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Short communication

Epinastine, a nonsedating histamine H_1 receptor antagonist, has a negligible effect on HERG channel

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Abstract

Terfenadine and astemizole rarely cause cardiac arrhythmias by suppressing the cardiac rapid delayed rectifier K^+ channel encoded by the human *ether-a-go-go-related gene* (HERG). Epinastine, however, has not been reported to have the adverse effect. We have therefore compared the effects of epinastine, terfenadine and astemizole on HERG channels expressed in *Xenopus* oocytes. Terfenadine and astemizole suppressed the HERG current with IC_{50} of 431 nM and 69 nM, respectively. In contrast, 100 μM epinastine inhibited the HERG current by only $11 \pm 2.1\%$. These results may provide an explanation for the difference in the cardiotoxity between different nonsedating histamine H_1 receptor antagonists.

Keywords: Histamine H_1 receptor antagonist; Epinastine; Terfenadine; Astemizole; HERG; K^+ channel

1. Introduction

Second generation nonsedating histamine H_1 receptor antagonists such as terfenadine and astemizole, which lack the adverse effect on the central nervous system, have been widely used clinically. However, it was recently reported that these second generation histamine H_1 receptor antagonists sometimes cause cardiac arrhythmias including *torsade de pointes*, which can cause sudden death (Monahan et al., 1990; Wiley et al., 1992; Sakemi and Van Natta, 1993). Previous studies demonstrated that terfenadine and astemizole block the rapid component of the delayed rectifier potassium current (I_{Kr}) in cardiac myocytes and the recombinant I_{Kr} channel, the human *ether-a-go-go*-related gene (HERG) (Salata et al., 1995; Carmeliet, 1998; Taglialatela et al., 1998). In cardiac myocytes I_{Kr} contributes to termination of the plateau phase of the action potential. Thus, suppression of I_{Kr} results in prolongation of action potential and the QT interval on the electrocardiogram (ECG), which can lead to ventricular tachycardia including *torsade de pointes* by inducing early after depolarization (Jurkiewicz and Sanguinetti, 1993).

Although epinastine is a nonsedating histamine H_1 receptor antagonist (Fuegner et al., 1988), the episodes of *torsade de pointes* or sudden death have not been reported in patients who have received this drug. Because another nonsedating histamine H_1 receptor antagonist, cetirizine, that has no cardiac side-effects, has been reported to have a weak inhibitory effect on HERG channels (Carmeliet, 1998; Taglialatela et al., 1998), we speculated that epinastine may also have little effect on HERG channels.

In this study we have compared the effects of epinastine, terfenadine and astemizole upon the recombinant I_{Kr} channel, HERG, expressed in *Xenopus* oocytes. We find that unlike terfenadine and astemizole, epinastine is a very ineffective blocker of HERG channel currents which may therefore explain the lack of cardiac side-effects obtained during treatment with this histamine H_1 receptor antagonist.

2. Materials and methods

The methods for oocyte expression has been described previously (Sanguinetti et al., 1995). The pSP64 plasmid including the HERG cDNA was kindly provided by Drs. M.T. Keating and M.C. Sanguinetti, University of Utah, Salt lake City, UT, USA.

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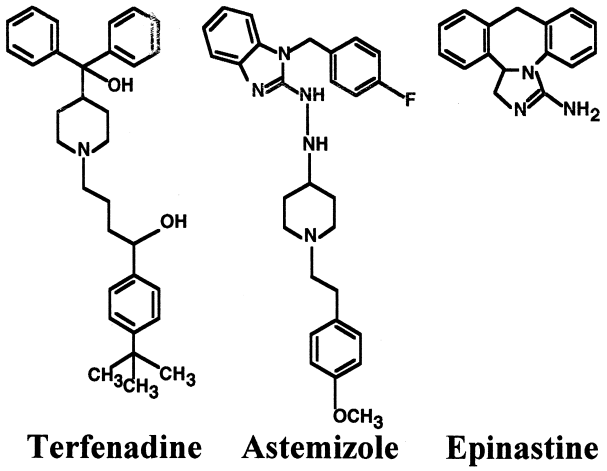


Fig. 1. Structural formulas of nonsedative histamine H_1 receptor antagonists.

