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Blockade of Multiple Human Cardiac Potassium Currents by the Antihistamine Terfenadine: Possible Mechanism for Terfenadine-Associated Cardiotoxicity

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SUMMARY

Use of the antihistamine terfenadine has been associated with QT prolongation and *torsade de pointes*. One possible mechanism is blockade of cardiac potassium channels. We therefore characterized the effects of terfenadine on potassium currents recorded from isolated human cardiac myocytes. We demonstrated terfenadine block of the transient outward current and a novel, ultra-rapidly activating, delayed rectifier K^+ current (I_{Kur}), which is very sensitive to 4-aminopyridine. I_{Kur} is probably produced by the protein product of Kv1.5a, a Shaker-like potassium channel cDNA cloned from human heart. We also compared terfenadine blockade of fHK (Kv1.5a) currents stably expressed in a human embryonic kidney cell line with terfenadine blockade of I_{Kur} in human atrial myocytes. Using the patch-clamp technique, we found that terfenadine produced a time-dependent reduction in Kv1.5a current that was consistent with blockade from the cytoplasmic side of the channel. The terfenadine-sensitive Kv1.5a current in human embryonic kidney cells was similar to the 4-aminopyridine-sensitive current in human atrial myocytes. In addition to blockade of the transient outward current and I_{Kur}, terfenadine at clinically relevant concentrations blocked both the rapidly and slowly activating components of the delayed rectifier in human atrial myocytes. Blockade of these K^+ currents may contribute to the cardiotoxicity associated with terfenadine usage.

Millions of prescriptions for terfenadine, a widely used antihistamine, are written each year. Recently, a number of cases of QT prolongation and *torsade de pointes* have been reported in association with the use of terfenadine. Many of the cases involved patients with no previous history of cardiac problems, taking recommended doses of terfenadine in conjunction with the antifungal agent ketoconazole (1, 2). Terfenadine normally undergoes extensive first-pass hepatic metabolism, by the cytochrome P-450C enzyme CYP3A4, to its acid metabolite terfenadine carboxylate (3). Ketoconazole is a well known inhibitor of cytochrome P-450 metabolic pathways (4–6). Accordingly, in these patients the plasma concentration of terfenadine was much higher than expected. The unusually high concentrations of terfenadine were believed to be the cause of the cardiotoxicity.

A likely mechanism for QT prolongation and *torsade de pointes* is blockade of cardiac potassium channels. This is supported by an association between drugs such as quinidine and sotalol, which block cardiac potassium currents, prolong QT intervals, and induce *torsade de pointes* (7). Quinidine and sotalol blocked I_K in guinea pigs and rabbits (8, 9), and quinidine was a potent inhibitor of a rapidly activating potassium channel cloned from human heart (10). The effects of quinidine raise the possibility that terfenadine may block more than one potassium current.

The blocking properties of terfenadine on human cardiac ion channels are completely unknown. In the only published work to date, Woosley *et al.* (11) demonstrated that concentrations of terfenadine that have been shown to be toxic in humans (~200 nM) reduced I_K in cat ventricular myocytes. However, the well known species differences among cardiac currents, together with the absence of information on terfenadine blockade of human cardiac potassium channels, prompted us to examine the effects of terfenadine on potassium currents of enzymatically isolated human cardiac myocytes. We examined the blocking effects of terfenadine on a highly 4-AP-sensitive potassium current, I_{Kur}, as well as I_{to} and I_K.

I_{Kur} appears to be produced by the protein product of a

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ABBREVIATIONS: I_K, delayed rectifier K^+ current; I_{to}, transient outward K^+ current; I_{Kur}, ultra-rapidly activating, delayed rectifier K^+ current; I_{Kr}, rapid component of the delayed rectifier K^+ current; I_{Ks}, slow component of the delayed rectifier K^+ current; 4-AP, 4-aminopyridine; I_{K1}, inward rectifier K^+ current; I_{Na}, Na^+ current; I_{Ca}, Ca^{2+} current; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; TEA, tetraethylammonium; HEK, human embryonic kidney.

Shaker-like potassium channel gene cloned from human heart, Kv1.5a (12). Kv1.5a is expressed in both the atria and ventricles of adult human heart (12). A similar current is produced by HK2, which has an amino acid sequence that is almost identical to the sequence of Kv1.5a (13). In keeping with its Shaker-like amino acid sequence, I_{Kur} is much more rapidly activating than delayed rectifier K^+ currents that have heretofore been considered in human cardiac electrophysiology. Because separation of K^+ currents in cardiac myocytes either may be incomplete or may involve drugs with nonspecific effects, we characterized the blocking properties of terfenadine on Kv1.5a channels uniquely and stably expressed in a HEK cell line. We used the patch-clamp method to compare terfenadine blockade of Kv1.5a currents in HEK cells (14) with terfenadine blockade of I_{Kur} in atrial myocytes. We found that terfenadine potently inhibits Kv1.5a currents in a time-dependent manner. For the first time, we demonstrate terfenadine blockade of I_{Kur} in human atrial myocytes. In addition, we confirmed terfenadine block of I_{Kr} in human myocytes and demonstrated for the first time that terfenadine blocks I_{to} as well as I_{Ks}.

Materials and Methods

Isolation of Cardiac Myocytes

Human myocytes were obtained from specimens of right atrial appendage obtained from patients undergoing cardiopulmonary bypass. All tissue was obtained in accordance with Baylor College of Medicine Institutional guidelines. All atrial specimens were grossly normal at the time of excision. The cell isolation procedure was similar to that described by Wang and co-workers (15), based on an earlier method reported by Escande *et al.* (16). Briefly, samples were quickly immersed in a cardioplegia solution consisting of 50 mM KH_2PO_4, 8 mM $MgSO_4$, 10 mM $NaHCO_3$, 5 mM adenosine, 25 mM taurine, 140 mM glucose, and 100 mM mannitol, titrated to pH 7.4 and bubbled with 100% O_2 at 0–4°. Specimens were minced into 0.5–1-mm cubes and transferred to a 50-ml conical tube containing an ultra-low calcium wash solution containing 137 mM NaCl, 5 mM KH_2PO_4, 1 mM $MgSO_4$, 10 mM taurine, 10 mM glucose, 5 mM HEPES, and 100 μM EGTA, pH 7.4 (22–24°). The tissue was gently agitated by continuous bubbling with 100% O_2 for 5 min. The tissue was then incubated in 5 ml of solution containing 137 mM NaCl, 5 mM KH_2PO_4, 1 mM $MgSO_4$, 10 mM taurine, 10 mM glucose, and 5 mM HEPES, supplemented with 0.1% bovine albumin, 2.2 mg/ml collagenase type V, and 1.0 mg/ml protease type XXIV (Sigma Chemical Co.), pH 7.4 (37°), and bubbled continuously with 100% O_2. The supernatant was removed after 40 min and discarded. The chunks were then incubated in a solution of the same ionic composition but supplemented with only collagenase and 100 μM $CaCl_2$. Microscopic examination of the medium was performed every 10–20 min, to determine the number and quality of the isolated cells. When the yield appeared to be maximal, the cell suspension was centrifuged for 2 min and the resulting pellet was resuspended in a modified Kraftbruhe solution containing 25 mM KCl, 10 mM KH_2PO_4, 25 mM taurine, 0.5 mM EGTA, 22 mM glucose, 55 mM glutamic acid, and 0.1% bovine albumin, pH 7.3 (22–24°). In general, the isolation procedure produced an initial yield of approximately 40–60% spindle-shaped, calcium-tolerant cells. Cells were used within 8 hr after isolation.

Transfection and Cell Culture

HEK 293 cells (American Type Culture Collection, Rockville, MD) were maintained in minimal essential medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (GIBCO). The cDNA for Kv1.5a was subcloned into the pRc/CMV expression vector (Invitrogen), purified by alkaline lysis/polyethylene glycol precipitation, and linearized at the *NotI* site. The Kv1.5a construct (1 μg) was incubated with 50 μg of Lipofectin (BRL) and 1×10^6 HEK 293 cells in 3 ml of serum-free medium for transfection. To allow expression of antibiotic resistance, the cells were incubated for 48 hr in normal medium and subcultured by 1/10 dilution in medium containing 500 μg/ml Geneticin (GIBCO). Antibiotic-resistant clones were selected at random after 2 weeks and plated either onto polylysine-treated (0.4%) coverslips for use in experiments or onto culture dishes to maintain stock cultures. Fresh medium containing Geneticin (500 μg/ml) was exchanged once each week, and cells were passaged by trypsin-EDTA treatment when confluent.

