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Mechanism of block of a human cardiac potassium channel by terfenadine racemate and enantiomers

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1 The cardiac toxicity of racemic terfenadine (marked QT prolongation and polymorphic ventricular arrhythmias) is probably due to potassium channel blockade. To test whether one of its enantiomers would be a less efficient potassium channel blocker, we compared the mechanism of action of the racemate with that of the individual enantiomers.

2 We synthesized the individual enantiomers of terfenadine and examined under whole cell voltage-clamp conditions the mechanism of action of the racemate, both enantiomers and a major metabolite on a cloned human cardiac potassium channel, hKv1.5. This delayed rectifier is sensitive to quinidine, clofilium and other 'class III' antiarrhythmic drugs at clinically relevant concentrations.

3 Upon depolarization, racemic terfenadine and its enantiomers induced a fast decline of hKv1.5 current towards a reduced steady state current level. During subsequent repolarization the tail currents deactivated more slowly than the control, resulting in a 'crossover' phenomenon.

4 The voltage-dependence of block was biphasic with a steep increase in block over the voltage range of channel opening (−30 to 0 mV), and a more shallow phase positive to 0 mV (where the channel is fully open). The latter was consistent with a binding reaction sensing 21% of the transmembrane electrical field (with reference to the cell interior).

5 The EC_{50} for hKv1.5 block by racemic terfenadine was 0.88 μM, while the values for **R**- and **S**-terfenadine were 1.19 μM and 1.16 μM, respectively. In contrast, the acid metabolite reduced hKv1.5 current by only 5% at a concentration of 50 μM.

6 These findings suggest that terfenadine blocks the hKv1.5 channel after it opens by entering into the internal mouth of the channel. We have previously shown that quinidine blocks hKv1.5 in a similar manner but with an apparent affinity of ~6 μM. Thus, terfenadine and its enantiomers are approximately equipotent open state blockers of this human K^+ channel and about 6 times more potent than quinidine. The similar state-, time-, and voltage-dependence of hKv1.5 block by both enantiomers also indicates that the chiral centre does not significantly constrain the orientation of critical binding determinants of terfenadine with respect to the receptor site.

Keywords: Potassium channels; arrhythmia; torsades de pointes; stereoisomers; histamine H_1 antagonist; pro-arrhythmia; Kv1.5

Introduction

Multiple types of potassium currents have been identified in the heart. These potassium currents maintain the resting potential, control action potential duration and modulate pacemaker activity. Inhibition of the delayed rectifier K^+ currents prolongs action potential duration, an effect which may be antiarrhythmic (Singh & Nademanee, 1985) but also carries a proarrhythmic potential. Indeed, excessive lengthening of repolarization is associated with abnormal electrical activity consisting of early afterdepolarizations triggering a polymorphic ventricular arrhythmia known as torsades de pointes (Roden, 1990). This arrhythmia appears to be a side-effect common to many antiarrhythmic drugs that block cardiac K^+ channels, such as quinidine, procainamide and sotalol.

Some cases of torsades de pointes occur in patients who receive non-antiarrhythmic drugs. An example is the histamine H_1-receptor antagonist, terfenadine which is widely used to alleviate allergic symptoms (McTavish *et al.*, 1990) and which was among the top 10 prescribed drugs in the U.S.A. in 1991 (Simonsen, 1992). Clinical situations in which terfenadine may accumulate have been associated with torsades de pointes (Davies *et al.*, 1989; Monahan *et al.*, 1990; Mathews *et al.*, 1991; Honig *et al.*, 1992). This is consistent with the concept that the drug (or an accumulating metabolite or enantiomer) blocks cardiac K^+ channels (Woosley *et al.*, 1993).

Drug-channel interactions in the heart have been studied largely in native cardiac myocytes of 'representative' mammalian species. However, this approach has some drawbacks such as the problem of the multiple overlapping ionic currents in the native myocytes. Usually a combination of extracellular ion substitution and channel blockers is used to eliminate all currents except the one of interest (Sanguinetti & Jurkiewicz, 1990; Balser *et al.*, 1991). These approaches have the disadvantages that blockers may not be highly specific and that detailed kinetic analysis of drug-channel interactions may not be feasible if drug-induced block of other ionic currents is not strictly time- and voltage-independent. Therefore, to quantify mechanisms of ionic channel block, a model system without contamination by other currents has certain advantages. The expression of cloned cardiac K^+ channels in tissue culture cells provides such a system. Recently, a cloned human cardiac K^+ channel, designated HK2 (Tamkun *et al.*, 1991) or hKv1.5, has been successfully expressed in a mouse L-cell line (Snyders *et al.*, 1993). This hKv1.5 current appears very similar to the fast activating potassium currents observed in rat atrial myocytes (Boyle & Nerbonne, 1991), canine neonatal epicardial ventricular myocytes (Jeck & Boyden, 1992) and human atrial myocytes (Wang *et al.*, 1993). The electrophysiological properties of the hKv1.5 have been characterized in detail

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(Snyders *et al.*, 1993) and this model system has allowed elucidation of the mechanism of block of this channel by quinidine (Snyders *et al.*, 1992).

