Inhibition of HERG1 K$^+$ channels by the novel second-generation antihistamine mizolastine

*,[1]Maurizio Taglialatela, [1]Anna Pannaccione, [1]Pasqualina Castaldo, [1]Giovanna Giorgio & [1]Lucio Annunziato

[1]Section of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples Federico II, Naples, Italy

1   Ventricular arrhythmias are rare but life-threatening side effects of therapy with the second-generation H$_1$ receptor antagonists terfenadine and astemizole. Blockade of the K$^+$ channels encoded by the Human Ether-à-go-go-Related Gene 1 (HERG1) K$^+$ channels, which is the molecular basis of the cardiac repolarizing current I$_{Kr}$, by prolonging cardiac repolarization, has been recognized as the mechanism underlying the cardiac toxicity of these compounds.

2   In the present study, the potential blocking ability of the novel second-generation H$_1$ receptor antagonist mizolastine of the HERG1 K$^+$ channels heterologously expressed in *Xenopus* oocytes and in HEK 293 cells or constitutively present in SH-SY5Y human neuroblastoma cells has been examined and compared to that of astemizole.

3   Mizolastine blocked HERG1 K$^+$ channels expressed in *Xenopus* oocytes with an estimated IC$_{50}$ of 3.4 μM. Mizolastine blockade was characterized by a fast dissociation rate when compared to that of astemizole; when fitted to a monoexponential function, the time constants for drug dissociation from the K$^+$ channel were 72.4$\pm$11.9 s for 3 μM mizolastine, and 1361$\pm$306 s for 1 μM astemizole.

4   In human embryonic kidney 293 cells (HEK 293 cells) stably transfected with HERG1 cDNA, extracellular application of mizolastine exerted a dose-related inhibitory action on I$_{HERG1}$, with an IC$_{50}$ of 350$\pm$76 nM. Furthermore, mizolastine dose-dependently inhibited HERG1 K$^+$ channels constitutively expressed in SH-SY5Y human neuroblastoma clonal cells.

5   The results of the present study suggest that the novel second-generation H$_1$ receptor antagonist mizolastine, in concentrations higher than those achieved *in vivo* during standard therapy, is able to block in some degree both constitutively and heterologously expressed HERG1 K$^+$ channels, and confirm the heterogeneity of molecules belonging to this therapeutical class with respect to their HERG1-inhibitory action.

British Journal of Pharmacology (2000) **131,** 1081–1088

Introduction

In the last two decades, second-generation H$_1$ receptor blockers have been developed for the symptomatic treatment of allergic reactions in order to overcome the marked antimuscarinic and sedative properties displayed by first-generation congeners (Sorkin & Heel, 1985). Due to their improved H$_1$ selectivity, absence of sedation, and possibly, antiallergic properties not deriving from their antihistaminic activity, second-generation H$_1$ receptor blockers are among the most prescribed drug families (Slater *et al.*, 1999).

Mizolastine (2-[[[1-[1-[(4-fluorophenyl)methyl]-1H-benzimidazol-2-yl]piperidinyl]methylamino]-4(3H)-pyrimidinone; SL 85.0324) is a new potent and selective second-generation H$_1$ receptor blocker available in Europe, which is effective in relieving the symptoms of allergic rhinitis (Leynadier *et al.*, 1996; Tasman & Weber, 1995) and urticaria (Brostoff *et al.*, 1996). This compound, structurally related to astemizole, is devoid of sedative (Patat *et al.*, 1995; Vuurman *et al.*, 1994) and antimuscarinic (Danjou *et al.*, 1992) properties, and

displays a more hydrophilic profile which confers a lower distribution volume, good bioavailability, and limited metabolic conversion by the hepatic CYPs 3A4 and 2A6 (Delauche-Cavallier *et al.*, 1999). These pharmacological properties have represented a distinct advantage over some other antihistamines.

Despite the obvious improvement in the treatment of allergic diseases represented by second-generation antihistamines, concerns over the risk/benefit profile of some members of this pharmacological class have recently been raised (Woosley, 1996). In fact, the rare occurrence of 'torsade de pointes', a form of polymorphic ventricular arrhythmia, has been described in patients taking intentional overdoses of the second-generation antihistamines astemizole or terfenadine (Craft, 1986; Davies *et al.*, 1989), or suffering from predisposing factors to the development of cardiac arrhythmias and undergoing therapy with these antihistamines (Monahnan *et al.*, 1990). These potentially fatal adverse cardiovascular reactions have increased the level of awareness in the medical community, leading to guidelines established by the European Agency for the Evaluation of Medicinal Products (EMEA) for pre-clinical and clinical

*Author for correspondence at: Section of Pharmacology, Dept. of Neuroscience, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy. E-mail: mtaglial@unina.it

studies aimed at reducing the risk for cardiovascular adverse effects by drugs to be introduced in the market (EMEA, 1997).

The QT prolongation and ventricular arrhythmia caused by terfenadine and astemizole have been related to the ability of these drugs to interfere with cardiac potassium channels involved in action potential repolarization (Berul & Morad, 1995), and particularly with the rapid component of the cardiac repolarizing potassium current (I_{Kr}) (Woosley *et al.*, 1993; Sanguinetti *et al.*, 1995; Salata *et al.*, 1995). The Human Ether-à-go-go-Related Gene 1 (HERG1) (Warmke & Ganetzky, 1994) seems to represent the molecular basis of I_{Kr}, since the K^+ currents encoded by HERG1 display biophysical and pharmacological characteristics similar to those of cardiac I_{Kr} (Trudeau *et al.*, 1995; Sanguinetti *et al.*, 1995).

The aim of the present study has been to investigate the potential interaction of increasing concentrations of mizolastine with HERG1 K^+ channels heterologously expressed in *Xenopus* oocytes and in human embryonic kidney 293 cells (HEK 293 cells) (Zhou *et al.*, 1998), or endogenously present in SH-SY5Y human neuroblastoma cells (Arcangeli *et al.*, 1995); and to compare the actions of the novel H_1 receptor blocker with those of astemizole, one of the second-generation antihistamines with well-known inhibitory actions on the K^+ channels encoded by HERG1 (Suessbrich *et al.*, 1996; Taglialatela *et al.*, 1998b).

