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Effects of Terfenadine and Its Metabolites on a Delayed Rectifier K^+ Channel Cloned from Human Heart

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SUMMARY

Use of the nonsedating antihistamine terfenadine has been associated with altered cardiac repolarization in certain clinical settings. For this reason we examined the effects of terfenadine, and its metabolites, on a rapidly activating delayed rectifier K^+ channel (fHK) cloned from human heart. fHK was stably expressed in human embryonic kidney cells, and both whole-cell currents and currents from excised inside-out patches were recorded. Terfenadine (3 μM) blocked whole-cell fHK current by 72 ± 6%. In inside-out patches, terfenadine applied to the cytoplasmic surface blocked fHK with an IC_{50} value of 367 nM. The main effect of terfenadine was to enhance the rate of inactivation of fHK current and thereby reduce the current at the end of a prolonged voltage-clamp pulse. The blockade displayed a weak voltage dependence, increasing at more positive potentials. The mechanism of action of terfenadine is therefore consistent with blockade of open channels. In contrast, the metabolites of terfenadine were weakly active on fHK. IC_{50} values for all of the metabolites tested ranged from 27-fold to 583-fold higher than that obtained for terfenadine. It is concluded that terfenadine, but not its metabolites, blocks at least one type of human cardiac K^+ channel at clinically relevant concentrations and that this activity may underlie the cardiac arrhythmias that have been associated with the use of this drug.

Terfenadine (Seldane) is a nonsedating antihistamine that ranks as one of the most widely prescribed drugs in the United States (1). Terfenadine is rapidly converted via the cytochrome P450 enzyme system into several metabolites, including an acid metabolite and an alcohol metabolite, both of which are structurally similar to terfenadine. The acid metabolite is thought to be the biologically active antihistamine (2). Although terfenadine is generally safe and effective, recent reports have appeared linking toxic doses of terfenadine with QT_c prolongation and serious cardiac ventricular arrhythmias (3, 4). Perhaps of more concern is the interaction between therapeutic doses of terfenadine and the antifungal agent ketoconazole. Ketoconazole slows terfenadine metabolism, resulting in markedly elevated plasma terfenadine concentrations. This in turn is thought to lead to QT prolongation and ventricular arrhythmias, such as torsades de pointes (4–6). The mechanism whereby terfenadine or its metabolites contribute to these untoward cardiac effects has been unclear. Recently, however it was shown that terfenadine, but not its acid metabolite, blocked an outward, voltage-dependent K^+ current in cat ventricular myocytes (7). To further explore this phenomenon, we examined the effects of terfenadine and its major metabolites on a rapidly activating delayed rectifier K^+ channel (designated fHK) that we have cloned from human heart and stably expressed in a human embryonic kidney cell line (8). fHK is expressed in both human atrial and ventricular myocytes and the K^+ current expressed by fHK is similar to a rapidly activating delayed rectifier K^+ current observed in human atrial myocytes (8).

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Materials and Methods

Cell culture. The human embryonic kidney cell line HEK-293 was used for all experiments. These cells were transfected with the cDNA encoding the fHK channel as described previously, and the subclone designated J14 was used for all experiments (8). These cells express fHK at approximately 50–100 times the level seen in untransfected cells (8). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO BRL, Grand Island, NY), in an atmosphere of 95% air/5% CO_2. This medium also contained penicillin/streptomycin/fungizone and G418 (GIBCO BRL).

Electrophysiology. Cells used for electrophysiological experiments were seeded on glass coverslips 48–72 hr before use. Currents were recorded from whole cells as well as from cell-free inside-out macropatches (9). Electrodes (1–4-MΩ resistance) were fashioned from TW150 glass capillary tubes (World Precision Instruments, New Haven, CT). For inside-out patches the electrodes were filled with the following solution (in mM): NaCl, 130; KCl, 5.0; sodium acetate, 2.8; $MgCl_2$, 1.0; HEPES, 10; glucose, 10; $CaCl_2$, 1.0; pH 7.4 with 1 N NaOH.

ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; QT_c, corrected QT interval; FHK, fetal human potassium channel.

