Mechanism of Block and Identification of the Verapamil Binding Domain to HERG Potassium Channels

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Abstract—Calcium channel antagonists have diverse effects on cardiac electrophysiology. We studied the effects of verapamil, diltiazem, and nifedipine on HERG K⁺ channels that encode Iₖᵃ in native heart cells. In our experiments, verapamil caused high-affinity block of HERG current (IC₅₀ = 143.0 nmol/L), a value close to those reported for verapamil block of L-type Ca²⁺ channels, whereas diltiazem weakly blocked HERG current (IC₅₀ = 17.3 μmol/L), and nifedipine did not block HERG current. Verapamil block of HERG channels was use and frequency dependent, and verapamil unbound from HERG channels at voltages near the normal cardiac cell resting potential or with drug washout. Block of HERG current by verapamil was reduced by lowering pH₀, which decreases the proportion of drug in the membrane-permeable neutral form. N-methyl-verapamil, a membrane-impermeable, permanently charged verapamil analogue, blocked HERG channels only when applied intracellularly. Verapamil antagonized dofetilide block of HERG channels, which suggests that they may share a common binding site. The C-type inactivation-deficient mutations, Ser620Thr and Ser631Ala, reduced verapamil block, which is consistent with a role for C-type inactivation in high-affinity drug block, although the Ser620Thr mutation decreased verapamil block 20-fold more than the Ser631Ala mutation. Our findings suggest that verapamil enters the cell membrane in the neutral form to act at a site within the pore accessible from the intracellular side of the cell membrane, possibly involving the serine at position 620. Thus, verapamil shares high-affinity HERG channel blocking properties with other class III antiarrhythmic drugs, and this may contribute to its antiarrhythmic mechanism. (Circ Res. 1999;84:989-998.)

Key Words: Ca²⁺ antagonist ■ HERG ■ rapidly activating delayed K⁺ channel ■ antiarrhythmic drug ■ arrhythmia

L-type Ca²⁺ channel antagonists (phenylalkylamines, benzothiazepines, and dihydropyridines, with respective prototype drugs verapamil, diltiazem, and nifedipine) have distinct effects on cardiac electrophysiology and arrhythmias. In heart cells, low concentrations of verapamil have been shown to prolong cardiac action potential duration, whereas high concentrations shorten it. In contrast, dihydropyridines cause only concentration-dependent shortening of cardiac action potential duration. Some forms of ventricular tachycardia are specifically sensitive to verapamil. Verapamil has recently been shown to prolong the atrial effective refractory period in humans and to prevent atrial tachycardia- and fibrillation-induced shortening of the atrial effective refractory period (“electrical remodeling”). Observations such as these suggest that in addition to blocking L-type Ca²⁺ channels, verapamil may modify repolarizing membrane currents at low drug concentrations.

Cardiac delayed rectifier K⁺ current is composed of 2 distinct currents, the rapidly (Iₖᵃ) and slowly (Iₖᵦ) activating components. Iₖᵃ channel protein is encoded by the human ether-a go-go-related gene (HERG). Suppression of HERG channels causes action potential and QT interval prolongation, which can be both antiarrhythmic and cause long-QT syndrome. We examined the effects of verapamil, diltiazem, and nifedipine on HERG channels heterologously expressed in a human embryonic kidney (HEK 293) cell line. Our data show that verapamil is a potent antagonist of HERG channel current, whereas diltiazem only weakly suppresses HERG current, and nifedipine has no effect. We then studied verapamil block of HERG channels, including its use- and frequency-dependent properties, membrane sidedness of action, and binding domain on the HERG channel. Our results indicate that externally applied verapamil enters the membrane in the neutral form to reach a binding domain within the pore that is accessible from the intracellular side of the membrane. Preliminary reports of this work have appeared.

Materials and Methods

DNA Constructs and Stable Transfection of HEK 293 Cells

The transfection techniques have been published previously. Briefly, HERG cDNA was subcloned into BamHI/EcoRI sites of the pCDNA3 vector (Invitrogen). HEK 293 cells were transfected with this construct using the lipofectamine method ( Gibco), and the stably transfected cells were subcloned to achieve a uniform HERG.
expression level. cDNA for the HERG mutations Ser631Ala and Ser620Thr (provided by Dr Gail Robertson, University of Wisconsin, Madison) was expressed transiently or stably in a similar manner. The cells were cultured in MEM supplemented with 10% FBS and 400 μg/mL G418. The cells were harvested from the culture dish by trypsinization, washed twice with standard MEM, and stored in this medium at room temperature for electrophysiological study.