Methods

Xenopus *oocytes isolation*

Ovarian lobes were surgically removed from adult female *Xenopus* frogs (Rettili di Schneider, Varese, Italy) and placed in 100-mm Petri dishes containing a Ca^{2+}-free solution of the following composition (in mM): NaCl 82.5, KCl 2, $MgCl_2$ 1, HEPES 5, piruvic acid 2.5, 100 u ml^{-1} penicillin, and 100 μg ml^{-1} 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^{-1}). At the end of the collagenase treatment, the oocytes were placed in a Ca^{2+}-containing solution of the following composition (in mM): NaCl 100, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, piruvic acid 2.5, 100 u ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin, pH 7.5 with NaOH. Dissociated oocytes were then placed in a 19°C incubator and used for the experiments on the following day.

Molecular biology and oocyte injection

The cloning of HERG1 (Warmke & Ganetzky, 1994), BEAG (Frings *et al.*, 1998), rKv2.1 (Frech *et al.*, 1989), and rKv3.1 (Taglialatela *et al.*, 1991) has already been described. These cDNAs were cloned into the following vectors: pSp64A$^+$ for HERG1, pCRII for BEAG, and pBluescript for rKv2.1 and rKv3.1. cDNAs were linearized with *Hind*III for BEAG, *Eco*RI for HERG1, and *Not*I for rKv2.1 and rKv3.1. cRNAs were transcribed *in vitro* from linearized cDNAs by means of a commercially available kit (mCAP, Stratagene), using T7 RNA polymerase for BEAG, rKv2.1, and rKv3.1, and SP6 RNA polymerase for HERG1. RNAs were quantified by the RiboGreen fluorescent kit (Molecular Probes) and stored in a stock solution (250 ng μl^{-1}) at -20°C in 0.1 M KCl. One day after isolation, *Xenopus* oocytes were microinjected with 46 nl

of the respective cRNA stock solution or appropriate dilution.

Cell culture

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), containing glucose (4.5 g l^{-1}) and 5% foetal calf serum (FCS), and incubated at 37°C in a humidified atmosphere with 10% CO_2 in 100-mm plastic Petri dishes. HEK 293 cells were cultured in modified eagle medium (MEM), supplemented with Earle's salts, nonessential amino acids (0.1 mM), penicillin (50 u ml^{-1}), steptomycin (50 μg ml^{-1}), G418 (0.4 mg ml^{-1}) and 10% foetal calf serum (FCS), and incubated at 37°C in a humidified atmosphere with 5% CO_2 in 100-mm plastic Petri dishes. For electrophysiological experiments, the cells were seeded on glass coverslips (Fisher) coated with poly-L-lysine (30 μg ml^{-1}). All the experiments were performed 1–4 days after seeding at room temperature (22–23°C).

Electrophysiology

Voltage-clamp with two microelectrodes The oocytes were voltage-clamped with a commercially available amplifier (Warner OC-725A, Warner Instr. Corp.). Current and voltage electrodes were filled with 3 M KCl, 10 mM HEPES (pH 7.4; ≈ 1 MΩ resistance). The bath solution contained (in mM): NaCl 88, KCl 10, $MgCl_2$ 2.6, $CaCl_2$ 0.18, HEPES 5, pH 7.5. This solution was perfused in the recording chamber at a rate of about 0.2 ml min^{-1}. 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 Instr., CA, U.S.A.) was used for data acquisition and analysis. Currents were recorded at room temperature.

Patch-clamp Currents from the human neuroblastoma SH-SY5Y and the HERG1-transfected HEK 293 cells were recorded at room temperature using a commercially available amplifier (Axopatch 200A, Axon Instruments, Foster City, CA, U.S.A.). 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 (in mM): KCl 100, EGTA 10 and HEPES 10, pH 7.3 with KOH (for the SH-SY5Y cells), or NaCl 150, KCl 10, $CaCl_2$ 3, $MgCl_2$ 1, HEPES 10, pH 7.4 with NaOH (for the HERG1-transfected HEK 293 cells). The pipettes were filled with (in mM) CsCl 110, tetraethylammonium-Cl 10 $MgCl_2$ 2, EGTA 10, glucose 8, Mg-ATP 2, cyclic AMP 0.25 and HEPES 10, pH 7.3 with NaCl KOH (for the SH-SY5Y cells), or K-Aspartate 130, NaCl 10, $CaCl_2$ 4, $MgCl_2$ 2, EGTA 10, Mg-ATP 2, cyclic AMP 0.25 and HEPES 10, pH 7.4 with NaOH (for the HERG1-transfected HEK 293 cells; free Ca^{2+}: 51 nM, calculated with MaxChelator Ver. 6.50).

Drugs and statistics

All the reagents were purchased from Sigma Chemicals (Italy). Astemizole was kindly provided by Janssen-Cilag (Rome, Italy). The H_1-receptor antagonists were dissolved in dimethylsulphoxide (DMSO) at concentrations between 5 and 50 mM, and stock solutions were kept at -20°C. Appropriate drug dilutions were prepared daily. The maximal DMSO concentration (0.6%) did not affect HERG1 K^+ channels recorded in *Xenopus* oocytes, HEK 293 cells, or SH-SY5Y

human neuroblastoma cells. Statistical significance between the data was obtained by means of the Student's *t*-test or by the ANOVA test followed by the Tukey test. When appropriate, data are expressed as the mean ± s.e.mean.

Results

Differential effect of mizolastine on the K^+ currents carried by four cloned K^+ channels expressed in Xenopus oocytes

Upon microinjection with HERG1 cRNA, *Xenopus* oocytes expressed a K^+ current having biophysical properties which resembled those of the I_{Kr} current (Trudeau *et al.*, 1995; Sanguinetti *et al.*, 1995). This K^+ current is activated by depolarization yet displays a pronounced inward rectification of the current-voltage relationship at positive potentials (>0 mV); furthermore, it shows rather slow kinetics of activation, and exhibits a large inward component upon repolarization to -100 mV, a membrane potential value below the equilibrium potential for K^+ ions. Extracellular perfusion with mizolastine (10 μM) inhibited HERG1 K^+ channels in *Xenopus* oocytes (Figure 1); the per cent of inhibition ($\approx 70\%$), was similar whether measured at the peak of the inward current elicited at -100 mV following long (1–2 s) depolarizing pulses to 0 mV or at the peak of the outward current during the depolarizing pulse. Analogously, 10 μM mizolastine also blocked by $\approx 70\%$ the outward K^+ currents carried by the channels encoded by the bovine isoform of the Ether-à-go-go Gene (BEAG; Frings *et al.*, 1998), when heterologously expressed in *Xenopus* oocytes. By contrast, the K^+ currents flowing through the channels encoded by $K_v2.1$ were insensitive to blockade by 10 μM mizolastine; whereas those carried by $K_v3.1$ were only slightly affected by the same concentration of the second-generation antihistamine (Figure 1).

