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Molecular Basis for the Lack of HERG K^+ Channel Block-Related Cardiotoxicity by the H_1 Receptor Blocker Cetirizine Compared with Other Second-Generation Antihistamines

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ABSTRACT

In the current study, the potential blocking ability of K^+ channels encoded by the human ether-a-go-go related gene (*HERG*) by the piperazine H_1 receptor antagonist cetirizine has been examined and compared with that of other second-generation antihistamines (astemizole, terfenadine, and loratadine). Cetirizine was completely devoid of any inhibitory action on HERG K^+ channels heterologously expressed in *Xenopus laevis* oocytes in concentrations up to 30 μM. On the other hand, terfenadine and astemizole effectively blocked HERG K^+ channels with nanomolar affinities (the estimated IC_{50} values were 330 and 480 nM, respectively), whereas loratadine was ~300-fold less potent ($IC_{50} \approx 100$ μM). In addition, in contrast to terfenadine, cetirizine did not show use-dependent blockade. In SH-SY5Y cells, a human neuroblastoma clone that constitutively expresses K^+ currents carried by HERG channels (I_{HERG}), as well as in human embryonic kidney 293 cells stably transfected with HERG cDNA, extracellular perfusion with 3 μM cetirizine did not exert any inhibitory action on I_{HERG}. Astemizole (3 μM), on the other hand, was highly effective. Terfenadine (3 μM) caused a marked (≈80%) inhibition of I_{HERG} in SH-SY5Y cells, whereas loratadine, at the same concentration, caused a 40% blockade. Furthermore, the application of cetirizine (3 μM) on the intracellular side of the membrane of HERG-transfected human embryonic kidney 293 cells did not affect I_{HERG}, whereas the same intracellular concentration of astemizole caused a complete block. The results of the current study suggest that second-generation antihistamines display marked differences in their ability to block HERG K^+ channels. Cetirizine in particular, which possesses more polar and smaller substituent groups attached to the tertiary amine compared with other antihistamines, lacks HERG-blocking properties, possibly explaining the absence of torsade de pointes ventricular arrhythmias associated with its therapeutical use.

Drugs that block the histamine receptor subtype H_1 are widely used to relieve the symptoms of allergic reactions (Babe and Serafin, 1996). During the past 20 years, second-generation H_1 receptor blockers have been developed to overcome the marked antimuscarinic and sedative properties displayed by first-generation antihistamines like diphenhydramine, promethazine, hydroxyzine, and pyrilamine (Sorkin and Heel, 1985). Because of their novel pharmacological profile, second-generation antihistamines such as terfenadine, astemizole, loratadine, cetirizine, and ebastine have been progressively replacing the older molecules on the market, thus becoming one of the most prescribed drug families in Western countries (Woosley, 1996).

Despite the enormous success of second-generation antihistamines, in the mid-1980s, ~10 years after their introduction into the market, several reports appeared in the literature indicating the rare occurrence of a form of polymorphic ventricular arrhythmia, the so-called torsade de pointes, after the administration of astemizole or terfenadine (Jackman *et al.*, 1988). This ventricular arrhythmia, which occurs in the setting of a marked prolongation of the QT interval on the surface electrocardiogram, has been described either in patients taking intentional overdoses of these second-generation antihistamines (Craft, 1986; Davies *et al.*, 1989) or in subjects with one or more predisposing factors to the development of cardiac arrhythmias (Monahan *et al.*, 1990). These latter conditions included a reduced drug-metabolizing capacity of the patient (liver diseases, simultaneous administration of drugs known to inhibit hepatic metabolism such as macrolide antibiotics and ketoconazole), congenital prolonga-

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ABBREVIATIONS: I_{HERG}, K^+ currents carried by HERG channels; HEK, human embryonic kidney; I_{kr}, Rapid component of the repolarizing K^+ current in cardiac cells, EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

tion of the QT interval, ischemic heart disease, congestive heart failure, and electrolyte imbalance, such as hypokalemia or hypomagnesemia (Woosley, 1996).

It has been suggested recently that the QT prolongation and ventricular arrhythmia caused by terfenadine and astemizole might be secondary to their ability to interfere with cardiac potassium channels involved in action potential repolarization (Berul and Morad, 1995) and in particular with the I_{Kr} component of the cardiac repolarizing current (Salata *et al*. 1995). The human ether-a-go-go related gene (*HERG*) (Warmke and Ganetzky, 1994) seems to represent the molecular basis of I_{Kr} because the K^+ currents encoded by HERG display biophysical and pharmacological characteristics similar to those of I_{Kr} (Trudeau *et al*., 1995; Spector *et al*., 1996). In addition, evidence has been provided to show that HERG mutations are responsible for one form of long QT syndrome (LQTS-2), a chromosome 7-linked form of human arrhythmia (Curran *et al*., 1995). Altogether, these observations suggest that HERG is a primary target for both congenital (mutation-induced) and acquired (drug-induced) action potential prolongation, delayed repolarization, and cardiac arrhythmias.

Because of the occurrence of torsade de pointes in some of the patients taking terfenadine and astemizole, some authors have speculated that other nonsedating antihistamines might induce similar cardiotoxic effects (Good *et al*., 1994; Woosley, 1996). Among second-generation antihistamines, the piperazine H_1 receptor blocker cetirizine, which has been available for several years in Europe and was recently approved by the Food and Drug Administration for use in the United States, seems to lack arrhythmogenic potential both in humans (Sale *et al*., 1994) and in experimental animals (Hey *et al*., 1996). The aim of the current study was to (1) investigate the potential interaction of cetirizine with HERG K^+ channels heterologously expressed in *Xenopus laevis* oocytes and in HEK 293 cells (Zhou *et al*., 1998), or endogenously present in SH-SY5Y human neuroblastoma cells (Arcangeli *et al*., 1995; Bianchi *et al*., 1998), and (2) compare the actions of the piperazine molecule with those of other second-generation antihistamines reported to be associated with (e.g., terfenadine, astemizole, and ebastine) (Roy *et al*., 1996; Suessbrich *et al*., 1996; Ko *et al*., 1997) or lacking in (e.g., loratadine) (Ko *et al*., 1997) HERG K^+ channel-blocking activity.

