Block of hERG K$^+$ Channel by Classic Histamine H$_1$ Receptor Antagonist Chlorpheniramine

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Chlorpheniramine is a potent first-generation histamine H$_1$ receptor antagonist that can increase action potential duration and induce QT prolongation in several animal models. Since block of cardiac human *ether-a-go-go*-related gene (*hERG*) channels is one of leading causes of acquired long QT syndrome, we investigated the acute effects of chlorpheniramine on hERG channels to determine the electrophysiological basis for its proarrhythmic potential. We examined the effects of chlorpheniramine on the hERG channels expressed in *Xenopus* oocytes using two-microelectrode voltage-clamp techniques. Chlorpheniramine induced a concentration-dependent decrease of the current amplitude at the end of the voltage steps and hERG tail currents. The IC$_{50}$ of chlorpheniramine-dependent hERG block in *Xenopus* oocytes decreased progressively relative to the degree of depolarization. Chlorpheniramine affected the channels in the activated and inactivated states but not in the closed states. The S6 domain mutations Y652A and F656A partially attenuated (Y652A) or abolished (F656A) the hERG current block. These results suggest that the H$_1$ antihistamine, chlorpheniramine is a blocker of the hERG channels, providing a molecular mechanism for the drug-induced arrhythmogenic side effects.

Key Words: Antihistamine, HERG channel, Chlorpheniramine, Rapidly-activating delayed rectifier K$^+$ channel

INTRODUCTION

Antihistamines are among the most commonly prescribed drugs in the world and have evolved considerably since the first generation of compounds was introduced >50 years ago. The first generation antihistamines are lipid-soluble drugs widely used to treat allergic symptoms through the blocking of peripheral H1 receptors (Katzung, 2003). These drugs freely cross the blood-brain barrier and can affect the central nervous system (Goldberg et al., 1987; Katzung, 2003). The main side effects of these compounds are sedation, drowsiness and impaired performance, although anticholinergic and antiserotonergic effects may also be observed. Chlorpheniramine, the p-chloro analogue of bromopheniramine, is a potent first-generation antihistamine which has been available for medical use since 1951. Over the past 30 years, second-generation antihistamines have been developed, which have less central activity and therefore do not cause the drowsiness. Overdose of both first- and second- generation antihistamines can cause severe cardiac arrhythmia (Hestand and Teske, 1977; Craft, 1986; Taglialatela et al., 2000) by directly inhibiting cardiac K$^+$ currents (Taglialatela et al., 1999). These findings led us to hypothesize that chlorpheniramine, one of first-generation antihistamines, may exert arrhythmic effects by affecting K$^+$ currents.

Among the various cardiac K$^+$ currents, the rapidly-activating delayed rectifier K$^+$ current (I$_{Kr}$) - mediated by an ion channel encoded by the human *ether-a-go-go*-related gene (*hERG*) - is important for the termination of the cardiac action potential and determines the shape of the repolarization phase (Tie et al., 2000). Therefore, the aim of the present study was to investigate the possibility for an effect of chlorpheniramine on hERG channels and the molecular determinants involved in the possible blocking effect of the drug using mutant hERG channels.

METHODS

Expression of hERG in oocytes

hERG (accession no. U04270) cRNA was synthesized by *in vitro* transcription from 1 μg of linearized cDNA using T7 message machine kits (Ambion, Austin, TX, USA) and stored in 10 mM Tris-HCl (pH 7.4) at -80°C. The amino acid mutations where generated by polymerase chain reaction (PCR) with synthetic mutant oligonucleotide primers. The mutations Y652A and F656A were verified by sequencing (ABI3100). Stage V-VI oocytes were surgically removed from female *Xenopus laevis* (Nasco, Modesto, CA, USA) anesthetized with 0.17% tricane methanesulphonate (Sigma, St. Louis, MO, USA). Using fine forceps, the theca and follicle layers were manually removed from the oocytes, and then

