Protriptyline block of the human ether-à-go-go-related gene (HERG) K⁺ channel

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Abstract

Protriptyline, a tricyclic antidepressant for psychiatric disorders, can induce prolonged QT, torsades de pointes, and sudden death. We studied the effects of protriptyline on human ether-à-go-go-related gene (HERG) channels expressed in Xenopus oocytes and HEK293 cells. Protriptyline induced a concentration-dependent decrease in current amplitudes at the end of the voltage steps and HERG tail currents. The IC_{50} for protriptyline block of HERG current in Xenopus oocytes progressively decreased relative to the degree of depolarization, from 142.0 μM at −40 mV to 52.9 μM at +40 mV. The voltage dependence of the block could be fit with a monoexponential function, and the fractional electrical distance was estimated to be δ=0.93. The IC_{50} for the protriptyline-induced blockade of HERG currents in HEK293 cells at 36 °C was 1.18 μM at +20 mV. Protriptyline affected channels in the activated and inactivated states, but not in the closed states. HERG blockade by protriptyline was use-dependent, exhibiting a more rapid onset and a greater steady-state block at higher frequencies of activation. Our findings suggest that inhibition of HERG currents may contribute to the arrhythmogenic side effects of protriptyline.

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Keywords: Protriptyline; Tricyclic antidepressant; HERG channel; Long QT syndrome; Arrhythmias

Introduction

Two major groups of psychotropic agents, the tricyclic antidepressants (TCAs) and phenothiazines, are among the most frequently prescribed medications in clinical use today. TCAs were introduced by Kuhn (1957) and have been given the common designation “tricyclics” because of their three-ring nucleus. Since TCAs are usually prescribed for depressed individuals who may have suicidal tendencies, these drugs are frequently implicated in cases of deliberate self-poisoning. TCA poisoning is characterized by increased neuromuscular excitability, abnormalities in the electrocardiogram (ECG), and in severe cases, cardiogenic shock and cardiac arrest (Serafimovski et al., 1975). The mortality associated with TCA overdose is substantial, and the cause of death is usually cardiovascular and related to arrhythmias (Marshall and Forker, 1982). Plasma TCA levels positively correlates with the QRS duration on ECG (Petit et al., 1977).

Protriptyline is a secondary amine that differs structurally from the other TCAs because of a minor modification of the tricyclic ring structure (Baldessarini, 2001). Fatal protriptyline poisoning can result in sinus tachycardia and increases in atrial conduction, QTc intervals, and the ventricular refractory period (Greenblatt et al., 1974; Magorien et al., 1979). The lengthening of the QT interval usually reflects a delayed repolarization of the action potential in ventricular myocytes (Tan et al., 1995). In addition, excessive prolonging of the QT interval is a marker for risk of torsades de pointes, a potentially fatal ventricular tachyarrhythmia (Haverkamp et al., 2000), which has led to the withdrawal of some QT-prolonging drugs from the market. Several ion currents (including inward Na⁺ and Ca^{2+} currents, etc.)
as well as outward K⁺ currents) contribute to the cardiac action potential duration, but the most common mechanism by which drugs delay repolarization and prolong the QT interval is by blocking one or more outward K⁺ currents (Witchel et al., 2003). In human cardiomyocytes, a specific blockage of rapidly activating components of delayed rectifier cardiac K⁺ current (I₉₋₅) appears to be the main mechanism whereby drugs act to produce acquired long QT syndrome and the associated ventricular arrhythmias (Brown and Rampe, 2000). The human ether-à-go-go-related gene (HERG) encodes the major protein underlying I₉₋₅, the pore-forming unit of the channel (Sanguinetti et al., 1995), and the inhibition of HERG currents by amitriptyline, a TCA, underlies the proarrhythmic effects of the drug in psychiatric patients (Jo et al., 2000). This raises the possibility that protriptyline may prolong the action potential duration in vivo, and may increase the QT interval by inhibiting the HERG current, which can eventually result in torsades de pointes and sudden cardiac death.

It is necessary to test chemical entities experimentally for potential blockade of the HERG channel because the drugs that inhibit HERG channels are structurally diverse (Haverkamp et al., 2000), making predictions based on structure alone difficult. In this study, we used the HERG channel expressed in Xenopus oocytes or HEK cells to test whether protriptyline would block the HERG channel and to examine inhibiting mechanisms.