Materials and Methods

X. laevis Oocyte Isolation

Ovarian lobes were surgically removed from adult female *X. laevis* frogs (Rettili di Schneider, Varese, Italy) and placed into 100-mm Petri dishes containing a Ca^{2+}-free solution composed of 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvic acid, 100 units/ml penicillin, and 100 μg/ml streptomycin, pH 7.5 with NaOH. After four extensive washes, the oocytes (stage V–VI) were dissociated at room temperature by collagenase treatment (type IA, 45–80 min at a concentration of 2 mg/ml). At the end of the collagenase treatment, the oocytes were placed in a Ca^{2+}-containing solution composed of 100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvic acid, 100 units/ml penicillin, and 100 μg/ml streptomycin, pH 7.5 with NaOH. Dissociated oocytes then were placed in a 19° incubator and microinjected on the next day.

Molecular Biology and Oocyte Injection

The cloning of HERG has been described previously (Warmke and Ganetzky, 1994). The engineering of HEK 293 cells stably transfected with HERG cDNA was described by Zhou *et al*. (1998). HERG cDNA was linearized with the restriction enzyme *Eco*RI, and RNA was transcribed *in vitro* from linearized cDNAs by means of commercially available kits (mCAP; Stratagene, La Jolla, CA) using the SP6 RNA polymerase. cRNA was stored in a stock solution (250 ng/μl) at −20° in 0.1 M KCl. One day after isolation, *X. laevis* oocytes were microinjected with 76 nl of cRNA stock solution or appropriate dilutions. At 2–10 days after the cRNA microinjection, HERG K^+ currents expressed in *X. laevis* oocytes were measured by the two-microelectrode voltage-clamp technique.

Cell Culture

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium, containing glucose (4.5 g/liter) and 5% fetal calf serum, and incubated at 37° in a humidified atmosphere with 10% CO_2 in 100-mm plastic Petri dishes. HEK 293 cells were cultured in minimal essential medium, supplemented with Earle's salts, nonessential amino acids (0.1 mM), penicillin (50 units/ml), streptomycin (50 μg/ml), G418 (0.4 mg/ml), and 10% fetal calf serum and incubated at 37° in a humidified atmosphere with 5% CO_2 in 100-mm plastic Petri dishes. For electrophysiological experiments, the cells were seeded onto glass coverslips (Fisher) coated with poly-L-lysine (30 μg/ml). All the experiments were performed 1–4 days after seeding at room temperature (22–23°).

Electrophysiology

Voltage-clamp with two microelectrodes. The oocytes were voltage-clamped with a commercially available amplifier (Warner OC-725A; Warner Instruments, Hamden, CT). Current and voltage electrodes were filled with 3 M KCl and 10 mM HEPES (pH 7.4; ≈1 MΩ resistance). The bath solution contained 88 mM NaCl, 10 mM KCl, 2.6 mM $MgCl_2$, 0.18 mM $CaCl_2$, and 5 mM HEPES, pH 7.5. This solution was perfused in the recording chamber at a rate of ~0.2 ml/min. Data were stored on the hard disk of a 486 IBM compatible computer for off-line analysis. The pCLAMP software (version 6.0.2: Axon Instruments, Burlingame, CA) was used for data acquisition and analysis. Currents were recorded at room temperature.

Patch-clamp. Currents from the human neuroblastoma SH-SY5Y and the HERG-transfected HEK 293 cells were recorded at room temperature using a commercially available amplifier (Axopatch 200A: Axon Instruments). The whole-cell configuration of the patch-clamp technique (Hamill *et al*., 1981) was adopted using glass micropipettes of 3–7 MΩ resistance. No compensation was performed for pipette resistance and cell capacitance. The cells were perfused with an extracellular solution containing 100 mM KCl, 10 mM EGTA, and 10 mM HEPES, pH 7.3 with KOH (for the SH-SY5Y cells), or 150 mM NaCl, 10 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.4 with NaOH (for the HERG-transfected HEK 293 cells). The pipettes were filled with 110 mM CsCl, 10 mM tetraethylammonium-Cl, 2 mM $MgCl_2$, 10 mM EGTA, 8 mM glucose, 2 mM Mg-ATP, 0.25 mM cAMP, and 10 mM HEPES, pH 7.3 with NaCl KOH (for the SH-SY5Y cells), or 130 mM K-Aspartate, 10 mM NaCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM EGTA, 2 mM Mg-ATP, 0.25 mM cAMP, and 10 mM HEPES, pH 7.4 with NaOH (for the HERG-transfected HEK 293 cells).

Drugs and Statistics

All the reagents were purchased from Sigma Chemical (Milan, Italy). Astemizole was kindly provided by Janssen-Cilag (Rome, Italy). Loratadine was kindly obtained from Schering-Plough (Milan, Italy). Cetirizine was generously donated by UCB Pharma (Torino, Italy). The H_1 receptor antagonists were dissolved in dimethylsulfoxide at concentrations between 5 and 50 mM, and stock solutions were kept at −20°. Appropriate drug dilutions were prepared daily.

The maximal dimethylsulfoxide concentration (0.6%) did not affect HERG K^+ channels recorded in *X. laevis* oocytes, HEK 293 cells, or SH-SY5Y human neuroblastoma cells. Statistical significance between the data was obtained with the Student's *t* test or an analysis of variance followed by Tukey's test. When appropriate, data are expressed as the mean ± standard error.