ABBREVIATIONS: I$_{Kr}$, rapidly-activating delayed rectifier K$^+$ current; hERG, human *ether-a-go-go*-related gene; APD$_{90}$, action potential duration at 90% repolarization; APD, action potential duration.

each oocyte was injected with 40 nl of cRNA (0.1~0.5 μg/μl). The injected oocytes were maintained in a modified Barth's Solution. The modified Barth's Solution contained (mM): 88 NaCl, 1 KCl, 0.4 CaCl$_2$, 0.33 Ca(NO$_3$)$_2$, 1 MgSO$_4$, 2.4 NaHCO$_3$, 10 HEPES (pH 7.4), and 50 μg/ml gentamicin sulphonate. Currents were studied two to seven days after injection. This study was performed according to the Research Guidelines of Kangwon National University IACUC.

Solutions and voltage-clamp recordings from oocytes

Normal Ringer's Solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, and 10 mM HEPES (pH adjusted to 7.4 with NaOH). Solutions were applied to oocytes by continuous perfusion of the chamber while recording. Solution exchanges were completed within three min, and the hERG currents were recorded 5 min after the solution exchange. The effects of several concentrations of chlorpheniramine on the hERG currents were determined after the currents showed reversibility when washed with normal Ringer's Solution. It took about 15 min to wash out ≤50 μM of the drug and about 30 min to wash out ≥100 μM of the drug. If the oocyte did not recover a current to its initial amplitude after 30 min of washing with a normal Ringer's Solution, it was not used further. Currents were measured at room temperature (20~23°C) with a two-microelectrode voltage clamp amplifier (Warner Instruments, Hamden, CT, USA). Electrodes were filled with 3 M KCl and had a resistance of 2~4 MΩ for voltage-recording electrodes and 0.6~1 MΩ for current-passing electrodes. Stimulation and data acquisition were controlled with an AD-DA converter (Digidata 1200, Axon Instruments) and pCLAMP software (v 5.1, Axon Instruments). The antihistamine chlorpheniramine and other reagents were purchased from Sigma (St. Louis, MO, USA). A stock solution of chlorpheniramine was prepared in distilled water and added to the external solutions at suitable concentrations shortly before each experiment.

Pulse protocols and analysis

To obtain concentration-response curves in the presence of chlorpheniramine, concentration-dependent inhibition was fitted with the equation:

$$I_{tail} = I_{tail \cdot max} / [1 + (IC_{50}/D)^n],$$

where I_{tail} indicates peak tail currents, $I_{tail \cdot max}$ is the maximum peak tail current, D is the concentration of the small molecule, n is the Hill coefficient, and IC_{50} is the concentration at which the half-maximal peak tail currents were inhibited.

Statistical evaluations

All data are expressed as mean±S.E.M. Unpaired or paired Student t tests, or ANOVA were used for statistical comparisons when appropriate, and differences were considered significant at $p < 0.05$.

