Block of human cardiac Kv1.5 channels by loratadine: voltage-, time- and use-dependent block at concentrations above therapeutic levels

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Abstract

Objective: The aim of this study was to analyze the effects of loratadine on a human cardiac K+ channel (hKv1.5) cloned from human ventricle and stably expressed in a mouse cell line. Methods: Currents were studied using the whole-cell configuration of the patch-clamp technique in Ltk− cells transfected with the gene encoding hKv1.5 channels. Results: Loratadine inhibited in a concentration-dependent manner the hKv1.5 current, the apparent affinity being 1.2 ± 0.2 μM. The blockade increased steeply between −30 and 0 mV which corresponded with the voltage range for channel opening, thus suggesting that the drug binds preferentially to the K+ open state of the channel. The apparent association and dissociation rate constants were 3.6 ± 0.5 = 10 P s and 3.7 ± 1.6 P s, respectively. Loratadine, 1 μM, increased the time constant of deactivation of tail currents elicited on return to −40 mV after 500 ms depolarizing pulses to +60 mV from 36.2 ± 3.4 to 64.9 ± 3.6 ms (n = 6, P < 0.01), thus inducing a ‘crossover’ phenomenon. Application of trains of pulses at 1 Hz lead to a progressive increase in the blockade reaching a final value of 48.6 ± 4.3%. Recovery from loratadine-induced block at −80 mV exhibited a time constant of 743.0 ± 78.0 ms. Finally, the results of a mathematical simulation of the effects of loratadine, based on an open-channel block model, reproduced fairly well the main effects of the drug. Conclusions: The present results demonstrated that loratadine blocked hKv1.5 channels in a concentration-, voltage-, time- and use-dependent manner but only at concentrations much higher than therapeutic plasma levels in man. © 1997 Elsevier Science B.V.

Keywords: Potassium channel, hKv1.5; Ltk− cells; Loratadine; Terfenadine; Astemizole; Patch-clamp; Mouse

1. Introduction

Very recently it has been reported that terfenadine and astemizole, two non-sedating H1-receptor antagonists, are potent cardiac K+ channels blockers. In fact, at clinically relevant concentrations, terfenadine and astemizole blocked the rapidly activating component of the delayed rectifier (I_{Kr}) in isolated cat [1,2] and guinea-pig ventricular myocytes [3] as well as the transient outward current and the rapid and slow components of the delayed rectifier current in human atrial myocytes [4,5]. In addition, terfenadine also blocked the Kv1.5 channels cloned from human ventricle and stably expressed in human embryonic kidney cells [6] or in a mouse L-cell line [7]. These K+ -channel-blocking properties have been considered responsible for the excessive prolongation of the electrocardiographic QT interval induced by both drugs which, in turn, reflects an excessive delay of ventricular repolarization.

Loratadine is a long-acting non-sedating histamine H1-receptor antagonist widely prescribed for treating acute coryza and chronic allergic rhinitis and skin disorders [8]. Loratadine did not prolong the QTc or QRS intervals and produced no arrhythmias even at doses several orders of magnitude greater than the recommended clinical dose in both experimental animals and healthy volunteers [8–11]. Moreover, serious ventricular arrhythmias or torsade de pointes have not been described with loratadine, either alone [8] or in combination with other drugs [8,9]. How-
ever, very recently several reports of supraventricular arrhythmias associated with loratadine use have been reported [8,12,13]. This suggests that loratadine may interact with a K⁺ current involved in atrial repolarization. One human cardiac target is the Kv1.5 channel which generates the ultrarapid delayed rectifier outward K⁺ current (I\textsubscript{Kur}) in human atria, whereas it appears to be absent from human ventricle, and contributes to repolarization of the human atrial action potential [14,15]. Because this possibility has not been tested yet, in the present study we have examined the effects of loratadine on hKv1.5 channels cloned from human ventricle and stably expressed in a Ltk\(^{-}\) cell line. This model provides a means for studying drug effects on a single human cardiac K⁺ channel subtype without contamination of other voltage-gated currents [16]. We present evidence that loratadine blocks open hKv1.5 channels in a concentration-, voltage-, time-, and use-dependent manner. However, the blockade was evident only at concentrations higher than the therapeutic plasma levels in man.