Materials and methods

Expression of HERG in oocytes

HERG (accession no. U04270) cRNA was synthesized by in vitro transcription from 1 µg of linearized cDNA using T7 message machine kits (Ambion, Austin, TX, USA) and stored in 10 mM Tris–HCl (pH 7.4) at −80 °C. Stage V–VI oocytes were surgically removed from female Xenopus laevis (Nasco, Modesto, CA, USA) anesthetized with 0.17% tricane methane-sulphonate (Sigma, St. Louis, MO, USA). Using fine forceps, the theca and follicle layers were manually removed from the oocytes, and then each oocyte was injected with 40 nl of cRNA (0.1–0.5 µg/µl). The injected oocytes were maintained in a modified Barth’s solution. The modified Barth’s solution contained (mM): 88 NaCl, 1 KCl, 0.4 CaCl₂, 0.33 Ca(NO₃)₂, 1 MgSO₄, 2.4 NaHCO₃, 10 HEPES (pH 7.4), and 50 µg/ml gentamicin sulphonate. Currents were studied two to seven days after injection. This study was performed according to the Research Guidelines of Kangwon National University.

Solutions and voltage-clamp recordings from oocytes

Normal Ringer’s solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES (pH adjusted to 7.4 with NaOH). The antidepressant protriptyline and all salts were purchased from Sigma (St. Louis, MO, USA). A stock solution of protriptyline was prepared in distilled water and added to the external solutions at suitable concentrations shortly before each experiment. Solutions were applied to oocytes by continuous perfusion of the chamber while recording. Solution exchanges were completed within 3 min, and the HERG currents were recorded 5 min after the solution exchange. The effects of several concentrations of protriptyline on the HERG currents were determined after the currents showed reversibility when washed with normal Ringer’s solution. It took about 15 min to wash out ≤50 µM of the drug and about 30 min to wash out ≥100 µM of the drug. If the oocyte did not recover a current to its initial amplitude after 30 min of washing with a normal Ringer’s solution, it was not used further. In general, each oocyte was treated with four to seven concentrations of protriptyline. Currents were measured at room temperature (20–23 °C) with a two-microelectrode voltage-clamp amplifier (Warner Instruments, Hamden, CT, USA). Electrodes were filled with 3 M KCl and had a resistance of 2–4 MΩ for voltage-recording electrodes and 0.6–1 MΩ for current-passing electrodes. Stimulation and data acquisition were controlled with an AD–DA converter (Digidata 1200, Axon Instruments) and pCLAMP software (v 5.1, Axon Instruments).

The fractional electrical distance (δ), i.e. the fraction of the transmembrane electrical field sensed by a single positive charge at the binding site, was determined with half-blocking concentrations (K₂D) obtained from the fractional current (fD) as the current with 100 µM protriptyline and under control conditions at the end of the voltage step with the equation $K_2D = f_D / (1 - f_D) \times 100$ (in µM). The value of δ was obtained by fitting the K₂D values with the equation $K_2D = K_0V_{mV} \times \exp(-z\delta FV/RT)$ where $K_0V_{mV}$ represents the half-blocking concentration at the reference potential of 0 mV. V represents the membrane potential and $z, R, F$ and $T$ have their usual meaning (Snyders et al., 1992).