Figure 2A shows the effect of different concentrations of mizolastine (1, 10 and 30 μM) on the inward K^+ currents carried by heterologously expressed HERG1 expressed in *Xenopus* oocytes recorded upon cell hyperpolarization to -100 mV following depolarization to 0 mV to activate the current. In Figure 2B, the per cent of control HERG1 K^+ tail currents blockade exerted by the different mizolastine concentrations (0.3–30 μM), calculated from the ratio $I_{drug}/I_{control}$ of the peak inward K^+ currents elicited at -100 mV following depolarization to 0 mV (without subtraction of the steady-state inward current measured at the end of the hyperpolarizing pulse), were normalized and fit to the following binding isotherm: $y = max/(1 + X/IC_{50})^n$, where X is the drug concentration and n the Hill coefficient. Fitted values for n were 0.93 ± 0.08. The IC_{50} for HERG1 blockade by mizolastine was 3.4 μM.

Since other second-generation antihistamines such as terfenadine and astemizole are known to potently inhibit HERG1 K^+ channels, in the next series of experiments the kinetics of HERG1 K^+ channels blockade by astemizole and mizolastine were compared. Figure 3 shows the time-course of the blockade of the inward HERG1 tail currents at -100 mV by increasing concentrations of mizolastine (0.3–3 μM), each perfused for 3 min followed by a washout period of variable duration (3–10 min). After achieving a steady-state block with each mizolastine concentration, the removal of the drug from the solution perfusing the oocytes caused a complete recovery of the K^+ currents. When the time-course of such recovery was fit to a monoexponential function, the
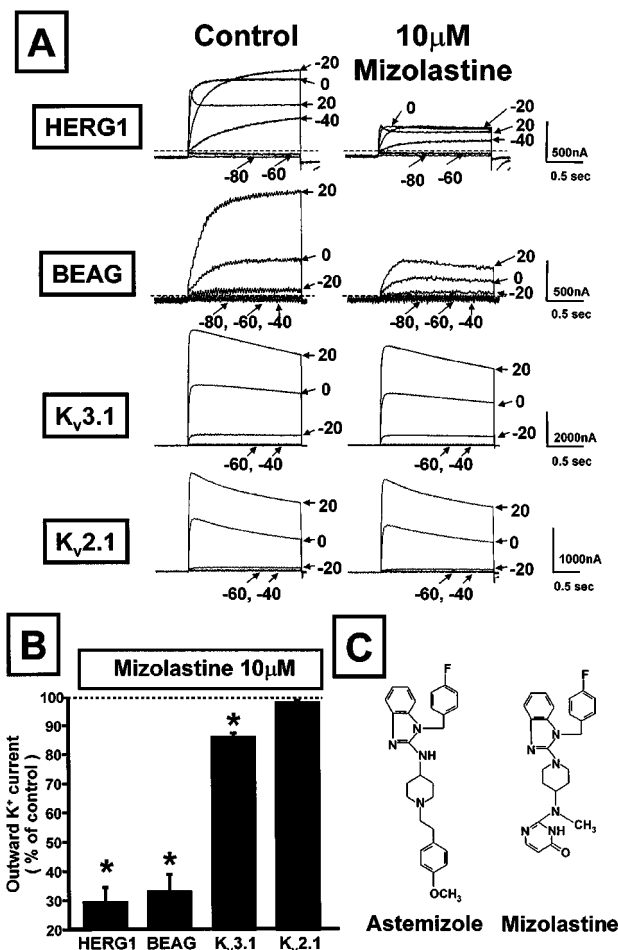

Figure 1 Effect of mizolastine on the K^+ currents carried by four cloned K^+ channels expressed in *Xenopus* oocytes. (A) Representative current traces recorded with the two-microelectrode voltage-clamp technique from four different oocytes expressing HERG1, BEAG, Kv3.1, and Kv2.1 recorded in control condition and after 5 min perfusion with 10 μM mizolastine. Holding potential: -90 mV (HERG1 and BEAG), -70 mV ($K_v3.1$ and $K_v2.1$); test potentials from -80 mV to $+20$ mV (HERG1 and BEAG) or from -60 mV to $+20$ mV ($K_v3.1$ and $K_v2.1$) in 20 mV steps; return potential: -100 mV (HERG1 and BEAG) or -70 mV ($K_v3.1$ and $K_v2.1$). When reported, the dashed lines in the panel indicate the zero current level. (B) The per cent variation of the outward K^+ current induced by 5 min perfusion with 10 μM mizolastine ($I_{drug}/I_{control}$) at voltages which maximally activated the conductance for each K^+ channel (0 mV for HERG1, $+20$ mV for BEAG, $K_v3.1$ and $K_v2.1$). Each experimental point is the mean ± s.e.mean of 3–8 separate experiments. Asterisks denote values significantly different from control values ($P<0.05$). (C) Chemical structures of mizolastine and astemizole.

time constants of this monoexponential function did not depend on mizolastine concentration. In the experiment shown in Figure 3, the time constants were 46.6 s, 31.2 s, and 48.4 s for 0.3, 1 and 3 μM mizolastine, respectively. These rates likely reflect the kinetics of drug dissociation from the channel and could not be accounted for by the time-course of solution exchange in the recording bath (≈ 15 s), as judged by the change in reversal potential of the HERG1 K^+ current occurring upon switching solutions with different K^+ concentrations (data not shown). On the other hand, the time-course of HERG1 K^+ current recovery after 1 μM astemizole was much slower than that occurring with mizolastine, resulting in a value of 2340 s in the experiment shown in Figure 3. When similar experiments and analysis
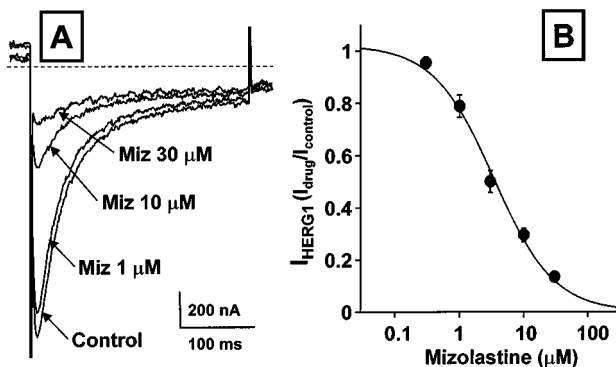
Figure 2 Dose-dependence of the inhibitory action of mizolastine on HERG1 K^+ currents expressed in *Xenopus* oocytes. (A) Representative current traces recorded with the two-microelectrode voltage-clamp technique from a single HERG1-expressing oocyte exposed to control condition, and after perfusion with 1, 10 and 30 μM mizolastine (5 min for each concentration). Holding potential: -90 mV; test potential: 0 mV; return potential: -100 mV. Only the tail currents recorded upon return to -100 mV are shown. The dashed line in the panel indicate the zero current level. (B) Dose-dependence of HERG1 K^+ currents inhibition by mizolastine. The inhibitory effect of the different concentration of the H_1 receptor antagonist on the peak of the inward HERG1 K^+ currents recorded at -100 mV was calculated for several cells and plotted against drug concentrations. The solid line represents the fit of the experimental data to the following binding isotherm: $y = max/(1 + X/IC_{50})^n$, where X is the drug concentration and n the Hill coefficient. The fitted value for n was between 0.72 and 0.96. Each point is the mean $\pm$ s.e.mean of 3–6 determinations.
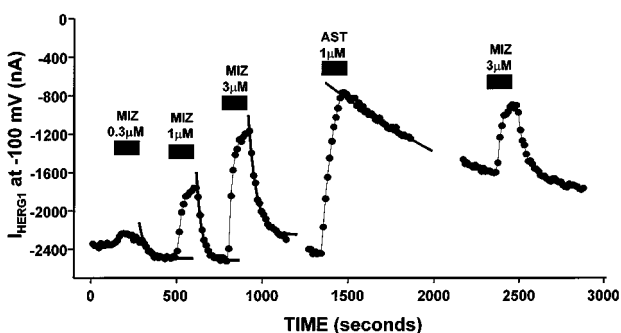