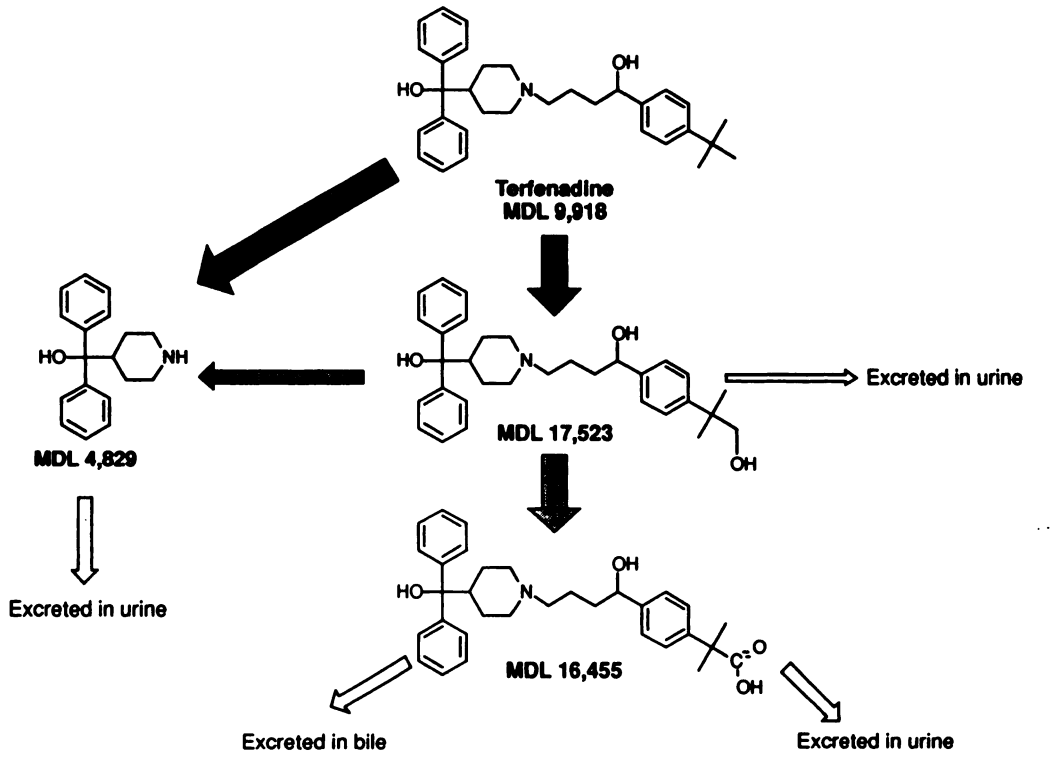

Fig. 1. Schematic representation of the metabolism of terfenadine, illustrating the structures of the major metabolites.

This solution served as the external solution for whole-cell recordings. The external recording solution used for inside-out patches contained the following (in mM): potassium aspartate, 120; KCl, 20; Na_2ATP, 4.0; HEPES, 5.0; $MgCl_2$, 1.0; pH 7.2 with KOH. This served as the internal solution for whole-cell experiments. Currents were recorded at room temperature using an Axopatch-1D amplifier (Axon Instruments, Burlingame, CA) and were conditioned by a four-pole low-pass filter with a cut-off frequency of 500 Hz. Currents were stored and analyzed using a Compaq Deskpro computer and pCLAMP software (Axon Instruments). Linear leakage and capacity currents were corrected on-line by using the $P/4$ subtraction method. The IC_{50} values of terfenadine and its various metabolites were obtained by nonlinear least-squares fits of the data (GraphPAD Software, San Diego, CA).

Chemicals. Terfenadine and its metabolites were synthesized at the Marion Merrell Dow Research Institute (Cincinnati, OH). The acid metabolite and the azacyclonol metabolite were synthesized as hydrochloride salts and designated at Marion Merrell Dow as MDL 16,455A and MDL 4,829A, respectively. When compounds are produced *in vivo* via metabolism the suffix A is not used. All other materials were obtained from commercial sources.

