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Comparative effects of nonsedating histamine H_1 receptor antagonists, ebastine and terfenadine, on human Kv1.5 channels

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Abstract

The effects of ebastine and terfenadine, long-acting nonsedating histamine H_1 receptor antagonists, were studied on hKv1.5 channels using the whole-cell voltage-clamp configuration of the patch-clamp technique in Ltk^- cells transfected with the gene encoding the hKv1.5 channel. Upon depolarization to +60 mV, terfenadine, 1 μM and 3 μM, inhibited the hKv1.5 current by 42.4 ± 6.4% and 69.3 ± 4.2% ($P < 0.01$). In contrast, at the same range of concentrations, ebastine-induced inhibition of this K^+ current averaged 6.5 ± 2.0% and 13.0 ± 2.0 ($P < 0.05$). At the highest concentration tested (3 μM) neither terfenadine carboxylate nor carebastine significantly modified hKv1.5 current. All these results suggest that ebastine could represent a safer alternative to terfenadine in the clinical practice.

Keywords: Antihistaminic agent; Terfenadine; Ebastine; Carebastine; K^+ current; hKv1.5 channel

1. Introduction

Recent evidences have indicated that the use of terfenadine, a long-acting nonsedating histamine H_1 receptor antagonist, can be associated with prolongation of the QTc interval and development of polymorphic ventricular arrhythmias (torsades de pointes) (Davies et al., 1989; Monahan et al., 1990). It has been reported that terfenadine blocks the rapidly activating component of the delayed rectifier (I_{Kr}) in isolated cat (Woosley et al., 1993; Chen and Woosley, 1994), and guinea pig ventricular myocytes (Salata et al., 1995), as well as the transient outward current (I_{TO}) and the rapid and slow components of the delayed rectifier current in human atrial myocytes (Crumb et al., 1995; Berul and Morad, 1995). However, terfenadine-induced QTc prolongation has been related to the block induced of hKv1.5 and HERG channels (Rampe et al., 1993; Yang et al., 1995; Crumb et al., 1995; Roy et al., 1996). Terfenadine is rapidly converted via cytochrome P450 CYP3A4 into several metabolites, including its active metabolite, terfenadine carboxylate (Leeson et al., 1982; Garteiz et al., 1982).

Ebastine is a newer long-acting nonsedating histamine H_1 receptor antagonist closely related to terfenadine, which, apparently, is devoid of QTc prolongation properties in human healthy volunteers and in patients with allergic rhinitis (Investigator's Brochure, 1995; Wiseman and Faulds, 1996). Coadministration of ebastine with drugs such as ketoconazole or erythromycin slowed its metabolism and increased the C_{max} values from an undetectable level to 43 nM and 120 nM (Wiseman and Faulds, 1996). Similarly to terfenadine, ebastine is rapidly and extensively metabolized by the cytochrome P450 CYP3A4 to its carboxylic acid active metabolite carebastine (Estelle et al., 1991; Wiseman and Faulds, 1996). The plasma concentrations of this metabolite vary between 4 nM and 20 nM (Wiseman and Faulds, 1996). Therefore, in the present study we compared the effects of terfenadine and terfenadine carboxylate with those produced by ebastine and carebastine on hKv1.5 channels expressed in Ltk^- cells without the complications of overlapping currents and limited human tissue availability. A preliminary report of this study has been published in abstract form (Valenzuela et al., 1996a).

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2. Materials and methods

2.1. Cell culture and solutions

The method to establish hKv1.5 expression in a clonal mouse Ltk^- cell line has been described previously (Snyders et al., 1992, 1993). Transfected cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% horse serum and 0.25 mg/ml G418, under a 5% CO_2 atmosphere. The cultures were passaged every 3–5 days, using a brief trypsin treatment. Before experimental use, subconfluent cultures were incubated with 2 μM dexamethasone for 24 h in order to induce the expression of hKv1.5 channels. The cells were removed from the dish with a rubber policeman, a procedure that left the vast majority of the cells intact. The cell suspension was stored at room temperature (21–23°C) and used within 12 h for all the experiments reported here.