Figure 3 Kinetic difference between HERG1 K^+ channel blockade by mizolastine and astemizole in *Xenopus* oocytes. The peak of the inward tail K^+ current carried by HERG1 channels, measured upon return to -100 mV after depolarizing pulses of 1 s duration to 0 mV elicited every 15–20 s from an holding potential of -90 mV, is plotted versus time during the exposure to the different experimental conditions indicated by the black boxes. The solid lines are the fits of the experimental points recorded upon washout of each of the drug concentrations indicated to single exponential equations of the following form: $y = A1*exp\{-(t-K)/\tau\} + C$, using the fitting routines of the Clampfit module of the pClamp suite (ver. 6.0.3), where A1 is the amplitude of the exponential function, $(t-K)$ is the time range used fot the fitting (taking into consideration the start of the fit region, K), τ is the time constant, and C is an offset to account for the steady-state asymptote.

were performed on five different cells, the time constants for HERG1 K^+ current recovery were 72.4 ± 11.9 s ($n=5$) for 3 μM mizolastine, and 1361 ± 306 s ($n=5$) for 1 μM astemizole. These results suggested that the lower potency for HERG1 K^+ channels blockade exhibited by mizolastine when compared to astemizole (the IC_{50s} were 3.4 μM vs 480 nM, respectively) (Taglialatela *et al.*, 1998b) could be mainly ascribed to the faster dissociation rate of mizolastine from HERG1 K^+ channels.

Effects of mizolastine on HERG1 K^+ channels heterologously expressed upon stable transfection of HEK 293 cells with HERG1 cDNA or constitutively expressed in SH-SY5Y human neuroblastoma cells

In order to verify that the HERG1 inhibitory action exerted by mizolastine was not a characteristic of the *Xenopus* oocyte expression system, HEK 293 cells stably transfected with HERG1 cDNA (Zhou *et al.*, 1998) were used. Figure 4 shows representative current traces from the same HERG1-transfected HEK 293 cell subsequently recorded in control conditions and after perfusion with increasing mizolastine concentrations (0.01–1 μM), each lasting about 4 min. From a holding potential of -80 mV, the K^+ channels were activated by 1-s depolarizing pulses at potentials from -60 mV to $+40$ mV. At the end of the depolarizing pulses, the cell was repolarized to -100 mV, giving rise to a large inward tail current. In line with the results obtained in *Xenopus* oocytes, mizolastine displayed a dose-dependent inhibitory action on the HERG1 K^+ currents heterologously expressed in HEK 293 cells, with an IC_{50} for such inhibitory action of 350 ± 76 nM ($n=5$ cells). After perfusing the cells with the highest concentration of mizolastine (1 μM), 1 μM astemizole perfusion caused a further and almost complete blockade of HERG1 inward K^+ currents ($91.8 \pm 2.5\%$; $n=8$). The small residual outward and inward K^+ currents recorded after astemizole perfusion are likely carried by endogenous outwardly-rectifying K^+ channels constitutively expressed in HEK 293 cells, since it was insensitive to blockade by extracellular or intracellular application of astemizole and was identical to the K^+ current recorded in untransfected HEK 293 cells (data not shown).