HEK cell culture and whole-cell patch recording

HEK293 cells stably expressing the HERG channel, a kind gift from Dr. C. January (Zhou et al., 1998), were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM non-essential amino acid solution, 100 U/ml penicillin–streptomycin, 100 g/ml streptomycin sulfate, and 100 g/ml zeocin in an atmosphere of 95% air and 5% CO₂. At 60%–80% confluence, cells were treated with media containing 0.25% trypsin and 0.02% EDTA for 3 min, washed with fresh media, and dispensed to new plastic culture dishes. For electrophysiological recording, cells were seeded on 5 mm diameter glass cover slips, incubated for 5–24 h in 24-well plates, and then transferred to the recording chamber. Whole cell currents were recorded using patch-clamp techniques. The external bath solution contained 136 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose and 10 mM HEPES, adjusted to pH 7.4 with NaOH. The intracellular pipette solution contained 130 mM KCl, 1 mM MgCl₂, 10 mM EGTA, 5 mM Mg-ATP and 10 mM HEPES, adjusted to pH 7.2 with KOH. The patch pipettes had resistances of 2–4 MΩ in the external bath solutions. All ionic currents were recorded using EPC10 amplifiers (HEKA electronic, Lambrecht-Pfalz, Germany). The Pulse + PulseFit computer program (HEKA...
electronic) was used for voltage-clamp amplifier control, data acquisition, and analysis. The current signals were filtered with a 10 kHz analog 3-pole Bessel filter followed by a 2.9 kHz analog 4-pole Bessel filter. The sampling frequency was set at 0.6 kHz. Solutions were applied to the HEK 293 cells by continuous perfusion of the chamber during recording. All experiments were performed at 36±1 °C.

Pulse protocols and analysis

To obtain concentration–response curves in the presence of protriptyline, dose-dependent inhibition was fitted with the equation:

\[ I_{\text{tail}} = I_{\text{tail max}} \left( \frac{[D]}{[D] + IC_{50}} \right)^n, \]

where \( I_{\text{tail}} \) indicates peak tail currents, \( I_{\text{tail max}} \) is the maximum peak tail current, \( D \) is the concentration of the small molecule, \( n \) is the Hill coefficient, and \( IC_{50} \) is the concentration at which the half-maximal peak tail currents were inhibited.

Virtual docking

A homology model of the HERG potassium channel was built on the basis of the 1.7 Å crystal structure (PDB ID code: 1R3J) of the KvAP channel (Zhou and MacKinnon, 2003), using the homology modeling program, MODELLER v8.0 (Sali and Blundell, 1993). A long stretch of amino acids (M579 to G603), located at the third extracellular loop of the HERG channel, was not included for the modeling because this region was not present in the template structure and does not appear to be involved in drug-induced inhibition of the HERG channel. Hydrogen atoms and Kollman-all charges were added to the homology model of the HERG channel using Sybyl v7.0 (Tripos Inc., St. Louis, MO). The three-dimensional structure of protriptyline was sketched using the Sybyl v7.0 molecular modeling software, and atomic charges were calculated using the Gasteiger–Huckel charge method. The conformation of protriptyline was refined with three consecutive optimization algorithms, the steepest descent, conjugate gradient, and quasi-Newton (Broyden, Fletcher, Goldfarb and Shanno; BFGS) methods, until we obtained convergences of 0.05, 0.01, and 0.001 kcal/mol Å, respectively. The classical molecular mechanical calculations were performed using the Tripos force field. We defined all atoms located within 10 Å of the hydroxyl oxygen atom of the Y652 residue in the HERG homology model as falling within the candidate site, and then performed virtual docking of protriptyline in the candidate site using GOLD v2.2, a program that applies stochastic genetic algorithms for conformational searching (Verdonk et al., 2003). The number of genetic operations was set to \( 1 \times 10^4 \) and the population size was set to \( 1 \times 10^3 \). A docked model with the best GOLD score was selected for the final complex structure. The Poseview program (Stierand et al., 2006) was used to investigate the interactions of the protriptyline molecule with the HERG channel. All structural figures were prepared using PyMol v0.98 (DeLano Scientific LLC, San Francisco, CA).

Statistical evaluations

All data are expressed as mean±S.E.M. Unpaired or paired Student t tests, or ANOVA were used for statistical comparisons when appropriate, and differences were considered significant at \( P<0.05 \).