Results

Differential effect of cetirizine on the K^+ currents carried by HERG channels expressed in *X. laevis* oocytes compared with astemizole, terfenadine, and loratadine. On microinjection with HERG cRNA, *X. laevis* oocytes expressed a K^+ current with biophysical properties that resembled those of I_{Kr} (Trudeau *et al.*, 1995; Spector *et al.*, 1996). This K^+ current is activated by depolarization but displays a pronounced inward rectification of the current-voltage relationship at positive potentials (>0 mV), displays rather slow kinetics of activation, and exhibits a large inward component on repolarization to −100 mV, a value of membrane potential below the equilibrium potential for K^+ ions (Fig. 1).

Extracellular perfusion (5 min) with cetirizine in concentrations ranging from 1 to 30 μM failed to affect HERG K^+ channels in *X. laevis* oocytes (Fig. 1A). By contrast, perfusion with another second-generation H_1 receptor blocker, astemizole, at the same concentrations (1–10 μM), and with terfe-

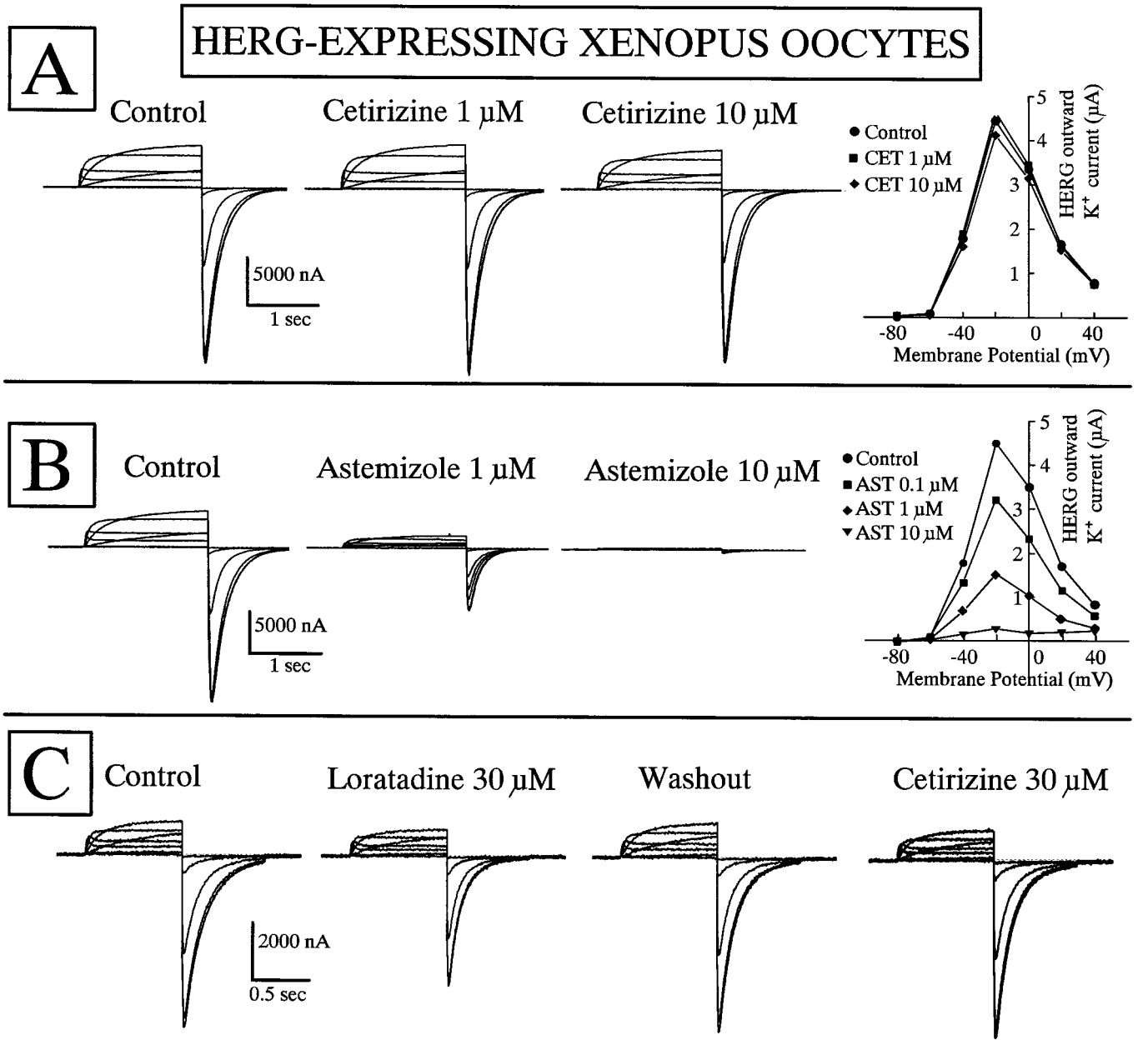

Fig. 1. Effect of cetirizine on HERG K^+ currents expressed in *X. laevis* oocytes showing a comparison of astemizole with loratadine. Representative current traces and current-to-voltage relationships were recorded from two different HERG-expressing oocytes under control conditions and after a 5-min perfusion with cetirizine (A, 1 and 10 μM) or astemizole (B, 1 and 10 μM). C, Representative current traces from a single HERG-expressing oocyte recorded under control conditions, after a 10-min perfusion with 30 μM loratadine, after a 20-min washout, and after a 10-min exposure to 30 μM extracellular cetirizine. Holding potential, −90 mV; test potentials, from −80 mV to +40 mV in 20-mV steps; return potential, −100 mV. *Dashed lines*, zero-current level.