In addition to the fundamental role of the I_{Kr} current in regulating action potential repolarization in cardiac cells, recent evidence suggests that HERG1 K^+ channels are also expressed in other excitable tissues such as the brain (Wymore *et al.*, 1997), in several neuroblastoma cell lines (Arcangeli *et al.*, 1995), as well as in most tumor cell lines of different histogenesis (I_{HERG1}) (Bianchi *et al.*, 1998). For this reason, the SH-SY5Y clone of human neuroblastoma cells was used to study the effect of mizolastine on mammalian cells constitutively expressing HERG1 K^+ channels. In order to avoid contamination by other K^+ currents also expressed in these cells, I_{HERG1} was studied by means of a voltage-clamp protocol in which the cell was depolarized for 10 s to 0 mV, a membrane potential which fully activated I_{HERG1} and completely inactivated the delayed rectifier K^+ current (Arcangeli *et al.*, 1995), and then repolarized to increasingly negative voltages (from 0 to -120 mV) for 365 ms. This voltage protocol allows to detect a K^+-selective inward current displaying the biophysical properties of I_{HERG1} (Arcangeli *et al.*, 1995; Bianchi *et al.*, 1998). Figure 5 shows representative current responses from a single SH-SY5Y human neuroblastoma cell exposed to increasing mizolastine concentrations (0.1–1 μM). Similarly to the results obtained in HEK 293 HERG1-transfected cells, 0.1 μM mizolastine causes approximately a 50% blockade of the inward HERG1 K^+ currents; and a further increase in I_{HERG1} blockade was observed by increasing the concentration of the second-generation H_1 receptor blocker to 1 μM. Pooling together the results obtained in five different cells in which experiments similar to that shown in Figure 5 were performed, the per cent of inhibition of the peak inward I_{HERG1} of current recorded at -120 mV after 0.1 and 1 μM mizolastine, respectively, were $60.9 \pm 9.9\%$ ($n=5$) and $78.5 \pm 2.6\%$ ($n=5$) of control currents. It should be underlined that the
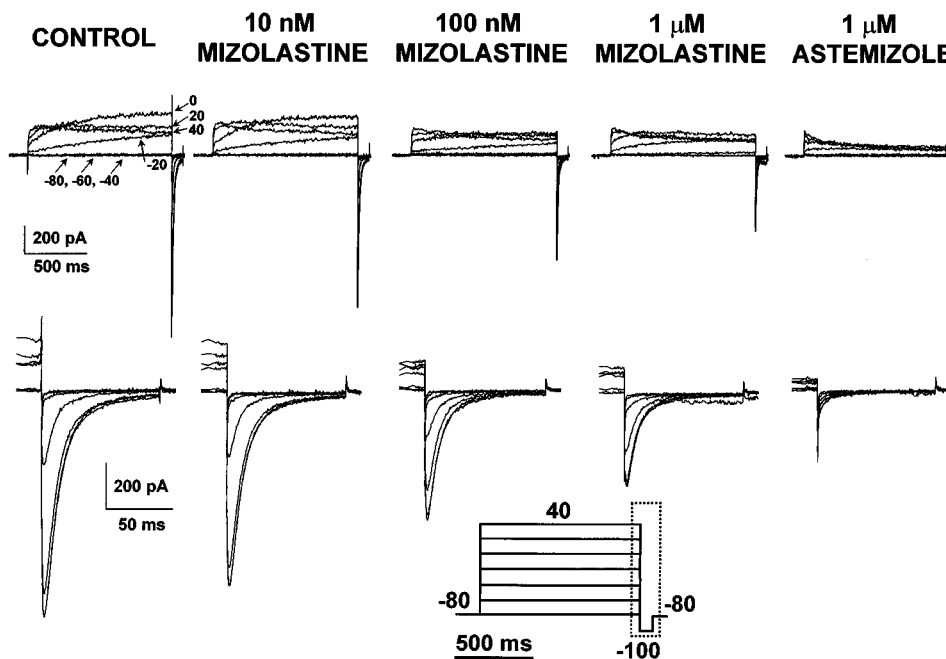
Figure 4 Effect of extracellular perfusion with mizolastine on I_{HERG1} heterologously expressed in HEK 293 cells stably transfected with HERG1 cDNA. Representative current traces recorded in the whole-cell configuration of the patch-clamp technique from a single HERG1-transfected HEK 293 cell. Records were obtained in control conditions, after 5 min perfusion with increasing concentrations of extracellular mizolastine (10 nM, 100 nM and 1 μM), and after 5 min exposure to 1 μM extracellular astemizole. The pulse protocol (shown at the bottom of the Figure) was the following: holding potential: -80 mV; test potentials from -80 mV to $+40$ mV in 20 mV steps (1 s duration); return potential: -100 mV. The bottom panels of the figure (corresponding to the time region indicated by the boxed area in the voltage pulse protocol) show the inward tail currents recorded upon repolarization to -100 mV on an expanded time and current scale.
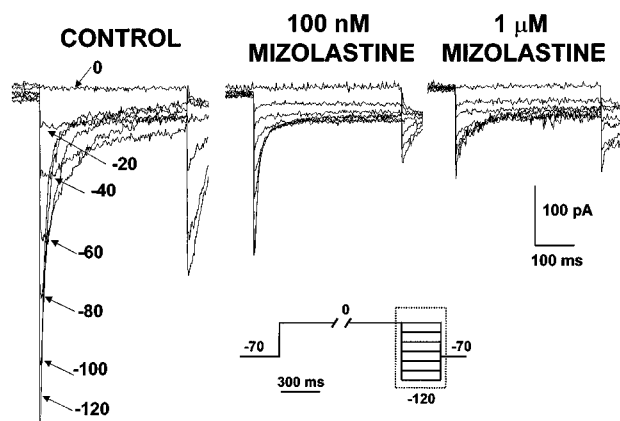

Figure 5 Effect of mizolastine on I_{HERG1} constitutively expressed in SH-SY5Y human neuroblastoma cells. Representative current traces recorded in the whole-cell configuration of the patch-clamp technique from an SH-SY5Y human neuroblastoma cell recorded in control conditions and after subsequent perfusion with 100 nM and 1 μM mizolastine (5 min each). The pulse protocol (shown at the bottom of the Figure) was the following: holding potential: -70 mV; depolarizing test potential: 0 mV for 10 s; hyperpolarizing return potentials: from 0 to -120 mV in -20 mV steps for 365 ms; return potential: -70 mV. Only approximately the last 450 ms of each pulses are shown, as indicated by the boxed area in the voltage pulse protocol.

intracellular solution for SH-SY5Y cells contained Cs^+ and TEA to minimize the contribution of the delayed rectifier current during the long depolarizing pulse (10 s at 0 mV) required to activate I_{HERG1}, as previously described (Taglialatela *et al.*, 1998b). Due to the intrinsic voltage-dependence and to the fast kinetics of dissociation for both TEA and Cs^+, the slow inward currents recorded at negative potentials

should be minimally affected by the blocking ions. In fact, similar inhibition by mizolastine was obtained using an intracellular solution for SH-SY5Y recording identical to that for HEK 293 cells (data not shown).

Discussion

The present study aimed to investigate the possible interference of mizolastine, a novel second-generation H_1 receptor antagonist recently available in Europe, with the K^+ currents carried by the HERG1 gene product. These channels, which provide the molecular basis for the cardiac repolarizing current I_{Kr} that plays a crucial role in controlling the duration of the cardiac action potential (Sanguinetti & Jurkiewicz, 1990; Sanguinetti *et al.*, 1995), have been focused on since several reports have appeared in the literature of the last decade warning about the potential pro-arrhythmic effects of drugs such as some first- and second-generation antihistamines (astemizole and terfenadine, in particular), prokinetics, antipsychotics, and antidepressants that derive from HERG1 K^+ channels blockade (Taglialatela *et al.*, 1998a).