Results

Protriptyline inhibits HERG current expressed in Xenopus oocytes

We assayed the effect of protriptyline on HERG currents using a Xenopus oocyte-expression system. Throughout the experiments, the holding potential was kept at −70 mV to obtain the minimum leak current, and the repolarization potential was held constant at −60 mV for the analysis of tail currents (\( I_{\text{tail}} \)). Fig. 1A shows an example of a voltage-clamp recording from a Xenopus oocyte and representative current traces both under control conditions and after exposure to 50 μM protriptyline. Under control conditions, the depolarizing steps activated time-dependent outward currents. The amplitude of the outward currents measured at the end of the pulse (\( I_{\text{HERG}} \)) increased with greater positive voltage steps, reaching a maximum value at −10 mV. The amplitude of \( I_{\text{HERG}} \) was normalized to the maximal amplitude of the \( I_{\text{HERG}} \) obtained under control conditions, and the amplitude in Fig. 1B was normalized to the potential of the step depolarization (\( I_{\text{HERG, nor}} \) Fig. 1B). Depolarizing steps to even greater positive values caused a current decrease, resulting in a negative slope of the \( I-V \) curve (Fig. 1B). Current–voltage relationships for \( I_{\text{HERG, nor}} \) obtained at various concentrations of protriptyline are plotted in Fig. 1B. As the concentration of protriptyline progressively increased, the amplitude of \( I_{\text{HERG, nor}} \) showed a concentration-dependent decrease.

After the depolarizing steps, repolarization to −60 mV induced an outward \( I_{\text{tail}} \) which had an amplitude even larger than that of \( I_{\text{HERG}} \) during depolarization. This is a characteristic property of HERG currents, and is due to a rapid recovery from inactivation and a slow deactivation mechanism (Sanguinetti et al., 1995). The amplitude of \( I_{\text{tail}} \) increased with depolarizing steps from −50 to +20 mV, and was then superimposed on further depolarizing steps to +40 mV. When 50 μM protriptyline was added to the perfusate, both \( I_{\text{HERG}} \) and \( I_{\text{tail}} \) were reduced (Fig. 1A, right panel). The amplitude of \( I_{\text{tail}} \) was normalized to the peak amplitude obtained under control conditions at maximum depolarization, and was plotted against the potential of the step depolarization (Fig. 1C). The normalized \( I_{\text{tail}} \) reflects the voltage-dependent activation of the HERG channels. Data obtained under control conditions were well-fitted by the Boltzmann equation, with a half-maximal activation (\( V_{1/2} \)) at −27.4 mV. As the concentration of protriptyline was increased, the peak \( I_{\text{tail}} \) amplitude decreased, indicating that the maximum conductance of HERG channels is decreased by protriptyline. Also, in the presence of protriptyline, \( I_{\text{tail}} \) does not reach the steady-state level, but declines at more positive potentials, indicating that blockade is more pronounced at positive potentials.
Fig. 1. The effect of protriptyline on human ether-a-go-go-related gene (HERG) currents (I_{HERG}) elicited by depolarizing voltage pulses. A: Superimposed current traces elicited by depolarizing voltage pulses (4 s) in 10 mV steps (left panel) from a holding potential of −70 mV in the absence of protriptyline (control, middle panel) and in the presence of 50 μM protriptyline (right panel). B: Plot of the normalized HERG current measured at the end of depolarizing pulses (I_{HERG,net}) against the pulse potential in the control and protriptyline conditions. The maximal amplitude of the I_{HERG} in the control was given a value of 1. C: Plot of the normalized tail current measured at its peak just after repolarization. The peak amplitude of the tail current in the absence of the drug was set as 1. Control data were fitted to the Boltzmann equation, \(y=1/(1+\exp[-x/V_{1/2}]/\delta])\), with \(V_{1/2}\) of −27.4 mV. D: Activation curves with values normalized to the respective maximum value at each concentration of protriptyline. Symbols with error bars represent mean±S.E.M. (n=8).

To examine whether protriptyline shifted the activation curve, we normalized the values in Fig. 1C to the respective maximum values at each concentration. The activation curves in the control oocytes, as well as those treated with 1, 3, 10, and 20 μM protriptyline basically overlapped, whereas the curves representing higher concentrations of the drug (50, 100, and 300 μM) were shifted downward without a significant leftward-shift (Fig. 1D). The \(V_{1/2}\) calculations were consistent with this finding, yielding values of −27.4±0.52, −26.0±0.60, −25.5±0.59, −25.5±0.92, −26.9±1.88, −26.7±3.12, −27.8±4.29, and −34.9±6.79 mV in the control and 1, 3, 10, 20, 50, 100 and 300 μM protriptyline-treated groups, respectively (n=8, P>0.05). Thus, the \(V_{1/2}\) values in the presence of 1–300 μM protriptyline were not significantly different, indicating that protriptyline does not change activation gating at this concentration range.

