulses to −120 mV from a holding potential of −40 mV. (B) Effects of 3 μM terfenadine or 1 μM astemizole on HERG channels. Current was evoked with 0.5 s depolarizing pulses to 40 mV from a holding potential of −80 mV every 3 s. Tail currents were recorded at −85 mV.

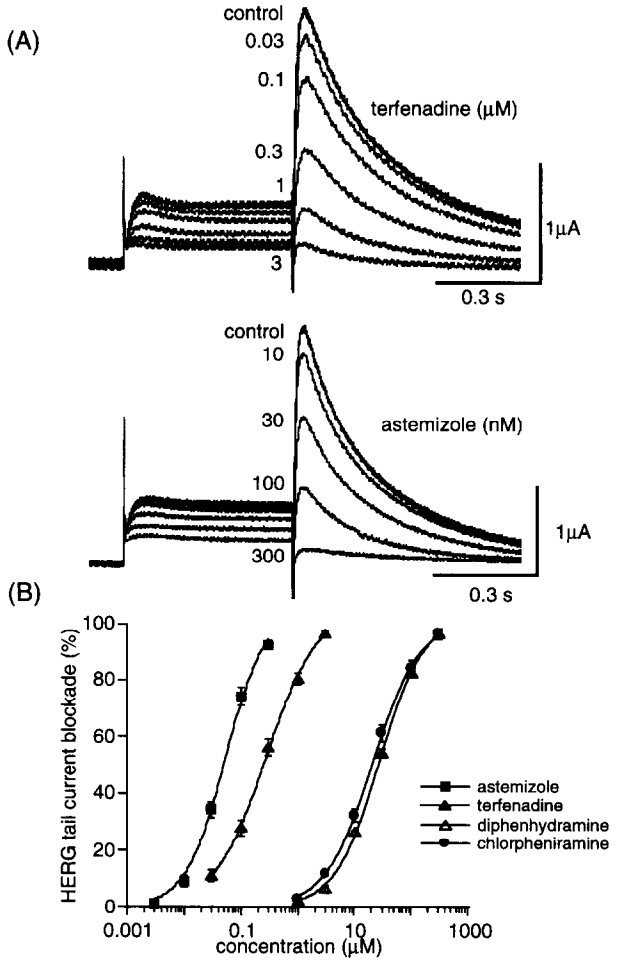

Fig. 2. (A) Original traces show the dose-dependent blockade of HERG channels by terfenadine and astemizole. (B) Concentration blockade correlation of astemizole, terfenadine, diphenhydramine and chlorpheniramine on HERG channel tail currents. Data are given as arithmetic means (± S.E.M.)

izations to −20 mV and 20 mV, respectively. Both terfenadine and astemizole mediated HERG blockade depended significantly on the voltage.

Finally, terfenadine and astemizole were also tested for their effects on HERG channel activation (at −20 mV) and deactivation kinetics (at −85 mV). Neither compound affected HERG channel rate of activation and deactivation at the described voltages (data not shown).

4. Discussion

Under certain conditions (overdose, liver disease or using simultaneously other drugs known to inhibit hepatic metabolism) terfenadine can produce QT interval prolongation in the ECG and syncope or cardiac arrest [17]. Similarly, astemizole has also been shown to produce manifest cardiac abnormalities in some rare, exceptional clinical cases associated with extreme astemizole overdose or inappropriate co-medications [2,3]. No reports exist about any adverse cardiovascular effects for chlorpheniramine and diphenhydramine.In this study we expressed representatives of distinct K^+ channel families in *Xenopus* oocytes and tested them for their sensitivity to terfenadine and astemizole. Both compounds blocked K^+ currents through HERG channels during depolarizations as well as repolarizations. This result supports the notion that blockade of HERG channels is the major reason for QT interval prolongation caused by terfenadine and astemizole. Most importantly, in this study both compounds blocked HERG channels expressed in *Xenopus* oocytes at concentrations similar to the plasma concentrations in patients with terfenadine or astemizole induced QT prolongation. Moreover, blockade of I_{Kr} (the conductance induced by HERG channels in human cardiac myocytes) by D-sotalol or its derivatives is also known to cause QT prolongation [12]. Finally, the genetic defect of HERG causes a QT syndrome [18]. These reports support that both HERG inhibition and dysfunction can indeed induce QT prolongation.

Several other studies have observed inhibitory effects of terfenadine also on other K^+ channels: the delayed rectifier fHK (Kv1.5), which was cloned from human heart and stably expressed in HEK-293 cells, was blocked with an IC_{50} of 0.4