Results

Fig. 1 illustrates the metabolism of terfenadine as it is known to occur in animals and in humans (10, 11). The parent compound is metabolized through an alcohol intermediate (MDL 17,523) to the biologically active acid metabolite MDL 16,455. Both MDL 17,523 and terfenadine can also be converted to the azacyclonol derivative MDL 4,829.

Fig. 2 shows the effects of 3 μM terfenadine on whole-cell fHK current elicited by a 1-sec test pulse to +30 mV from a holding potential of −80 mV. Terfenadine enhanced the rate of current decay, resulting in a decrease in current amplitude

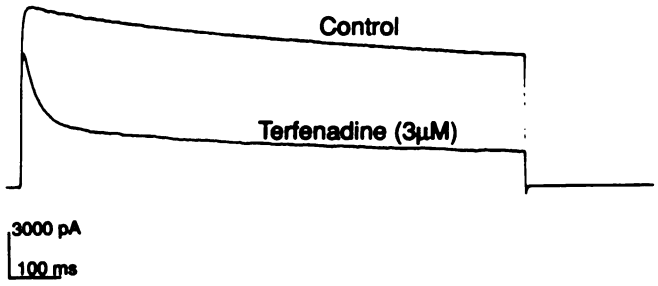

Fig. 2. Effects of terfenadine on whole-cell fHK current. Currents were elicited by a 1-sec pulse to +30 mV from a holding potential of −80 mV, in the presence and absence of 3 μM terfenadine. Under these conditions terfenadine dramatically accelerated current decay and inhibited steady state fHK current by 72 ± 6% (n = 3).

at the end of the 1-sec pulse (steady state). Terfenadine (3 μM) decreased steady state current by 72 ± 6% (n = 3). These inhibitory effects of terfenadine on whole-cell fHK current took approximately 5 min or more to develop.

Because of the prolonged equilibration times and the very large current amplitudes encountered in the whole-cell configuration (8), we further examined the effects of terfenadine on fHK using inside-out macropatches. Fig. 3A shows the effects of 300 nM terfenadine on fHK from an inside-out membrane patch. Current was elicited by a 1-sec clamp pulse to +50 mV from a holding potential of −80 mV. As was the case for the whole-cell currents, terfenadine accelerated the decay rate of fHK in inside-out patches. This effect was even more dramatic

when higher concentrations (10 μM) of terfenadine were used (Fig. 3B). The effects of terfenadine were apparent within 1-2 min and largely reversible upon washing of the patch with drug-free solution for the same period of time (Fig. 3A). When currents were measured at steady state, terfenadine potently inhibited fHK, with an IC_{50} value of 367 nM (Fig. 3C). Under control conditions at +50 mV the inactivation of fHK current was well fitted to a single-exponential function with a time constant of 484 ± 52 msec ($n = 5$). After addition of terfenadine (300 nM), inactivation followed a biexponential decay with fast and slow time constants of 42 ± 3 msec and 433 ± 46 msec, respectively ($n = 5$). The amplitude of the fast inactivating component comprised 46 ± 5% of the total current. Fig. 4 illustrates the effects of terfenadine on fHK current inactivation.

Fig. 5 shows the effects of 300 nM terfenadine on the fHK current-voltage (I-V) relationship. When measured at steady state, terfenadine reduced current over the entire voltage range tested (Fig. 3C). This block showed a tendency to increase with increasing voltage, from 35 ± 6% at +20 mV to 40 ± 5% at +60 mV ($n = 5$). However, these values were not significantly different from each other.