Protriptyline blocks HERG current voltage-dependently

To test whether the effect of protriptyline is voltage-dependent, we compared the protriptyline-induced decrease of \(I_{\text{tail}}\) at different potentials (Fig. 2). Our results revealed that a higher degree of blockade was present at more positive voltages (Fig. 2A). At −40 mV, 50 μM protriptyline reduced the amplitude of normalized \(I_{\text{tail}}\) by 10.2% (from 0.15±0.04 to 0.13±0.04; n=8, P<0.05), while at 0 and +40 mV, the same concentrations of protriptyline reduced the amplitude of \(I_{\text{tail}}\) by 27.7% (from 0.94±0.02 to 0.68±0.01; n=8, P<0.05) and 50.2% (from 1.00±0.01 to 0.50±0.04; n=8, P<0.05), respectively. Dose–response relationships were obtained at +40 mV, 0 mV and −40 mV. The data were fitted by Hill equations (Fig. 2B), giving IC_{50} values of 142.0±4.45 μM, 91.7±5.85 μM and 52.9±2.42 μM, respectively, and Hill coefficients of 1.90±0.11, 1.71±0.18 and 1.27±0.07 at −40 mV, 0 mV and +40 mV, respectively (n=8). The HERG-current inhibition by 50 μM protriptyline at different voltages (−40, −20, 0, +20, and +40 mV) was 10.2%±10.1%, 20.6%±6.1%, 27.7%±2.4%, 35.1%±2.7%, and 50.2%±6.7%, respectively, suggesting that the protriptyline-induced blockade of HERG currents progressively increases with increasing depolarization. Collectively, these findings indicate that the protriptyline-induced blockade of HERG current is voltage-dependent.

For further analysis, the relative current under protriptyline (100 μM) was calculated for each potential (Fig. 3; filled squares; n=8). Then, we normalized relative currents with the drug as fractions of the control current, and fitted it with a monoeponential function (Fig. 3, solid line). Since the relative conductance of the HERG control current reached more than 90% of its maximal value at potentials above 0 mV (Fig. 3, dashed line; mean open probability at 0 mV obtained by Boltzmann fit: 0.94), the range between 0 and +40 mV was used to estimate the fractional electrical distance (δ), i.e. the fraction of the transmembrane electrical field sensed at the receptor site of protriptyline. Half-blocking concentrations (K_D) were calculated from the fraction of control current achieved with protriptyline. Fitting the mean K_D values in the potential range from 0 to +40 mV with the mean K_D at the reference potential of 0 mV (K_D 0 mV=87.0 μM) yielded a fractional electrical distance of δ=0.93.
Protriptyline blocks HERG channels mainly in the open state and in the inactivated state

Next, to investigate whether the channel was blocked in the closed or activated (i.e. open and/or inactivated) state, we activated currents using a protocol containing a single depolarizing step to 0 mV for 8 s from a holding potential of −90 mV (Fig. 4A). After obtaining the control measurement, we applied 50 μM protriptyline and then made recordings. The degree of inhibition (i.e. (1 − protriptyline current/control current) × 100 (in %)) is displayed in Fig. 4B. Analysis of the test pulse after protriptyline application revealed a time-dependent increase of blockage to 55% at 4 s in this representative cell (Fig. 4B). Additionally, at the beginning of the pulse, the fractional sustained current (obtained by normalizing the currents with protriptyline relative to control currents) was 0.87 ± 0.05 of the control (n=8), indicating that HERG channels are only slightly blocked by protriptyline while remaining at the holding potential. In this series of experiments, 50 μM protriptyline reduced HERG outward currents at the end of the 0 mV pulse by 45.1% ± 5.0% (n=8).

To address the question of whether HERG channels are also blocked by protriptyline in its inactivated state, a long test pulse to +80 mV (4 s) from a holding potential of −90 mV was applied to inactivate the channels, followed by a second voltage step (0 mV, 3.5 s) to open HERG channels (n=8). Typical current traces under control conditions and after the application of 50 μM protriptyline are displayed in Fig. 4C. Fig. 4D depicts the normalized relative blockage upon channel opening during the second voltage pulse (0 mV), indicating that pronounced...
inhibition of HERG channels had already been reached during the previous inactivating +80 mV pulse. No additional time-dependent blockage of open channels were observed during the 0 mV pulse. Currents at the end of the second voltage step (0 mV) were reduced by 34.1% ±3.4% (n = 8). Taken together, protriptyline inhibits HERG channels predominantly in the open and inactivated state rather than in the closed state.