Solutions

For recording from human myocytes, cells were perfused with an "external" solution that consisted of 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.8 mM sodium acetate, 1.8 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.3 with NaOH. Glass pipettes (electrodes) were filled with an "internal" solution that consisted of 120 mM potassium aspartate, 20 mM KCl, 4 mM NaATP, 5 mM EGTA, and 5 mM HEPES, adjusted to pH 7.2 with KOH. For recording from transfected HEK cells, cells were perfused with a depolarizing solution containing 120 mM KCl, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.3 with KOH. Pipettes were filled with a solution containing 120 mM NaCl, 2.5 mM KCl, 2 mM $MgCl_2$, and 10 mM HEPES, adjusted to pH 7.3 with NaOH. Drugs were applied in the bath solution for recording from either myocytes or HEK cells. Terfenadine and terfenadine carboxylate were gifts from Marion Merrell Dow.

Voltage-Clamp Recording

Myocytes. Acceptable atrial myocytes were rod-shaped, with well defined striations, and lacked any visible blebs on the surface. Currents were measured using the whole-cell variant of the patch-clamp method (17). Pipette tip resistances were approximately 1.0–2.0 mΩ when the pipettes were filled with the internal solution. Experiments were performed in cells in which the estimated voltage drop across the uncompensated series resistance was <5 mV. Data were not compensated for liquid junction potentials, which were typically small (<3 mV). Bath temperature was measured with a thermistor placed near the cell under study and was maintained at approximately 22° or 33° (± 0.5°) with a thermoelectric device.

HEK cells. All recordings from HEK cells were made in the excised inside-out patch configuration. The inside-out patch configuration was chosen because preliminary data suggested that certain drugs (e.g., terfenadine) diffuse very slowly across the HEK cell membrane. Thus, to more accurately study apparent steady state drug effects, the inside-out configuration was chosen. Apparent steady state drug block was routinely reached within 3 min using this configuration. In addition, upon washout of drug, current returned to approximately 80% of the predrug value, suggesting that diffusion-limited spaces are not a substantial problem with this configuration. Pipettes used for recording from HEK cells had tip resistances between 2 and 4 mΩ. Kv1.5a currents recorded in the patch configuration from HEK cells varied from approximately 40 pA to 400 pA (holding potential, −80 mV; test potential, +60 mV). This dramatic difference in current amplitude probably reflects differences in channel expression. The reasons for these differences in channel expression are unknown. All experiments using HEK cells were performed at 22°. The pH reported in the text refers to the pH of the solution bathing the inside of the excised patch (i.e., bath solution).

Delivery of voltage-clamp pulses and data acquisition were controlled by a Compaq Deskpro computer running pClamp software (Axon Instruments, Burlingame, CA). The computer was interfaced to the amplifier with a Labmaster digital-analog interface (Scientific Solutions, Solon, OH). Unless otherwise indicated, currents were sampled at 5 kHz and filtered at 1 kHz. All currents were capacity and leak subtracted by subtraction of a current linearly scaled and opposite in polarity to the current response to a sequence of 5-mV hyperpolarizing pulses from either −40 mV (for recording from myocytes) or −80 mV (HEK cells), through the use of software routines incorporated in

pClamp. All values are given as mean ± standard error. The voltage dependence of terfenadine block was analyzed by linear regression fits to the following equation:

$$\ln K_d = \ln K_d(0\ \text{mV}) - (zFV/RT)\delta \quad (1)$$

where $K_d(0\ \text{mV})$ is the extrapolated K_d at 0 mV, δ is the fractional electrical distance, $z = 1$, and F, V, R, and T have their usual meanings.

Results

Effects of terfenadine on human atrial potassium currents. Fig. 1 illustrates the effects of terfenadine on two potassium currents recorded from isolated human atrial myocytes. In Fig. 1A, the current elicited by a 320-msec depolarizing pulse to +60 mV from a holding potential of −40 mV, in the presence and absence of 1 μM terfenadine, is shown. This holding potential was chosen to inactivate I_{Na}, and experiments were performed at 22° to minimize I_K and I_{Ca}. The pulse to +60 mV elicited a rapidly activating outward current, which decayed to nearly steady state levels by the end of the pulse. The peak of this current probably reflects both I_{to} and the highly 4-AP-sensitive I_{Kur} (18), whose protein is thought to be produced by the K^+ channel gene fHK (12), whereas the current measured at the end of the pulse reflects primarily I_{Kur}. fHK is one of three isoforms expressed in humans and in the standard nomenclature may be referred to as Kv1.5a (12).

Upon addition of 300 nM terfenadine to the bath solution, a concentration near the range associated with cardiotoxicity in humans (19), the peak current amplitude was reduced by 18.4 ± 3.6% and the current measured at the end of the voltage pulse was reduced by 14.5 ± 2.3% (V_{test} = +60 mV, n = 5), whereas the addition of 1 μM terfenadine (Fig. 1A, *lower trace*) reduced the peak current by 15.1 ± 2.2% (n = 5) and the current measured at the end of the voltage pulse by 42.2 ± 6.5% (n = 6). In contrast, terfenadine (1 μM) had no effect on I_{K1} (data not shown). The current-voltage relationship for the terfenadine-sensitive current, measured at the end of the voltage pulse, showed that the current activates at approximately −20 mV (Fig. 1B), as does I_{Kur} (12, 18). This reduction in current amplitude measured at the end of the pulse could be abolished by the addition of 200 μM 4-AP to the bath solution. The only target in human atrial myocytes for 4-AP at this concentration is I_{Kur} (12, 18).

Fig. 2A illustrates a family of current traces elicited by voltage pulses from −60 mV to +60 mV (holding potential, −40 mV) in the presence of 200 μM Cd^{2+}, 1 mM Ba^{2+}, 10 mM TEA, and 200 μM 4-AP. Cd^{2+}, Ba^{2+}, and TEA were added to block I_{Ca}, I_{K1}, and I_K, respectively, but did not reduce the amplitude of I_{Kur}. The addition of these blockers did not alter the ability of terfenadine to block the current measured at the end of the pulse (38.8 ± 6.7% reduction, n = 4). In the presence of 200 μM 4-AP, the addition of 1 μM terfenadine (Fig. 2, B and D) did not affect the residual current measured at the end of the voltage pulse (percentage reduction = 4.2 ± 2.4%, n = 5), whereas the peak current was still reduced by 12 ± 2.0%. This indicates that terfenadine blocks a highly 4-AP-sensitive current (I_{Kur}) and that the reduction in peak current reflects primarily a reduction in I_{to}. In Fig. 2C, the current-voltage relationship for the 200 μM 4-AP-sensitive current recorded in human atrial myocytes is plotted. As illustrated, this current activates at approximately −20 mV and shows a current-voltage relationship very similar to that for the terfenadine-sensitive current recorded in human atrial myocytes (Fig. 1B).

A representative family of 200 μM 4-AP-sensitive current traces recorded from a human atrial myocyte is shown in Fig. 3A. These currents were measured as difference currents and are the currents from which the current-voltage relationship in Fig. 2C was constructed. As illustrated, the currents activate rapidly and show very little inactivation. For comparison, a family of 1 μM terfenadine-sensitive difference currents recorded from HEK cells expressing Kv1.5a is shown in Fig. 3B, and the normalized current-voltage relationships for both currents are shown in Fig. 3C. These two current families illustrate the striking similarity between the highly 4-AP-sensitive current recorded in human atrial myocytes (I_{Kur}) and the Kv1.5a current recorded in a mammalian expression system.

In addition to blocking cardiac potassium currents, we have recently shown that terfenadine dose-dependently blocks I_{Na} and I_{Ca} recorded in isolated guinea pig ventricular myocytes.