RESULTS

Concentration-dependence of WT hERG channel block by chlorpheniramine

This study examined the effect of chlorpheniramine on hERG currents (I_{HERG}) using a *Xenopus* oocyte expression system. Throughout these experiments, the holding potential was maintained at −70 mV, and tail currents (I_{tail}) were recorded at −60 mV after depolarizing pulses from −50 to +40 mV (Fig. 1). Fig. 1A gives the representative current traces of a voltage-clamp recording obtained at −60 mV after depolarizing pulse to +30 mV both under the control conditions and after exposure to 1~300 μM chlorpheniramine. After the depolarizing steps, repolarization to −60 mV induced an outward I_{tail}, which had an amplitude even greater than that of I_{HERG} during depolarization, which is due to rapid recovery from inactivation and a slow deactivation mechanism (Zhou et al., 1998). The amplitude of I_{tail} showed a concentration-dependent decrease with increasing chlorpheniramine concentration (Fig. 1A). The amplitude of I_{tail} was normalized to the peak amplitude obtained under the control conditions at the maximum depolarization, and was plotted against the potential of the step depolarization (n=6, Fig. 1B). The data obtained under the control con-
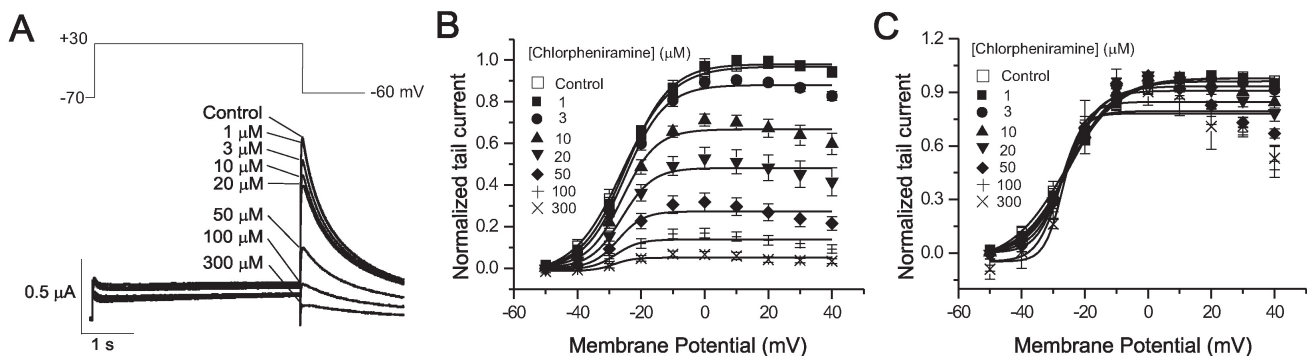

Fig. 1. The effect of chlorpheniramine on *human-ether-a-go-go*-related gene (*hERG*) currents (I_{HERG}) elicited by depolarizing voltage pulses. (A) Superimposed current traces elicited by depolarizing voltage pulses (4 s) to +30 mV steps (upper panel) from a holding potential of −70 mV in the absence of chlorpheniramine (control) and in the presence of 1~300 μM chlorpheniramine (lower panel). (B) Plot of the normalized tail current measured at its peak just after repolarization (n=6). The peak amplitude of the tail current in the absence of the drug was set as 1. Control data were fitted to the Boltzmann Equation, $y=1/\{1+\exp[(-V+V_{1/2})/dx]\}$, with $V_{1/2}$ of −25.7 mV. (C) Activation curves with values normalized to the respective maximum value at each concentration of chlorpheniramine. *Symbols* with error bars represent mean±S.E.M. (n=3~5).

ditions were well-fitted by the Boltzmann Equation, with a half-maximal activation ($V_{1/2}$) at -26.2 mV. The peak I_{tail} amplitude decreased with increasing chlorpheniramine concentration, which indicates that the maximum conductance of the hERG channels is decreased by chlorpheniramine. In addition, in the presence of chlorpheniramine, I_{tail} does not reach the steady-state level but decreases at more positive potentials, indicating that the blockade is more pronounced at the positive potentials.

The values shown in Fig. 1B were normalized to the respective maximum values at each concentration to determine if chlorpheniramine shifts the activation curve (Fig. 1C). The activation curves in the control oocytes, as well as those treated with 1, and 3 μM chlorpheniramine overlapped, whereas the curves representing the higher concentrations of the drug ($10 \sim 300$ μM) were shifted downward without a significant leftward-shift. The $V_{1/2}$ calculations are consistent with this finding, yielding values of -26.2 ± 0.62 mV in the control group and, -25.0 ± 0.63, -25.1 ± 0.77, -25.7 ± 1.41, $-26.0 \pm .87$, -27.2 ± 2.67, -27.8 ± 3.51, and -26.9 ± 3.18 mV in the 1, 3, 10, 20, 50, 100 and 300 μM chlorpheniramine-treated groups, respectively ($n=3 \sim 5$, $p > 0.05$). Therefore, the $V_{1/2}$ values in the presence of $1 \sim 300$ μM chlorpheniramine were similar, indicating that chlorpheniramine does not alter the activation gating at this concentration range.