Protriptyline block is frequency-dependent

To examine the use-dependence of the protriptyline effect, HERG channels were activated by 0.3 s depolarizing steps up to +20 mV from a holding potential of −90 mV followed by a repolarizing step to −40 mV (300 ms) to elicit outward tail currents. Pulses were applied at intervals of 1 s or 10 s under control conditions and in the presence of 30 μM protriptyline, with each cell studied only at one stimulation rate (n = 5). The development of current reduction was plotted versus time (Fig. 5). We found that the time course of the channel blockade was dependent on the activation frequency, with HERG blockade by protriptyline occurring more quickly at higher activation frequencies. Also, we found that the steady-state block by treatment of 30 μM protriptyline for 10 min was stronger at a higher rather than at a lower activation frequency, indicating that the binding of protriptyline was favored at higher frequencies.

Protriptyline inhibits HERG currents expressed in HEK cells

The IC50 values of many HERG channel blockers differ depending on whether the HERG channels are expressed in

![Fig. 4. Blocking of activated HERG channels by protriptyline. A: An original recording of currents under control conditions (control) and after exposure to 50 μM protriptyline (for 13 min, without any intermittent test pulse). B: The degree of HERG-current inhibition in percentages (%). Current inhibition increased time-dependently to 55% at 4 s in this representative cell, indicating that mostly open and/or inactivated channels were blocked. C: Inhibition of inactivated channels by 50 μM protriptyline. HERG channels were inactivated by a first voltage step to +80 mV, followed by channel opening at 0 mV. D: The corresponding relative block during the 0 mV step is displayed. Maximum inhibition was achieved in the inactivated state during the first step, and no further time-dependent blockage occurred upon channel opening during the second voltage step.

![Fig. 5. Use-dependent HERG channel blockade by protriptyline. Tail currents were recorded at −40 mV after a 0.3 s depolarizing pre-pulse to +20 mV from a holding potential of −90 mV every 1 s (■) or 10 s (●), after exposure to 30 μM protriptyline. The resulting mean relative tail-current amplitudes are plotted versus time. For the purpose of clear presentation, not all measurements are displayed. Symbols with error bars represent mean ± S.E.M. (n = 5 each).]
Protriptyline is a TCA indicated for the treatment of patients with major depressive illness. The therapeutic site of the antidepressive action of TCAs is predominantly in the central nervous system, and consists of the 5-HT_{2} serotonin receptors, norepinephrine reuptake inhibition, and α_{2}-adrenergic antagonism (Baldessarini, 2001). Besides their use for depressive illness, TCAs, including protriptyline, are important for patients with neuropathic pain syndrome (Monks and Merskey, 1984; Collins et al., 2000). The therapeutic plasma concentrations of protriptyline typically range from 0.4 to 1.43 μM (Risch et al., 1979). When the rate of plasma-protein binding is up to 95% for all tricyclics (Marshall and Forker, 1982), the free plasma concentration may be assumed to be in the range from 20 to 71.5 nM. Therapeutic doses of protriptyline have been associated with fatal arrhythmias, such as QTc prolongation and ventricular tachycardia (Magorien et al., 1979). A case report of sudden death related to a protriptyline overdose has also been reported (Greenblatt et al., 1974). The drug is oxidized by hepatic cytochrome—P-450 microsomal oxidase and by conjugation processes to give an elimination half-life of ~80 h, which is relatively longer than other TCAs (Baldessarini, 2001). Also, the half-life may be prolonged in patients either with hepatic disease and renal insufficiency or when used in combination with other anticancer or antipsychotic drugs (Buckley and Sanders, 2000). We found that protriptyline blocked HERG in a mammalian cell line with an IC\textsubscript{50} of ~1.1 μM (Fig. 6), which is at the supra-therapeutic level compared with the reports of both the therapeutic serum concentration (Risch et al., 1979) and that in which the drug causes QTc prolongation and ventricular tachycardia (Magorien et al., 1979). However, there are several hypotheses regarding the threshold for the prediction of clinically important HERG channel blockades. It has been implicated that drugs for which the IC\textsubscript{50} is at least 30-fold greater than the highest achievable free plasma concentration will be free of liability for torsades de pointes (Redfern et al., 2003). Considering the IC\textsubscript{50} value (1.1 μM) obtained from HEK cell is about 15-fold higher than the expected the highest achievable free plasma concentration (71.5 nM), our findings suggest that the inhibition of HERG channels by the drug could contribute to its proarrhythmic effects.