We used this *in vitro* system to study the mechanism of terfenadine inhibition of this human cardiac K^+ channel. In a recent study it was suggested that terfenadine acts as an open channel blocker of fHK, a cloned channel related to hKv1.5 (Rampe *et al.*, 1993). However, no voltage-dependence of block was demonstrated. We specifically investigated the voltage-dependence of block at depolarized potentials, and the time-dependence of deactivating tail currents at negative potentials to provide evidence for the open channel block mechanism. In the same study the benzhydryl-piperidine part of the molecule displayed low affinity, while hydroxylation of the phenylbutyl part of the molecule reduced the affinity moderately. Because the chiral centre is located in the phenylbutyl end of the molecule it is of interest to explore possible stereoselective channel block. Terfenadine is prescribed as a racemate and its H_1 receptor antagonism does not display stereoselectivity (Zhang *et al.*, 1991). For optically active antiarrhythmic drugs, K^+ channel block may (Mirro *et al.*, 1981) or may not (Carmeliet, 1985) be stereoselective. In the case of terfenadine, identification of stereoselectivity in block might imply that prescription of a non-K^+ channel blocking enantiomer would decrease the risk of torsades de pointes. Therefore, we synthesized the enantiomers of terfenadine and compared their effects to those of racemic terfenadine and of its major acid metabolite in voltage-clamped L-cells.

Methods

Cell culture

The method to establish hKv1.5 expression in clonal L-cell lines has been described previously (Snyders *et al.*, 1992; 1993). Transfected cells were cultured in DMEM supplemented with 10% horse serum and 0.25 mg mg^{-1} G418 (geneticin, Gibco BRL, Grand Island, NY, U.S.A.) in a 5% CO_2 atmosphere. The cultures were passed every 3–5 days using a brief trypsinization. Before electrophysiological experiments, subconfluent cultures were incubated with 2 μM dexamethasone for 24 h, as expression of the channel is under control of a dexamethasone-inducible promoter. The cells were removed from the dish with a cell scraper, and the cell suspension was stored at room temperature (22–23°C) and used within 12 h for all the experiments described here.

Electrical recording

Glass micropipettes were pulled from starbore borosilicate glass (Radnoti, Monrovia, CA, U.S.A.) and heat polished. They were filled with 'intracellular' solution (see below) and connected to the headstage of an Axopatch-1A patch clamp amplifier (Axon Instruments Inc., Foster City, CA, U.S.A.). All currents were recorded at room temperature (22–23°C) and were sampled at 3–10 times the anti-alias filter setting using a 12-bit analog-to-digital converter (Labmaster, Scientific Solutions, Solon, Ohio, U.S.A.). A commercial software package (pClamp, Axon Instruments) was used for data acquisition and command potentials Microelectrode resistance averaged 2.7 ± 0.7 MΩ ($n = 30$, range 1.5–5). The microelectrodes were gently lowered onto the cells and Gigaohm seal formation (16 ± 9 GΩ, range 6–33 GΩ, $n = 26$) was achieved by suction. Following 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 to −70 mV were recorded at 50 kHz (filtered at 10–20 kHz) for subsequent calculation of capacitive surface area, access resistance and input impedance. Thereafter capacitance and series resistance compensation were optimized; 80% compensation of the effective access resistance (9.1 ± 0.9 MΩ) was usually obtained. With an average current of 1.3 nA at +50 mV the voltage error induced by the residual series resistance was less than 3 mV.

Synthesis of terfenadine enantiomers

The method of chiral synthesis via organoboranes (Brown *et al.*, 1988) was used to synthesize the terfenadine enantiomers as illustrated in Figure 1. Racemic terfenadine was oxidized to its corresponding ketone (4-(1,1-dimethylethyl)phenyl-4-(hydroxy-diphenylmethyl)-1-piperidinebutanone). This ketone was purified, crystallized and its structure was confirmed by n.m.r. This ketone was reduced to **R**(+)-terfenadine using (+)-diisopinocampheyl-chloroborane ((+)-Ipc_2BCl) as a

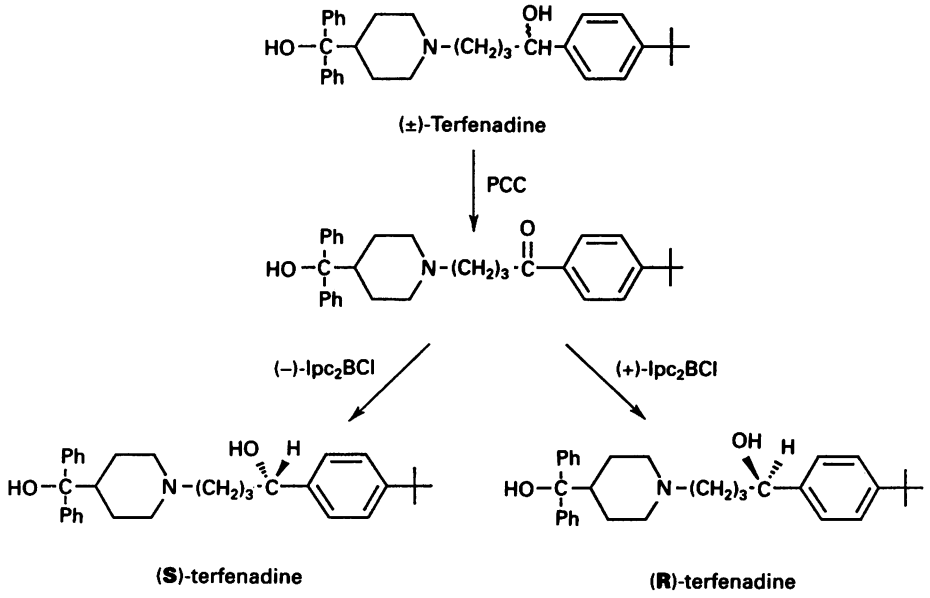

Figure 1 Synthesis of terfenadine enantiomers: racemic terfenadine was oxidized to the corresponding ketone using pyridinum chlorochromate (PCC). The appropriate chiral reducing agents (+)- or (−)-Ipc_2BCl, were used to impose the desired chiral configuration.