Voltage-dependent block of WT hERG channel by chlorpheniramine

The chlorpheniramine-induced decrease in I_{tail} at different potentials was compared in order to determine if the effect of chlorpheniramine was voltage dependent (Fig. 2A). Concentration-response relationships were constructed at 0 mV, $+20$ mV and $+40$ mV. The percentage inhibition in the hERG current by 20 μM chlorpheniramine at 0, $+10$, $+20, +30$, and $+40$ mV was $40.6 \pm 6.3\%$, $45.9 \pm 6.1\%$, $50.0 \pm 5.8\%$, $52.1 \pm 5.8\%$, and $53.0 \pm 6.1\%$, respectively ($n=6$, data not shown). This suggests that the chlorpheniramine-induced blockade of the hERG currents progressively increases with increasing depolarization. Concentration-response relationships were constructed at $+40$ mV, $+20$ mV and 0 mV. The data was modeled using the Hill equation (Fig. 2B), giving IC$_{50}$ values of 25.5 ± 1.09 μM, 19.2 ± 0.59 μM and 15.6 ± 0.44 μM, respectively, and Hill coefficients of 1.10 ± 0.05, 1.12 ± 0.04 and 1.13 ± 0.03 at 0 mV, $+20$ mV and $+40$ mV, respectively ($n=6$). These findings indicate that the chlorpheniramine-induced blockade of the hERG current is voltage-dependent.
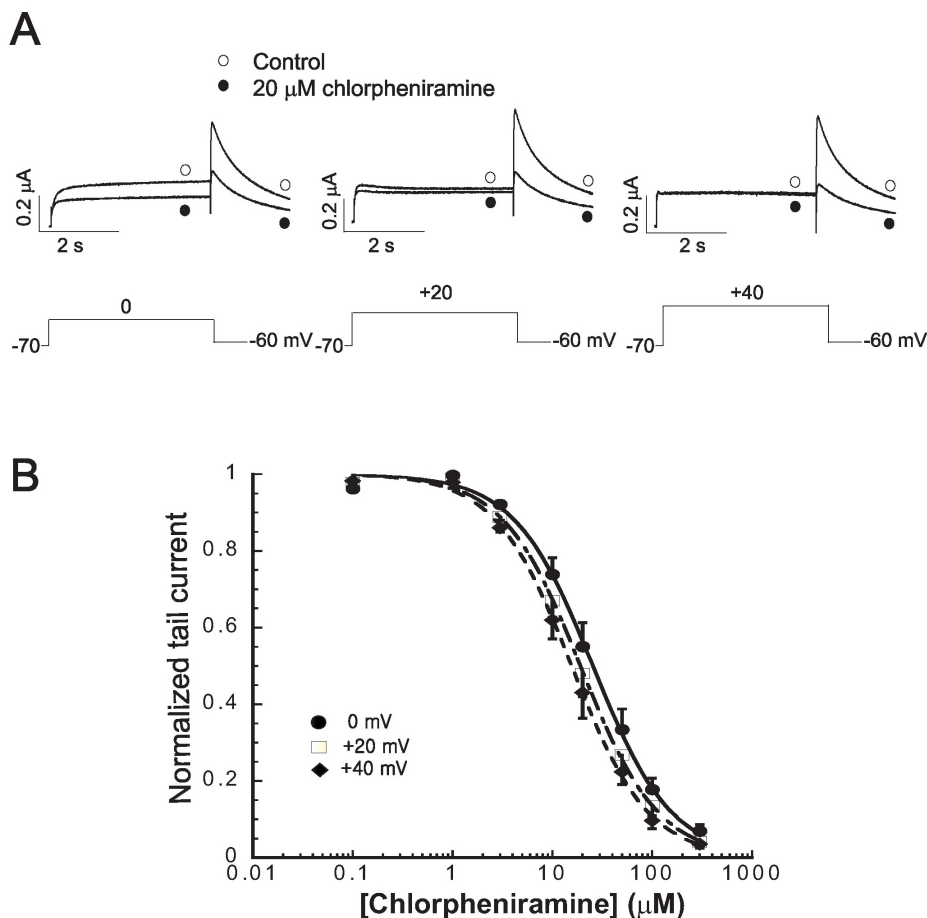