Other drugs that cause long QT syndrome and torsades de pointes block HERG channels in a voltage-dependent manner,
suggesting that these drugs bind to the open or inactivated state of HERG channels. For example, two histamine-receptor antagonists, terfenadine and astemizole (Suessbrich et al., 1996), preferentially bind to the inactivated state of HERG, whereas the gastrointestinal prokinetic agent, cisapride, blocks the channel in its open state (Rampe et al., 1997). We have shown here that protriptyline decreased the amplitudes of the maximum outward current and the maximum peak tail current, and the magnitude of the blockade increased with greater positive voltages, which increased the open probability and enhanced inactivation (Fig. 1). Our results also indicate that protriptyline blockade is state-dependent: HERG channels were predominantly blocked in the open and inactivated states but not in the closed state (Fig. 4). Finally, protriptyline did not significantly change \( V_{1/2} \) values of the activation curve, which indicates that the drug blocks HERG channels without changing activation properties (Fig. 1).

One of the major side effects of TCAs is cardiotoxicity (Baldessarini, 2001). Cholinergic, sympatholytic, and direct “quinidine-like” membrane actions appear to interplay in the heart (Stimmel, 1979; Risch et al., 1981a,b; Marshall and Forker, 1982; Baldessarini, 2001). Increased heart rate is due primarily to the anticholinergic action, whereas ECG changes and conduction disturbances largely result from direct membrane effects (Frommer et al., 1987). Indeed, TCAs block a variety of other ion channels, including cardiac Na\(^+\) channels (Sudoh et al., 2003), delayed rectifier K\(^+\) channels and transient outward K\(^+\) channels in sympathetic neurons (Wooltorton and Mathie, 1995), Ca\(^{2+}\)-activated K\(^+\) channels in spinal cord neurons (Kamatchi and Ticku, 1991), and L-type Ca\(^{2+}\) channels in dorsal root ganglia (Choi et al., 1992). Since protriptyline can affect various action potential parameters, including QTc prolongation (Dumovic et al., 1977; Magorien et al., 1979; Zbinden et al., 1980), atrioventricular conduction (Bianchetti et al., 1977), and ventricular tachycardia (Magorien et al., 1979), protriptyline can probably modulate several ion channels involved in cardiac action potential, although the drug does not seem to inhibit ion channels in the heart other than Na\(^+\) channels (Sudoh et al., 2003). We have shown here that protriptyline inhibited the major component of \( I_{Kr} \) HERG, suggesting that protriptyline-induced cardiac arrhythmias may also result from HERG inhibition. In addition, the relatively low incidence of arrhythmia during protriptyline treatment could be explained by inhibition of voltage-sensitive Ca\(^{2+}\) channels, which might counteract the potential arrhythmogenic effects of HERG blockade, although it has not been reported that protriptyline blocks cardiac L-type Ca\(^{2+}\) channels. Therefore, to understand the mechanisms underlying protriptyline-induced electrophysiological changes in the action potential and ECG, it is important to determine the effects of this drug on ion channels other than HERG.