The results of this study suggest that mizolastine is able to block in some degree both constitutively and heterologously expressed HERG1 K^+ channels. In fact, the benzimidazole blocked HERG1 K^+ channels either heterologously expressed in amphibian (*Xenopus* oocytes) or in mammalian (HEK 293) cells, as well as constitutively present in SH-SY5Y human neuroblastoma cells. The existence of a 10 fold difference in the IC_{50} values obtained for mizolastine blockade of HERG1 K^+ channels expressed in *Xenopus* oocytes and in HEK 293 cells could be explained by the different properties of the amphybian membrane when compared to that of mammalian

cells. In fact, the follicular cell layer and the vitelline membrane surrounding the oocytic membrane might to a certain degree impede the access of compounds at the level of the ion channels expressed in the amphybian membrane. Furthermore, it should be reminded that this difference is known to exist also for other HERG1 K^+ channel blockers; in fact, the antiarrhythmic methanesulphonanilide dofetilide displayed an IC_{50} value for HERG1 in whole *Xenopus* oocytes of 320 nM (Ficker *et al.*, 1998), whereas in HEK-transfected mammalian cells this value is approximately 30 times lower, being 12 nM (Snyders *et al.*, 1996). In addition, the potency of mizolastine in inhibiting HERG1 K^+ channels appears to be at least 10 times lower than that of the well-known cardiotoxic molecule astemizole. In fact, the IC_{50}s for HERG1 inhibition in *Xenopus* oocytes were 3 μM for mizolastine and 300 nM for astemizole; furthermore, in HERG1-transfected HEK 293 cells, the IC_{50}s for the two second-generation antihistamines were 300 and 1 nM, respectively (Zhou *et al.*, 1998). The lower potency of HERG1 inhibition by mizolastine could be at least in part ascribed to its faster dissociation rate from its receptor site on the channel when compared to astemizole.

The potential clinical relevance of a certain inhibitory action by mizolastine on HERG1 K^+ channels is worth considering, since the drug reached a C_{MAX} of 0.3–1 μM during standard therapy (10–20 mg die^{-1}) (Chosidow *et al.*, 1996; Chaufour *et al.*, 1999), a concentration range which clearly showed significant HERG1 inhibitory action, particularly in eukaryotic cells. However, it should be noted that the steady-state levels achieved by the drug in therapeutic settings are much lower than the C_{MAX}, and that the potency for HERG1 K^+ channels blockade by mizolastine is much lower than that exerted by the same drug at the level of H_1 receptors *in vitro* (the K_D for mizolastine antagonism at H_1 receptors is 1 nM) (Benavides *et al.*, 1995).

In addition, the present study also shows that mizolastine is able to interfere with the K^+ channels encoded by the Ether-à-go-go (EAG) and by the $K_v3.1$ genes; the potency of mizolastine in inhibiting EAG channels seems to be comparable to that displayed by the same molecule on the K^+ channels encoded by HERG1, whereas $K_v3.1$ was only inhibited by 15% at the maximal drug concentration tested (10 μM). However, it should be underlined that, in several species including humans (Pardo *et al.*, 1999), EAG K^+ channels seem not to be expressed in cardiac tissue (Ludwig *et al.*, 1994), but limited to the brain. It is therefore possible to speculate that EAG blockade is unlikely to cause an increased risk of arrhythmic events. On the other hand, the K^+ channels encoded by the $K_v3.1$ gene also do not seem to play a major role in the control of cardiac excitability, even though one study has suggested the participation of $K_v3.1$-like channels in the ultrarapid delayed rectifier K^+ current found in canine atrial myocytes (Yue *et al.*, 1996). More importantly, mizolastine (up to 10 μM) was unable to affect the potassium channels encoded by the $K_v2.1$ gene, which is expressed in the heart and participates in the control of cardiac repolarization (Xu *et al.*, 1999).

In consideration of the rare, yet serious adverse cardiac effects occurring with terfenadine and astemizole, European and US Health Regulating Authorities have recommended a careful examination of the cardiac safety of newly introduced drugs in preclinical and clinical studies, and in particular the study of their possible interference with ion channels controlling the cardiac action potential (EMEA, 1997). *In vitro* studies performed on guinea-pig ventricular myocytes have suggested that mizolastine is unable to lengthen cardiac

action potential duration, even at concentrations 10–30 times above the standard therapeutic range (Biton *et al.*, 1998). Furthermore, clinical studies in both healthy volunteers (Chaufour *et al.*, 1999) and in allergic patients (Delauche-Cavallier *et al.*, 1999) have shown that mizolastine showed no significant effect on cardiac repolarization. Therefore, the results of both animal and clinical studies argue against the possible occurrence of cardiotoxic events during standard therapy with the novel H_1 receptor blocker. However, it should be underlined that the recognition of the potential for cardiotoxicity exerted by terfenadine and astemizole required more than 10 years of extensive world-wide clinical use of these drugs. Interestingly, the initial warnings issued by the Regulatory Agencies against the potential risks associated to terfenadine and astemizole assumption were based on the reviews of only a couple of dozen clinical cases (among the millions of daily drug doses sold per year) in which the correlation between drug assumption and adverse cardiotoxic effects had been clearly demonstrated (Honig & Baraniuk, 1996).

A direct comparison of HERG1-blocking properties by different H_1 receptor blockers also allows to provide further insight into the structure-activity relationships for these molecules. In fact, it has been suggested that the HERG1 K^+ channel-blocking properties of terfenadine and its structural analogue ebastine are at least in part related to the tertiary amine substituent of the molecule rather than to the presence of the piperidine ring, or to the aromatic ring structures conferring H_1 receptor blocking activity (Zhang, 1997); no correlation has been recently found between the ability to prolong the cardiac action potential duration, an effect possibly related to HERG1 K^+ channels blockade, and the H_1 antagonistic activity by several antihistamines (Zhang, 1997; Taglialatela *et al.*, 2000). Lipophilicity and bulkiness appear to be the two crucial parameters in the tertiary amine group substituent conferring HERG1 K^+ channel-blocking capacity to the antihistaminic molecule. In fact, the second-generation antihistamine cetirizine, which appears to lack of significant HERG1-inhibitory properties (Taglialatela *et al.*, 1998b), has polar and smaller substitutions at the nitrogen atom (amido and carboxyl groups, respectively); on the other hand, terfenadine, astemizole, and ebastine, the H_1 receptor antagonists most effective in inhibiting HERG1 K^+ channels, have less polar and bulkier phenyl rings in the substituting side chains. Mizolastine, on the other hand, is characterized by a lower lipophylicity and thus by a low tissular penetration and distribution volume (Delauche-Cavallier *et al.*, 1999). The lower lipophilicity of mizolastine could explain the lower potency of this novel congener in inhibiting HERG1 K^+ channels when compared to astemizole or terfenadine. Furthermore, HERG1 K^+ channels blockade by terfenadine (Roy *et al.*, 1996) and astemizole (Taglialatela *et al.*, 1998b), as well as by the antiarrhythmic dofetilide (Kiehn *et al.*, 1996), seems to occur at a site located on the cytoplasmic side of the channel. However, the more hydrophilic profile, together with the relatively fast dissociation from the receptor site on the HERG1 K^+ channels when compared to astemizole, seems to suggest the possible extracellular location of the blocking site for mizolastine on the channel protein.