Fig. 6 shows the effects of the acid metabolite of terfenadine,

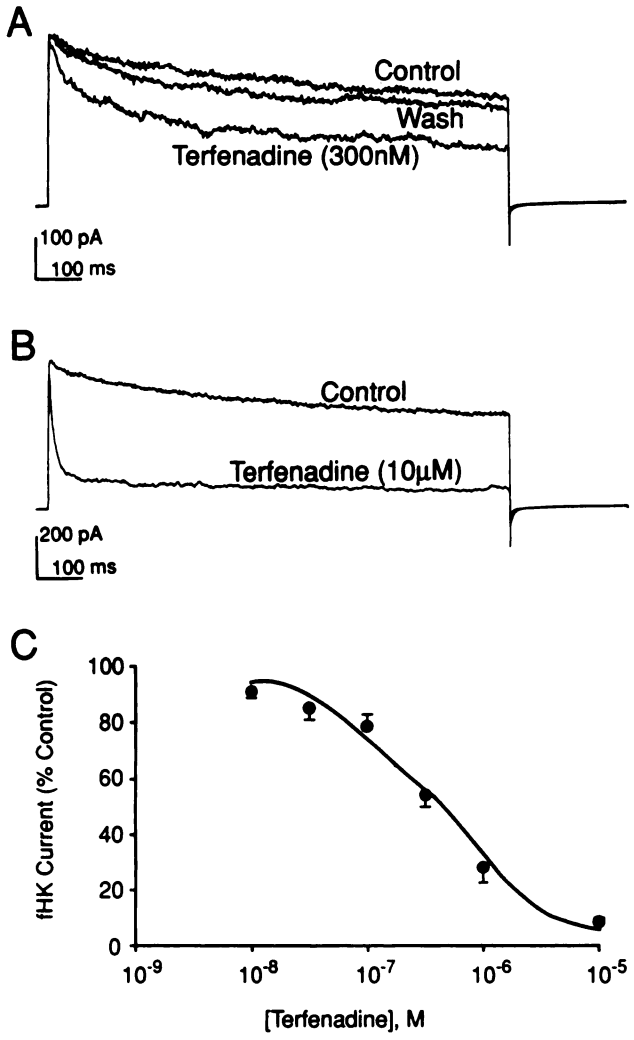

Fig. 3. Effects of terfenadine on fHK current recorded from cell-free patches. A, fHK current from an inside-out membrane patch was elicited by a 1-sec pulse to +50 mV from a holding potential of −80 mV. Control current, current in the presence of 300 nM terfenadine, and current after washout of the drug are indicated. Drug effects and washout took approximately 90 sec for the data shown. Note the enhancement of current inactivation in the presence of terfenadine. B, Effects of 10 μM terfenadine on fHK current. Currents were elicited as described for A. C, Dose-response relationship for terfenadine inhibition of fHK current. Currents were measured at the end of a 1-sec test pulse to +50 mV to generate the dose-response curve. The IC_{50} value for terfenadine was 3.67×10^{-7} M. *Error bars*, standard error ($n = 3$–8).