2. Methods

2.1. Cell culture

The experiments were performed in mouse L-cell line expressing hKv1.5 channels. Cell culture has been described in detail elsewhere [16]. Transfected cells were cultured in DMEM supplemented with 10% horse serum and 0.25 mg/ml G418 (a neomycin analog, Gibco, Grand Island, NY, USA) in a 5% CO\(_2\) atmosphere. The cultures were passaged every 4–5 days using a brief trypsin treatment. Prior to experimental use, subconfluent cultures were incubated with 2 \(\mu\)M dexamethasone for 24 h as expression of the channel is under control of a dexamethasone-inducible promoter [16]. The cells were removed from the dish with a cell scraper, a procedure that leaves the vast majority of the cells intact. The cell suspension was stored at room temperature and used within 12 h for electrophysiological experiments.

2.2. Electrical recording

A small aliquot of cell suspension was placed in a 0.5 ml chamber mounted on the stage of an inverted microscope (Nikon TMS, Nikon Co., Tokyo, Japan). After settling to the bottom of the chamber, cells were perfused at 1 ml·min\(^{-1}\) with an external solution containing (in mM): NaCl 130, KCl 4, CaCl\(_2\) 1, MgCl\(_2\) 1, HEPES 10 and glucose 10; pH was adjusted to 7.4 with NaOH. Recording pipettes were filled with an ‘internal’ solution containing (in mM): K-aspartate 80, KCl 42, KH\(_2\)PO\(_4\) 10, MgATP 5, phosphocreatine 3, HEPES 5 and EGTA 5 (adjusted to pH 7.2 with KOH). All the experiments were performed at 24–25°C.

2.3. Recording techniques

hKv1.5 currents were measured using the whole-cell configuration of the patch-clamp technique [17] using an Axopatch-1D patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Recording pipettes were pulled from Narishige (GD1) (Narishige Co., Ltd Tokyo, Japan) borosilicate capillary tubes using a programmable patch micropipette puller (Model P-87 Brown-Flaming, Sutter Instruments Co., Novato, CA, USA) and were heat-polished with a microforge (Model MF-83, Narishige). To ensure voltage-clamp quality, micropipette resistance was kept below 3.5 M\(\Omega\) when filled with the internal solution and immersed in the external solution. The micropipettes were gently lowered onto the cells to get a gigaohm seal after applying suction. After seal formation, the 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, access resistance and input impedance. Thereafter, capacitance and series resistance compensation were optimized and \(\approx 80\%\) compensation was usually obtained. Maximum outward currents amplitudes at +60 mV averaged 1.5 ± 0.1 nA (\(n = 22\)). Thus, no significant voltage errors (< 5 mV) due to series resistance were expected with the electrodes used and this was confirmed by the calculated access resistance (\(R_a\)). Moreover, the low capacitance (10.2 ± 0.9 pF) enabled fast clamp control. Voltage-clamp command pulses were generated by a 12-bit digital-to-analog converter. The current records were sampled at 3–10 times the antialias filter setting and stored on the hard disk of a Tandon 386/25 computer for subsequent analysis. Data acquisition and command potentials were controlled by pClamp 5.5.1. software (Axon Instruments, Foster City, CA, USA).

2.4. Pulse protocols and analysis

After control data were obtained, bath perfusion was switched to drug-containing solution. The effect of drug infusion or removal was monitored with test pulses from −80 to +30 mV applied every 30 s until steady-state was obtained (within 15 min). Therefore, an equilibration period of 20 min was allowed to elapse before measuring the drug effects.

The holding potential was maintained at −80 mV and the cycle time for any protocol was 10 s in order to avoid accumulation of block. The protocol to obtain current–voltage (\(I–V\)) relationships and activation curves consisted of 500 ms pulses that were imposed in 10 mV increments between −80 and +70 mV, with additional interpolated pulses to yield 5 mV increments between −30 and +10 mV (activation range of hKv1.5) [16,18,19]. The ‘steady-state’ \(I–V\) relationships were obtained by
plotting the current level after 500 ms as a function of the membrane potential. 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 on return to −40 mV. The activation curve was constructed by plotting normalized values of tail currents elicited on return to −40 mV after 500 ms depolarizations to various test potentials (from −80 to +60 mV) as a function of the membrane potential.