In conclusion, the results of the present study suggest that the new second-generation H_1 receptor antagonist mizolastine displays a certain degree of inhibitory action on HERG1 K^+ channels, which underlie the cardiac repolarizing current I_{Kr} and represent a major target for the arrhythmogenic action exerted by other antihistamines such

as terfenadine and astemizole. Although caution should be exercised when extrapolating the possible *in vivo* consequences of the results achieved from *in vitro* studies, and in consideration of the relatively high concentrations of mizolastine required to inhibit HERG1 K$^+$ channels, which appear to be above the steady-state plasma levels required to exert its therapeutical action, the present results might be of some clinical significance for those patients at risk of developing cardiac arrhythmias undergoing therapy with mizolastine.

References

ARCANGELI, A., BIANCHI, L., BECCHETTI, A., FARAVELLI, L., CORONNELLO, M., MINI, E., OLIVOTTO, M. & E. WANKE. (1995). A novel inward-rectifying K$^+$ current with a cell cycle-dependence governs the resting potential of neuroblastoma cells. *J. Physiol. (London)*, **489**, 455–471.

BENAVIDES, J., SCHOEMAKER, H., DANA, C., CLAUSTRE, Y., DELAHAYE, M., PROTEAU, M., MANOURY, P., ALLEN, J., SCATTON, B. & LANGER, S.Z. (1995). In vivo and in vitro interaction of the novel selective histamine H1 receptor antagonist mizolastine with H$_1$ receptors in the rodent. *Arzneimittelforschung*, **45**, 551–558.

BERUL, C.I. & MORAD, M. (1995). Regulation of potassium channels by nonsedating antihistamines. *Circulation*, **91**, 2220–2225.

BIANCHI, L., WIBLE, B., ARCANGELI, A., TAGLIALATELA, M., MORRA, F., CASTALDO, P., CROCIANI, O., ROSATI, B., FARAVELLI, L., OLIVOTTO, M. & WANKE, E. (1998). Herg encodes a K$^+$ current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? *Cancer Res.*, **58**, 815–822.

BITON, B., MAITRE, S., GODET, D., DEPOORTERE, H., ARBILLA, S., O'CONNOR S.E., AVENET, P. & SCATTON, B. (1998). Effect of the novel non-sedative histamine H$_1$ receptor antagonist mizolastine on cardiac potassium and sodium currents. *Allergy*, **43** (Suppl.): 160.

BROSTOFF, J., FITZHARRIS, P., DUNMORE, C., THERON, M. & BLONDIN, P. (1996). Efficacy of mizolastine, a new antihistamine, compared with placebo in the treatment of chronic idiopathic urticaria. *Allergy*, **51**, 320–325.

CHAUFOUR, S., CAPLAIN, H., LILIENTHAL, N., L'HERITIER, C., DESCHAMPS, C., DUBRUC, C. & ROSENZWEIG, P. (1999). Study of cardiac repolarization in healthy volunteers performed with mizolastine, a new H1-receptor antagonist. *Br. J. Clin. Pharmacol.*, **47**, 515–520.

CHOSIDOW, O., DUBRUC, C., DANJOU, P., FUSEAU, E., ESPAGNE, E., BIANCHETTI, G., THENOT, J.P., HERSON, S., ROSENZWEIG, P. & REVUZ, J. (1996). Plasma and skin suction-blister-fluid pharmacokinetics and time course of the effects of oral mizolastine. *Eur. J. Clin. Pharmacol.*, **50**, 327–333.

CRAFT, T.M. (1986). Torsade de pointes after astemizole overdose. *Br. Med. J.*, **292**, 660.

DANJOU, P., MOLINIER, P., BERLIN, I., PATAT, A., ROSENZWEIG, P. & MORSELLI, P.L. (1992). Assessment of the anticholinergic effect of the new antihistamine mizolastine in healthy subjects. *Br. J. Clin. Pharmacol.*, **34**, 328–331.

DAVIES, A.J., HARINDA, V., McHEWAN, A. & GHOSE, R.R. (1989). Cardiotoxic effect with convulsions in terfenadine overdose. *Br. Med. J.*, **302**, 1469.

DELAUCHE-CAVALLIER, M.C., CHAUFOUR, S., GUERALT, E., LACROUX, A., MURRIETA, M. & WAJMAN, A. (1999). QT interval monitoring during clinical studies with mizolastine, a new H1 antihistamine. *Clin. Exp. Allergy*, **29**, 206–211.

EUROPEAN AGENCY FOR THE EVALUATION OF MEDICINAL PRODUCTS: COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS. (1997). Points to consider: the assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products. CPMP/986/96.

FICKER, E., JAROLIMEK, W., KIEHN, J., BAUMANN, A. & BROWN, A.M. (1998). Molecular determinants of dofetilide block of HERG K + channels. *Circ. Res.*, **82**, 386–395.

FRECH, G.C., VanDONGEN, A.M.J., SCHUSTER, G., BROWN, A.M. & JOHO R.H. (1989). A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning. *Nature*, **340**, 642–645.

FRINGS, S., BRULL, N., DZEJA, C., ANGELE, A., HAGEN, V., KAUPP, U.B. & BAUMANN A. (1998). Characterization of ether-a-go-go channels present in photoreceptors reveals similarity to IKx, a K$^+$ current in rod inner segments. *J. Gen. Physiol.*, **111**, 583–599.

HAMILL, O.P., MARTY, E., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membranae patches. *Pflügers Arch.*, **391**, 85–100.

HONIG, P. & BARANIUK, J.N. (1996). In: *Histamine and H$_1$-receptor antagonists in allergic diseases*. ed. Estelle, F., Simons, R. pp.383–412, New York: Marcel Dekker Inc.

KIEHN, J., LACERDA, A., WIBLE, B. & BROWN, A.M. (1996). Molecular physiology and pharmacology of HERG. Single channel currents and block by dofetilide. *Circulation*, **94**, 2575–2579.

LEYNADIER, F., BOUSQUET, J., MURRIETA, M. & ATTALI, P. (1996). Efficacy and safety of mizolastine in seasonal allergic rhinitis. The Rhinase Study Group. *Ann. Allergy Asthma Immunol.*, **76**, 163–168.