The intracellular pipette filling solution contained (in mM): K-aspartate 80, KCl 50, KH_2PO_4 10, MgATP 3, HEPES 10, EGTA 5, and was adjusted to pH 7.25 with KOH. The bath solution contained (in mM): NaCl 130, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and glucose 10 and was adjusted to pH 7.35 with NaOH. Terfenadine, terfenadine carboxylate, ebastine and carebastine were kindly provided by Lab. Almirall (Barcelona, Spain). Drugs as a powder were dissolved in dimethyl sulfoxide (DMSO) to yield stock solutions of 10 mM. Further dilutions in external solution were made to obtain the desired final concentration.

2.2. Electrical recording

Experiments were performed in a small volume (0.5-ml) bath mounted on the stage of an inverted microscope (Nikon model TMS, Garden City, NY, USA), continuously perfused at a flow rate of 0.5–1.0 ml/min. The hKv1.5 currents were recorded at room temperature (21–23°C) using the whole-cell voltage-clamp configuration of the patch-clamp technique (Hamill et al., 1981) with an Axopatch-1C patch-clamp amplifier (Axon Instruments, Foster City, CA, USA). Currents were filtered at 2 kHz (4-pole Bessel filter), sampled at 4 kHz and stored on the hard disk of a Hewlett-Packard Vectra VL/100 Pentium computer for subsequent analysis. Data acquisition and command potentials were controlled by the pClamp 5.5.1 software (Axon Instruments, Foster City, CA, USA).

Micropipettes were pulled from borosilicate glass capillary tubes (Narishige, GD-1, Tokyo, Japan) on a programmable horizontal puller (Sutter Instrument, San Rafael, CA, USA) and heat polished with a microforge (Narishige, Tokyo, Japan). When filled with the intracellular solution and immersed into the bath (external) solution, the pipette tip resistance ranged between 1–3 MΩ. The micropipettes were gently lowered onto the cells to obtain a gigaohm seal (16 ± 6 GΩ; $n = 21$) after applying suction. After seal formation, cells were lifted from the bottom of the perfusion bath and the membrane patch was ruptured with brief additional suction. The capacitive transients elicited by symmetrical 10-mV steps from −80 mV were recorded at 50 kHz (filtered at 10 kHz) for subsequent calculation of capacitative surface area (18 ± 3 pF, $n = 23$), access resistance and input impedance. Thereafter, capacitance and series resistance compensation were optimized and 80% compensation of the effective access resistance was usually obtained.

2.3. Pulse protocol and analysis

After control data were obtained, bath perfusion was switched to drug-containing solution. The holding potential was maintained at −80 mV. The cycle time for any protocol was 10 s in order to avoid accumulation of block or incomplete deactivation of the current.

The protocol to obtain current-voltage (I–V) relationships and activation curves consisted of 250-ms pulses that were imposed in 10-mV increments between −80 and +60 mV, with additional interpolated pulses to yield 5-mV increments between −30 and +10 mV, i.e., the activation range of the hKv1.5 channels (Snyders et al., 1993; Valenzuela et al., 1995). The 'steady-state' I–V relationships were obtained by measuring the current at the end of the 250-ms depolarizations. Between −80 and −40 mV, only passive linear leak was observed and least-squares fits to these data were used for passive leak correction. Deactivating 'tail' currents were recorded at −40 mV. The activation curve was obtained from the tail current amplitude immediately after the capacitive transient. Measurements were performed using the Clampfit program of pClamp 5.5.1 and by a custom-made analysis program.

Activation curves were fitted with a Boltzmann equation:

$$y = 1/\{1 + \exp[-(E - E_h)/s]\} \quad (1)$$

in which s represents the slope factor, E the membrane potential and E_h the voltage at which 50% of the channels are open. The time course of tail currents and the slow inactivation were fitted with the sum of exponentials. The activation kinetics were determined with the dominant time constant of activation approach in which a single exponential was fitted to the latter 50% of activation (White and Bezanilla, 1985; Snyders et al., 1993; Valenzuela et al., 1994). The curve fitting procedure used a nonlinear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot. Goodness of the fit was judged by the χ^2 criterion and by inspection for systematic nonrandom trends in the difference plot.