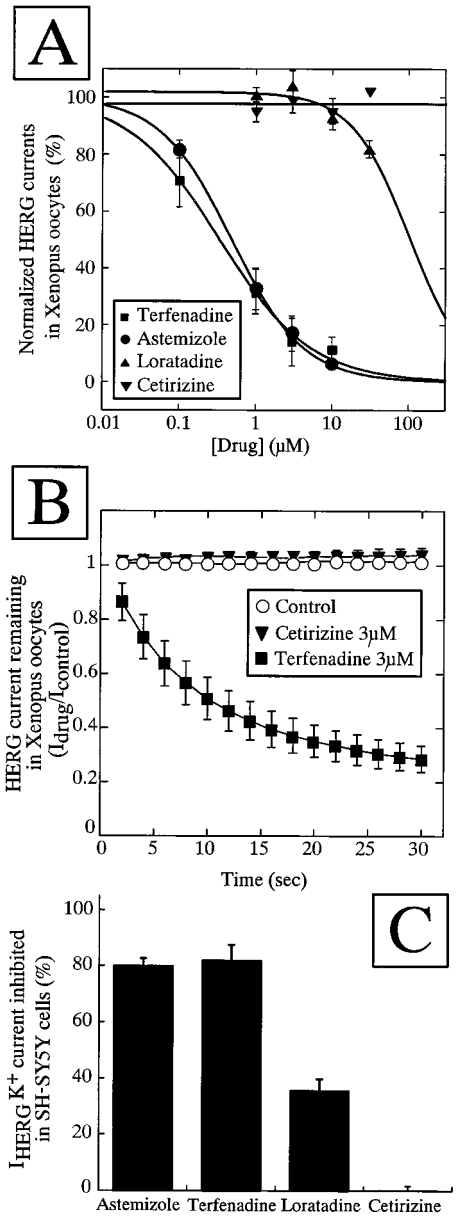

Fig. 2. Lack of effect of cetirizine in inhibiting HERG K^+ channels in *X. laevis* oocytes and SH-SY5Y human neuroblastoma cells. A, Dose-response curve for HERG K^+ channels block by four second-generation antihistamines. The inward HERG K^+ tail currents recorded in *X. laevis* oocytes on repolarization to −100 mV after depolarizing pulses of 2 sec to 0 mV were normalized to the control value and expressed as a function of drug concentration. *Solid lines*, fits of the experimental data to the binding isotherm $y = \max/(1 + X/IC_{50})^n$, where X is the drug concentration, and n is the Hill coefficient. Fitted values for n were between 0.72 and 0.96. Each point is the mean ± standard error of three to six determinations. B, Cumulative block of HERG K^+ currents expressed in *X. laevis* oocytes by terfenadine and lack of effect of cetirizine. The same experimental protocol (holding potential, −90 mV, 15 pulses to 0 mV for 300 msec followed by a 100-msec return to −120 mV; 0.5-Hz pulsing frequency) was repeated in the same HERG-expressing oocytes under control conditions, after a 6-min perfusion with 3 μM cetirizine, and after a 6-min exposure to 3 μM extracellular terfenadine. The peak inward K^+ currents carried by HERG channels at −120 mV were normalized to the current value obtained in the first pulse of the control condition (no drug perfusion) for each cell and expressed as a function of time. C, Effect of the four different H_1 receptor antagonists astemizole, terfenadine, loratadine, and cetirizine on I_{HERG} constitutively expressed in SH-SY5Y human neuroblastoma cells. The same experimental protocol (holding potential, −60 mV; test potential, 0 mV for 10 sec; return potentials, from 0 to −140/−180 mV in −20-mV steps for 100 msec) was performed in several SH-SY5Y cells under control conditions and after a 5-min perfusion with each H_1 receptor antagonist (3 μM). Drug effects are reported as percent of inhibition of the inward I_{HERG} current at −140 mV, without leak subtraction. *Values,* mean ± standard error of four determinations for each drug.

nadine (see below) dose-dependently inhibited HERG K^+ channels (Fig. 1B). On the other hand, loratadine, like cetirizine, failed to interfere with HERG K^+ currents in concentrations of 1–10 μM, whereas it caused a certain degree of inhibition of HERG K^+ currents (≈20%) at a higher concentration (30 μM) (Fig. 1C).

Fig. 2A shows the dose-response curves for HERG K^+ channels blockade by cetirizine and the other three second-generation H_1 receptor antagonists that were studied for comparison. Astemizole and terfenadine blocked HERG K^+ channels in a concentration-dependent manner with IC_{50} values in the nanomolar range (480 and 330 nM, respectively). Loratadine was ~300 times less potent than astemizole and terfenadine in inhibiting HERG K^+ channels (IC_{50} = 101 μM). In contrast, cetirizine was completely devoid of any inhibitory action on HERG K^+ channels even at the highest concentration tested (30 μM). It should be noted that although the estimated IC_{50} value for terfenadine overlapped with those previously described (Roy *et al.*, 1996; Suessbrich *et al.*, 1996), the potency of astemizole in inhibiting HERG K^+ channels in the current study was ~10 times lower than previously reported (Suessbrich *et al.*, 1996). This might be due to longer incubation times used by Suessbrich *et al.* (1996), which allowed to achieve higher intracellular drug concentrations.

To investigate whether cetirizine displayed use-dependent blockade of HERG K^+ channels, oocytes were superfused with cetirizine for 6 min while the membrane potential was held at −90 mV, a hyperpolarized value that does not allow the opening of the HERG K^+ channels; then, the cells were pulsed at high frequencies (0.5 Hz) to 0 mV, a depolarized potential that maximally activates the K^+ conductance. Using this voltage protocol, cumulative channel blockade can be revealed if the interpulse time is shorter than the dissociation rate of the blocking drug from the receptor site (Spector *et al.*, 1996; Suessbrich *et al.*, 1996). However, even under this experimental condition, no effect of cetirizine (3 μM) on the inward tail currents carried by HERG K^+ channels could be detected (Fig. 2B). By contrast, using an identical voltage-clamp protocol, terfenadine (3 μM) caused a cumulative block of HERG K^+ channels (Fig. 2B), suggesting that at −100 mV, the drug does not completely dissociate during the interval between successive pulses.