The expressed channel currents were recorded by conventional two-microelectrode voltage-clamp with a commercially available amplifier (Turbo Clamp TEC 01C, Tamm, Germany). The glass microelectrodes had a resistance of 0.5–1.5 MΩ when filled with 3 M KCl. Oocytes were bathed in a solution containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$ and 5 HEPES (pH was adjusted to 7.6 with NaOH). The currents were recorded at room temperature, and the membrane potential was held at −80 mV. The voltage protocols were described in figure legends.

Astemizole and terfenadine were purchased from Sigma (St. Louis, USA). Epinastine was obtained from Boehringer Ingelheim (Biberach, Germany). The chemical structures of these compounds are shown in Fig. 1. Astemizole and terfenadine were dissolved in dimethylsulfoxide as 10 mM stock solutions; the vehicle at the final concentrations did not affect the HERG currents. Epinastine was dissolved in distilled water at 10 mM.

3. Results

Xenopus oocytes injected with HERG cRNA expressed K^+ channel currents whose properties were very similar to those of I_{Kr} (Sanguinetti et al., 1995). Fig. 2A (control) shows the HERG currents elicited by voltage steps ranging from −120 to +40 mV applied in 10 mV increments from the holding potential of −80 mV. HERG channel currents were activated in a time-dependent manner with voltage steps positive to −80 mV. The current–voltage relationship of HERG channel currents evoked by rectangular voltage steps was bell-shaped (Fig. 2B): Between −80 and −10 mV, voltage steps evoked an increasing HERG channel current, while those more positive than −10 mV evoked progressively smaller HERG channel currents due to the development of C-type inactivation (Sanguinetti et al., 1995). Upon repolarization to −60 mV, the HERG channel tail current rapidly increased and then gradually decreased to a steady-state value (Fig. 2A). The former is due to the prompt removal of C-type inactivation upon repolarization, while the latter reflects the deactivation of HERG channels (Sanguinetti et al., 1995). When the peak of the tail current recorded at −60 mV was plotted against the membrane potential to which the preceding voltage step had been applied, there was no reduction of cell current following depolarization to positive membrane potentials. We used the same voltage-clamp protocol to examine the effects of epinastine, astemizole and terfenadine on the HERG channel currents.

Oocytes were superfused with each drug for 10 min during which time 4 s depolarizing pulses to +20 mV from the holding potential of −80 mV were applied every 20 s. The application of 1 μM astemizole or 1 μM terfenadine decreased both steady-state and peak tail currents of HERG, which reached a steady-state level within 8 min. 10 min after their application, 1 μM terfenadine and 1 μM astemizole had inhibited the peak HERG tail currents by $68 \pm 7.4\%$ ($n = 5$) and $93 \pm 2.8\%$ ($n = 5$), respectively. Neither astemizole nor terfenadine significantly altered the HERG current–voltage relationship (Fig. 2B). In contrast, epinastine had much weaker effects on the HERG steady state and tail currents even at 10 and 100 μM. The inhibition of HERG tail current caused by 100 μM epinastine was only $11 \pm 2.1\%$ ($n = 5$). We further examined the possibility that epinastine may inhibit HERG channels in a use-dependent manner. The oocytes were superfused with 100 μM epinastine for 10 min (holding potential, −80 mV), and then the voltage pulse to 0 mV for 500 ms followed by that to −60 mV for 100 ms were