Activation curves have been fitted with a Boltzmann distribution according to the following equation:

\[ y = \frac{1}{1 + \exp\left(\frac{V_h - V_m}{k}\right)} \]  

where \( V_h \) is the half-point of activation (in mV), \( V_m \) is the test potential and \( k \) represents the slope factor for the activation curve (in mV). In order to describe the time course of currents during depolarizing pulses and tail currents upon repolarization, an exponential analysis was used as an operational approach, fitting these processes to an equation of the form:

\[ y = C + A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + \ldots + A_n \exp(-t/\tau_n) \]

where \( \tau_1, \tau_2 \) and \( \tau_n \) are the system time constants, \( A_1, A_2 \) and \( A_n \) are the amplitudes of each component of the exponential, and \( C \) is the baseline value. The curve-fitting procedure used a non-linear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot. Goodness of fit was judged by the \( \chi^2 \) criterion and by inspection for systematic non-random trends in the difference plot. The activation kinetics of hKv1.5 have been described as a sigmoidal process, indicating that the channel has multiple closed states [16]. However, in the present study and in order to describe the dominant time constant of this process and the effects of loratadine on it, the latter part of the activating current was fitted with a single exponential, following a procedure previously described and used for the same purpose [16,18,19].

Fractional block was defined as:

\[ f = 1 - \frac{I_{\text{Drug}}}{I_{\text{Control}}} \]

A first-order blocking scheme was used to describe drug–channel interaction kinetics; apparent affinity constant, \( K_D \) (concentration for 50% block or EC_{50}), and Hill coefficient, \( n_H \), were obtained from fitting the fractional block (\( f \)) at various drug concentrations [\( D \)] to the equation:

\[ f = 1/[1 + (K_D/\{D\})^{n_H}] \]  

and apparent rate constants for binding (\( k \)) and unbinding (\( l \)) were obtained from fitting:

\[ \tau_{\text{Block}} = 1/(k \times \{D\} + l) \]  

in which \( \tau_{\text{Block}} \) is the time constant of development of block.

2.5. Drugs

Loratadine was kindly provided by Almirall, SA (Barcelona, Spain). Drug as a powder was initially dissolved in dimethyl sulfoxide (Sigma Chemical Co., London) to yield 1 mM stock solutions. Further dilutions were carried out in external solution to obtain final concentrations between 0.1 and 20 \( \mu \)M. Control solutions contained the same concentrations of dimethyl sulfoxide as the test solution.

2.6. Statistical methods

Data obtained under control conditions were compared with those obtained after drug exposure in a paired manner. For comparisons at a single voltage or drug concentration differences were analyzed using the Student \( t \)-test. To analyze block at multiple voltages or drug concentrations, two-way analysis of variance was used. Results are expressed as mean ± S.E.M. A \( P \)-value of less than 0.05 was considered as significant. More details on each procedure are given under Section 3.

3. Results

3.1. Concentration-dependent hKv1.5 block by loratadine

Fig. 1 shows superimposed potassium current recordings from a mouse L-cell expressing hKv1.5 channels in control conditions, in the presence of loratadine and after washout of the drug. The upper panel shows that the hKv1.5 current rose rapidly to a peak and then slowly and partially inactivated as previously described [20,21]. Outward currents were followed by decaying outward tail currents upon repolarization to −40 mV. The middle panel shows that loratadine, 3 \( \mu \)M, reduced the peak outward current elicited by pulses to +60 mV by 18.5 ± 2.2% \( (n = 6) \), which was reached at earlier times, and altered the time course of the current during depolarization, so that the current declined much faster and to a larger extent than the slow inactivation observed in control. Thus, the reduction of the hKv1.5 current measured at the end of the 500 ms depolarizing pulses to +60 mV was 67% (from 794 to 262 pA). The bottom panel in Fig. 1 shows current traces obtained after 20 min perfusion with drug-free solution. Currents were restored to 85% of control value, indicating that the effects of loratadine were largely reversible upon washout, although the recovery was slow and incomplete.