We have shown here that the protriptyline blockade of HERG, and possibly \( I_{Kr} \), was more prominent at higher frequencies (Fig. 5), which may not be consistent with the reverse use-dependent repolarization lengthening by other \( I_{Kr} \) blockers in cardiac cells (Hondeghem and Sniders, 1990). Reverse use-dependence plays a role in bradycardia-dependent proarrhythmic effects of various class-III antiarrhythmic agents, as well as with several \( I_{Kr} \) blocking agents (Hondeghem and Sniders, 1990). Therefore, it would be useful to determine whether protriptyline induces frequency-dependent increases in action potential duration in cardiomyocytes and ventricular muscle (Ducroq et al., 2005). The molecular mechanism underlying use-dependence is not fully understood. Mitcheson et al. (2000) demonstrated that drugs may be trapped in the HERG channel pore upon closure of the activation gate,
whereas Kiehn et al. (1999) suggested that positive frequency-dependence may indicate an accumulation of blockade resulting from slow dissociation properties in the open channel state. The regulatory β-subunit MiRP1 combines with HERG to reconstitute native I\textsubscript{Kr} (Abott et al., 1999), but Scherer et al. (2002) showed that the effects of terfenadine and fexofenadine were not altered by co-expression with MiRP1. In addition, Kamiya et al. (2001) did not detect significantly different IC\textsubscript{50} values when HERG channel inhibition by vesnarinone was compared in the presence and absence of MiRP1. Weerapura et al. (2002) revealed that the co-expression of HERG with wild-type MiRP1 does not alter its sensitivity to HERG-blocking drugs. They raised doubts about the contention that the co-assembly of HERG and MiRP1 is required to reassemble native I\textsubscript{Kr}, since a lack of MiRP1 did not affect the resemblance of currents to native I\textsubscript{Kr}.

Computer simulation of HERG blocking by protriptyline suggested that protriptyline binds the HERG channel at the internal chamber and makes a hydrogen bond to the carbonyl oxygen of Leu622 (Fig. 7). The protonated nitrogen of HERG channel blockers makes a hydrogen bond to the carbonyl oxygen of Thr623 (Choe et al., 2006). Since the carbonyl oxygens of Leu622 and Thr623 in the HERG channel model are close, they may play the same role of accepting hydrogen from the tertiary amine of the blockers. In addition, Tyr652 is close to protriptyline, suggesting the aromatic side chain of the residue may be involved in aromatic- aromatic interactions with the aromatic rings of the blocker. Several amino acids also have hydrophobic contacts with protriptyline (Fig. 7A and B). However, Phe656, which has hydrophobic interactions with blockers (Choe et al., 2006; Fernandez et al., 2004), did not contact protriptyline in the simulation, suggesting that a Phe656 mutation would not affect blockade. Our result shows that the electrical distance of the protriptyline block in the HERG channel is 0.93 (Fig. 3). The value is not incompatible with our modeling result because the electrical distance is not related to the physical distance proportionally.

TCAs are associated with LQT of the ECG. Several TCAs have been reported to block the HERG channel with different IC\textsubscript{50}s and mechanisms (Cavalli et al., 2002; Duncan et al., 2007). For example, amitriptyline, imipramine, and doxepin block the HERG channel with the IC\textsubscript{50}s of 10 μM, 3.4 μM, and 6.5 μM, respectively (Cavalli et al., 2002; Duncan et al., 2007). In this study, protriptyline is shown to be another TCA that blocks the HERG channel with the IC\textsubscript{50} of 1.18 μM. Currently, it is difficult to correlate the blocking potency and the structures of the drugs used for treatment, however, it’s interesting to note that although the only difference between the structure of protriptyline and amitriptyline is location of a double bond, protriptyline is approximately 10-fold more potent than amitriptyline. Additionally, imipramine and amitriptyline have been shown to have a relatively weak and a strong voltage-dependence for the HERG channel block, respectively, whereas the inhibition of the HERG channel that occurs as a result of doxepin treatment occurs voltage-independently (Jo et al., 2000; Teschemacher et al., 1999; Duncan et al., 2007). The results of this study indicated that the blockade of HERG current by protriptyline has a relatively strong voltage-dependence (Fig. 2). Taken together, these results indicate that it is necessary to test each TCA experimentally to evaluate its potential to block the HERG channel since the HERG-blocking potencies and inhibitory mechanisms of TCAs vary widely and cannot be predicted based on structure alone.

Conclusion

In the present work we have shown that the antidepressant drug, protriptyline, blocks HERG channels in Xenopus oocytes and HEK cells, suggesting that the drug-induced arrhythmias observed in protriptyline-treated patients may be due, at least in part, to protriptyline inhibition of I\textsubscript{Kr}.

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