Fig. 2. Voltage dependence of chlorpheniramine-induced hERG current block. (A) Current traces from a cell depolarized to 0 mV (left panel), +20 mV (middle panel) and +40 mV (right panel), before and after exposure to 20 μM chlorpheniramine, showing increased block of hERG current at more positive membrane potentials. The protocol consisted of 4 s depolarizing steps to 0 mV, +20 mV or +40 mV from a holding potential of -70 mV, followed by repolarization to -60 mV. (B) Concentration-dependent block of I$_{HERG}$ by chlorpheniramine at different membrane potentials. At each depolarizing voltage step (0 mV, +20 mV or +40 mV), the tail currents in the presence of various concentrations of chlorpheniramine were normalized to the tail current obtained in the absence of drug, and then plotted against chlorpheniramine concentrations. *Symbols* with error bars represent mean$\pm$S.E.M ($n=6$). The line represents the data fits to the Hill equation.
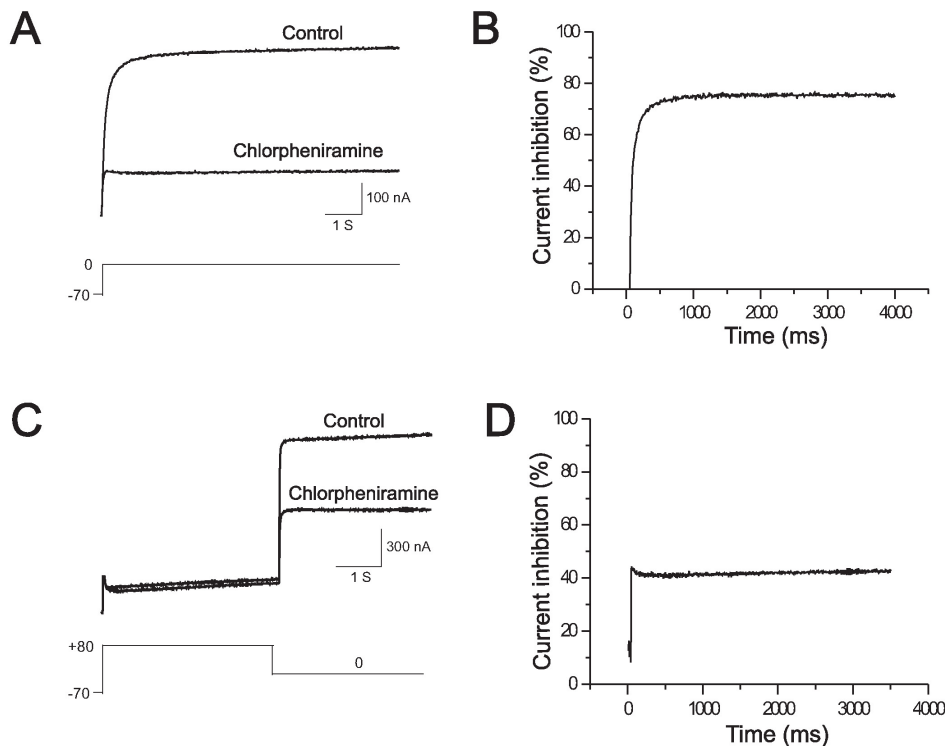
Fig. 3. Blocking of activated hERG channels by chlorpheniramine. (A) An original recording of currents under control conditions (control) and after exposure to 50 μM chlorpheniramine (for 13 min, without any intermittent test pulse). (B) The degree of hERG-current inhibition in percentages (%). Current inhibition increased time-dependently to 76% at 1 s in this representative cell, indicating that mostly open and/or inactivated channels were blocked. (C) Inhibition of inactivated channels by 20 μM chlorpheniramine. hERG channels were inactivated by a first voltage-step to +80 mV, followed by channel opening at 0 mV. (D) The corresponding relative block during the 0 mV step is displayed. Maximum inhibition was achieved in the inactivated state during the first step, and no further time-dependent block occurred upon channel opening during the second voltage step.