Comparison of the effects on HERG K^+ channels constitutively expressed in SH-SY5Y human neuroblastoma cells (I_{HERG}) between cetirizine and other second-generation antihistamines. Beside the fundamental role of the I_{Kr} current in regulating action potential repolarization in cardiac cells, recent evidence suggests that HERG K^+ channels (I_{HERG}) are also expressed in other excitable tissues such as the brain (Wymore *et al.*, 1997), in several neuroblastoma cell lines (Arcangeli *et al.*, 1995), and in other tumor cell lines such as TE671 human rhabdomyosarcoma, the human mammary gland adenocarcinoma SK-BR-3, the monoblastic leukemia line FLG29.1, the pituitary cell lines GH_3, GH_4, and MMQ, and others (Bianchi *et al.*,

1998). For this reason, the SH-SY5Y clone of human neuroblastoma cells was used to compare the effects of the four different H_1 receptor blockers on mammalian cells that constitutively express HERG K^+ channels.

Due to the simultaneous expression of various classes of K^+ channels in these cells, I_{HERG} was studied by means of a voltage-clamp protocol in which the cell was depolarized for 10 sec to 0 mV, a membrane potential that fully activated I_{HERG} and completely inactivated the delayed rectifier K^+ current and then repolarized to increasingly negative voltages (from 0 to −140/−180 mV) for ~100 msec. Using this voltage protocol, it is possible to detect a K^+-selective inward tail current displaying the biophysical properties of I_{HERG} (Arcangeli *et al*., 1995; Bianchi *et al*., 1998). The relatively small density of HERG K^+ channels in SH-SY5Y human neuroblastoma cells required the use of a high (100 mM) external K^+ concentration as a charge carrier. Fig. 2C summarizes the percent of inhibition of I_{HERG} by the same concentration (3 μM) of the four second-generation antihistamines examined in several SH-SY5Y human neuroblastoma cells. In analogy to the results obtained in oocytes, superperfusion with astemizole or terfenadine for 3–8 min caused an almost complete suppression of the inward tail current of I_{HERG} (≥80% blockade). On the other hand, the same concentration of loratadine caused a 40% blockade of I_{HERG}, whereas cetirizine (3 μM) was completely devoid of any inhibitory action.

Differential effect of the intracellular and extracellular applications of astemizole and cetirizine on I_{HERG} heterologously expressed after stable transfection of HEK 293 cells with HERG cDNA. The hypothesis that the lack of inhibitory action of cetirizine on I_{HERG} in both *X. laevis* oocytes and SH-SY5Y human neuroblastoma cells was caused by the relatively poor access of the drug to its putative intracellular receptor site on the channel has been investigated in a subsequent series of studies. To this aim, HEK 293 cells stably transfected with HERG cDNA (Zhou *et al*., 1998) were used. In fact, in these cells the ~50 times higher density of I_{HERG} compared with the SH-SY5Y human neuroblastoma cells (Taglialatela M, unpublished observations) allows the adequate recording of I_{HERG} with lower concentrations of extracellular K^+ (10 mM). Using this extracellular K^+ concentration, it is possible to obtain stable recording conditions lasting several minutes (up to 1 hr) and to resolve outward K^+ currents carried by HERG channels.

Fig. 3A shows representative current traces from the same HERG-transfected HEK 293 cell subsequently recorded in control conditions and after 5 min of extracellular 3 μM cetirizine perfusion. In accordance with the results obtained in *X. laevis* oocytes and SH-SY5Y human neuroblastoma cells, cetirizine failed to affect inward and outward I_{HERG}. After a 5-min perfusion with 3 μM cetirizine, the inward component of I_{HERG} in HERG-transfected HEK 293 cells was blocked by 1 ± 4.6% (five determinations; $p > 0.05$). By contrast, 3 μM astemizole blocked 91.8 ± 2.5% of the control inward current recorded before astemizole superfusion (eight determinations; $p < 0.05$). It should be emphasized that the small residual outward and inward K^+ currents recorded after prolonged (>5 min) perfusion with astemizole are likely to be carried by outwardly rectifying K^+ channels constitutively expressed in HEK 293 cells, presumably of the delayed-rectifier type. This current does not seem to be related to I_{HERG} expression because it remained unchanged after prolonged exposure to astemizole and was identical to the K^+ current recorded in untransfected HEK 293 cells, which also was unaffected by external perfusion with 3 μM astemizole (data not shown). Because the inward component of the K^+ current in HERG-transfected HEK 293 cells was almost entirely accounted for by I_{HERG}, whereas the outward component was largely contaminated by endogenous K^+ channels, the analysis of the effect of second-generation antihistamines was restricted to the inward K^+ current component.

To investigate whether cetirizine could be an effective blocker of I_{HERG} when applied on the intracellular side of the membrane, we studied the time course of the inward component of I_{HERG} in HERG-transfected HEK 293 cells after gaining access to the cell interior in the presence and absence of the drug inside the recording pipette (Fig. 3B). Under control conditions (no drug in the pipette), a 20% decrease of the inward K^+ current occurred after 20 min. When cetirizine (3 μM) was included in the patch pipette, the time-dependent reduction in I_{HERG} in HERG-transfected HEK 293 cells was superimposable to that observed in controls. By contrast, if the same concentration of astemizole was added to the pipette solution, I_{HERG} showed a marked time-related decline, being almost completely blocked after 20 min (≥95% blockade), which suggested that an effective exchange between the pipette solution and the cell cytoplasm was occurring because astemizole was able to diffuse out of the pipette and to reach its binding site, presumably located on the cytoplasmic surface of the HERG K^+ channels. Fig. 3C shows the complete time course of the inward I_{HERG} decline under control conditions and with 3 μM cetirizine or astemizole in the recording pipette. It should be noted that under our experimental conditions, cetirizine is unlikely to diffuse rapidly out of the pipette and to cause immediate internal block because the inward I_{HERG} recorded at time 0 min in the three experimental groups (control, 3 μM astemizole in the pipette, and 3 μM cetirizine in the pipette) did not differ ($p > 0.05$) among each other, being 2324 ± 265 pA (eight determinations), 2146 ± 263 pA (six determinations), and 2451 ± 630 pA (six determinations), respectively.