LUDWIG, J., TERLAU, H., WUNDER, F., BRÜGGEMANN, A., PARDO, L.A., MARQUARDT, A., STUHMER, W. & PONGS, O. (1994). Functional expression of a rat homologue of the voltage gated ether a go-go potassium channel reveals differences in selectivity and activation kinetics between the Drosophila channel and its mammalian counterpart. *EMBO J.*, **13**, 4451–4458.

MONAHAN, B.P., FERGUSON, C.L., KILLEAVY, E.S., LLOYD, B.K., TROY, J. & CANTILENA, L.R. (1990). Torsade de pointes occurring in association with terfenadine use. *J.A.M.A.*, **264**, 2788–2790.

PARDO, L.A., DEL CAMINO, D., SANCHEZ, A., ALVES, F., BRUGGE-MANN, A., BECKH, S. & STUHMER, W. (1999). Oncogenic potential of EAG K(+) channels. *EMBO J.*, **18**, 5540–5547.

PATAT, A., PERAULT, M.C., VANDEL, B., ULLIAC, N., ZIELENIUK, I. & ROSENZWEIG, P. (1995). Lack of interaction between a new antihistamine, mizolastine, and lorazepam on psychomotor performance and memory in healthy volunteers. *Br. J. Clin. Pharmacol.*, **39**, 31–38.

ROY, M-L. DUMANIE, R. & BROWN, A.M. (1996). HERG, a primary human ventricular target of the non sedating antihistamine terfenadine. *Circulation*, **94**, 817–823.

SALATA, J.J., JURKIEWICZ, N.K., WALLACE, A.A., STUPIENSKI, R.F.III, GUINOSSO, P.J. & LYNCH Jr., J.J. (1995). Cardiac electrophysiological actions of the histamine H$_1$-receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine. *Circ. Res.*, **76**, 110–119.

SANGUINETTI, M.C., JIANG, C., CURRAN, M.E. & KEATING, M.T. (1995). A mechanistic link between an inherited and acquired cardiac arrhythmia: HERG encodes the I$_{Kr}$ potassium channel. *Cell*, **81**, 299–307.

SANGUINETTI, M.C. & JURKIEWICZ, N.K. (1990). Two components of cardiac delayed rectifier K$^+$ current: differential sensitivity to block by class III antiarrhythmic agents. *J. Gen. Physiol.*, **96**, 835–863.

SLATER, J.W., ZECHNICH, A.D. & HAXBY, D.G. (1999). Second-generation antihistamines: a comparative review. *Drugs*, **57**, 31–47.

SNYDERS, D.J. & CHAUDHARY, A. (1996). High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol. Pharmacol.*, **49**, 949–955.

SORKIN, E.M. & HEEL, R.C. (1985). Terfenadine: a review of its pharmacodynamic properties and therapeutic efficacy. *Drugs,* **29,** 34 – 56.

SUESSBRICH, H., WALDEGGER, S., LANG, F. & A.E. BUSCH. (1996). Blockade of HERG channels expressed in Xenopus oocytes by the histamine receptor antagonists terfenadine and astemizole. *FEBS Lett.,* **385,** 77 – 80.

TAGLIALATELA, M., CASTALDO, P., PANNACCIONE, A., GIORGIO, G. & ANNUNZIATO, L. (1998a). HERG K$^+$ channels as pharmacological targets: present and future implications. *Biochem. Pharmacol.,* **55,** 1741 – 1746.

TAGLIALATELA, M., PANNACCIONE, A., CASTALDO, P., GIORGIO, G., ZHOU, Z., JANUARY, C.T., GENOVESE, A., MARONE, G. & ANNUNZIATO, L. (1998b). 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. *Mol. Pharmacol.,* **54,** 113 – 121.

TAGLIALATELA, M., TIMMERMAN, H. & ANNUNZIATO, L. (2000). Cardiotoxic potential and CNS effects of first-generation antihistamines. *Trends Pharmacol. Sci.,* **21,** 52 – 56.

TAGLIALATELA, M., VanDONGEN, A.M.J., DREWE, J.A., JOHO, R.H., BROWN, A.M. & KIRSCH, G.E. (1991). Patterns of internal and external tetraethylammonium block in four homologous K$^+$ channels. *Mol. Pharmacol.,* **40,** 229 – 307.

TASMAN, A.J. & WEBER P. (1995). A double blind placebo controlled multicentered study on mizolastine, a new non- sedative H1-receptor antagonist, in patients suffering from perennial allergic rhinoconjunctivitis. *Allergy,* **50,** 79.

TRUDEAU, M.C., WARMKE, J.W., GANETZKI, B. & ROBERTSON, G.A. (1995). HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science,* **269,** 92 – 95.

VUURMAN, E.F., UITERWIJK, M.M., ROSENZWEIG, P. & O'HAN-LON, J.F. (1994). Effects of mizolastine and clemastine on actual driving and psychomotor performance in healthy volunteers. *Eur. J. Clin. Pharmacol.,* **47,** 253 – 259.

WARMKE, J.W. & GANETZKY, B. (1994). A family of potassium channel genes related to eag in *Drosofila* and mammals. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 3438 – 3442.

WOOSLEY, R.L., CHEN, Y., FREIMAN, J.P. & GILLIS, R.A. (1993). Mechanism of the cardiotoxic action of terfenadine. *J.A.M.A.,* **269,** 1532 – 1536.

WOOSLEY, R.L. (1996). Cardiac actions of antihistamines. *Annu. Rev. Pharmacol. Toxicol.,* **36,** 233 – 252.

WYMORE, R.S., GINTANT, G.A., WYMORE, R.T., DIXON, J.E., MCKINNON, D. & I.S. COHEN. (1997). Tissue and species distribution of mRNA for the I$_{Kr}$-like K$^+$ channel, erg. *Circ. Res.,* **80,** 261 – 268.

XU, H., BARRY, D.M., LI, H., BRUNET, S., GUO, W. & NERBONNE, J.M. (1999). Attenuation of the slow component of delayed rectification, action potential prolongation, and triggered activity in mice expressing a dominant-negative Kv2 alpha subunit. *Circ. Res.,* **85,** 623 – 633.

YUE, L., FENG, J., LI, G.R. & NATTEL, S. (1996). Characterization of an ultrarapid delayed rectifier potassium channel involved in canine atrial repolarization. *J. Physiol. (London).,* **496,** 647 – 662.

ZHANG, M-Q. (1997). Chemistry underlying the cardiotoxicity of antihistamines. *Curr. Med. Chem.,* **4,** 187 – 200.

ZHOU, Z., GONG, Q., YE, B., FAN, Z., MAKIELSKI, J.C., ROBERTSON, G.A. & JANUARY, C.T. (1998). Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys. J.,* **74,** 230 – 241.