State-dependence of WT hERG channel block by chlorpheniramine

hERG currents were activated using a protocol containing a single depolarizing step to 0 mV for 8 s to determine if the channel was blocked in the closed or activated (i.e. open and/or inactivated) state (Fig. 3A). After obtaining the control measurement, 50 μM chlorpheniramine was applied and the recordings were made. Fig. 3B shows the percent inhibition calculated by: (1-chlorpheniramine current/control current) ×100. Analysis of the test pulse after the application of chlorpheniramine revealed a time-dependent blockade; a representative cell showing 76% block at 1 s is shown in Fig. 3B. At the beginning of the pulse, the fractional sustained current, which was obtained by normalizing the currents with chlorpheniramine relative to control currents, was 0.83±0.03 of control (n=6). This indicates that the hERG channels were only slightly blocked by chlorpheniramine while remaining at the holding potential. In this series of experiments, 50 μM chlorpheniramine reduced the hERG outward currents at the end of the 0 mV pulse by 68.2±3.7% (n=6).

In order to address the question as to whether the hERG channels are also blocked by chlorpheniramine in its inactivated state, a long test pulse to +80 mV (4 s) was applied to inactivate the channels, which was followed by a second voltage step (0 mV, 3.5 s) to open the hERG channels (n=5). Fig. 3C shows typical current traces under the control conditions and after the application of 20 μM chlorpheniramine. Fig. 3D shows the normalized relative blockage upon channel opening during the second voltage pulse (0 mV), indicating that the pronounced inhibition of the

hERG channels had already been reached during the previous inactivating +80 mV pulse. No additional time-dependent blockade of the open channels was observed during the 0 mV pulse. The currents at the end of the second voltage step (0 mV) were decreased by 37.3±4.3% (n=5). Overall, chlorpheniramine inhibits the hERG channels mainly in the open and inactivated state rather than in the closed state.