Fig. 2 shows the concentration-dependence of hKv1.5 inhibition. The suppression of the current after 500 ms at +60 mV was used as an index of block. A non-linear
least-squares fit of the concentration–response equation (Eq. (2), see Section 2) to the individual data points yielded an apparent $K_D$ of $1.2 \pm 0.2 \ \mu M$ and a Hill coefficient of $0.92 \pm 0.11$. When the data were fitted with the Hill coefficient constrained to 1, a similar apparent affinity was obtained ($K_D = 1.2 \pm 0.1 \ \mu M$). These results suggest that binding of one molecule per channel is sufficient to block potassium permeation.

3.2. Voltage-dependence of drug–channel interaction

Fig. 3A shows the current–voltage ($I–V$) relations in the presence and in the absence of loratadine $1 \ \mu M$ obtained in one typical experiment. The control $I–V$ relationship was almost linear for depolarizations positive to $+10 \ \text{mV}$. Loratadine, $1 \ \mu M$, reduced hKv1.5 current over the whole voltage range over which this current is activated. To quantify the effects of the voltage on the drug–channel interaction, the relative current ($I_{loa}/I_{con}$) was plotted as a function of the membrane potential (Fig. 3B). The dotted line shows the activation curve of the hKv1.5 channels in this particular experiment. The current activated at $−30 \ \text{mV}$ and the conductance of the channels was fully saturated at $0 \ \text{mV}$. The midpoint and the slope factor from Boltzmann equation (Eq. (1), see Section 2) yielded values of $−16.4 \pm 0.5$ and $4.6 \pm 0.2 \ \text{mV} \ (n = 8)$, respectively. In the presence of loratadine the blockade increased steeply between $−30$ and $0 \ \text{mV}$ which corresponded with the voltage range for channel opening [16]. This result suggests that loratadine binds preferentially to the open state of the hKv1.5 channels. Between $0$ and $+60 \ \text{mV}$ the blockade decreased with a shallow voltage dependence despite the fact that all channels are open over this voltage range. In fact, in 12 cells the blockade induced by loratadine $1 \ \mu M$ decreased from $63.6 \pm 4.2\%$ at $−10 \ \text{mV}$ to $54.3 \pm 3.5\%$ at $+60 \ \text{mV} \ (P < 0.01)$. Loratadine’s $pK_a$ is 4.9 and therefore at the intracellular pH of 7.2 loratadine predominates in its uncharged form. Thus, the voltage-dependence of block could not be due to the effect of the

![Image](https://example.com/image1.png)

**Fig. 1.** Effects of loratadine on hKv1.5 channels expressed in a mouse L-cell. Superimposed current traces are shown for 500 ms depolarizing pulses from $−80 \ \text{mV}$ to voltages between $−60$ and $+60 \ \text{mV}$ in steps of $20 \ \text{mV}$ in control conditions (the upper panel), in the presence of $3 \ \mu M$ loratadine (the middle panel) and after washout of the drug (the bottom panel). Data filtered at 1 kHz (4-pole Bessel) and digitized at 5 kHz. Cell capacitance, 9 pF.

![Image](https://example.com/image2.png)

**Fig. 2.** Concentration dependence of loratadine-induced block of hKv1.5. Reduction of current (relative to control) after 500 ms depolarizations to $+60 \ \text{mV}$ was used as index of block. The continuous line represents the fit of the experimental data to Eq. (2) (see Section 2). For comparison, the dashed line represents the fit of the experimental data for a Hill coefficient ($n_H$) of 1. Each point represents the mean ± s.e.m. of 5–10 experiments.