chiral reducing agent. Purification on a silica gel column using 1% methanol/methylelne chloride provided the **R**-isomer of terfenadine (reaction yield: 65%). The structure was confirmed by n.m.r. The S(−)-enantiomer was similarly synthesized using (-)-Ipc_2BCl. The purity of each enantiomer was assessed by h.p.l.c. using an Ultron ES-OVM column with methanol/25 mM phosphate buffer (pH 4.6) (25:75) as mobile phase. The **R**-enantiomer was 97.5% pure, and S(-)-terfenadine was 95.2% pure. n.m.r. spectra were recorded in $CDCl_3$ on a Bruker/IBM NR 300.

Other solutions and drugs

The intracellular pipette filling solution contained (in mM): KCl 110, HEPES 10, K_4BAPTA 5, K_2ATP 5, $MgCl_2$ 1, and was adjusted to pH 7.2 with KOH, yielding a final intracellular K^+ concentration of approximately 145 mM. The bath solution contained (mM): NaCl 130, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, glucose 10, and was adjusted to pH 7.35 with NaOH. Racemic terfenadine (mol.wt. = 472) was purchased from Sigma Chemical CO., St. Louis, MO, U.S.A. Terfenadine carboxylate was provided by Marion Merrell Dow Inc., Cincinnati, OH, U.S.A. All compounds were dissolved in dimethyl sulphoxide (DMSO, Sigma Chemical Co.) to yield stock solutions of 10 mM. This vehicle had no measurable effects on the hKv1.5 current in final working concentrations up to 0.1%. Control solutions contained the same concentrations of DMSO as the test solution.

Pulse protocols and analysis

After obtaining control data, bath perfusion was switched to drug-containing solution. Drug infusion or removal was monitored with test pulses from −80 mV to +50 mV, applied every 30 s until steady-state was obtained (within about 10 min). The holding potential was −80 mV unless indicated otherwise, and the interval between depolarizations for any protocol in control or in drug was 10 s or slower. The protocol to obtain current-voltage (*IV*) relationships and activation curves consisted of 250 ms pulses that were imposed in 10 mV increments between −80 and +60 mV. 'Steady state' *IV* relationships were obtained by measuring currents at the end of the 250 ms depolarizations. Between −80 and −40 mV, only passive linear leak was observed; least squares fit to these data were used for passive leak correction. Deactivating tail currents were recorded upon repolarization to −30 to −50 mV. The activation curve was obtained from the tail current amplitude immediately after the capacitive transient. For steady-state measurements, raw data points were averaged over a small time window (5–10 ms). Activation curves were fitted with a Boltzmann equation of the form $y = 1/(1 + \exp[-(E-E_h)/k])$ where E_h represents the half-activation voltage and k the slope factor. The time course of tail currents and drug-induced 'inactivation' was fitted with a sum of exponentials. The curve-fitting procedure used a non-linear least squares (Gauss-Newton) algorithm. The fit results were displayed in linear and semilogarithmic format together with a plot of the residual deviation of the data from the fitted curve (difference plot). Goodness of the fit was judged by the χ^2_1 criterion (*F*-test) and by inspection for systematic non-random trends in the difference plot.

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

$$f = 1/(1 + (K_D/[D])^n) \quad [1]$$

Voltage-dependence of block was determined as follows: leak subtracted current in the presence of drug was normalized to matching control to yield fractional block at each voltage ($f = 1\text{-}I_{terfenadine}/I_{control}$). The voltage-dependence of block was described by a Woodhull (1973) model and was fitted to the equation:

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

where z represents the valency, F the Faraday constant, R the gas constant, T the absolute temperature, and δ represents the fractional electrical distance, i.e. the fraction of the transmembrane field sensed by a single charge at the receptor site. K_D^* represents the apparent affinity at a reference voltage (0 mV).

Statistical methods

Results are expressed as mean ± s.e.mean. A paired *t* test was used to compare the effect of each test compound to its control; $P < 0.05$ was considered significant.

Results

Concentration-dependent block of the hKv1.5 current by terfenadine

Figure 2a shows representative recordings of the K^+ current through hKv1.5 channels expressed in mouse L-cells during depolarizations from a holding potential of −80 mV to voltages between −30 and +50 mV in steps of 10 mV. Under control conditions (Figure 2a), the hKv1.5 current rose rapidly with a sigmoidal time course to a peak and then declined slowly (slow and partial inactivation) as reported