Voltage dependence of block was determined as follows: leak-corrected current in the presence of drug was normalized to matching control to yield the fractional

block at each voltage ($f = 1 - I_{drug}/I_{control}$). The voltage dependence of block was fitted to:

$$f = [D]/\{[D] + K_d^* \times \exp(-\delta zFE/RT)\} \quad (2)$$

where z, F, R and T represent the valence, the Faraday constant, the universal gas constant and the absolute temperature, respectively and δ the fractional electrical distance, i.e., the fraction of the transmembrane electrical field sensed by a single charge at the receptor site and K_d^* represents the apparent dissociation constant at the reference potential (0 mV).

2.4. Statistical methods

Results are expressed as mean ± S.E.M. Paired Student's *t*-test was used to compare the effects of each drug with the control values. Statistical significance was taken as $P < 0.05$.

3. Results

3.1. Effects of ebastine and terfenadine on hKv1.5 currents

The top panel of Fig. 1 shows superimposed tracings of potassium current through hKv1.5 channels expressed in mouse Ltk$^-$ cells following the application of 500 ms depolarizing pulses from −80 mV to different test potentials between −60 and +60 mV. Under control conditions, the hKv1.5 current rose rapidly with a sigmoidal time course to a peak and then declined slowly (slow and partial inactivation). Outward tail currents were observed upon repolarization to −40 mV. Activation time constants ranged from 22.3 ± 1.2 ms at −10 mV ($n = 16$) to 2.0 ± 0.3 ms at +60 mV ($n = 16$), as previously described (Snyders et al., 1992, 1993; Valenzuela et al., 1995). Following the exposure to ebastine (1 μM) or terfenadine (1 μM), induction of block progressed with a time constant of 3–4 min, which was about 5 times slower than the effect of changing extracellular K^+ concentration at similar flow rates. This delay suggested an intramembrane or intracellular site of action and, therefore, 10–15 min of equilibration were allowed before assessment of drug effects.

The middle and bottom panels of Fig. 1 illustrate the differential effectiveness of ebastine and terfenadine to block hKv1.5 channels. It is evident that terfenadine displayed a higher potency than ebastine to inhibit the hKv1.5 current. In fact, terfenadine not only reduced the current amplitude but also altered the time course of the current during depolarization, without modifying the initial activation time course of the current. The terfenadine-induced accelerated decline of hKv1.5 current to the reduced steady-state level proceeded with a time constant of 32 ± 4 ms ($n = 5$). At the end of a 500-ms step to +60 mV terfenadine 1 μM and 3 μM reduced the hKv1.5 current

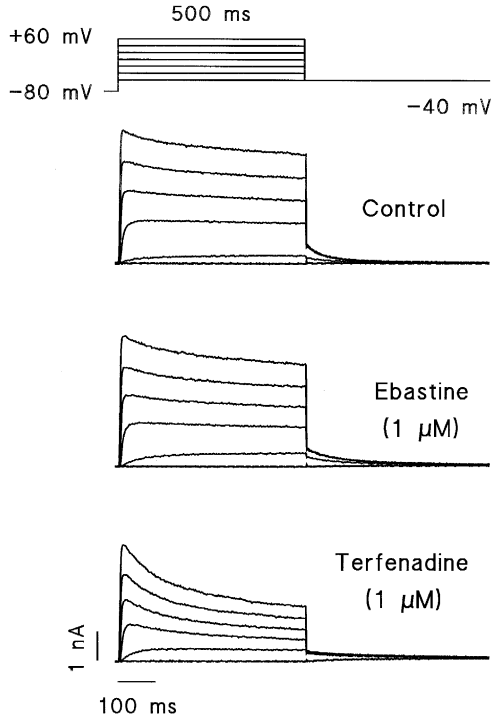

Fig. 1. Effects of ebastine and terfenadine on hKv1.5 currents obtained in the same cell. In the upper panel currents for depolarizations from −80 mV to voltages between −60 and +60 mV in steps of 20 mV are shown. Tail currents were obtained on return to −40 mV. The middle panel shows the effects of ebastine (1 μM) on hKv1.5 and the bottom panel shows the effects of terfenadine on this K^+ current at the same concentration. Cell capacitance, 22 pF. Data filtered at 2 kHz (four-pole Bessel) and digitized at 10 kHz; additional digital filtering at 1 kHz.