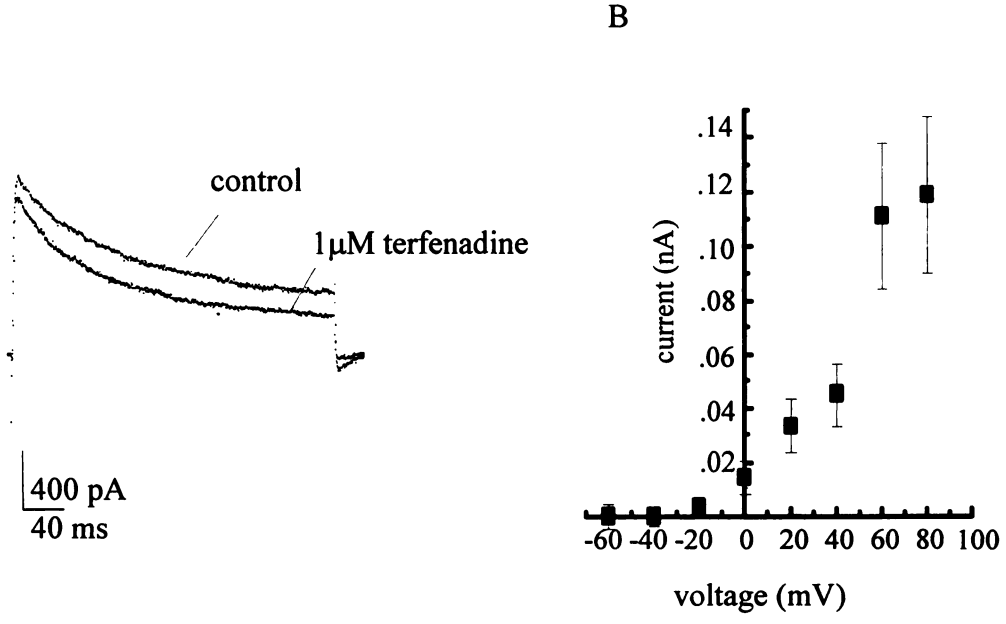

Fig. 1. Effects of 1 μM terfenadine on potassium currents recorded from human atrial myocytes. A, Current elicited by a 320-msec depolarizing pulse to +60 mV from a holding potential of −40 mV, in the absence and presence of 1 μM terfenadine. Experiments were performed in Tyrode's buffer at 22°. B, Current-voltage relationship for the terfenadine-sensitive current (n = 5). Pulse duration and holding potential were the same as in A. Plot was constructed from measurements of currents, at the end of the pulse, recorded in control solution minus those recorded in the presence of terfenadine. *Symbols*, mean ± standard error.

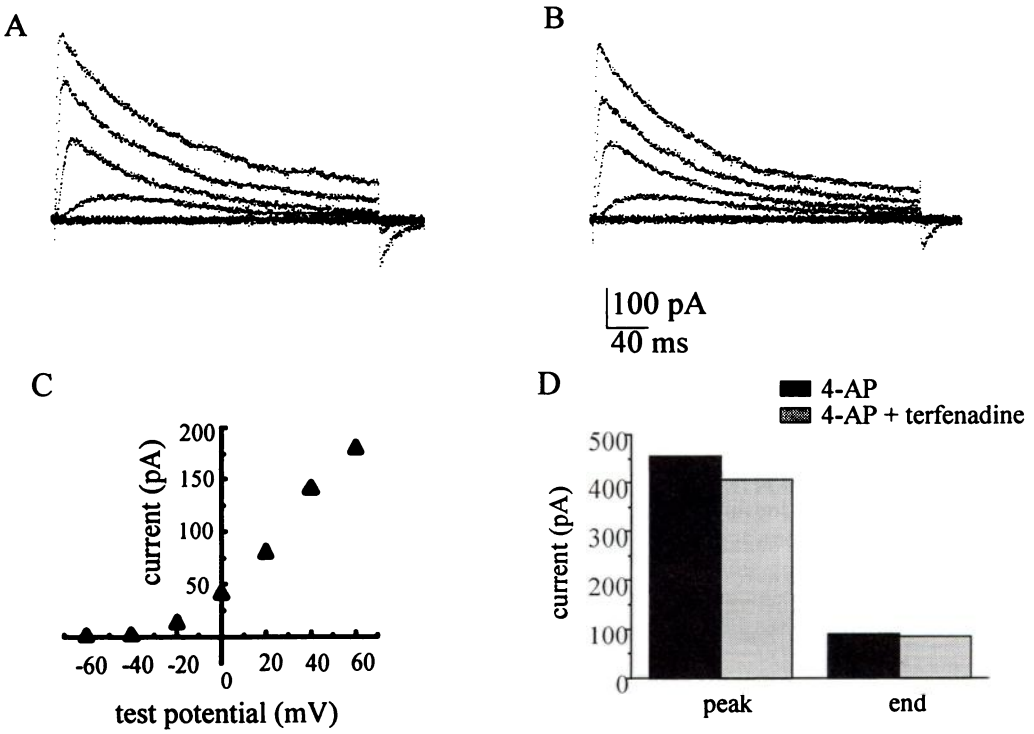

Fig. 2. Effect of terfenadine on currents recorded from human atrial myocytes in the presence of 4-AP. Currents were elicited by a series of 320-msec voltage pulses from −60 mV to +60 mV (holding potential, −40 mV). A, Currents recorded in the presence of 200 μM Cd^{2+}, 1 mM Ba^{2+}, 10 mM TEA, and 200 μM 4-AP. B, Same cell after the addition of 1 μM terfenadine. Note the lack of effect on the current measured at the end of the pulse. C, Current-voltage relationship for the 200 μM 4-AP-sensitive current. Plot was constructed from measurements obtained from the end of the 320-msec voltage pulse in the absence of 4-AP minus those obtained in the presence of 200 μM 4-AP (see Fig. 3A). Note the similarity to the current-voltage relationship shown in Fig. 1C. D, Graph indicating the reduction in peak and end of pulse currents in the absence and presence of 1 μM terfenadine, for the cell illustrated in A and B.

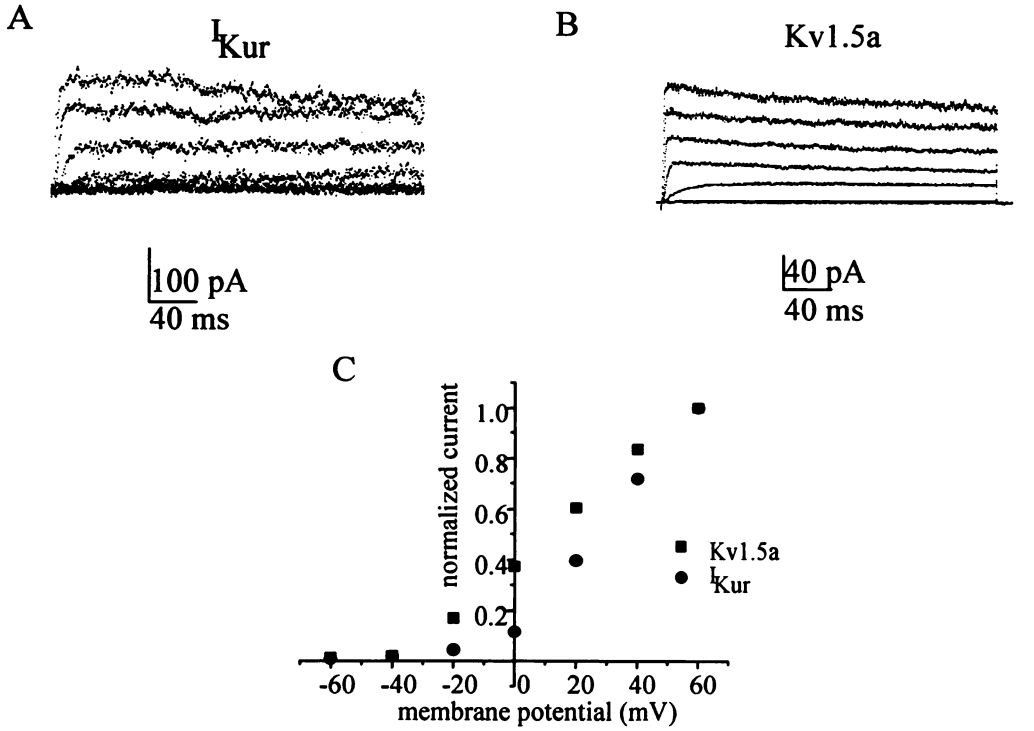

Fig. 3. 4-AP- and terfenadine-sensitive currents. A, 200 μM 4-AP-sensitive current recorded from a human atrial myocyte. Currents were elicited by a series of 320-msec voltage pulses from −60 mV to +60 mV in 20-mV steps (holding potential, −40 mV). Currents represent a family of difference currents (i.e., currents measured in the absence of 4-AP minus currents measured in the presence of 200 μM 4-AP). B, Terfenadine (1 μM)-sensitive current recorded from HEK cells expressing Kv1.5a. Currents represent difference currents. Currents were elicited by a series of 320-msec voltage pulses from −60 mV to +60 mV (holding potential, −80 mV). Note the similarity to the 4-AP-sensitive currents recorded in human atrial myocytes (A). C, Current-voltage relationship for the family of currents illustrated in A and B. Currents were normalized to peak current and plotted as a function of membrane potential.