![Image](https://example.com/image3.png)

**Fig. 3.** (A) Effect of $1 \ \mu M$ loratadine on the steady-state current–voltage ($I–V$) relationships (500 ms isochronal). (B) Relative current expressed as $1−I_{loa}/I_{con}$ from data shown in panel A. The dotted line shows the activation curve of the hKv1.5 channel for this particular experiment. Block increased steeply between $−30$ and $−10 \ \text{mV}$. For membrane potentials more depolarized than $0 \ \text{mV}$ a more shallow voltage dependence was observed. Data of both panels were obtained from the same cell.
transmembrane electrical field on the interaction between the charged loratadine molecule and the channel receptor.

3.3. Time-dependent effects of loratadine on hKv1.5 channels

The activation process was fitted to a monoexponential function following the procedure described in Section 2. In the absence of loratadine, the time constant of activation of hKv1.5 currents elicited by 500 ms depolarizing pulses from −80 to +60 mV averaged 1.7 ± 0.1 ms, while in the presence of 3 μM loratadine it was 1.6 ± 0.1 ms, which indicated that the drug did not significantly modify the time course of activation (n = 5, P > 0.05).

As shown in the middle panel of Fig. 1, loratadine induced a falling phase in the current traces which superimposed on the slow process of inactivation which characterizes the hKv1.5 current at positive potentials (τ = 257.9 ± 15.4 ms). Thus, in the presence of loratadine current traces positive to +40 mV were fitted with a biexponential function so that two time constants were obtained. The time constant of the fast falling phase was considered to represent the time constant of development of block (τ<sub>Block</sub>), whereas the slow time constant reflects the slow and partial process of inactivation. In Fig. 4, the τ<sub>Block</sub> was plotted as a function of loratadine concentration and the experimental data of 15 experiments were fitted to a hyperbolic function (see Eq. (3)). Following this approach we were able to calculate the apparent association (k) and dissociation rate constants (l) which averaged (3.6 ± 0.5) × 10<sup>6</sup> M<sup>−1</sup>·s<sup>−1</sup> and 3.7 ± 1.6·s<sup>−1</sup>, respectively. Following the previous assumption of a first-order reaction between drug and receptor, the ratio between the l- and k-values would give the apparent dissociation constant (l/k = K<sub>D</sub>). This estimate is independent of the apparent K<sub>D</sub> obtained from steady-state current suppression (Fig. 2). Nevertheless, the derived value (1.03 μM) is in close agreement with that calculated from the fit of the concentration–response curve. Binding rates can also be derived from the apparent K<sub>D</sub> and the average value of τ<sub>Block</sub> at 3 μM (0.069 ± 0.001 s). Using this procedure, the calculated k and l-values were 3.4 × 10<sup>6</sup>·M<sup>−1</sup>·s<sup>−1</sup> and 4.1·s<sup>−1</sup>, respectively.

The effects of loratadine on the time constant of deactivation of tail currents elicited on return to −40 mV after 500 ms depolarizing pulses to +60 mV were also analyzed. In control conditions the deactivation process mainly reflects the irreversible closing of the channels at negative potentials [16]. In the absence of drug, the deactivation process was fitted to a monoexponential function and the time constant of tail currents averaged 36.2 ± 3.4 ms (n = 6) at −40 mV. However, as it is represented in a typical experiment shown in Fig. 5, in the presence of 1 μM loratadine, the peak tail current amplitude decreased and the subsequent time course of the tail current was significantly slowed, so that the calculated time constant of deactivation averaged 64.9 ± 3.6 ms (n = 5, P < 0.01). As a consequence of the slowing of the time constant of deactivation a crossing of the tail currents was observed (i.e., the crossover phenomenon).