by 42.4 ± 6.4% ($n = 5$) and 69.3 ± 4.2% ($n = 5$), whereas at the same range of concentrations, ebastine inhibited much less ($P < 0.01$) the current by 6.5 ± 2.0% ($n = 6$, $P < 0.05$ versus control) and 13.0 ± 2.0% ($n = 6$, $P < 0.05$, versus control). The effects of higher concentrations of ebastine could not be tested since the drug precipitated in the external solution. After perfusion with terfenadine or ebastine, the amplitude of the current was restored to 89 ± 3.1% ($n = 24$) of its initial control value after 20 min of perfusion with drug-free solution.

3.2. Voltage- and time-dependent block of the hKv1.5 current by terfenadine

Fig. 2A shows the *I–V* relationship of hKv1.5 current in the absence and in the presence of terfenadine and ebastine (1 μM). Terfenadine inhibited the hKv1.5 current over the whole range of potentials tested, whereas the percentage of block induced by ebastine measured at the end of depolarizing pulses of 500 ms in duration from −80 mV to +60 mV, averaged only a 6.5 ± 2.0% ($n = 6$). Terfenadine induced a voltage-dependent inhibition of the hKv1.5 current which increased as the test potential become more positive. In order to quantify the voltage dependence of terfenadine-induced block, we represented

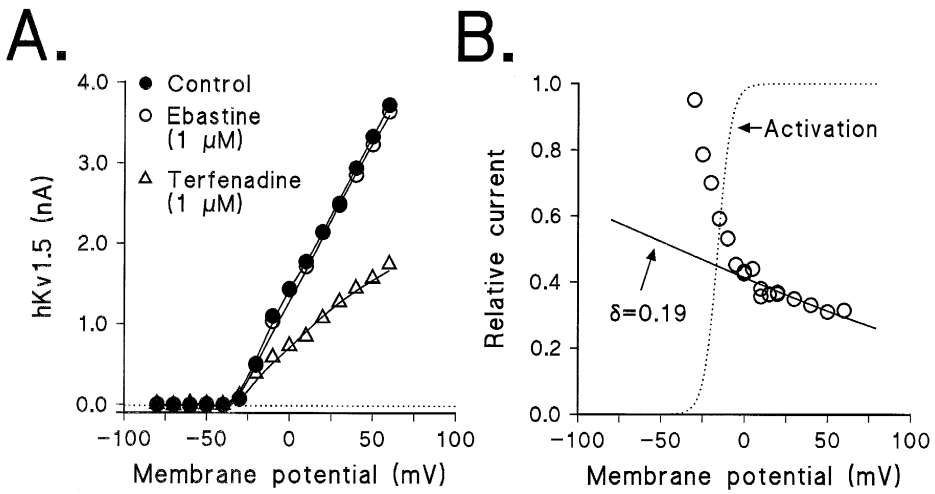

Fig. 2. *I–V* relationship of hKv1.5 in the absence and in the presence of ebastine (1 μM) and terfenadine (1 μM). (A) *I–V* relationship (500 ms isochronal) in control conditions (●) and in the presence of 1 μM ebastine (○) and terfenadine (1 μM) (△). (B) Voltage-dependence block of hKv1.5 channels induced by terfenadine 1 μM. Relative current expressed as $I_{terfenadine}/I_{control}$ from data shown in panel A. The dashed line represents the activation curve for this experiment. Block increased steeply between −20 mV and 0 mV, which corresponds to the voltage range of activation of hKv1.5. For membrane potentials positive to 0 mV, a continued but more shallow voltage dependence was observed which represents the effect of the membrane electrical field on the interaction between terfenadine and the open state of hKv1.5 channel. This voltage dependence was fitted (continuous line) with Eq. (2) (see Section 2) and yielded $\delta = 0.19$.

the fractional current ($I_{terfenadine}/I_{control}$) as a function of voltage (Fig. 2B). The dotted line in Fig. 2B shows the activation curve of the current in this particular experiment. Block increased steeply in the voltage range of channel activation (between −30 mV and 0 mV), which suggests that terfenadine-induced block did not occur until the channel opens. Between 0 mV and +60 mV, block still increased, but with a shallower voltage dependence.