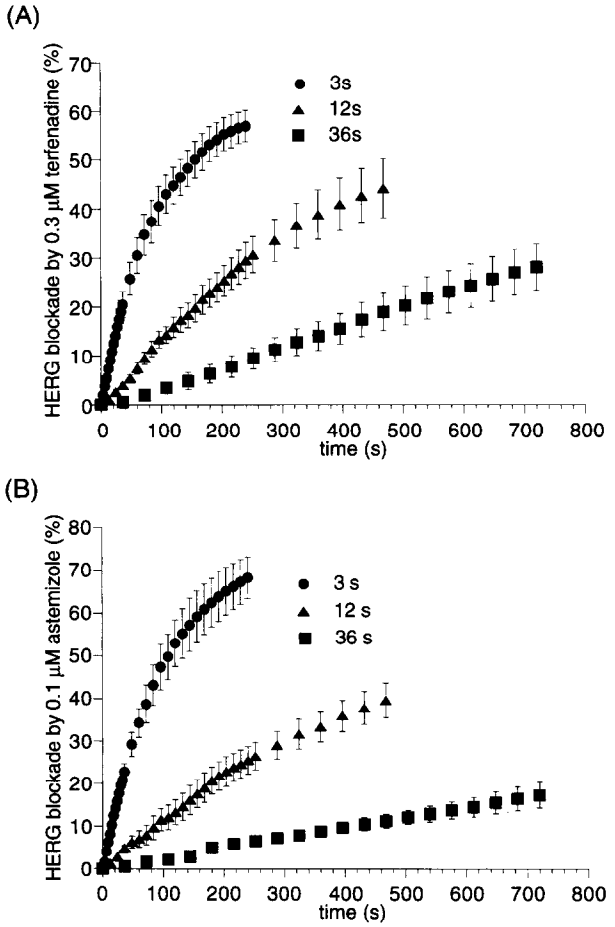

Fig. 3. Use-dependent HERG channel blockade by 0.3 μM terfenadine (A) or 0.1 μM astemizole (B). Tail currents were recorded at −85 mV after a 0.5 s depolarizing prepulse to 40 mV from a holding potential of −80 mV every 3 s, 12 s or 36 s. The solution flow rate was 20 ml/min, ensuring a complete exchange of the bath solution within 10–15 s. Before starting with the first depolarizing pulse (time = 0) the oocytes were superfused for 30 s with the drug containing solution to ensure complete and safe exchange of the bath. The drug effects were recorded in distinct sets of experiments with different intervals (3 s, 12 s and 36 s) between the depolarizing steps. For clarity not all data points for 3 s and 12 s intervals are given in the graph. For the set of experiments with 36 s intervals between the depolarizing steps all data are given. Data are given as arithmetic means (± S.E.M.).

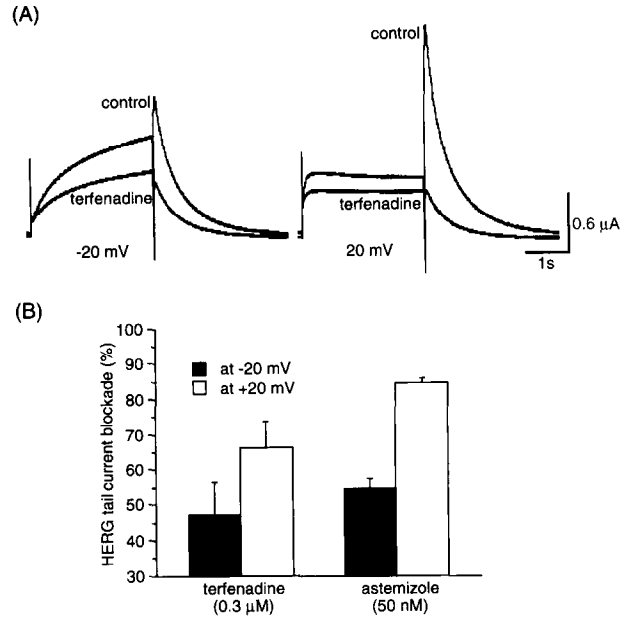

Fig. 4. (A) Original trace showing the voltage dependence of terfenadine on HERG. Currents were evoked with 3 s depolarizing pulses to −20 mV and 20 mV, respectively, from a holding potential of −80 mV every 5 s. Only the effects on the tail currents were calculated. (B) Voltage-dependent block of 0.3 μM terfenadine and 50 nM astemizole on HERG tail currents ($n = 6$; $P < 0.05$).

μM [19], an IC_{50} similar to the value for HERG channels in the present study. However, in contrast to the clearly defined role of HERG channels in human heart, the effects of Kv1.5 inhibition on the ECG are not clear yet. In any case, these studies suggest that terfenadine may interfere with more than one type of K^+ channel. However, terfenadine blocks HERG with relatively high potency. In the present study the terfenadine sensitivity of HERG channels exceeded that of other K^+ channels by two orders of magnitude. Astemizole was clearly more specific for HERG channels with a more than 1000-fold higher potency compared to I_{sK}, Kv1.1 and IRK1.

The extreme use dependence of terfenadine and astemizole suggests that drug binding can only occur to the open or inactivated state of HERG channels. Two observations favor binding to the inactivated state of HERG channels. (1) If terfenadine and astemizole bound and unbound only to and from open channels one would predict a smaller τ_{act} and a greater τ_{deact}. However, both terfenadine and astemizole had no effects on the rate of HERG channel activation and deactivation. (2) Steady-state blockade of HERG by terfenadine and astemizole was voltage-dependent and greater at more positive potentials. Because at more positive potentials more HERG channels transfer to the inactivated state [12], this result supports a preferred binding of terfenadine and astemizole to inactivated HERG channels.

In summary, the present study demonstrates potent blockade of HERG channels by the histamine receptor antagonists terfenadine and astemizole. This channel blockade is likely to be involved in the prolonged QT intervals observed in certain patients.

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