At concentrations greater than 10 μM, terfenadine markedly reduced both I_{Na} and I_{Ca} (20). In contrast, the addition of 1 μM terfenadine had no effect on I_{Na} and I_{Ca}.[1] The effects of terfenadine on I_{Na} and I_{Ca} recorded in human myocytes were not examined in the present study.

Terfenadine block of Kv1.5a. Fig. 4A shows recordings of potassium current through Kv1.5a channels stably expressed in a HEK cell line. The Kv1.5a current rose rapidly to a peak and then slowly and partially inactivated over the time course of the voltage pulse (320 msec) to an apparent steady state. In Fig. 4B, current traces elicited by a voltage step to +60 mV (holding potential, −80 mV) in the absence and presence of 1 μM terfenadine are shown. As indicated, upon addition of drug there was a marked reduction in current amplitude, with the current at the beginning of the pulse not being blocked at all, whereas the current at the end of the pulse was reduced by 29.9

[1] Y. Atsuko, W. Crumb, B. Wible, unpublished observations.

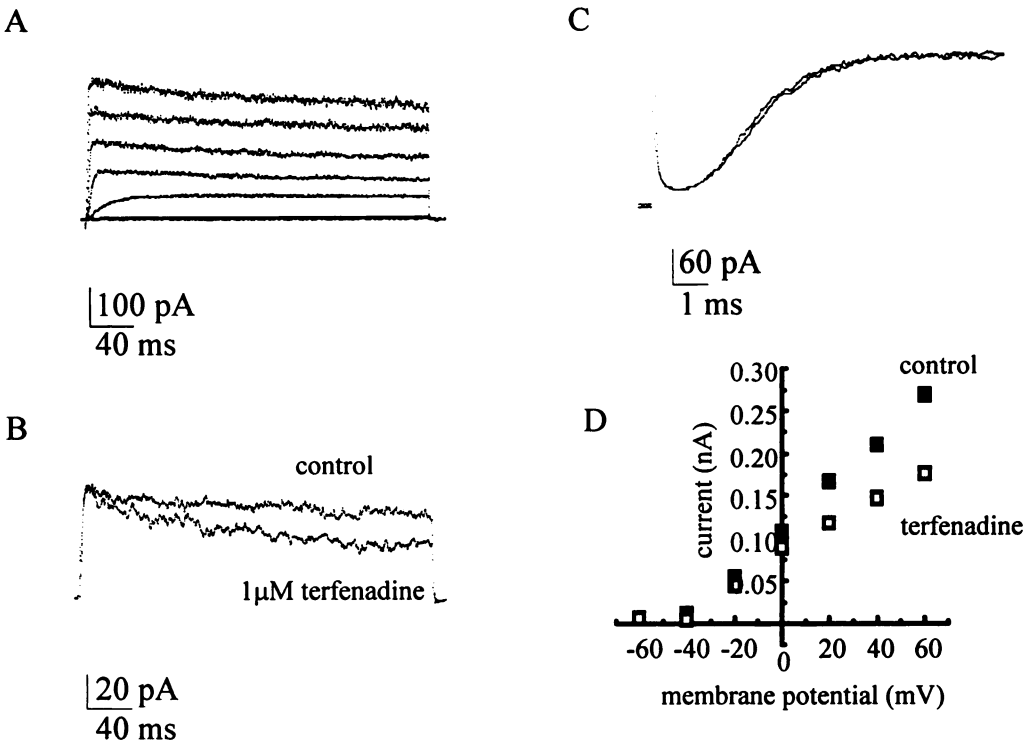

Fig. 4. Effect of terfenadine on Kv1.5a current recorded from HEK 293 cells. Currents were recorded in the excised inside-out patch configuration. A, Family of Kv1.5a currents recorded from an HEK cell stably expressing Kv1.5a message. Currents were elicited by a series of 320-msec voltage pulses from −60 mV to +60 mV (holding potential, −80 mV). B, Effect of 1 μM terfenadine on Kv1.5a current. Currents were elicited by a pulse to +60 mV. Note the reduction in Kv1.5a current amplitude produced at the end of the pulse and the lack of effect at the beginning of the pulse. C, Superimposed current traces recorded from an HEK cell in the presence and absence of 1 μM terfenadine. Currents were elicited by a 320-msec voltage pulse to +60 mV (holding potential, −80 mV). Currents were sampled at 50 kHz and filtered at 10 kHz. D, Current-voltage relationship recorded from an HEK cell in the absence and in the presence of 1 μM terfenadine. Currents were measured at the end of the voltage pulse.

± 2.8% (n = 7). Similar results have recently been reported (14).

The time course of current activation before and after addition of terfenadine was also examined (Fig. 4C). In the absence of drug, current activation could be best fit by a single-exponential function with a value of 1.3 ± 0.2 msec (n = 10). In the presence of terfenadine, this value was identical, 1.3 ± 0.1 msec (n = 7).

Fig. 4D shows the effect of 1 μM terfenadine on the steady state current-voltage relationship for Kv1.5a. In the presence of terfenadine, current values were reduced at potentials positive to −20 mV, with a similar degree of current reduction occurring at more depolarized potentials (percentage reduction at +20 mV, 16.68 ± 3.35%; +40 mV, 20.8 ± 1.80%; +60 mV, 29.9 ± 2.8%; n = 7) ($p > 0.05$). This lack of significant voltage dependence in terfenadine block of Kv1.5a current is reflected in the shallow slope of the fit to eq. 1 (see Materials and Methods). Fits to this equation yield an electrical distance, δ, which represents the fraction of the membrane electric field sensed by a singly charged ion at the receptor site. A fit of this equation to mean data points (n = 7) positive to +10 mV gave a δ value of 0.06. Over this voltage range Kv1.5a opening probability, P_o, was maximal (12). This suggests that the cationic form of terfenadine penetrates only slightly (6%) into the membrane electric field (from the cytoplasmic side) to reach its binding site.

The effects of pH on terfenadine blockade of Kv1.5a channels were also examined. In contrast to pH 7.3 (Fig. 4B), when the pH of the solution bathing the internal side of the membrane was made more acidic (pH 6.9), terfenadine (1 μM) markedly reduced the peak current (34.6 ± 5.5% reduction, n = 3) (Fig. 5). Block continued to develop throughout the 320-msec voltage pulse, with the reduction at the end of the pulse being significantly greater than that measured at the beginning of the pulse

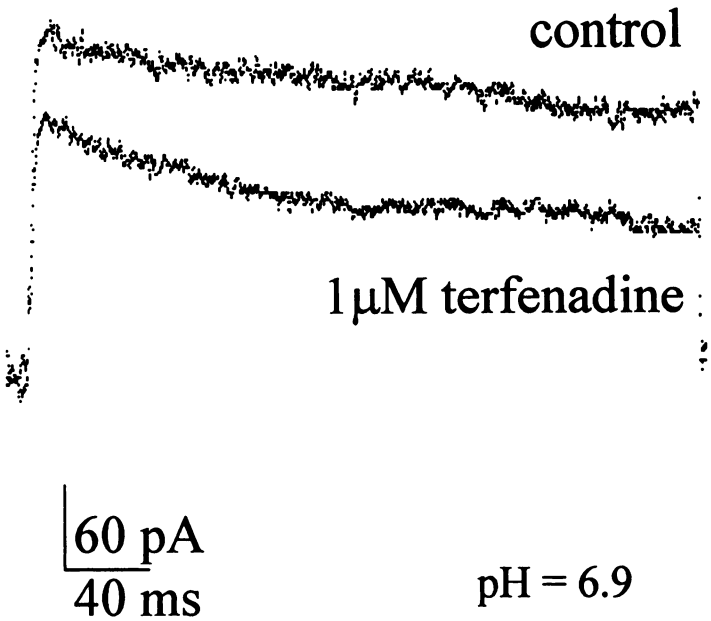

Fig. 5. Effect of pH on terfenadine block of Kv1.5a channels expressed in HEK cells. Recordings were made in the inside-out patch configuration. Current traces were elicited by a 320-msec voltage pulse to +60 mV in the absence and presence of 1 μM terfenadine. The pH of the solution bathing the interior of the patch was 6.9. Current represents the first pulse after a 2–3-min rest period at −80 mV. Note the reduction in peak current.