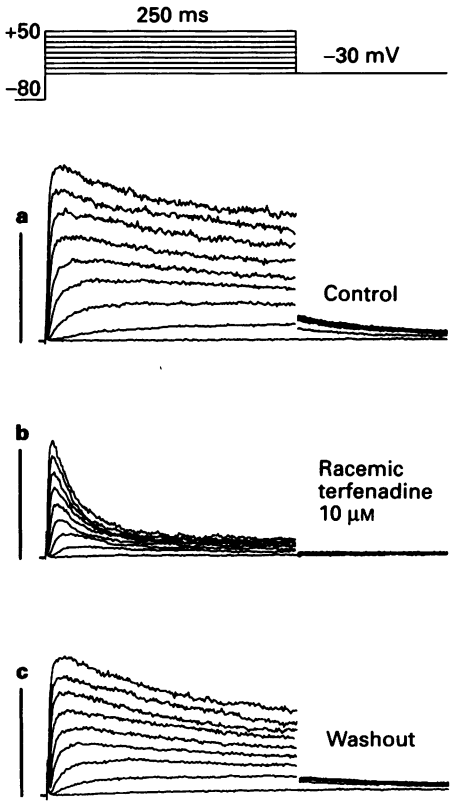

Figure 2 Suppression of hKv1.5 current by terfenadine. Currents are shown for depolarizations from −80 mV to voltages between −30 and +50 mV in steps of 10 mV; tail currents were obtained upon repolarization to −30 mV. Panels (a–c) represent control, 10 μM racemic terfenadine, and washout (20 min), respectively. After addition of terfenadine, the currents at the end of the depolarization were reduced at all levels of voltages. The effect was largely reversed after a 20 min washout. Cell capacitance, 14 pF. Vertical calibration: 0.5 nA. Data filtered at 2 kHz (4 pole Bessel) and digitized at 10 kHz; digital leak subtraction and additional digital filtering at 1 kHz.

previously (Snyders *et al.*, 1992; 1993). The steady-state current amplitude (at the end of 250 ms step) was 1280 ± 120 pA (or 94 ± 9 pA/pF, $n = 30$) at +50 mV. Decaying outward tail currents were observed upon repolarization to −30 mV. Figure 2b shows that this outward K^+ current was markedly suppressed by application of 10 μM racemic terfenadine. The effect was largely reversible after a 20-min wash-out period (Figure 2c). Terfenadine not only reduced the current amplitude, but also altered the time course of the current during depolarization. The current initially activated as in control, but subsequently declined markedly. This decline occurred much faster and to a larger extent than the slow inactivation observed in control. Analysis of this time course of block (see below) indicated that a steady level was achieved within 250 ms, while only a limited degree of slow inactivation occurred over this time. Therefore, the reduction of the hKv1.5 current at end of a-250 ms depolarization ($I_{terfenadine}/I_{control}$) was used as an index of block. In the experiment shown in Figure 2, the K^+ current at +50 mV was reduced to 10% of control in the presence of 10 μM racemic terfenadine. This steady-state reduction of hKv1.5 current was concentration dependent as shown in Figure 3. A non-linear least squares fit of the concentration-response equation [*1*] (see methods) to these data was used to obtain the apparent affinity K_D and Hill coefficient n. In Figure 3, the dotted line represents the fit to the racemic terfenadine data with an apparent K_D of 0.88 μM and n = 0.95. When the data were fit assuming a Hill coefficient of 1, a similar apparent affinity was obtained (K_D of 0.81 μM). Either approach suggested that binding of one terfenadine molecule per channel was sufficient to inhibit potassium permeation.

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

Figure 4 shows that suppression of hKv1.5 current was observed over the whole voltage range over which this current is activated. To determine and quantitate the voltage-dependence of this inhibition, the relative current ($I_{terfenadine}/I_{control}$) was plotted as a function of voltage (Figure 4b). Block increased steeply between −20 mV and 0 mV, coinciding with the voltage range of channel opening (Snyders *et al.*, 1993). This suggests that terfenadine binds primarily to the open channel. Between 0 mV and +60 mV, terfenadine-induced block continued to increase with a more shallow voltage-dependence. It is unlikely that this shallow voltage-dependence was due to channel gating because hKv1.5 activation has reached saturation over this voltage range (Snyders *et al.*, 1993). Terfenadine is a weak base ($pK_a \sim 10$) and is therefore predominantly present in its charged form at the intracellular pH of 7.4. Thus, the voltage-dependence of block could be due to the effect of the transmembrane electrical field on the terfenadine-channel interaction, similar to that reported for the interaction of quinidine with this hKv1.5 channel (Snyders *et al.*, 1992). If terfenadine reaches the receptor from the inside, then channel block is expected to increase in a voltage-dependent manner according to equation [2] (Methods), which incorporates the effect of the transmembrane electrical field (Woodhull, 1973). The parameter δ in this equation represents the fractional electrical distance, i.e., the fraction of the membrane field sensed by the positive charge at the receptor site. The dashed line in Figure 4b represents the fit of this equation to the data points positive to 0 mV (solid symbols). A fractional electrical distance $\delta = 0.24$ was obtained in this experiment with 10 μM racemic terfenadine. The average value ($\delta = 0.21$, see Table 1) was similar to the value $\delta = 0.18$ obtained in previous experiments with quinidine (Snyders *et al.*, 1992).