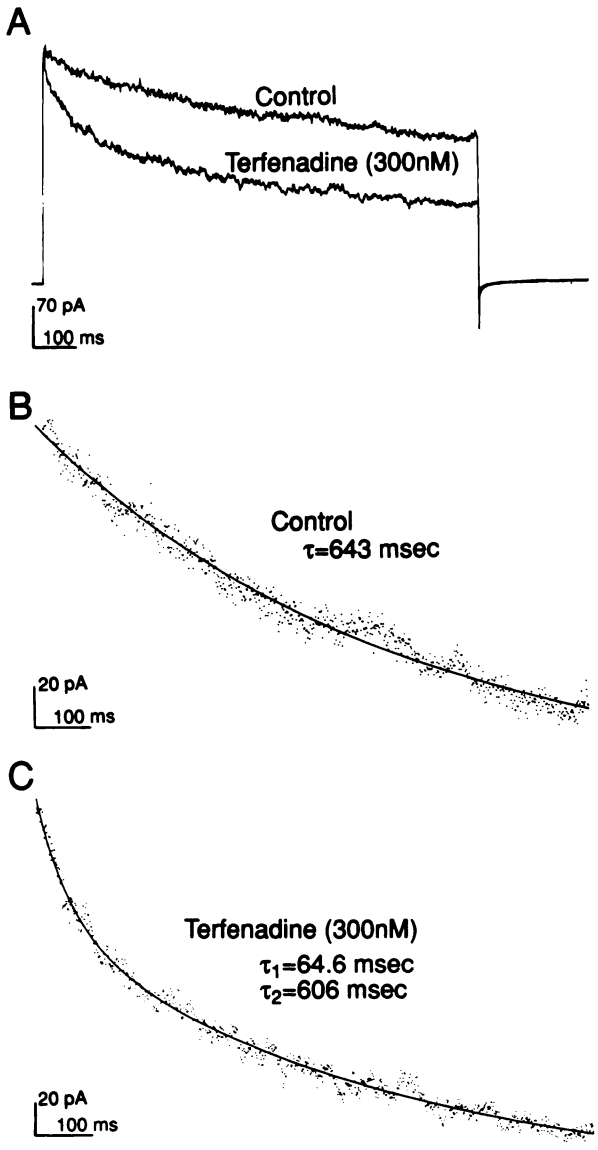

Fig. 4. Effects of terfenadine on fHK current kinetics. A, fHK currents from an inside-out macropatch were elicited by a 1-sec test pulse to +50 mV from a holding potential of −80 mV, under control conditions and after the addition of 300 nM terfenadine. B, Inactivation in the absence of drug was well fitted to a single-exponential decay, displaying a time constant of 643 msec. C, After exposure to terfenadine, current inactivation was greatly enhanced and was fitted as the sum of two exponential functions, with fast and slow time constants of 64.6 msec and 606 msec, respectively.

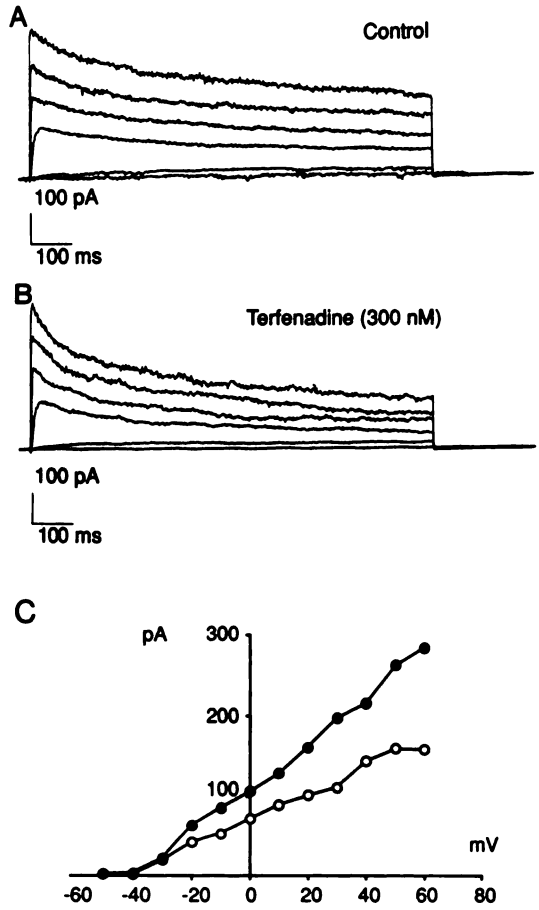

Fig. 5. Effects of terfenadine on fHK current-voltage (I-V) relationship. Currents from inside-out patches were elicited by 1-sec test pulses to potentials between −50 and +60 mV and are shown under control conditions (A) and after the addition of 300 nM terfenadine (B). The resultant I-V relationships (C) were constructed by averaging the last 100 msec of each pulse. ●, Control data; ○, current after the addition of terfenadine.

MDL 16,455A, on fHK current from an inside-out patch. Like terfenadine, MDL 16,455A accelerated current decay. Under control conditions the inactivation time constant measured 379 ± 30 msec (n = 5). After addition of 300 μM MDL 16,455A inactivation was biexponential, with fast and slow time constants of 25.4 ± 2.3 msec and 418 ± 48 msec, respectively. The fast component of inactivation comprised 51 ± 2% of the total current (Fig. 4A). However, MDL 16,455A was a much less potent inhibitor of steady state current than was terfenadine, displaying an IC_{50} value of 214 μM.