(52.7 ± 4.1%, n = 3) ($p < 0.05$). The kinetics of channel activation at this pH were slower, compared with pH 7.3 (≈1.3 msec). In the absence of drug, the time constant describing channel activation was 1.9 ± 0.2 msec (n = 5). The kinetics of activation were not changed by the addition of drug (1.9 ± 0.1 msec, n = 3). The reduction in the peak current seen at a more

acidic pH could therefore reflect more terfenadine binding to the channel due to the slower rate of channel opening.

Effects of terfenadine carboxylate on potassium currents. In comparison with terfenadine, its acid metabolite terfenadine carboxylate is far less potent. In the excised inside-out patch configuration, the addition of 10 μM terfenadine carboxylate reduced the Kv1.5a current by 13.9 ± 1.9% (n = 4; test potential, +60 mV; pulse duration, 320 msec). This blocking effect is in contrast to our observations in human atrial myocytes, in which 10 μM terfenadine carboxylate did not change I_{K1} (measured at −100 mV) or peak and steady state outward potassium currents (Fig. 6). This difference in blockade may reflect membrane diffusion limitations encountered in myocytes, which would not be present in excised membrane patches. The decreased blocking potency that occurs with the substitution of a carboxylic acid for a methyl group suggests that this portion of the molecule is important for drug binding.

Terfenadine block of potassium currents at 33°. Woosley *et al.* (11) recently reported that concentrations of terfenadine associated with cardiotoxicity in humans dramatically reduced I_K recorded from cat ventricular myocytes. I_K has been reported to consist of two components, I_{Kr} and I_{Ks} (21). We therefore further characterized terfenadine blockade of I_K, to define the relative sensitivities of its two components for terfenadine. In Fig. 7, A and B, the effects of 200 nM terfenadine on I_K are shown. These experiments were performed at 33°, because I_K is virtually absent at room temperature (22°). Cells were pretreated with 1 mM 4-AP to block Kv1.5a (IC_{50} for 4-AP, 50 μM) (12). This concentration of 4-AP has been reported to have no effect on I_K recorded in human atrial myocytes (22). As indicated in Fig. 7B, terfenadine markedly reduced the current measured during the voltage pulse. The mean current-voltage relationship for the 200 nM terfenadine-sensitive current (n = 5) is plotted in Fig. 7C. The current-voltage curve depicts a relationship that shows outward rectification overall but an inflection between 0 and +20 mV. The curve is consistent with a current that begins to inwardly rectify between 0 and +20 mV and then increases in amplitude at +40 mV. This suggests that both components of I_K are blocked by terfenadine. Support for this is given in Fig. 7, D and E, in which representative terfenadine-sensitive currents are shown. At +40 mV, where the contribution of I_{Kr} is reduced, the terfenadine-sensitive current is a slowly activating current very similar to I_{Ks}. At +20 mV, the terfenadine-sensitive current is more rapidly activating and shows little inactivation, similar to I_{Kr} (21, 22). The current blocked by terfenadine at +40 mV was reduced by 34.9 ± 3.4% (n = 5), whereas the current at +20 mV was reduced by 49.1 ± 5.1% (n = 4). This decrease in current measured during a voltage pulse to +20 mV is similar to the reduction in tail current amplitude produced by terfenadine in cat ventricular myocytes (11).

Because I_{Kr} has been shown to be highly sensitive to dofetilide (K_d = 39 nM) (23), one would expect the absence of an inwardly rectifying component of the current-voltage relationship for the 200 nM terfenadine-sensitive current in cells pretreated with dofetilide. Results of such an experiment are shown in Fig. 8, in which the effects of 200 nM terfenadine in a cell pretreated with 1 μM dofetilide are shown. This experiment was also performed in the presence of 1 mM 4-AP to eliminate Kv1.5a and I_{to}. The current-voltage relationship for the terfenadine-sensitive current measured in this cell does not show any inwardly rectifying component similar to that seen in the absence of dofetilide (compare Figs. 8A and 7C). However, terfenadine blockade of I_{Ks} was still present (Fig. 8, C and D).

The effects of 200 nM terfenadine on I_{Kur} were also examined at 33°. Experiments were performed with 200 μM Cd^{2+}, which blocked I_{Ca}, and 2 μM dofetilide. The cells used in these experiments did not manifest I_{Ks} (22). With the addition of terfenadine, the current measured at the end of a voltage pulse to +60 mV was reduced by 12.6 ± 1.0% (n = 4). Because an increase in temperature to 33° markedly increased the activation kinetics of I_{to}, making a discrimination of capacitance and ionic current dificult, the effects of terfenadine on peak current at 33° were not examined.

Discussion

Background of Terfenadine. Terfenadine is one of the most commonly prescribed antihistamines in this country (24).

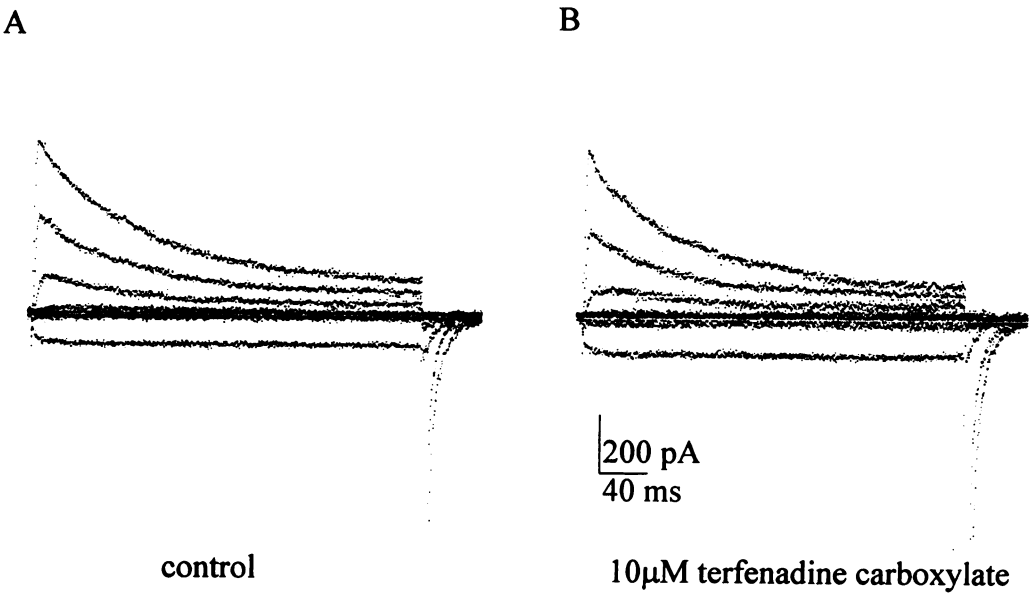

Fig. 6. Lack of effect of terfenadine carboxylate on currents recorded from human atrial myocytes. Holding potential was −40 mV, voltage steps were from −100 mV to +40 mV, and pulse duration was 320 msec. A, Family of currents recorded from a human atrial myocyte in the absence of terfenadine carboxylate (control); B, currents recorded in the presence of 10 μM terfenadine carboxylate.