Change of hKv1.5 current time course by terfenadine

If terfenadine can access its receptor only when the channel is on the open state, then inhibition of the K^+ current would only develop as channels start to open, and block development should be visible if the blocking rate is slower than the opening rate. Figure 5a shows superposition of the currents obtained at +50 mV before and after application of 10 μM racemic terfenadine. Under control conditions, the K^+ current rapidly reached its peak and then inactivated slowly. The time course of this partial inactivation was well fitted by a monoexponential function, yielding a time constant of 188 ± 41 ms ($n = 6$). In the presence of terfenadine, however, the peak current was smaller and reached at an earlier time, and the subsequent current decay displayed a combination of fast and slow phases that were well-fitted with a biexponential function. In these experiments, no significant differences

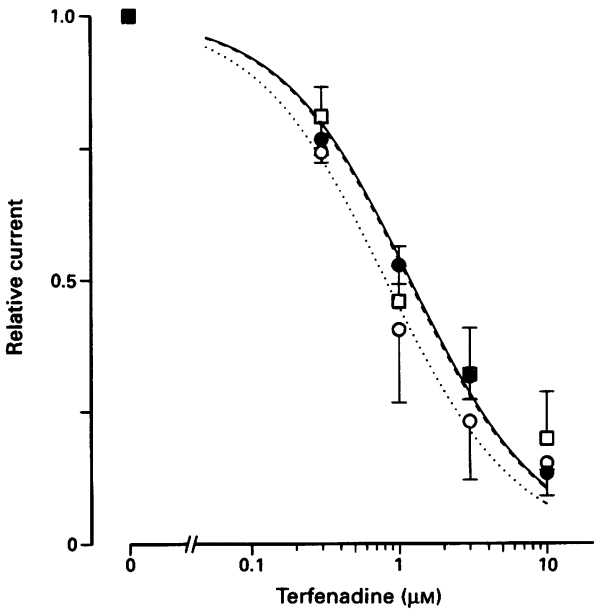

Figure 3 Concentration-dependence of suppression of hKv1.5 current by terfenadine and its enantiomers. Suppression of the steady-state hKv1.5 current (relative to control) at the end of depolarizations to +50 mV was used as index block. Data are shown as mean ± s.e.mean from 3–5 determinations at each concentration. The dotted line represents the fit of the Hill equation [1] to the racemic terfenadine data (○) which yielded an apparent K_D of 0.88 μM and a Hill coefficient of 0.95. The solid and dashed lines represent the fit for the R-(●) and S-enantiomer (□), respectively.

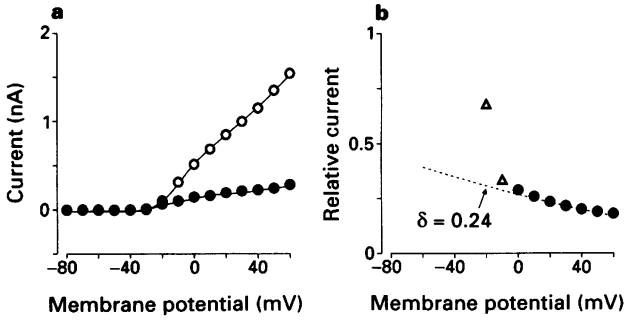

Figure 4 Voltage-dependence of hKv1.5 block by 10 μM terfenadine. (a) Current-voltage relationships (250 ms isochronal) in the absence (open symbols) and presence of drug (●). (b) Normalized block, shown as relative current ($I_{drug}/I_{control}$) from data in (a). Block increased steeply between −20 mV and 0 mV (Δ), corresponding to the voltage range of activation of the hKv1.5 channel. At levels positive to 0 mV, a more shallow voltage-dependence was observed (●). The dotted lines represent the result of the fit of equation [2] to these data, yielding an equivalent electrical distance $\delta = 0.24$ in this case.

were observed for the slow time constants (176 ± 42 vs 188 ± 41, $n = 6$, $P > 0.05$), while the time constant of the fast component was 26 ± 3 ms ($n = 6$). Thus, the fast time constant (τ_{block}) was considered to be a reasonable approximation of the drug-channel interaction kinetics because it is a new component which was sufficiently faster than the slow one to be resolved clearly.

Time course of tail currents

In the absence of drug, the hKv1.5 current deactivated completely at −50 mV, with a time constant of 31 ± 3 ms ($n = 5$). This time constant reflects the closing of the channel, which at this voltage is essentially irreversible (Snyders *et al.*, 1993). If terfenadine interacts only with the open channel, then dissociation of terfenadine from the blocked channel should result in a transient conducting channel, which subsequently could close. Figure 5b shows the superposition of the tail current obtained at −50 mV after a 250 ms depolarization to +50 mV in the absence and presence of racemic terfenadine. In the control experiment shown, the tail current declined with a time constant of 28 ms (Figure 5b). After exposure to 1 μM racemic terfenadine, the initial tail current amplitude was markedly reduced and the time course of the decline of the tail current was slower compared to control ($\tau = 40$ ms). The average values were 23 ± 3 ms and 33 ± 2 ms for control and 1 μM terfenadine, respectively ($n = 3$, $P = 0.04$). Consequently, the superposition of these tails with those in control resulted in a 'crossover' phenomenon (arrow in Figure 5b), compatible with transient unblocking, and providing additional evidence for open channel block.

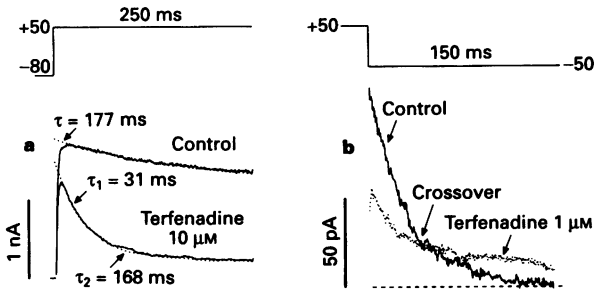

Figure 5 Time-dependent modification by terfenadine of hKv1.5 current during depolarization and repolarization. (a) Superimposed tracings from a holding potential of −80 mV to +50 mV are shown for control and 10 μM terfenadine. In the presence of terfenadine, a fast decline was superimposed on the slow inactivation observed in control. Time constants obtained using a biexponential fit, were $\tau_1 = 31$ ms and $\tau_2 = 168$ ms. The latter was similar to the slow time constant in control (177 ms). (b) Tail currents obtained at −50 mV after a 250 ms depolarization to +50 mV. In the presence of terfenadine (dotted tracing), the initial amplitude was reduced, reflecting block. The subsequent slower decline results in the 'crossover' phenomenon with the control tracing.