Fig. 7 shows the effects of MDL 17,523 and MDL 4,829A on fHK current from inside-out membrane patches. These compounds, like MDL 16,455A, were only weakly active at inhibiting fHK. When measured at steady state the IC_{50} values for MDL 17,523 and MDL 4,829A were 18 μM and 141 μM, respectively. Like terfenadine and MDL 16,455A, MDL 17,523 induced a rapid component of current decay that measured 7.6 ± 0.2 msec in the presence of 30 μM concentrations of this metabolite (n = 3) (Fig. 5A). This fast component of decay is likely related to a fast on-rate of the drug due to the high concentration used and a rapid dissociation rate from the channel. In contrast, the major effect of MDL 4,829A was to simply reduce fHK current, without having significant effects on its inactivation kinetics (Fig. 5B).

Discussion

The exact mechanism whereby terfenadine or one of its metabolites exerts proarrhythmic tendencies is not well understood. An important new finding has shown that terfenadine, but not its acid metabolite, can block a voltage-dependent outward K^+ current in cat ventricular cells having properties of the rapid delayed rectifier I_{Kr} (7). The purpose of the present study was to examine the effects of terfenadine and all of its metabolites on a delayed rectifier K^+ channel, fHK, cloned from human heart and to determine their relative potencies for blocking this channel. This model system provides a means of examining drug effects on a single human K^+ channel subtype. The current expressed by this fHK channel is similar in voltage dependence, kinetics, and 4-aminopyridine sensitivity to an ultra-rapid delayed rectifier current recorded from human atrial myocytes (8). It is likely that this current is also expressed in human ventricular myocytes, because fHK transcripts are present in these cells (8).

The results show that terfenadine is a potent inhibitor of fHK. The primary effect of terfenadine is to accelerate current decay, an activity that suggests an interaction with the open channel state. Terfenadine is predominantly positively charged at physiological pH (pK_a = 8.58). Its shallow positive voltage dependence and its ability to rapidly and reversibly block channels from inside-out membrane patches suggest that terfenadine accesses its binding site from the intracellular face of the channel. The IC_{50} value of terfenadine for blocking fHK (367 nM) is similar to that obtained for the blockade of K^+-dependent tail currents in cat ventricular cells (150 nM) (7). Serum plasma levels of terfenadine after overdose and associated with QT_c prolongation approach 100 nM (3). The concomitant administration of terfenadine with ketoconazole also results in prolongation of the QT_c interval and even higher (up to 172 nM) plasma terfenadine levels (5). Thus, concentrations of terfenadine needed to block fHK *in vitro* are achieved in various clinical settings. These data suggest a role for terfenadine as a blocker of voltage-dependent cardiac K^+ channels in humans. Blockade of fHK should prolong the action potential. 4-Aminopyridine at 50 μM specifically blocks the fHK-equivalent K^+ current in human atrial myocytes and produces a 66% prolongation in the action potential duration (12).

The active acid metabolite of terfenadine, MDL 16,455A, was also shown to inhibit fHK. Like terfenadine, MDL 16,455A accelerated fHK current decay. However, the IC_{50} value for MDL 16,455A (214 μM) was 583-fold higher than that of terfenadine, despite a close structural similarity between the two drugs. After an overdose of terfenadine, the levels of the acid metabolite reach only into the low micromolar range (2.8 μM) (3). Even under these circumstances the levels of MDL 16,455A would not be expected to significantly block fHK-like K^+ channels in human heart.

We also examined the effects of the terfenadine metabolites MDL 17,523 and MDL 4,829A on fHK. Neither of these compounds was a potent inhibitor of fHK, relative to terfenadine.