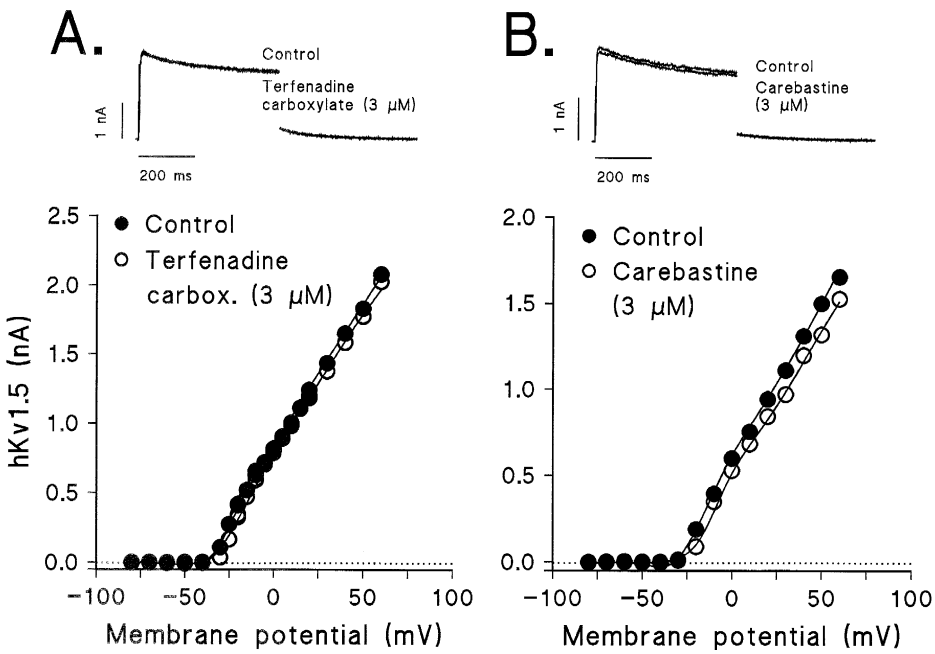

Fig. 3. Effects of the main metabolites of terfenadine and ebastine, terfenadine carboxylate and carebastine, respectively, on hKv1.5 current. (A) The top panel shows original records of hKv1.5 current obtained during application depolarizing steps from −80 mV to +60 mV during 500 ms in the absence and in the presence of terfenadine carboxylate 3 μM. The bottom panel shows the *I–V* relationship under control conditions and in the presence of terfenadine carboxylate (3 μM). (B) The top panel shows original records of hKv1.5 current obtained during the application depolarizing steps from −80 mV to +60 mV during 500 ms in the absence and in the presence of carebastine (3 μM). The bottom panel shows the *I–V* relationship under control conditions and in the presence of carebastine (3 μM).

This voltage dependence cannot be attributed to the activation of the channel, since at this range of membrane potentials the activation curve reached saturation. Terfenadine is a weak base (pK_a approx. 10) (Yang et al., 1995) and, therefore, at the intracellular pH of 7.25, it is predominantly present in its charged form. Thus, the voltage dependence of block could be the result of the effect of the transmembrane electrical field on the interaction between terfenadine and the receptor in the channel. If terfenadine reaches its receptor from the inside, then the block is expected to increase in a voltage-dependent manner according to Eq. (2) (Section 2), which incorporates the effect of the transmembrane electrical field (Woodhull, 1973). The parameter δ in this equation represents the fractional distance, i.e. the fraction of the membrane field sensed by a positive charge at the receptor site. The solid line in Fig. 2B represents the fit of this equation to the data points positive to 0 mV. In this particular experiment the δ value was of 0.19. The average δ value obtained in 5 cells was 0.18 ± 0.02.