Effect of R(+)- and S(−)-enantiomers of terfenadine on hKv1.5 current

To test for potential stereoselectivity in the interaction of terfenadine with the hKv1.5 channel, we used the individual R(+)- and S(−)-enantiomers (Figure 1), synthesized as described above. Figure 6 shows the suppression of hKv1.5 currents by 10 μM of R(+)-terfenadine (Figure 6a, b) and S(−)-terfenadine (Figure 6c, d). As in the case of the racemate, a marked time-dependent reduction of outward current was obtained in the presence of each enantiomer. The steady-state levels of block were similarly high for each enantiomer at this concentration.

The concentration-dependence was assessed from the steady-state suppression (250 ms isochronal values) of hKv1.5 current with concentrations between 0.3 and 10 μM. Averaged data are shown in Figure 3, for comparison with the corresponding results for the racemic mixture. These experiments indicated little difference in the affinity between both enantiomers, with apparent K_D values close to those obtained for the racemic mixture (Figure 3 and Table 1). The time-dependence of block during depolarization was similar for each enantiomer (Figure 6b, d). The time constants for the fast exponential component of current decline at +50 mV were 27 and 29 ms with 10 μM R- and S-terfenadine, respectively (Table 1). Thus, no significant difference was observed for the time constant of block between racemic terfenadine and its enantiomers. The suppression of hKv1.5 current by the individual enantiomers showed the same biphasic voltage-dependence as shown in Figure 4 for the racemate. The average values for the fractional electrical distance δ were 0.19 ± 0.01 for R-terfenadine,

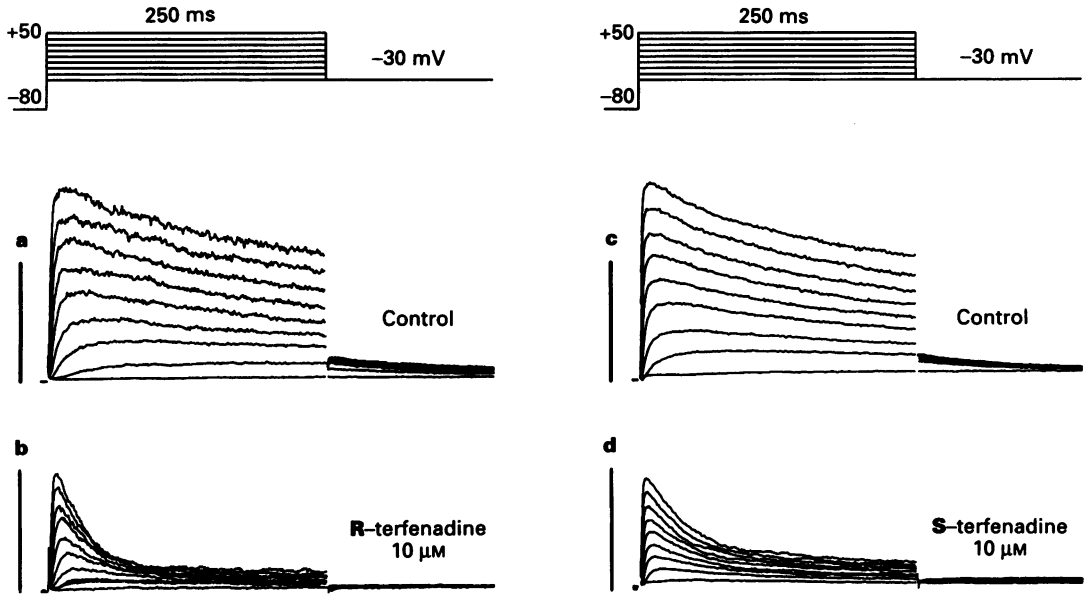

Figure 6 Suppression of hKv1.5 current by R- and S-terfenadine. Tracings for step depolarizations (pulse protocol: top). (a, b) Control and 10 μM R-terfenadine, respectively; (c, d) control and 10 μM S-terfenadine. As for racemic terfenadine, a time-dependent decline of current was observed for both enantiomers. Vertical calibration: 0.5 nA.

Table 1 Fractional electrical distance, affinity and kinetics for terfenadine block

	Racemic	R-*enantiomer*	S-*enantiomer*
δ	0.21 ± 0.02 (5)	0.19 ± 0.01 (5)	0.21 ± 0.02 (6)
τ_B (ms)	26 ± 3 (6)	27 ± 6 (4)	29 ± 5 (4)
K_D (μM)	0.81 ± 1.10	1.19 ± 0.09	1.16 ± 0.20

Mean ± s.e.mean (*n*). δ: Fractional electrical distance (see equation 2, methods), τ_B: time constant of fast decline of current at +50 mV induced by 10 μM terfenadine or its enantiomers. K_D: apparent affinity derived as shown in Figures 3 and 7, with Hill coefficient n = 1.

and 0.21 ± 0.02 for the S-enantiomer (see Table 1 for summary data). Thus neither concentration-, time-, nor voltage-dependence of block indicated stereoselectivity in the binding of the enantiomers to the hKv1.5 channel. The results indicated a similar mechanism consistent with open channel block. This was further confirmed by the observation of a typical tail current 'cross-over' for each enantiomer (data not shown).