3.4. Use-dependent effects of loratadine on hKv1.5 channels

Fig. 6A shows current traces elicited in the absence and in the presence of 1 μM loratadine when applying a train of 16 depolarizing pulses of 200-ms duration from a holding potential of −80 to +60 mV at a frequency of 1 Hz. In control conditions the current amplitude remained almost unaffected by the repetitive application of pulses during a train, whereas in the presence of loratadine the peak amplitude progressively decreased until a new
steady-state level is reached. Fig. 6B shows the ratio of the peak amplitude of the currents in the presence and in the absence of loratadine ($I_{\text{lor}}/I_{\text{con}}$) as a function of the number of pulses in the train. In the presence of the drug, there was a reduction in peak current amplitude elicited by the first depolarizing pulse applied after a rest period (i.e., ‘tonic block’) which averaged $12.6 \pm 2.0\%$ ($n = 6$). Moreover, during the application of the train of pulses the blockade of Kv1.5 channels induced by loratadine progressively increased reaching a final value of $48.6 \pm 4.3\%$ (i.e., phasic block) and most of this additional block was apparent within the first two pulses of the train. When trains of pulses at 2 Hz were applied, both the tonic ($15.8 \pm 2.9\%$) and the phasic loratadine-induced block ($46.6 \pm 4.1\%$) was not significantly different from that produced at 1 Hz. To characterize the onset kinetics of this

![Diagram of loratadine effects on Kv1.5 channels](image)

Fig. 7. Analysis of the recovery from loratadine-induced block on Kv1.5 currents. (A) Superimposed current traces obtained in the absence and in the presence of 1 μM loratadine. A double pulse protocol was applied. A 1-s conditioning prepulse from $-80$ to $+60$ mV was followed by a test pulse of 30 ms duration of the same amplitude at various interpulse intervals (from 30 ms to 10 s). (B) Graph showing the peak amplitude of the currents elicited by the test pulse as a function of the interstimulus interval in the absence (●) and in the presence (○) of loratadine 1 μM. Data were obtained from the same cell as in panel A. Continuous lines represent the biexponential fit of the peak current amplitude as a function of the interstimulus interval.
use-dependent block an exponential analysis was performed. The relationship between the relative current ($I_{ba}/I_{con}$) and the consecutive number of pulses in the 1 Hz train was well fitted to a monoexponential function from which the onset rate constant of block development ($K$) was calculated. In 6 cells, the $K$-value averaged $0.41 \pm 0.05 \cdot \text{pulse}^{-1}$.

Recovery from loratadine-induced block was measured by using a double pulse protocol in which a 1-s conditioning prepulse from $-80 \text{ to } +60 \text{ mV}$, which was enough to fully develop the steady-state blockade, was followed by a 30 ms test pulse of the same amplitude. The interpulse interval between both pulses was progressively increased from 30 ms to 10 s. Fig. 7A shows current traces elicited in the absence and in the presence of 1 $\mu M$ loratadine and illustrates that the amplitude of hKv1.5 currents was progressively restored as the interpulse interval was increased. Fig. 7B shows the peak current amplitudes elicited by the test pulse as a function of the time interval for the experiment shown in panel A. Under control conditions, the increase can be described by a monoexponential function, the time constant of this process being 549.0 ± 54.3 ms ($n = 7$). In contrast, in the presence of 1 $\mu M$ loratadine the current recovery was better fitted by a biexponential function, from which two time constants were calculated. The time constants of the fast and slow components averaged $422.3 \pm 69.7$ and $2900.4 \pm 509.2$ ms, respectively. It is interesting to note that the time constant of current recovery under control conditions was not statistically different from the time constant of the fast component obtained in the presence of loratadine ($P > 0.05$).

3.5. Mathematical simulation

Fig. 8 shows the results of a mathematical simulation of the effects of 1 and 3 $\mu M$ loratadine, based on an open-channel block model. Incorporation of the experimental values for $k$ and $l$ reproduced fairly well the main effects of loratadine: (a) the reduction of peak current without altering the initial sigmoidal activation time course of the current (Fig. 1); (b) the decline of hKv1.5 current during the depolarizing step (Fig. 1); and (c) the crossover phenomenon of the tail currents (Fig. 5). Since the model assumes that loratadine binds only to the open channel, simulation further suggested that the effects of the drug might be explained by preferential binding to the open state of the hKv1.5 channels.