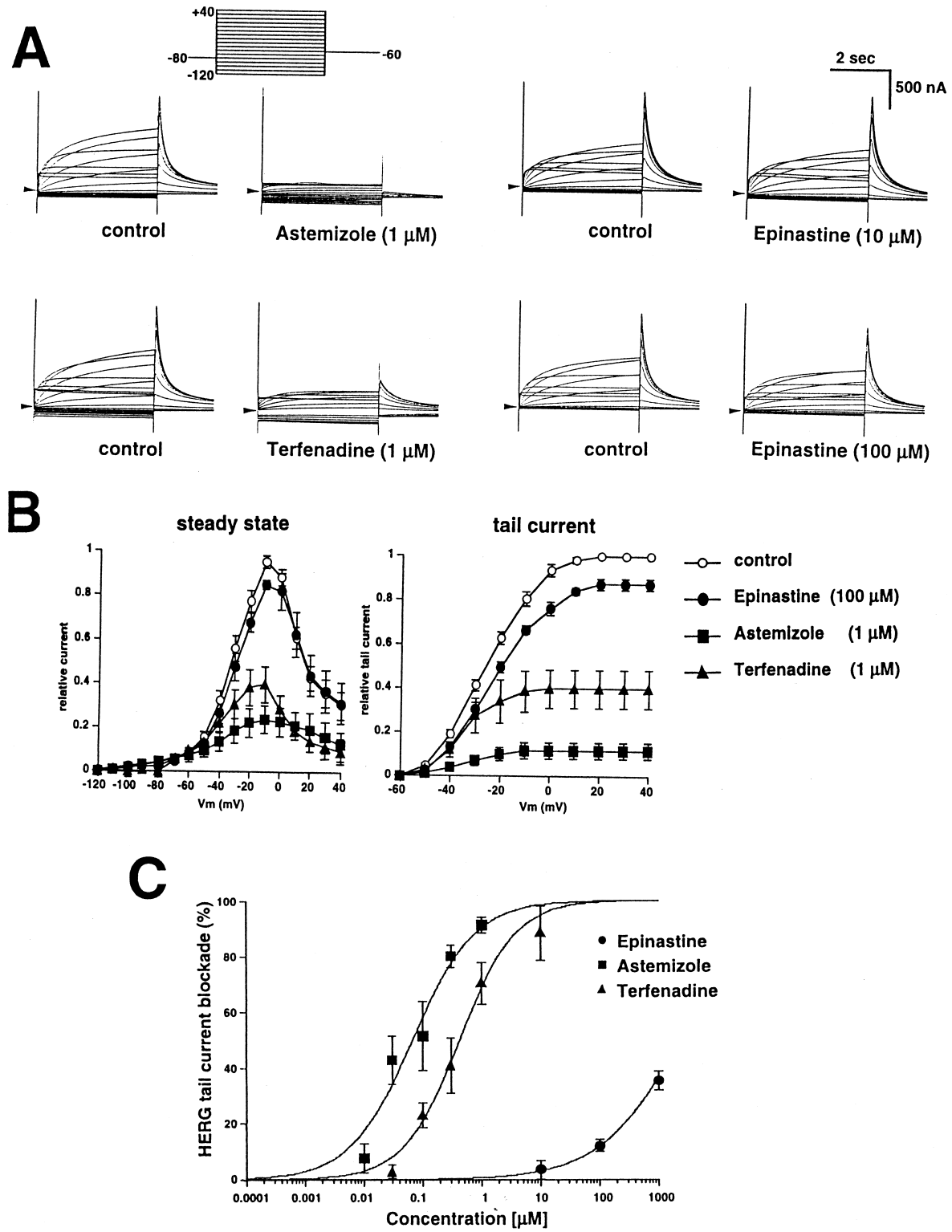
Fig. 2. Effects of terfenadine, astemizole and epinastine on the HERG currents expressed in *Xenopus* oocytes. (A) Representative HERG current recordings in the absence (control) and the presence of the agents indicated beneath each set of current traces. Each set of the traces was obtained by applying 4 s command pulses ranging from −120 to +40 mV from the holding potential of −80 mV every 20 s. Each command pulse was immediately followed by a 6 s step to −60 mV in order to record HERG channel tail currents. In the presence of the agents, the current traces were obtained 10 min after application of each agent. In all the experiments, extracellular K^+ concentration was 2 mM. (B) Voltage dependency of the effects of epinastine (100 μM), astemizole (1 μM) and terfenadine (1 μM). The amplitude of HERG channel currents was measured in the presence and the absence of each agent at the end of 4 s command pulses (left graph: steady state) or at the peak of tail current at −60 mV (right graph). The current amplitude was normalized to the maximum obtained under the control conditions and plotted against the membrane potential of the test pulse. Symbols and bars indicate the means ± S.E.M. ($n = 5$ for each point). (C) The relationship between concentration of astemizole, terfenadine and epinastine and HERG channel tail currents recorded at −60 mV following a depolarization to +20 mV. The inhibitory effect of each agent was normalized to the maximum effect of terfenadine, astemizole and epinastine. Symbols and bars indicate the means ± S.E.M. ($n = 5$ for each point) while lines represent the fit of each set of the data to the Hill equation (see text).

applied at 0.5 Hz. Even with the high frequent voltage steps, HERG channels were not depressed significantly by epinastine (data not shown).