Effect of terfenadine carboxylate on hKv1.5 current

Figure 7 shows representative current records for 250 ms depolarizations to +50 mV from a holding potential of −80 mV in the absence and presence of either terfenadine carboxylate or racemic terfenadine in the same cell. Compared to terfenadine (10 μM), a higher concentration of terfenadine carboxylate (50 μM) only slightly reduced the hKv1.5 current. In 6 experiments, 50 μM terfenadine carboxylate reduced the steady state current at +50 mV only by 5 ± 2% (NS), without detectable change of the hKv1.5 channel kinetics. Because this was 50 fold above the apparent K_D for hKv1.5 inhibition by the parent compound and its enantiomers, and at least 100 times above clinically relevant concentrations (Woosley *et al.*, 1993), we did not study the effect of higher concentrations.

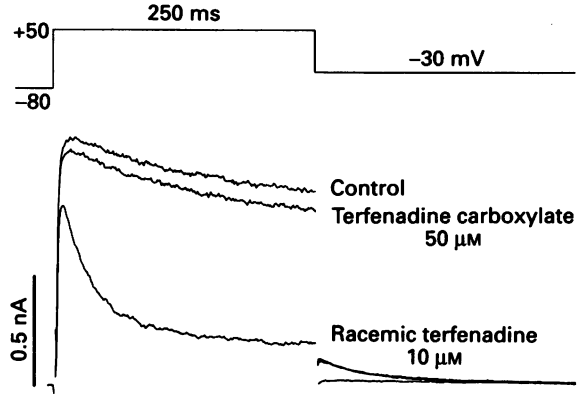

Figure 7 Comparison of the effects of terfenadine and its acid metabolite on hKv1.5 current. Shown are typical current records (voltage clamp protocol on top) in control, and after sequential application of the metabolite, and the parent compound in the same cell. Application of 50 μM terfenadine carboxylate reduced the current by less than 10%, but after washout, subsequent application of terfenadine resulted in the typical response.

Discussion

In this study, we have used our recently developed model system that allows direct study of human cardiac potassium channels (Snyders *et al.*, 1992; 1993) to analyze the mechanism of potassium channel block by terfenadine in detail. The human hKv1.5 channel activates positive to −30 mV, consistent with involvement in the control of cardiac action potential duration (Snyders *et al.*, 1993), as was shown in human atrial myocytes by selective block of this current (Wang *et al.*, 1993). We have previously shown that this channel is sensitive to quinidine in micromolar concentration (Snyders *et al.*, 1992). The results presented in this paper indicate (1) that terfenadine interacts with hKv1.5 in a time-, voltage-, and state-dependent fashion, (2) that both enantiomers are approximately equipotent, and (3) that the carboxylate metabolite is considerably less active, if active at all.

Terfenadine inhibits hKv1.5 current by acting as an open channel blocker

In the presence of terfenadine, the hKv1.5 current was reduced in a time-dependent manner. Upon depolarization, the channel initially opened as in control, but subsequently the current was reduced to a lower steady-state level (Figures 2, 5, 6 and 7). A similar observation was made for the interaction of terfenadine with fHK, a cloned channel similar to hKv1.5 (Rampe *et al.*, 1993). However, in that study, the increase of block with voltage was not significant. Drug-induced time-dependent decline of current can be due to several mechanisms, including block of open channels. Compelling evidence for open channel block includes voltage-dependence of block and the tail current cross-over phenomenon, as was shown for quinidine block of this channel (Snyders *et al.*, 1992). As shown in Figure 4 and Table 1, we observed voltage-dependent open channel block that can be described with the equivalent electrical distance δ. The value for δ was significantly different from 0, and indicates that terfenadine (and its enantiomers) sense about 20% of the applied transmembrane electrical field, referenced from the cytoplasmic side. This analysis assumes that terfenadine approaches the receptor from the inside, consistent with the effect of terfenadine in inside-out patches (Rampe *et al.*, 1993). Another argument for the open channel block model comes from the modification of the kinetics of the tail currents. For the deactivating tail currents at −50 mV, the time course was slowed with respect to control, resulting in the 'cross-over' effect shown in Figure 5.

While the modification of open channel current is consistent with open channel block, the demonstration of voltage-dependent block and tail current cross-over provides further strong evidence for this proposed mechanism of action. Both features indicate that the channel has to open before drug can reach the receptor and inhibit ion flow, and that the drug has to leave the channel before it can fully close. This is very similar to the mechanism of quinidine block of the hKv1.5 channel, but the affinity is about 6 fold higher. This can be understood if we assume that the 29 ms time constant (Table 1) reflects the time course of drug binding to the channel. For a bimolecular reaction (Snyders *et al.*, 1992), the time constant of block corresponds to $1/(k \times [D] + l)$. Together with the apparent affinity of 0.88 μM ($= l/k$), this yields values of $k = 3.5 \times 10^6\ s^{-1}M^{-1}$ and $l = 3\ s^{-1}$. Thus the apparent association rate constant k is only slightly smaller than that of quinidine ($4.5 \times 10^6\ s^{-1}M^{-1}$) obtained with a similar approach (Snyders *et al.*, 1992). The main difference is that the apparent dissociation rate constant l is about 10 fold slower compared to quinidine ($34\ s^{-1}$). Because this dissociation rate constant should reflect the stability of the drug-receptor complex, the slower dissociation may reflect a stronger hydrophobic component of binding, consistent with the high hydrophobicity of this agent. At the molecular level, this mechanism of block indicates that the drug moves some distance into the internal mouth of the channel before reaching the binding site. A hydrophobic component has recently also been observed for binding of tetraethylammonium at the internal quaternary ammonium site in related *Shaker* potassium channels (Choi *et al.*, 1993). In the carbox-