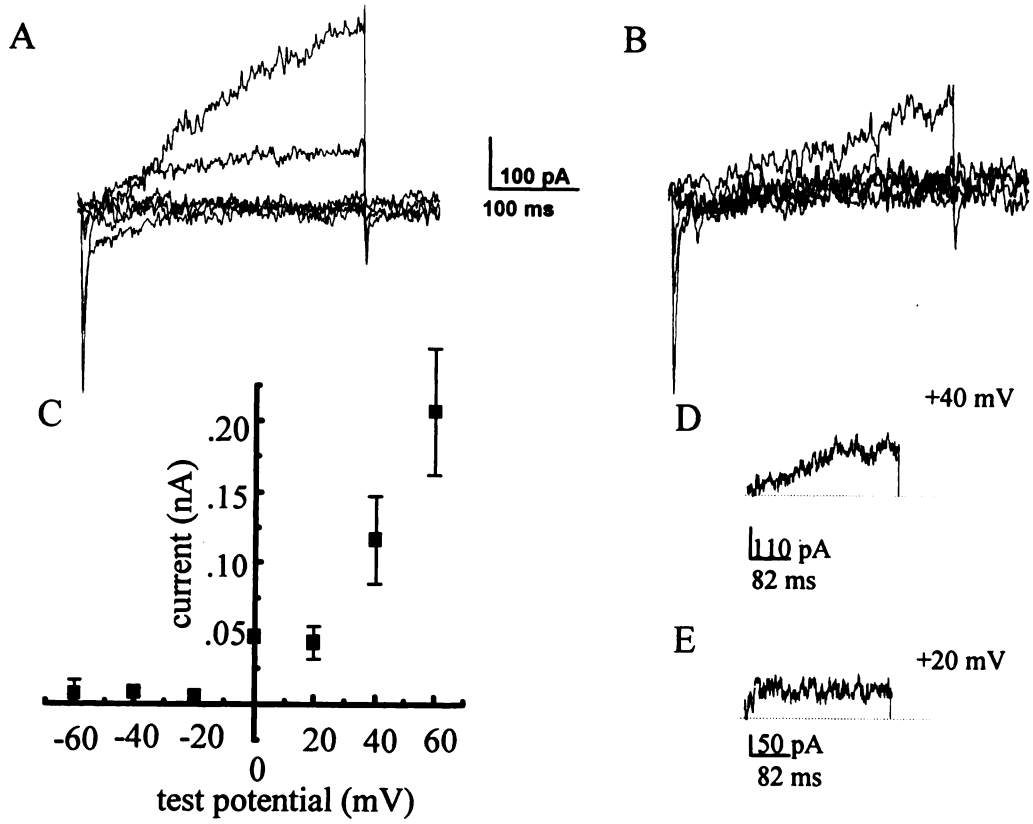

Fig. 7. Effects of 200 nM terfenadine on I_K recorded from human atrial myocytes. Currents were recorded at 33° in the presence of 1 mM 4-AP. The pulse protocol consisted of a series of 320-msec voltage pulses from −60 mV to +40 mV (holding potential, −40 mV). A, Family of currents recorded in the absence of terfenadine. B, Currents recorded in the presence of terfenadine. C, Mean current-voltage relationship for the 200 nM terfenadine-sensitive current (n = 5). Pulse protocol was the same as in A. Plot was constructed from terfenadine difference currents. Measurements were taken at the end of the voltage pulse. *Symbols*, mean ± standard error. D and E, Examples of the 200 nM terfenadine-sensitive currents recorded at +40 mV (D) and +20 mV (E). Currents represent difference currents (i.e., currents recorded in the absence of terfenadine minus currents recorded in the presence of 200 nM terfenadine). *Straight line*, zero current level.

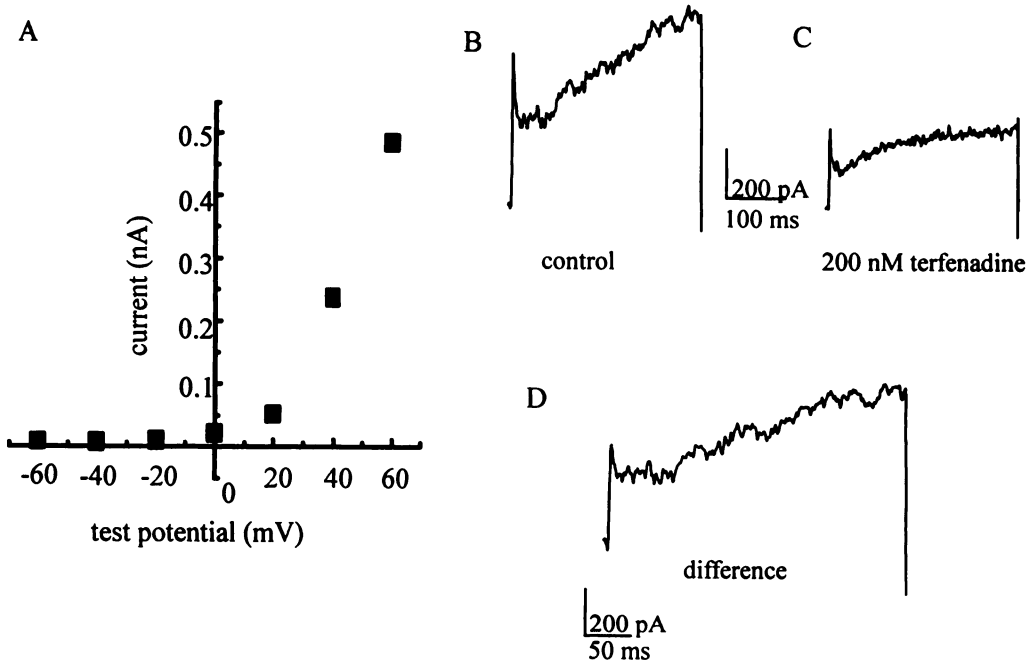

Fig. 8. Effect of 200 nM terfenadine on a human atrial myocyte pretreated with 1 μM dofetilide. Experiments were performed at 33° in the presence of 1 mM 4-AP. A, Current-voltage relationship for the terfenadine-sensitive current in this cell. Measurements were taken at the end of a 320-msec voltage pulse. B and C, Examples of currents elicited by a 320-msec voltage pulse to +60 mV in the absence (B) and presence (C) of terfenadine. D, Example of the terfenadine-sensitive difference current recorded at +60 mV. The rapidly decaying component at the beginning of the pulse represents unblocked I_{to}.

A small but growing number of cases of QT prolongation and a serious cardiac arrhythmia, *torsade de pointes*, have been reported in association with terfenadine use (1, 2). The exact mechanism whereby terfenadine exerts its arrhythmogenic effects is not known. In this study, we show that terfenadine blocks I_{to}, I_{Kur}, and both components of I_K in human atrial myocytes at concentrations associated with cardiotoxicity in humans. I_{K1}, which controls the resting potential and contributes to late repolarization of the cardiac action potential, was unaffected. The characterization of terfenadine blockade of Kv1.5a in the present investigation is the first comparative pharmacological study in which a human cardiac potassium channel stably expressed in a human cell line has been used to provide further insight into the mechanism of drug blockade of the current that this channel produces in human cardiac myocytes.

State and time dependence of terfenadine block of Kv1.5a. Terfenadine blocked the human cardiac potassium channel, Kv1.5a, in a state-and time-dependent manner. The data presented in this paper indicate that 1) the initial time

course of activation was not modified by terfenadine (Fig. 4C) and 2) terfenadine blockade (pH 7.3) occurred only after the channel opened (Fig. 4B). Taken together, these data suggest that Kv1.5a channels must open before terfenadine can bind. The resulting reduction in current measured at the end of the voltage pulse could be the result of either terfenadine block of open channels or a terfendine-induced modification of slow inactivation. The present study could not distinguish between these two mechanisms.

In contrast to block at pH 7.3, block at pH 6.9 reduced not only the steady state current but also the peak current. A reduction in the maximum peak current attained during a depolarizing pulse could be a measure of either rested channel block or rapid open channel block. Because the kinetics of channel opening at a more acidic pH are slower than at a neutral pH (1.9 msec versus 1.3 msec), thus allowing more time for drug binding, one possible mechanism for the reduction in peak current involves terfenadine binding to channels in open or pre-open conformations. If binding continued even after all channels were open, then this could account for the reduction in peak current as well as the progressive decrease in current amplitude occurring over the duration of the voltage pulse (Fig. 5). Alternatively, we cannot rule out the possibility of terfenadine binding to rested channels, thus resulting in a smaller fraction of conducting channels and the observed reduction in peak current.