4. Discussion

In the present study we have analyzed the effects of loratadine, a long-acting non sedating antihistamine agent on a cardiac K$^+$ channel cloned from human ventricle and stably expressed in a mouse L-cell line. In an attempt to overcome some of the inherent difficulties associated with the study of K$^+$ channels in human cardiac myocytes, we examined the effects of loratadine on 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 [14] which can contribute to repolarization of the human atrial action potential [14]. Moreover, very recently the hKv1.5 channel protein has been located in human atrial and ventricular myocardium explanted from newborn and adult patients [22]. The present results provided evidence for concentration-, time-, voltage-, and use-dependent blockade of hKv1.5 channels by loratadine.

4.1. Loratadine blocks hKv1.5 channels

Loratadine induces a concentration-dependent block of hKv1.5 channels which was at least as potent as the racemate and R- and S-terfenadine [7] ($K_D = 0.8, 1.2$ and 1.1 $\mu M$, respectively). In contrast, terfenadine was 3 times more potent than loratadine [21] ($K_D = 367$ nM) on fHK channels cloned from human heart [14] and expressed in the embryonic kidney cell line HEK-293. Moreover, loratadine was more potent than quinidine [20] ($K_D = 6$ $\mu M$) and at least as potent as zatebradine [19] ($K_D = 1.8$ $\mu M$).
µM) to block hKv1.5 channels. Although the concentrations need to block hKv1.5 are well above that for the clinical antihistamines (see below), the micromolar affinity is of interest in comparison with other drugs affecting this channel.

4.2. Voltage-dependence of block

The interaction of loratadine with the hKv1.5 channels was also voltage-dependent. The blockade increased steeply in the voltage range of channel activation (between −30 and 0 mV), which suggested that this drug binds preferentially to the open state of the channel (i.e., O ↔ OB transition). Thus, as previously described with quinidine [20], terfenadine [7] and some bradycardic agents [18,19], loratadine also appears to act as an open channel blocker of hKv1.5 channels. Moreover, most of the blocking properties of antiarrhythmic drugs on other delayed rectifier K+ currents (like I_K1 and I_Kv) are also linked to an open channel block [20,23–25]. At potentials positive to 0 mV, when the channel activation reached saturation, loratadine-induced block decreased progressively with a more shallow voltage-dependence. This behavior differentiates loratadine from the drugs previously studied on hKv1.5 channels, which also produce a voltage-dependent block but in the opposite direction [20,26]. The voltage dependence of open channel block produced by quinidine, terfenadine, bupivacaine enantiomers or zatebradine was considered to be the consequence of the effects of the transmembrane electrical field on the interaction between the drugs in their cationic form and the receptor at the channel level [18–20,26]. This explanation could not be applied for loratadine since at physiological pH it predominates in its uncharged form. In contrast, it is conceivable that the affinity of the open channel receptor displays itself an intrinsic and continuous voltage dependence. Alternatively, additional binding to activated channel states that predominate below 0 mV cannot be ruled out.

Loratadine induced a decline of the current during the application of the depolarizing pulse which reflected the transition to a channel state with a higher affinity for the drug (C ↔ O). The k- and l-values obtained in the presence of loratadine were very similar to those previously reported for terfenadine (3.5 × 10^6 · M⁻¹ · s⁻¹ and 3 · s⁻¹, respectively) [7]. Moreover, the k-value obtained for loratadine is quite similar to that reported for quinidine [20] (4.5 × 10^6 · M⁻¹ · s⁻¹) or R(+) bupivacaine [26] (4.7 × 10^6 · M⁻¹ · s⁻¹), while the l-value appears to be 8–10 times slower than that of quinidine [20] (34 · s⁻¹) or R(+) bupivacaine [26] (24 · s⁻¹).

Open channel block can affect not only the time course of the currents during the depolarizing pulse but also the time course of deactivation of the tail currents. This is actually a direct prediction of the model shown in Fig. 8. Under control conditions, tail currents reflected the rapid and irreversible closing of the channels upon repolarization. If a large fraction of channels are blocked (OB) and the unbinding kinetics is fast enough, then the tail current may initially display a rising phase reflecting the OB → O unblocking. Subsequently, the tail current should decline more slowly than in control conditions because channels that become unblocked during the tail will generate outward current and, thus, slow the decay resulting in a ‘crossover phenomenon’. Loratadine decreases the rate of decay of the tail and induces a crossover phenomenon, indicating that drug unbinding is required before channels can close and provides further evidence for the proposed open channel interaction.