ylate metabolite, the introduction of a formal negative charge reduces the hydrophobicity of part of the molecule, and may also counteract the effect of the positive charge on the tertiary amine. Both effects are potential explanations for the much lower potency of the carboxylate metabolite.

Comparison of in vitro *affinity with* in vivo *pharmacokinetics*

Terfenadine is normally not present in detectable values in human plasma (Woosley *et al.*, 1993), but toxicity has been associated with elevated levels (Coutant *et al.*, 1991). In some cases this was due to (intentional) overdosage (Davies *et al.*, 1989; Kintz & Mangin, 1992), but in most cases the toxicity appeared to result from drug interactions (Cortese & Bjornson, 1992; Peck *et al.*, 1993; Pohjola-Sintonen *et al.*, 1993; Hirschfeld & Jarosinski, 1993; Honig *et al.*, 1993). Terfenadine is metabolized to the active H_1-receptor antagonist, terfenadine carboxylate. This biotransformation is achieved by a specific hepatic enzyme CYP 3A4. When other agents such as erythromycin and ketoconazole compete for this system, the biotransformation is inhibited, resulting in elevated levels of the parent compound (Honig *et al.*, 1992; 1993). Plasma levels of 100 ng ml^{-1} ($\sim$0.2 μM) have been reported in cases of torsades de pointes. Although this level is below the apparent K_D for hKv5.1 block, it should be noted that control of the duration of the cardiac action potential relies on a delicate balance of small currents and that even partial block of one of these currents can result in marked changes in action potential duration. For instance, the human atrial action potential duration was increased by 30% by a concentration of 4-aminopyridine which only blocked 50% of the hKv1.5 current (Wang *et al.*, 1993). Thus partial potassium channel block during the plateau phase may be sufficient to cause substantial changes in action potential duration. In addition, the effective concentration in the myocytes may not be accurately reflected by the plasma concentration, given the lipophilic character of this drug, and our data (obtained at room temperature) may underestimate the potency of terfenadine at physiological temperatures. Moreover, at least one preliminary report suggests that racemic terfenadine may block other potassium channels (Crumb & Brown, 1993).

Relevance of relative potency to clinical toxicity

The biotransformation of racemic terfenadine is not stereoselective (Zamani *et al.*, 1991; Chan *et al.*, 1991), and receptor binding studies have shown a similar affinity of both enantiomers for histamine H_1 receptors (Zhang *et al.*, 1991). In this situation, a less toxic enantiomer might be beneficial without loss of therapeutic efficiency. However, the present *in vitro* data indicate that this possibility is not likely to be realized. Both enantiomers are approximately equipotent, and do not differ significantly in their effects from racemic terfenadine. In contrast, the carboxylate metabolite is not effective as a blocker of hKv1.5 in our study, as in others (Woosley *et al.*, 1993; Rampe *et al.*, 1993). Thus the results of our *in vitro* study do not support the idea of using either enantiomer in an attempt to reduce terfenadine-associated toxicity, but the results do support the approach of using the active metabolite as an H_1-receptor antagonist rather than the parent precursor drug to this end (Woosley *et al.*, 1993).

Multiple ion currents are involved in control of cardiac action potential duration. In human atrium, various components of the delayed rectifier (I_{Ks}, I_{Kr}, hKv1,5-like) have been described recently (Wang *et al.*, 1993). In contrast, information on delayed rectifiers in human ventricle is largely inferential. For example, dofetilide and other methanesulphonanilide antiarrhythmic drugs, which specifically block I_{Kr}, induce QT prolongation, but the I_{Kr} current itself has not yet been identified in human ventricle. Similarly, although the hKv1.5 current has not yet been reported in human ventricle, mRNA for this channel has been demonstrated in human ventricle, and the channel was cloned from a human ventricular cDNA library (Tamkun *et al.*, 1991).

We have used an *in vitro* system that allows the study of an identified human potassium channel. This allowed us to study the blocking mechanism in detail, revealing open channel block as the mechanism of action. Quinidine blocks at least two cardiac delayed rectifiers (hKv1.5 and I_{Ks}) with an open channel block mechanism (Balser *et al.*, 1991; Snyders *et al.*, 1992). It also blocks another delayed rectifier component (I_{Kr}), for which block by other class III agents involves an open channel block mechanism (Furukawa *et al.*, 1989; Follmer & Colatsky, 1990; Sanguinetti & Jurkiewicz, 1990). Therefore it is not unreasonable to expect that the terfenadine open channel block model may generally apply to its interaction with potassium channels other than the hKv1.5 channel. However, because the I_{Kr} and I_{Ks} currents activate on a slower time scale, it may be impossible to resolve the time-dependence of block for these channels. To the extent that the hKv1.5 channel is representative of other human cardiac K^+ channels, the system can serve as a model to analyze mechanisms of cardiac K^+ channel block and associated toxicity.

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