Fig. 2C compares the concentration-dependent effect of terfenadine, astemizole and epinastine on HERG tail currents. The relationships between the percent block of the HERG tail current and the concentrations of astemizole, terfenadine and epinastine were fit with a Hill equation:

$$\text{relative current} = 1/\left\{\left([\text{drug}]/IC_{50}\right)^{n_H} + 1\right\}$$

Analysis of the data with the Hill equation gave IC_{50} values of 431 nM and 69 nM, and Hill coefficients of 1.03



and 0.83 for terfenadine and astemizole, respectively. On the other hand, epinastine had an almost negligible effect on HERG channels at a concentration of 10 μM, and even 1 mM of the drug inhibited the currents by less than 50%.

4. Discussion

Terfenadine and astemizole, nonsedating histamine H_1 receptor antagonists, have been reported in some cases to induce QT prolongation on ECG and a life-threatening ventricular tachycardia, *torsade de pointes*. These adverse effects of terfenadine and astemizole have been shown to be related to block of cardiac I_{Kr} (Salata et al., 1995; Carmeliet, 1998; Taglialatela et al., 1998). Epinastine is a novel nonsedating histamine H_1 receptor antagonist that was launched on the market in countries including Japan, Korea, Mexico and Brazil, from 1994 (Fuegner et al., 1988). There has been no report that this drug causes prolongation of the QT interval on ECG and it is not clear why epinastine, unlike terfenadine and astemizole, provides no visible adverse effect upon the heart. I_{Kr} is coded by HERG, and when HERG is expressed in *Xenopus* oocytes, the current exhibits the properties similar to cardiac I_{Kr}. In this study we showed that epinastine exhibited a negligible effect on the HERG current. On the other hand, terfenadine and astemizole blocked HERG channels in a concentration-dependent manner with IC_{50} of 431 nM and 69 nM, respectively, which are compatible with the recorded plasma levels of these drugs in the patients who developed the drug-associated QT prolongation (Monahan et al., 1990; Wiley et al., 1992). In binding experiments for histamine H_1 receptors, the pK_i values ($-\log$ dissociation constant) of epinastine, terfenadine and astemizole were 8.9, 7.1 and 8.3, respectively (Ter Laak et al., 1993). In in vivo study on histamine-induced skin wheal in mice, the IC_{50} doses (mg/kg, p.o.) were 0.41, 0.71 and 0.22 for epinastine, terfenadine and astemizole, respectively (Komune et al., 1999). It was, therefore, concluded that epinastine is relatively selective histamine H_1 receptor antagonist, with negligible effects on HERG channels. The IC_{50} concentration of epinastine to block HERG channels was 1000 times higher than that needed to block histamine H_1 receptors.

Structural differences between epinastine, astemizole and terfenadine may be related to the different magnitudes of suppression of HERG channel currents by these compounds (Fig. 1). Astemizole and terfenadine have large carbon chains at the tertiary amine substitutant (Fig. 1). The other nonsedative histamine H_1 receptor antagonists, cetirizine and loratadine, that has been reported to have a weak effect on cardiac I_{Kr} or HERG channels, have a small substitute at the tertiary amine substitutant (Carmeliet, 1998; Taglialatela et al., 1998). Epinastine has no amine substitutant. These data suggest that the amine substitutant is not necessary for the blockade of histamine H_1 receptor, but seems to be related to the ability of the compound to block the cardiac I_{Kr}. If this is the case, the tertiary amine moiety interacts with HERG channels and various modifications of this side chain may develop not only specific histamine H_1 receptor antagonists but also new drugs affecting HERG channels.

In conclusion, this study demonstrates that epinastine has a negligible suppressive effect on HERG channel currents compared with astemizole and terfenadine. This may at least partially explain the absence of arrhythmogenic effects of the drug. Thus, epinastine is a useful histamine H_1 receptor antagonist for the treatment of allergic diseases because it is not only nonsedative but also devoid of cardiotoxic actions.

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