3.3. Effects of carebastine and terfenadine carboxylate on hKv1.5 channels

The effects of 3 μM terfenadine carboxylate and carebastine, the main metabolites of terfenadine and ebastine, respectively, were studied in 9 cells. This was the highest concentration at which carebastine did not precipitate in the external solution. The upper panel of Fig. 3 shows representative current records and the lower panel the $I–V$ relationship obtained in the absence and in the presence of either terfenadine carboxylate (A) or carebastine (B), respectively. The figure shows that after applying 500 ms depolarizing pulses from a holding potential of -80 mV to $+60$ mV in steps of 10 mV neither terfenadine carboxylate nor carebastine significantly modifies the amplitude of the hKv1.5 current at any membrane potential tested or the activation time course of activation of the hKv1.5 current. Thus, the percentage of suppression of the current measured at $+60$ mV averaged $0.03 \pm 0.01\%$ ($P > 0.05$; $n = 4$) and $5 \pm 1\%$ ($P > 0.05$; $n = 5$), respectively.

4. Discussion

In the present study we have analyzed and compared the effects of ebastine and terfenadine, as well as the effects of their main metabolites, carebastine and terfenadine carboxylate, respectively, on a human Kv1.5 channel stably expressed in Ltk^- cells. This current activates at voltages positive to -30 mV, consistent with its involvement in the control of cardiac action potential duration (Wang et al., 1993; Deal et al., 1996). The main finding of the present study is that ebastine is much less potent than terfenadine to block hKv1.5 channels.

The current generated by hKv1.5 channels is similar in voltage dependence, kinetics and pharmacological sensitivity to the very rapidly activating delayed rectifier K^+ current recorded in human atrial myocytes (I_{Kur}) (Wang et al., 1993), dog ventricle (Jeck and Boyden, 1992) and rat atria (Boyle and Nerbonne, 1991). All these results suggest that I_{Kur} is the native counterpart to hKv1.5 channels cloned from human ventricle (Wang et al., 1993; Tamkun et al., 1991; Snyders et al., 1993; Deal et al., 1996) and therefore, it can contribute to the repolarization process of the human atrial action potential (Wang et al., 1993). Block of cardiac K^+ channels has been considered to be the mechanism by which class III antiarrhythmic drugs slow repolarization and prolong the action potential duration (Hondeghem and Snyders, 1990; Colatsky et al., 1990; Roden, 1993). Importantly, selective block of the hKv1.5-like current in human atrial myocytes results in significant prolongation of the action potential duration (Wang et al., 1993), and therefore it represents a potential molecular target for class III antiarrhythmic drugs (Wang et al., 1993; Snyders and Yeola, 1995).

In this study the effects of ebastine and terfenadine have been analyzed on hKv1.5 channels, the most cardiac-specific in terms of both tissue-specific and developmental expression (Roberds et al., 1993). In fact, very recently the hKv1.5 channel protein has been located in human atrial and ventricular myocardium explanted from newborn and adult patients (Mays et al., 1995). However, electrophysiological studies (Konarzewska et al., 1995; Li et al., 1996) have shown the absence of hKv1.5-like current in human ventricular myocytes. The observed discrepancies between immunohistochemical (Mays et al., 1995) and electrophysiological studies (Konarzewska et al., 1995; Li et al., 1996) are not resolved yet and, therefore, future studies are clearly necessary to determine the mechanisms and functional importance of the cellular localization of cardiac hKv1.5 protein in human ventricle.

As previously described (Rampe et al., 1993; Yang et al., 1995), terfenadine blocked the open state of hKv1.5 channels in a voltage-dependent manner, consistent with an electrical distance (δ) of 0.18 ± 0.02. This δ value is similar to that previously described for quinidine (Snyders et al., 1992), bupivacaine enantiomers (Valenzuela et al., 1995), zatebradine (Valenzuela et al., 1996b) and the enantiomers of a new bradycardic agent (Delpón et al., 1996). This voltage dependence can be explained by the molecular structure of the drug. Terfenadine is a weak base with a pK_a of 10 (Yang et al., 1995), which indicates that at the physiological pH it is predominantly in its cationic form. Therefore, the voltage dependence of block observed at membrane potentials positive to 0 mV can be due to the effects of the transmembrane electrical field on the interaction between cationic terfenadine and the channel and suggests that the drug has to cross 18% of the transmembrane electrical field to reach the receptor in the channel, bind to it and block it. Terfenadine-induced block was time-dependent, in such a way that the drug induces a fast

decline of the current during the application of a depolarizing pulse as previously reported (Rampe et al., 1993; Yang et al., 1995). In contrast, ebastine-induced block was so less marked at the concentrations tested that we could not quantify its possible voltage- or time-dependent block.