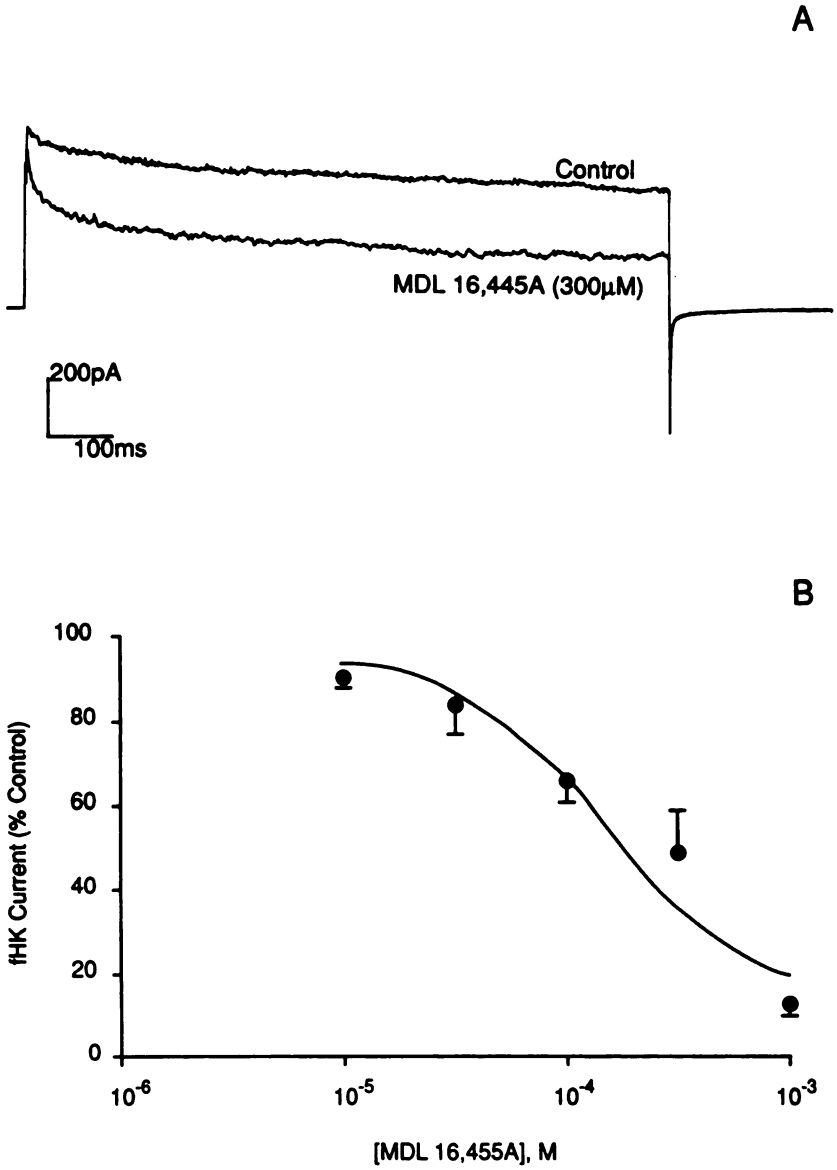

Fig. 6. Effects of the terfenadine acid metabolite MDL 16,455A on fHK current. A, fHK current from an inside-out patch was induced by a 1-sec test pulse to +50 mV from a holding potential of −80 mV, in the presence and absence of 300 μM MDL 16,455A. Current after washout of the drug is also shown. B, Dose-response relationship for MDL 16,455A inhibition of fHK current is shown. Currents were sampled at the end of a 1-sec test pulse to +50 mV to generate the dose-response relationship curve. The IC_{50} value for MDL 16,455A was 214 μM. *Error bars*, standard error (n = 3–6).

This finding was especially interesting in the case of MDL 17,523, which, like MDL 16,455A, has structural similarity to terfenadine. It is apparent that, as the tertiary butyl moiety of terfenadine is sequentially oxidized to increasingly more polar compounds, a corresponding decrease in the ability to block fHK is observed. This limited comparison suggests that hydrophobic interactions in this area of the molecule may help determine binding affinity for the channel. The short-lived alcohol metabolite is present in only low concentrations (<10 ng/ml) in serum after therapeutic doses in humans.[1] Under similar conditions, plasma levels of MDL 4,829 average approximately 200 nM (13). The concentrations of these metabolites have not been determined under extreme conditions such as in cases of terfenadine overdose. However, given the current information it seems unlikely that these compounds could achieve plasma concentrations similar to those needed to block fHK.