Discussion

The results of the current study suggest that the four second-generation H_1 receptor antagonists terfenadine, astemizole, loratadine, and cetirizine display considerable heterogeneity in blocking constitutively and heterologously expressed HERG K^+ channels. In fact, whereas cetirizine was completely devoid of any inhibitory action on these K^+ channels, astemizole and terfenadine both inhibited HERG K^+ channels with nanomolar affinities, whereas loratadine interfered with HERG K^+ channels only at the highest concentrations used.

The observation that different H_1 receptor antagonists display marked differences in their ability to inhibit HERG K^+ channels is of crucial clinical relevance considering that on one hand, these drugs are among the most frequently prescribed drugs in Western countries (Woosley, 1996), and on the other hand, these K^+ channels have a crucial role in controlling the duration of the cardiac action potential (Curran *et al*., 1995; Trudeau *et al*., 1995; Spector *et al*., 1996). In fact, the induction of cardiac arrhythmias by terfenadine and

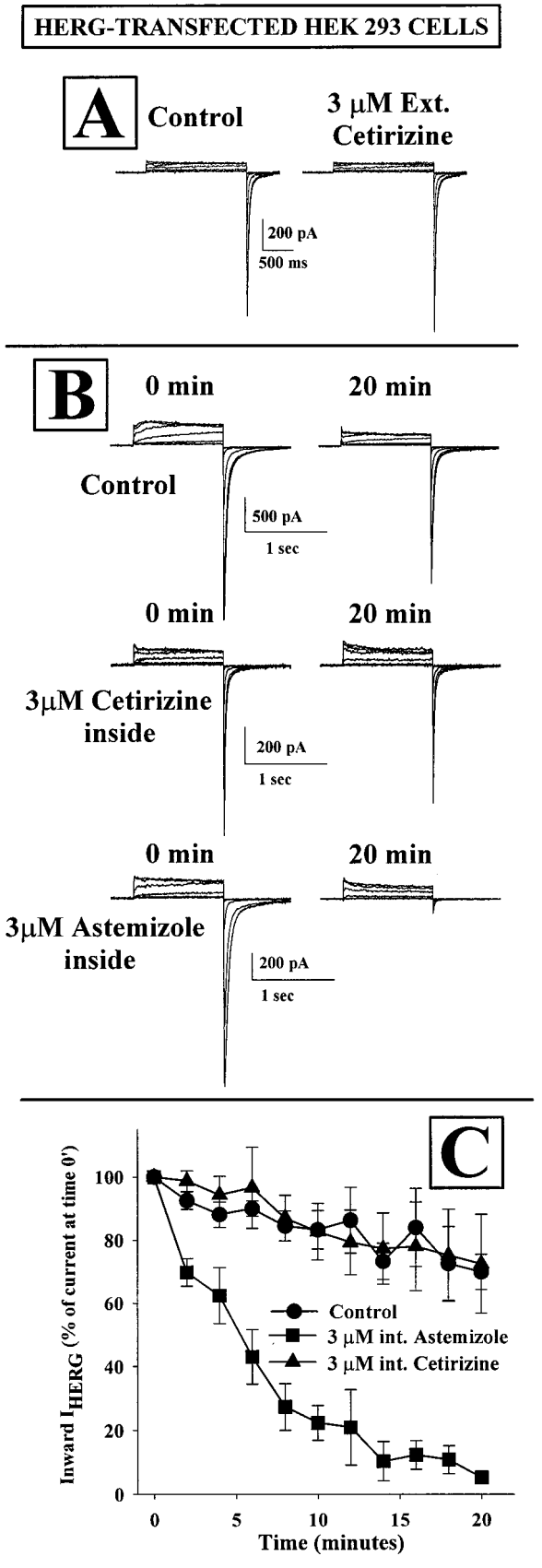

Fig. 3. Comparative effect of cetirizine and astemizole on I_{HERG} heterologously expressed in HEK 293 cells stably transfected with HERG cDNA. A, Effect of extracellular perfusion with cetirizine. Representative current traces were recorded in the whole-cell configuration of the patch-clamp technique from a single HERG-transfected HEK 293 cell. Records were obtained under control conditions and after a 5-min perfusion with 3 μM cetirizine. Holding potential, −85 mV; test potentials, from −60 mV to +60 mV in 20-mV steps; return potential, −100 mV. B, Effect of intracellular exposure to cetirizine or astemizole: currents at 0 and 20 min. Representative current traces were recorded at time 0 min and after 20 min in three different HERG-transfected HEK 293 cells. *Top*, control cell (K-aspartate in the pipette). *Middle*, K-aspartate plus 3 μM cetirizine. *Bottom*, K-aspartate plus 3 μM astemizole. Holding potential, −85 mV; test potentials, from −60 mV to +60 mV in 20-mV steps; return potential, −100 mV. C, Effect of intracellular exposure to cetirizine or astemizole: time course of I_{HERG} during the 20 min after breaking into the cell (time 0 min) under three different experimental situations: ●, control K-aspartate solution inside the pipette; ▲, K-aspartate plus 3 μM cetirizine; and ■, K-aspartate plus 3 μM astemizole. The tail current values obtained in each experimental group were normalized to the current recorded at time 0 min (100%) (see Results).