Evidence that blockade of Kv1.5a underlies the reduction in steady state current in myocytes at room temperature. In this study, we have shown that terfenadine (1 μM) dramatically reduces the current measured at the end of a 320-msec pulse in human atrial myocytes assayed at room temperature. In the presence of low concentrations of 4-AP (200 μM), terfenadine did not produce any reduction in the current measured at the end of the voltage pulse. This suggests that the terfenadine-induced reduction in the current measured at the end of the voltage pulse was due to block of a highly 4-AP-sensitive current. The target in human atrial myocytes for 4-AP at this concentration has been shown to be I_{Kur} (12, 18). I_{Kur} is produced by the protein product of a potassium channel gene cloned from human heart, designated Kv1.5a (12). Recently, I_{Kur} and Kv1.5a have been shown to share marked similarities in voltage dependence, channel gating, and sensitivity to 4-AP (12, 18). This study suggests that terfenadine blockade of Kv1.5a underlies the reduction in the current measured at the end of a voltage pulse in human atrial myocytes. This is supported by the following: 1) the current-voltage relationship for Kv1.5a (Fig. 4D) is identical to the current-voltage relationships for the 200 μM 4-AP-sensitive current (Fig. 2C) and for the terfenadine-sensitive current recorded from human atrial myocytes (Fig. 1B), 2) the kinetics of the 4-AP-sensitive current in human myocytes are similar to those of Kv1.5a (Figs. 3, A and B, and 4A), and 3) terfenadine inhibition of cloned Kv1.5a channels (30%) is similar to its inhibition of the current measured at the end of the pulse in human myocytes (40%).

Structural implications for terfenadine binding to Kv1.5a. The conversion of terfenadine to its acid metabolite, terfenadine carboxylate, involves substituting a carboxylic acid group for a methyl group. This simple substitution, however, yields a molecule that, despite being very similar, is far less potent than the parent compound. Terfenadine carboxylate at 10 μM produced only a 13% reduction in Kv1.5a current (versus 30% for 1 μM terfenadine). Interestingly, the alcohol metabolite of terfenadine, in which an alcohol group replaces the methyl group, is also far less potent than terfenadine (14). This suggests that hydrophobic groups, such as $-(CH_3)_3$, present in terfenadine are required for a stable drug/channel interaction. Hydrophobic binding has been postulated for quaternary ammonium and local anesthetic binding to sodium channels (25, 26), quinidine binding to HK2 channels (10), adenosine binding to adenosine receptors (27), and tetra-alkyl ammonium cation block of sarcoplasmic reticulum Ca^{2+} release channels (28). It is possible that hydrophobic interactions are responsible for terfenadine binding to Kv1.5a channels, as well as the channels that conduct I_{Ks}, I_{Kr}, and I_{to}. Alternatively, the differences in block between terfenadine and its acid metabolite may reflect more global changes in molecular charge and/or lipid solubility. A better understanding of the physical and chemical properties of terfenadine and its metabolites is reqiured before the merits of either of these possibilities can be assessed.

Based upon the results of our experiments, we speculate that terfenadine blocks Kv1.5a channels from the cytoplasmic side of the membrane. This speculation is supported by the following observations. 1) In excised (inside-out) patches, upon addition of 1 μM terfenadine to the bath solution block of Kv1.5a current recorded in HEK cells developed rapidly. 2) In the excised inside-out patch configuration, terfenadine blockade of Kv1.5a channels increased when the drug-containing solution bathing the inside of the membrane was made more acidic (compare Figs. 4B and 5). Thus, under conditions where a greater percentage of the terfenadine molecules were charged and less likely to traverse the membrane, terfenadine more potently inhibited Kv1.5a. 3) The δ value for terfenadine blockade of Kv1.5a channels was 0.06, suggesting that the protonated amine moiety of terfenadine binds to a site only slightly within the membrane electric field, about 6% inward from the cytoplasmic side of the membrane. The shallow voltage dependence is not due to channel gating, because Kv1.5a gating had reached saturation over the voltage range tested (12), but may be the result of molecular size limitations or steric effects.

Terfenadine, despite being highly charged at physiological pH, was able to block I_{Kur} in human atrial myocytes. We therefore propose a mechanism for terfenadine blockade of I_{Kur} in which the neutral form of the drug permeates the cell membrane, gaining access to the cell interior. Once inside the cell, terfenadine becomes protonated and we propose that it is the protonated form of the drug that blocks I_{Kur}. This mechanism for terfenadine blockade of I_{Kur} is similar to that proposed for local anesthetic blockade of sodium channels (29). Due to the shallow voltage dependence of block, we speculate that the protonated amine moiety of terfenadine interacts with some negatively charged group located outside of the membrane electric field (e.g., at the inner vestibule of the channel) and the hydrophobic carbon-rich portion of the molecule in some manner blocks the channel.

Blockade of I_{Kr} and I_{Ks}. Recently, Woosley *et al.* (11) showed in cat ventricular myocytes that concentrations of terfenadine associated with conduction disturbances in humans dramatically reduced I_K. Because I_K has been shown to consist of two components (I_{Ks} and I_{Kr}) (21), it was of interest to determine the specificity of terfenadine block of I_K. Our results suggest that terfenadine blocks both components. As indicated

in Fig. 7C, the current-voltage relationship for the terfenadine-sensitive current appears similar to the current-voltage relationship for I_K recorded in guinea pig ventricle (21). The rectification seen at +20 mV probably reflects I_{Kr}, which begins to rectify at approximately −20 mV in guinea pig ventricle. In support of this, in the presence of 1 μM dofetilide the terfenadine-sensitive, current-voltage relationship did not show any inward rectification (Fig. 8A). This concentration of dofetilide should completely abolish I_{Kr} (IC_{50} = 39 nM) (23). This suggests that the rectification seen in the terfenadine-sensitive, current-voltage relationship reflects the contribution of I_{Kr}. Furthermore, as indicated in Fig. 7E, the terfenadine-sensitive difference current measured at +20 mV is a relatively rapidly activating, noninactivating current. This current is not Kv1.5a, because these experiments were performed in the presence of 1 mM 4-AP, but is similar to I_{Kr} measured in human atrial as well as guinea pig ventricular myocytes (21, 22).

Because of the rectifying properties of I_{Kr}, this current is virtually absent at +40 mV. Therefore, the current increasing in amplitude at +40 and +60 mV in Fig. 7C is a current other than I_{Kr}. This current reflects I_{Ks}. Support for this is given in Figs. 7D and 8D, in which the terfenadine-sensitive difference currents are shown, illustrating a slowly activating current characteristic of I_{Ks} (21).

Limitations of study. Terfenadine cardiotoxicity has been associated with the occurrence of a particular type of ventricular arrhythmia, *torsade de pointes* (1, 2). Although atrial tissue was the only source of human cardiac tissue available to these investigators, the ionic currents present in human atrial myocytes share many similarities to those found in human ventricular myocytes. For example, the I_{Na} recorded from myocytes isolated from human ventricle (30) is very similar in its voltage dependence and kinetics to that found in human atrial myocytes (31). There are also similarities in voltage dependence, inactivation kinetics, and 4-AP sensitivity between the I_{to} recorded in myocytes isolated from human atria (15) and ventricle (32). In addition, I_K recorded from human atrial myocytes (22) shares many similarities with the I_K recorded from isolated ventriciular myocytes of many other species (21, 33). Although the use of "normal" ventricular myocytes would have been more desirable, we feel that, in light of the similarities between the atrial currents examined in the present study and their ventricular counterparts, the use of isolated human atrial myocytes is a reasonable alternative.