Chlorpheniramine block of WT and mutant hERG channels expressed in oocytes

Previous studies reported that two aromatic residues, Tyr-652 and Phe-656, which are located in S6 domain and face the pore cavity of the channel, are important components of the binding site for a number of compounds (Sanchz-Chapula et al., 2002). The potency of a channel block for the wild type and two mutant hERG channels (Y652A and F656A) were compared in order to determine if these key residues are also important in the chlorpheniramine-induced blocking of the hERG channel. As shown in Fig. 4, the inhibitory effect of chlorpheniramine (100 μM) was partially attenuated by a Y652A mutation (panel A) or abolished by an F656A mutation (panel B). The IC$_{50}$ values were consistent with this finding. The IC$_{50}$ values were 17.1±0.1 μM (n=5) in WT (obtained using protocol of panel A), 102.7±27.7 μM (n=7) in Y652A mutant channels, 61.1± 37.8 μM (n=7) in WT (obtained using protocol of panel B), and 2,153.2±3.1 μM (n=7) in F656A mutant hERG channels (panel C, D). This indicates that a mutation of Phe-656 located in the S6 domain of the hERG channel reduced the potency of the channel block by chlorpheniramine more than a mutation of Tyr-652 in the same region.
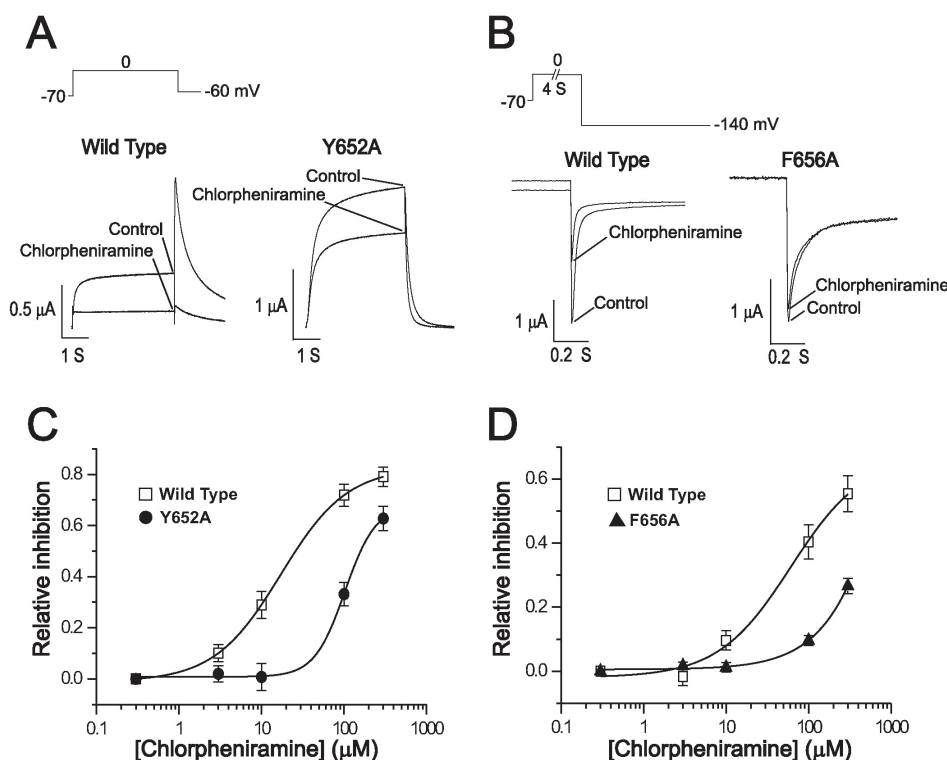
Fig. 4. Concentration-dependent inhibition of WT and mutant hERG channels expressed in oocytes. (A, B) Representative traces for WT and mutant HERG channel currents in the presence and absence of indicated concentrations of chlorpheniramine. The effect of the drug on WT and Y652A was quantified during a 4 s activating pulse to 0 mV from a holding potential of −90 mV. To increase the amplitude of poorly expressed F656A mutant channels, tail currents were recorded at −140 mV instead of −60 mV after the 4 s activating pulses. (C, D) The concentration-response curves were fitted with a logistic dose-response equation to obtain the IC$_{50}$ values of 17.1±0.1 μM (n=5), 102.7±27.7 μM (n=7), 61.1±37.8 μM (n=7), and 2,153.2±3.1 μM (n=7) in WT (obtained using protocol of panel A), Y652A, WT (obtained using protocol of panel B), and F656A HERG channels, respectively. The potency of chlorpheniramine block was reduced in the mutant channels. Data were expressed as mean±S.E.M..

DISCUSSION

The results of this study suggest that chlorpheniramine inhibits hERG channels expressed heterologously in *Xenopus* oocytes. A relatively high extracellular concentration of the drug was required to block the hERG channels, with an IC$_{50}$ value of ~20 μM (at +40 mV). This may be due to the properties of the *Xenopus* oocyte-expression system. The concentration of chlorpheniramine at the cell membrane may be decreased by the vitelline membrane and egg yolk. The block of hERG by trifluoperazine (Choi et al., 2005) resulted in IC$_{50}$ values 10 times higher when the drug was applied to *Xenopus* oocytes rather than to mammalian cells. Therefore, the putative IC$_{50}$ value for the block of hERG channels by chlorpheniramine in mammalian cells such as human embryonic kidney (HEK) and cardiomyocytes might be in the range of ~2 μM.