astemizole has been documented extensively (Craft, 1986; Davies *et al.*, 1989; Monahan *et al.*, 1990). The cardiotoxic effects exerted by these two molecules has been mostly related to their ability to prolong cardiac repolarization and therefore to induce early afterdepolarizations, which are thought to be one of the mechanisms for the genesis of torsade de pointes (Singh, 1993). More recently, after the discovery that the K^+ channels encoded by HERG represent the molecular basis of I_{Kr} (Sanguinetti *et al.*, 1995), terfenadine- and astemizole-induced cardiotoxicities have been tightly associated with their ability to block HERG K^+ channels (Roy *et al.*, 1996; Suessbrich *et al.*, 1996), although blockade of other cloned K^+ channels has also been reported for terfenadine (Rampe *et al.*, 1993; Crumb *et al.*, 1995). The existence of a tight correlation between the cardiotoxic effects of H_1 receptor antagonists and HERG K^+ channel blockade also is suggested by the observation that the IC_{50} values for HERG K^+ channels blockade by terfenadine and astemizole are close to the plasma concentration range (30–300 nM) measured in humans when ventricular arrhythmias occur (Hoppu *et al.*, 1991; Yun *et al.*, 1993; Woosley, 1996). Furthermore, it should be noted that the adverse cardiovascular effects of terfenadine, astemizole, and ebastine occur at plasma concentrations similar to those required to block peripheral H_1 receptors in guinea pigs (Hey *et al.*, 1996).

The cardiac side effects of astemizole and terfenadine have led to the suggestion that other second-generation H_1 receptor antagonists also might display similar untoward cardiac effects (Good *et al.*, 1994; Woosley, 1996). However, the observation in the current study that cetirizine was completely devoid of any interference with endogenously or heterologously expressed HERG K^+ channels seems to suggest that torsade de pointes is not likely to occur during conventional therapy with this drug. This conclusion seems to be confirmed by the observation that cetirizine did not display significant prolongation of the QT interval in experimental animals (Hey *et al.*, 1996) or humans (Sale *et al.*, 1994) and that no study has yet appeared in the literature reporting cardiac arrhythmias or QT prolongation associated with its use (Woosley, 1996). Furthermore, in a recent pharmacosurveillance study in which the risk profile for heart rhythm disorders and cardiac deaths was determined for some of the most common nonsedating antihistamines, cetirizine displayed the lowest adverse drug reaction report rate per million defined daily doses (Lindquist and Edwards, 1997).

The lack of inhibitory effect of cetirizine on HERG K^+ channels seems not to be the consequence of the poor perme-

ability of the *X. laevis* oocyte membrane where HERG channels have been expressed because it was also observed in SH-SY5Y human neuroblastoma cells constitutively expressing I_{HERG} (Arcangeli *et al*., 1995; Bianchi *et al*., 1998), as well as in HERG-transfected HEK 293 cells (Zhou *et al*., 1998). Furthermore, the results showing that cetirizine did not block HERG K^+ channels even at relatively high frequencies of stimulation (0.5 Hz) seems to rule out the possibility that cetirizine caused use-dependent blockade. In addition, it should be noted that the concentration of cetirizine (3 μM) used in the current study to evaluate the possible inhibition of I_{HERG} in SH-SY5Y and HERG-transfected HEK 293 cells was comparable to the levels of the drug observed in the plasma of normal subjects (1–5 μM) after the administration of doses two to six times higher than the commonly recommended daily therapeutical dose (Sale *et al*., 1994).

Loratadine inhibited HERG K^+ channels only at the highest concentrations tested (30 μM in *X. laevis* oocytes and 3 μM in the SH-SY5Y human neuroblastoma cells). The fact that loratadine was more effective in the SH-SY5Y human neuroblastoma cells compared with oocytes can be accounted for by (1) the lower membrane permeability of the frog oocyte membrane with respect to that of mammalian cells, (2) the expression in mammalian cells of HERG splice variants (London *et al*., 1997; Bianchi *et al*., 1998) not present in the oocyte expression system, which might slightly influence loratadine binding, or (3) the different contents of monovalent and divalent cations in the extracellular solutions used in the two cell preparations. Our data confirm those of a recent study in which loratadine (up to 10 μM) failed to affect HERG K^+ channels expressed in *X. laevis* oocytes but inhibited I_{HERG} stably expressed in a mammalian cell line with an IC_{50} value of 3 μM (Lacerda *et al*., 1997). However, it should be mentioned that 3 μM loratadine did not inhibit I_{Kr} in guinea pig ventricular myocytes (Ko *et al*., 1997), whereas in our study, loratadine was found to effectively block I_{HERG} in human neuroblastoma cells (3 μM) and in *X. laevis* oocytes (30 μM). Although the discrepancy between the two studies remains unresolved at the moment, possible explanations might be found in the expression of different HERG splice variants or in differences in recording conditions. In conclusion, the current results clearly suggest that loratadine is at least 300 times less potent than astemizole or terfenadine in inhibiting HERG K^+ channels. This observation might explain the lack of cardiac side effects associated with its use in humans (Woosley and Darrow, 1994; Brannan *et al*., 1995) and experimental animals (Hey *et al*., 1995), especially if one considers that after a single 40-mg dose, the C_{max} value of loratadine did not exceed 0.1 μM (Haria *et al*., 1994), a concentration at least 30 times lower than those used in the current study.