Conclusions. In summary, we have provided evidence for time- and state-dependent blockade of Kv1.5a by terfenadine. Kv1.5a is a potassium channel gene expressed in both the atria and ventricles of human heart, and its gene product is believed to be the channel through which I_{Kur}, a novel potassium current present in human atrial myocytes, is conducted (12, 18). Due to the pH dependence of terfenadine blockade of Kv1.5a, under conditions in which intracellular pH becomes more acidic, such as metabolic acidosis, terfenadine blockade of Kv1.5a would be markedly enhanced. One would therefore expect a greater effect of terfenadine in settings of infarcted or ischemic myocardium. In addition to block of I_{Kur}, we have shown that terfenadine blocks I_{to} and potently blocks both components of I_k (i.e., I_{Kr} and I_{Ks}). Blockade of these currents is produced by concentrations of terfenadine that have been associated with arrhythmias in humans (19). Although I_{to} and I_{Kur} were less sensitive to terfenadine than I_{Kr} and I_{Ks}, block of I_{to} and I_{Kur} may significantly contribute to terfenadine-induced cardiotoxicity. We therefore propose a molecular mechanism for terfenadine cardiotoxicity in which blockade of these cardiac potassium currents can lead to cardiac action potential prolongation and, under appropriate conditions, *torsade de pointes*. A possible contribution of terfenadine block of other human cardiac ion channels (i.e., Na^+ and Ca^{2+} channels) in the observed incidence of terfenadine-associated *torsade de pointes* requires further study.

Acknowledgments

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References

1. Monahan, B. P., C. L. Ferguson, E. S. Kileavy, B. K. Lloyd, J. Troy, and L. R. Cantilena. *Torsades de pointes* occurring in association with terfenadine use. *JAMA* **264**:2788–2790 (1990).
2. Zimmermann, M., H. Duruz, O. Guinand, O. Broccard, P. Levy, D. Lactis, and A. Bloch. *Torsades de pointes* after treatment with terfenadine and ketoconazole. *Eur. Heart J.* **13**:1002–1003 (1992).
3. Yun, C., R. A. Okerholm, and F. P. Guengerich. Oxidation of the antihistamine drug terfenadine in human microsomes: role of cytochrome P450 3A4 in *N*-dealkylation *C*-hydroxylation. *Drug Metab. Dispos.*, in press.
4. Brown, M. W., A. L. Maldonado, C. G. Meredith, and K. V. Speeg, Jr. Effect of ketoconazole on hepatic oxidative drug metabolism. *Clin. Pharmacol. Ther.* **37**:290–297 (1985).
5. Loose, D. S., P. B. Kan, M. A. Hirst, R. A. Marcus, and D. Feldman. Ketoconazole blocks adrenal steroidogenisis by inhibiting cytochrome P-450 dependent enzymes. *J. Clin. Invest.* **71**:1495–1499 (1983).
6. Meredith, C. G., A. L. Maldonado, and K. V. Speeg, Jr. The effect of ketoconazole on hepatic oxidative drug metabolism in the rat *in vivo* and *in vitro*. *Drug Metab. Dispos.* **13**:156–162 (1985).
7. Zehener, M., S. H. Hohnloser, and H. Just. QT-interval prolonging drugs: mechanisms and clinical relevance of their arrhythmogenic hazards. *Cardiovasc. Drug Ther.* **5**:515–530 (1991).
8. Carmeliet, E. Electrophysiologic and voltage clamp analysis of the effects of sotalol on isolated cardiac muscle and Purkinje fibers. *J. Pharmacol. Exp. Ther.* **232**:817–825 (1985).
9. Colatsky, T. Mechanisms of action of lidocaine and quinidine on the action potential duration of rabbit cardiac Purkinje fibers. *Circ. Res.* **50**:17–27 (1982).
10. Snyders, D. J., K. M. Knoth, S. L. Roberds, and M. M. Tamkun. Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.* **41**:322–330 (1992).
11. Woosley, R. L., Y. Chen, J. P. Frieman, and R. A. Gillis. Mechanism of the cardiotoxic actions of terfenadine. *JAMA* **269**:1532–1536 (1993).
12. Fedida, D., B. Wible, Z. Wang, B. Fermini, F. Faust, S. Nattel, and A. M. Brown. Identity of a novel delayed rectifier current from human heart with a cloned K^+ channel current. *Circ. Res.* **73**:210–216 (1993).
13. Snyders, D. J., M. M. Tamkun, and P. B. Bennett. A rapidly activating and slowly inactivating potassium channel cloned from human heart. *J. Gen. Physiol.* **101**:513–543 (1993).
14. Rampe, D., B. Wible, A. M. Brown, and R. C. Dage. Effects of terfenadine and its metabolites on a delayed rectifier K^+ channel cloned from human heart. *Mol. Pharmacol.* **44**:1240–1245 (1993).
15. Fermini, K., Z. Wang, D. Duan, and S. Nattel. Differences in rate dependence of transient outward current in rabbit and human atrium. *Am. J. Physiol.* **263**:H1747–H1754 (1992).
16. Escande, D., A. Coulombe, J. F. Faivre, E. Deroubaix, and E. Coraboeuf. Two types of transient outward currents in adult human atrial cells. *Am. J. Physiol.* **252**:H142–H148 (1987).
17. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. *Pflugers Arch.* **391**:85–100 (1981).
18. Wang, Z., B. Fermini, and S. Nattel. Sustained depolarization-induced outward current in human atrial myocytes: evidence for the role of a novel, ultra-rapid delayed rectifier potassium current. *Circ. Res.* **73**:276–285 (1993).
19. Honig, P. K., D. C. Wortham, K. Zamani, D. P. Conner, J. C. Mullin, and L. R. Cantilena. Terfenadine-ketoconazole interaction. *JAMA* **269**:1513–1518 (1993).
20. Morganroth, J., A. M. Brown, S. Critz, W. J. Crumb, D. L. Kunze, A. E. Lacerda, and H. Lopez. Variability of the QT_c interval: impact on defining drug effect and low-frequency cardiac event. *Am. J. Cardiol.* **72**:26B–31B (1993).
21. Sanguinetti, M. C., and N. K. Jurkiewicz. Two components of cardiac delayed rectifier current: differential sensitivity to block by class III antiarrhythmic agents. *J. Gen. Physiol.* **96**:195–215 (1990).

22. Wang, Z., B. Fermini, and S. Nattel. Delayed rectifier outward current and repolarization in human atrial myocytes. *Circ. Res.* **73**:276–285 (1993).
23. Jurkiewicz, N. K., and M. C. Sanguinetti. Rate-dependent prolongation of cardiac action potentials by a methanesulfonilide class III antiarrhythmic agent: specific block of rapidly activating delayed rectifier K^+ current by dofetilide. *Circ. Res.* **72**:75–83 (1993).
24. Simonsen, L. L. What are pharmacists dispensing most often? *Pharm. Times* **47**:65 (1992).
25. Wang, G. K., R. Simon, and S. Y. Wang. Quaternary ammonium compounds as structural probes of single batrachotoxin-activated Na^+ channels. *J. Gen. Physiol.* **98**:1005–1024 (1991).
26. Wang, G. K. Binding affinity and stereoselectivity of local anesthetics in single batrachotoxin-activated Na^+ channels. *J. Gen. Physiol.* **96**:1105–1127 (1990).
27. Dooley, M. J., and R. J. Quinn. The three binding domain model of adenosine receptors: molecular modeling aspects. *J. Med. Chem.* **35**:211–216 (1992).
28. Tinker, A., A. R. G. Lindsay, and A. J. Williams. Block of the sheep cardiac sarcoplasmic reticulum Ca^{2+} release channel by tetra-alkyl ammonium cations. *J. Membr. Biol.* **1127**:149–159 (1992).
29. Hille, B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* **69**:497–515 (1977).
30. Sakakibara, Y., T. Furukawa, D. H. Singer, H. Jia, C. L. Backer, C. E. Arentzen, and J. A. Wasserstrom. Sodium current in isolated human ventricular myocytes. *Am. J. Physiol.* **265**:H1301–H1309 (1993).
31. Sakakibara, Y., J. A. Wasserstrom, T. Furukawa, H. Jia, C. E. Arentzen, R. S. Hartz, and D. H. Singer. Charaterization of the sodium current in single human atrial myocytes. *Circ. Res.* **73**:535–546 (1992).
32. Nabauer, M., D. J. Beuckelmann, and E. Erdmann. Characteristics of transient outward current in human ventricular myocytes from patients with terminal failure. *Circ. Res.* **73**:386–394 (1993).
33. McDonald, T. F., and W. Trautwein. The potassium current underlying delayed rectification in cat ventricular muscle. *J. Physiol. (Lond.)* **274**:217–246 (1978).

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