The therapeutic plasma concentrations of chlorpheniramine typically range from 12.5 to 30 nM (Tagawa et al., 2001; Tagawa et al., 2002), The toxic plasma concentrations of chlorpheniramine has been shown to be 500 nM in the case report, which is ~10 times greater than the mean peak plasma level measured after a single therapeutic dose (Baselt and Cravey, 1995). Blood concentrations reported in two overdose fatalities were 1.25 and 2.75 μM (Reed, 1981; Baselt and Cravey, 1995). In guinea pig isolated papillary muscle, chlorpheniramine prolonged the action potential duration at 90% repolarization (APD$_{90}$) at 1 μM (Ki et al., 1996). Also, the rapid and extensive uptake of chlorpheniramine by various organs was reported in a rabbit model, with concentrations 30~160 fold higher in the lung, kidney and brain than the blood plasma level (Huang and Chiou, 1981). It has been suggested that drugs with an IC$_{50}$ at least 30 times greater than the highest achievable free plasma

concentration will not cause *torsades de pointes*, a potentially fatal ventricular tacharrhythmia (Redfern et al., 2003). In this context, chlorpheniramine could cause ventricular arrhythmia by blocking the hERG channel in overdose. Otherwise, chlorpheniramine could make the heart prone to fatal arrhythmia when it is used in conjunction with other drugs; indeed, case reports of life-threatening prolongation of QT$_c$ and QRS complex, as well as polymorphic ventricular tachycardia have been reported in the combinational use of chlorpheniramine with other compounds (Hung and Chang, 2006; Venkatraman et al., 2008).

This report shows that chlorpheniramine decreases the amplitudes of the maximum outward current and maximum peak tail current (Fig. 1). Moreover, the magnitude of the block was enhanced with increasing positive voltage, which increased the open probability and enhanced inactivation (Fig. 2). In addition, chlorpheniramine block may be state-dependent: the hERG channels are blocked mainly in the open and inactivated states but not in the closed state (Fig. 3). Finally, chlorpheniramine did not significantly alter the V$_{1/2}$ values of the activation curve, which suggests that the drug blocks the hERG channels without altering the activation properties (Fig. 1). In view of such a voltage-dependence of chlorpheniramine block of the hERG channels, the drug may well increase the action potential duration (APD) and make the heart more prone to arrhythmia, particularly under the pathological conditions associated with rapid heart actions and partially depolarized membranes.

It is known that hERG mRNA are abundantly present in the brain as well as in the heart (Wymore et al., 1996). The role of hERG channels in neuronal cells has been implicated in the regulation of the resting membrane potential, the cell cycle, the neurite outgrowth, and the spike-fre-

quency adaptation (Arcangeli et al, 1993; Arcangeli et al., 1995; Chiesa et al., 1997), Therefore, the hERG channel block by chlorpheniramine could change neural conduction activity and neuronal cell differentiation.

A large number of drugs from a range of classes, including antihistamines, antipsychotics, antiarrhythmics, antibiotics and gastrointestinal prokinetic agents, have been shown to block the hERG channels. It has been reported that the non-sedating histamine receptor antagonists, terfenadine and astemizole, inhibit hERG channels at nanomolar concentrations in a use- and voltage-dependent manner (Suessbrich et al., 1996). It has been shown that the inner cavity of the hERG channels is larger than that in the other voltage-gated K^+ channels, and can accommodate a wide range of chemical structures (Mitcheson, 2003). Particularly, the two aromatic residues in the S6 transmembrane domain, Tyr-652 and Phe-656, have been shown to be important because they could interact with structurally diverse drugs through hydrophobic interactions (Mitcheson et al., 2000). The present results (Fig. 4) indicate that mutations of the protein channel pore, Y652A and F656A, significantly attenuate the drug-induced hERG block, showing the direct action of chlorpheniramine on hERG channels.

In conclusion, chlorpheniramine, the H_1-receptor antihistamine, blocks the hERG channels underlying I_{Kr} when expressed in *Xenopus* oocytes. The inhibition of the channel shows voltage-, time- and state-dependencies; residues Tyr-652 and Phe-656 in the S6 domain are also important molecular determinants for the blocking interaction. Although the IC_{50} value for the blocking effect of chlorpheniramine for the hERG channel is a supra-therapeutic concentration, we have nevertheless shown that a conventional antihistamine could decrease I_{Kr}, which may contribute to drug-induced ventricular tachyarrhythmia.

ACKNOWLEDGEMENTS

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