In summary, this report is the first to examine the effects of terfenadine and its metabolites on a voltage-dependent K^+ channel cloned from human heart. We find that terfenadine potently inhibits this channel and the concentrations required to block fHK are similar to those that have been shown to produce QT_c prolongation in humans. In contrast, the metabolites of terfenadine were only weakly active as inhibitors of fHK, despite the fact that both MDL 16,455A and MDL 17,523 show close structural similarity to terfenadine. These results suggest that the metabolites of terfenadine are unlikely to block fHK-like K^+ currents in human heart at clinically relevant concentrations. Although we have established one type of human cardiac K^+ channel as a target for terfenadine, we cannot exclude possible effects on other channels such as that subserv-

[1] Marion Merrell Dow Inc., unpublished observations.

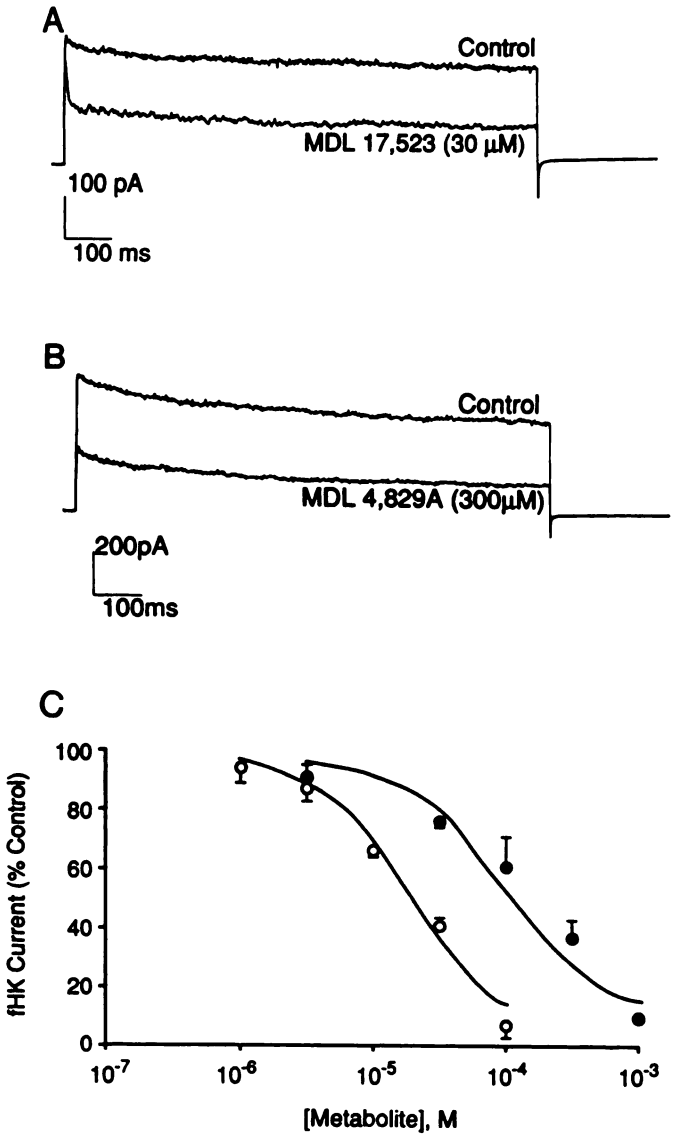

Fig. 7. Effects of MDL 17,523 and MDL 4,829A on fHK current. Current was induced by a 1-sec clamp pulse to +50 mV from a holding potential of −80 mV. The effects of 30 μM MDL 17,523 (A) or 300 μM MDL 4,829A (B) are shown. C, Dose-response relationships for MDL 17,523 (○) and MDL 4,829A (●). The IC_{50} values for MDL 17,523 and MDL 4,829A were 18 μM and 141 μM, respectively. *Error bars*, standard error (n = 3 or 4).

ing I_{Kr}. We conclude that terfenadine, but not its metabolites, can block human cardiac K^+ channels in certain clinical settings and that this mechanism may underlie its reported proarrhythmic tendencies. Cloned channels, like fHK, provide a new tool for studying drug interactions at human cardiac K^+ channels.

Acknowledgments

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