A direct comparison of HERG-blocking properties by the four H_1 receptor blockers also gives further insight into the structure-activity relationships for these molecules (Fig. 4). In fact, it has been suggested that the HERG K^+ channel-blocking properties of terfenadine and its structural analogue ebastine are at least in part related to the substituting groups attached to the tertiary amine of the molecule rather than to the presence of the piperidine ring (Salata *et al*., 1995; Ko *et al*., 1997). This view seems to be confirmed by the observation that loratadine, which also exhibits a piperidine ring in its structure, was at least 300 times less potent than terfenadine in inhibiting HERG K^+ channels. Furthermore, the aromatic ring structures common to most second-generation H_1 receptor antagonists seem not to be relevant for HERG K^+ channels blockade. In fact, this region of the molecule confers H_1 receptor-blocking activity (Babe and Serafin, 1996); however, no correlation has been recently found between the ability to prolong the cardiac action potential

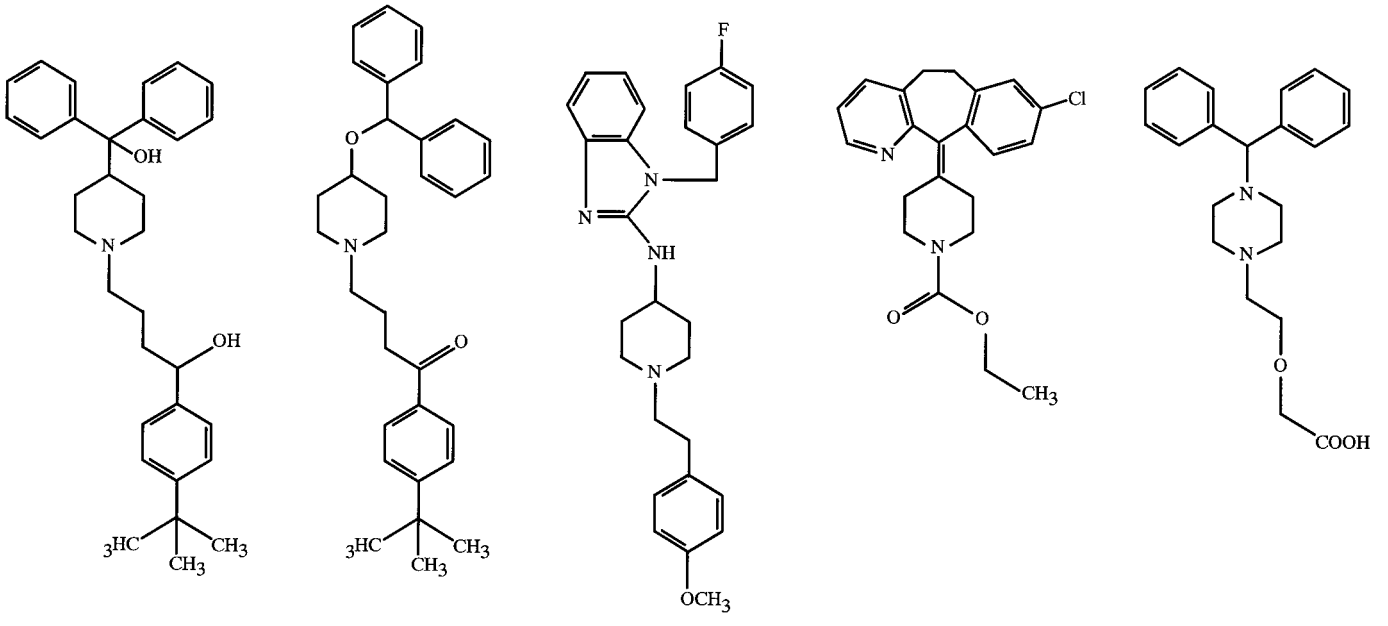

Fig. 4. Chemical structures of five different second-generation H_1 receptor blockers.

duration, an effect possibly related to HERG K^+ channels blockade, and the H_1 antagonistic activity by several antihistamines (Zhang, 1997).

Lipophilicity and bulkiness seem to be the two crucial parameters in the substituting groups attached to the tertiary amine conferring HERG K^+ channel-blocking capacity to the antihistaminic molecule (Zhang, 1997). In fact, both cetirizine and loratadine have polar and smaller substitutions at the nitrogen atom (amido and carboxyl groups, respectively), whereas terfenadine, astemizole, and ebastine, the H_1 receptor antagonists most effective in inhibiting HERG K^+ channels, have less polar and bulkier phenyl rings in the substituting side chains. This hypothesis is supported further by the observation that the more polar metabolites of terfenadine and astemizole, terfenadine carboxylate and norastemizole, respectively, do not display cardiotoxic potential (Hey *et al*., 1996). Furthermore, terfenadine carboxylate has been shown to be devoid of HERG K^+ channel-blocking ability (Roy *et al*., 1996).

Because it has been demonstrated that HERG K^+ channel blockade by terfenadine (Roy *et al*., 1996), as well as by the antiarrhythmic dofetilide (Kiehn *et al*., 1996), occurs at a site located on the cytoplasmic side of the channel, it seems possible to hypothesize that the lack of effect of cetirizine and the low potency of loratadine in inhibiting HERG K^+ channels might be due to (1) a lower membrane permeability caused by their higher polarity or (2) their inability to interact with the terfenadine/astemizole receptor site on the channel molecule. The observation that internally applied cetirizine failed to inhibit I_{HERG} in HERG-transfected HEK 293 cells seems to suggest that the intracellular side of the channel molecule is insensitive to the drug, at least at the cytosolic concentrations reached in the current experiments. Therefore, it seems plausible to conclude that cetirizine lacks the ability to optimally interact with the terfenadine/astemizole receptor site on the intracellular side of the HERG K^+ channel molecule.

In conclusion, the results of the current study suggest that second-generation H_1 receptor antagonists display marked heterogeneity in their blocking ability of HERG K^+ channels. In particular, loratadine and cetirizine, which lack HERG-blocking ability, do not seem to induce ventricular arrhythmias such as torsade de pointes, whereas terfenadine and astemizole are potent blockers of HERG K^+ channels and display significant arrhythmogenic potential. This conclusion might be of therapeutical significance for patients at risk of developing cardiac arrhythmias who require therapy with H_1 receptor blockers.

Finally, the observation that antihistamines greatly differ in their ability to interfere with HERG K^+ channels and, consequently, to determine cardiotoxic effects emphasizes the importance of an evaluation of the possible blockade of HERG K^+ channels, either constitutively present or heterologously expressed, during the early developmental phases of novel compounds belonging to this therapeutical class.

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