Ebastine and terfenadine undergo extensive first-pass metabolism to their active metabolites, carebastine and terfenadine carboxylate, respectively (Leeson et al., 1982; Garteiz et al., 1982; Wiseman and Faulds, 1996). Therefore, we also examined the effects of both metabolites on hKv1.5 currents. However, neither carebastine nor terfenadine carboxylate induced a significant block of hKv1.5 channels.

After oral administration, ebastine is rapidly and extensively metabolized to its active metabolite, carebastine, by the cytochrome P450 enzyme family and, therefore, plasma concentrations of the parent drug are negligible (Wiseman and Faulds, 1996). In healthy volunteers and in patients with allergic rhinitis, peak plasma concentrations (C_{max}) of carebastine increased in a dose-dependent manner. Following a single oral dose of 10–40 mg, the C_{max} of ebastine ranged between 0.1 mg/l and 0.4 mg/l, respectively (Vincent et al., 1988; Estelle et al., 1991; Yamaguchi et al., 1994; Matsuda et al., 1994; Wiseman and Faulds, 1996). Similar C_{max} values were obtained during repeated administration of 10–20 mg/day for 7 days in young and elderly healthy volunteers (for review, see Wiseman and Faulds, 1996). Since carebastine is highly bound to plasma proteins (approx. 98%), these C_{max} values would correspond to 4 nM and 20 nM, respectively, very far from those required to inhibit hKv1.5 current, as it is shown in the present study.

Severe cardiovascular adverse effects have been described following the coadministration of terfenadine with drugs (e.g., ketoconazole, erythromycin) that interfere with its hepatic metabolism via inhibition of cytochrome P450 CYP3A4 (Spatzenegger and Jaegger, 1995). Coadministration of ebastine with drugs such as ketoconazole or erythromycin slowed its metabolism and increased the C_{max} values from an undetectable level to 20.3 and 55.7 μg/l (43 nM and 120 nM) (Wiseman and Faulds, 1996). These concentrations of ebastine are again much lower than those producing a significant block of hKv1.5 channels. This may explain why no reports of serious adverse cardiovascular events in patients treated with ebastine have been reported (Wiseman and Faulds, 1996) and thus, the results of the present study suggest that ebastine may represent a safer alternative to terfenadine in the clinical practice.

The mechanism by which antihistaminics are able to induce cardiac arrhythmias is still uncertain, but it has been thought to be related to the blockade of several components of the cardiac delayed rectifier potassium current and other K^+ currents that control cardiac repolarization (Woosley et al., 1993; Rampe et al., 1993; Chen and Woosley, 1994; Berul and Morad, 1995; Crumb et al., 1995; Salata et al., 1995; Yang et al., 1995). Recently, it has been proposed that HERG channels could be the primary target for the antihistaminics-induced acquired long QT syndrome on the basis that sensitivity of HERG was 10 times greater than Kv1.5 to the effects of terfenadine when expressed in *Xenopus* oocytes (Roy et al., 1996). Since the expression of hKv1.5 channels is higher in human atrium than in ventricular tissue (Wang et al., 1993; Mays et al., 1995; Li et al., 1996), hKv1.5 channels could represent the molecular target of supraventricular arrhythmias induced by antihistaminics. Therefore, further studies evaluating the effects of ebastine on other cardiac K^+ currents that contribute both to the repolarization of the cardiac action potential and to the QTc prolongation (e.g., I_{Ks}, I_{TO}, HERG) are needed to confirm the hypothesis that ebastine could be a safer alternative to terfenadine in the clinical practice.

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