Our results demonstrated that loratadine (1 µM) slightly decreased (≈ 12%) the peak current elicited by the first depolarization of each train of stimuli (i.e., ‘tonic block’). This tonic block could be considered a measure of rested-state block. However, block observed at the time of the peak includes also open-state block developed before the time of the peak and block of intermediate activated but non-conducting states. In fact, the mathematical simulation of the effects of 1 µM loratadine, based on an open-channel block model (Fig. 8) predicts a small reduction of the peak current. Moreover, since loratadine-induced block develops slowly during depolarization, repetitive application of 200 ms depolarizing pulses to +60 mV at a frequency of 1 Hz led to a progressive increase in the block to a new steady-state level within ≈ 3 pulses (i.e., loratadine produced use-dependent block of hKv1.5 currents). A similar phasic blockade was observed following the application of trains of stimuli at 2 Hz, which indicated that at frequencies higher than 1 Hz loratadine did not induce frequency-dependent block of the hKv1.5 current. It is interesting to note that the steady-state block achieved in the presence of loratadine was statistically not different (P > 0.05) to that obtained when trains of pulses at normal cardiac rates (1 Hz) were applied, thus indicating that the use-dependence would not change the apparent affinity calculated under steady-state conditions. The use-dependent block induced by loratadine when trains of stimuli were applied can be understood if it is considered that in the presence of the drug the transition OB → O → C is slower than the transition between O and C states (the time constant of recovery from block ≈ 2900 ms). Finally, to the extent that the time constant of recovery reflects the rate of dissociation, it would appear that the dissociation is voltage-dependent since the derived rate constant t = −80 mV (0.34 · s⁻¹) is different to that obtained at +60 mV.

4.3. Clinical implications of this study

The possible extrapolation of the present results may be limited by two methodological problems. First, the effects of loratadine on hKv1.5 channels were assessed in a mammalian cell line. Thus, it is possible that differences in phospholipid composition of the cell membrane between Ltk− cells and human native cardiac myocytes may influ-
ence the results. However, highly lipophilic drugs like quinidine or terfenadine produced similar effects in mammalian cell lines and in human cardiac myocytes [4,6,7,20,27], which confirmed that Ltk− cells are an appropriate tool for studying the effects of drugs on human cardiac channels. Second, to resolve the time-dependent activation of hKv1.5 current, it is necessary to study the current at room temperature, since its activation is so rapid at normal body temperatures that its time dependence is very difficult, if not impossible, to resolve. Furthermore, association and dissociation binding rates are probably modified at low temperatures. Therefore, it is possible that in the present experiments we might underestimate the extent of the blocking effects of loratadine on hKv1.5 channels under physiological conditions.

In young healthy volunteers peak plasma concentrations (Cmax) after single oral doses of loratadine of 10 and 40 mg or multiple once-daily doses of 40 mg ranged between 4.7 and 27 μg/l (0.01–0.07 μM) [8,28,29]. At these plasma levels there was no evidence of prolongation of the QTc interval or any other ECG abnormality [8,9] and in the present study loratadine blocked hKv1.5 current by less than 10%. Loratadine is rapidly metabolized in the liver by CYP3A4, but when this enzyme is inhibited, it can also be metabolized by the alternative enzyme, CYP2D6 [30]. This could explain why in healthy volunteers when co-administered with drugs that inhibit CYP3A4 (i.e., ketoconazole, erythromycin) Cmax values increased to 39 μg/l (0.1 μM) [8]. However, these Cmax values are about 10-fold below the Kd for hKv1.5 block in the present study.

In conclusion, to our knowledge this study is the first to examine the effects of loratadine on a voltage-gated delayed rectifier K+ channel cloned from human ventricle. We demonstrated that loratadine inhibits in a concentration-, voltage-, time- and use-dependent manner the hKv1.5 channels but only at concentrations much higher than therapeutic plasma levels in man. This is consistent with a much wider therapeutic window for loratadine than for other antihistamine drugs.

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