**Patch-Clamp Recording Method**

Aliquots of cells were allowed to settle on the bottom of a <0.5 mL cell bath mounted on an inverted microscope (Diaphot, Nikon). Cells were superfused with Tyrode solution containing (in mM/L) 137 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (normal pH 7.4 adjusted with NaOH). Solution exchanges in the cell bath were completed within 1 minute. Membrane currents were recorded in the whole-cell patch clamp configuration. The pipette had inner diameters of 1 to ~1.5 μm and had resistances of 2 to 4 MΩ when filled with the internal pipette solution. The internal pipette solution contained (in mM/L) 130 KCl, 1 MgCl₂, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 adjusted with KOH). A Dagan 3911A patch clamp amplifier was used to record membrane currents.

**Drugs and Chemicals**

Verapamil, diltiazem, and nifedipine were purchased from Sigma. Verapamil and diltiazem were dissolved in distilled water to make 1 mM/L and 10 mM/L stock solutions, respectively. Nifedipine was dissolved in DMSO to give a stock concentration of 50 mM/L. N-methyl-verapamil, a quaternary, permanently charged, membrane-impermeable verapamil analogue, was obtained from Dr G. Paul (Knoll Pharmaceuticals, Ludwigshafen, Germany) as N-methyl-verapamil hydrochloride and was dissolved in distilled water to make a 10 mM/L stock solution. Dofetilide [{N-[4-[(methanesulfonylamo-phenoxy)-N-methylthylamino]ethylphenylo]-methanesulfonamide} was obtained from Pfizer Central Research and was dissolved in acidic water to make a 100 μmol/L stock solution. Final drug concentrations were made by diluting stock solution with Tyrode or pipette solution. DMSO at a concentration (0.1%) equivalent to the highest drug dilution studied had no effect on HERG current (n=4 cells).

**Curve Fitting and Statistical Methods**

Data are given as mean±SEM. Curve fitting was done using multiple nonlinear least-squares regression analysis (Origin, Microcal Software; Clampfit, Axon Instruments). Concentration effects were quantified by fitting the Hill equation \[ I_{\text{rest}}/I_{\text{max}} = 1/[1 + (D/IC_{50})^n] \], where \( D \) is the drug concentration, IC_{50} is the drug concentration for 50% block, and \( n \) is the Hill coefficient) to the results. Statistical significance was analyzed using a Student t test or ANOVA, where appropriate. \( P<0.05 \) was considered statistically significant.

**Results**

**Verapamil, Diltiazem, and Nifedipine Effects on HERG Channels**

Figure 1A shows control HERG current elicited by depolarizing steps to between −70 and 60 mV for 4 seconds applied from a holding potential of −80 mV. Tail current was recorded after the repolarizing step to −50 mV. Figure 1B shows the effect of 2 μmol/L verapamil on HERG current in the same cell. Figure 1C shows averaged current-voltage (I-V) relations for HERG current measured at the end of depolarizing steps and for the subsequent tail current peak amplitude for control conditions and with 2 μmol/L verapamil in the same cells (n=4 cells). Under control conditions, HERG current activated at voltages positive to −50 mV, maximum current was reached for steps to −10 mV, and at more positive voltages inward rectification was present because of voltage-dependent rapid inactivation.\(^{18,19} \) The tail current was maximal after voltage steps positive to 0 mV. At a concentration of 2 μmol/L, verapamil block of HERG current was nearly complete at all voltages. Figures 1D and 1E show the reversibility of verapamil block. In panel D,
HERG current was activated with a step to 20 mV, and tail current was recorded after the step to –50 mV. The voltage protocol was repeated at 15-second intervals, and HERG tail current peak amplitude plotted versus time is shown in panel E. Exposure to verapamil (500 nmol/L) caused a rapid reduction in current amplitude to a steady level of block that was rapidly reversed after drug washout. To study the concentration dependence relation for block of HERG current by verapamil, 4 to 9 cells were studied at each drug concentration, with each cell exposed to only 1 drug concentration. Verapamil block of HERG current at the end of the depolarizing step to 20 mV and the tail current peak amplitude were normalized to the respective control values and are plotted as relative current amplitude in panel F. Data points were fit with the Hill equation. The half-maximal inhibition concentration (IC50) for block of tail current was 143.0 nmol/L with a Hill coefficient of 1.06, whereas the IC50 for block of current measured at the end of the depolarizing step to 20 mV was 160.0 nmol/L with a Hill coefficient of 0.91. As shown in panel F, these concentration dependence relations were nearly identical. The Hill coefficients are consistent with the binding of verapamil to a single site.

The concentration dependence for block of HERG current by diltiazem was studied in 5 to 8 cells at each drug concentration using a protocol similar to that shown in Figure 1D. Diltiazem blocked HERG current in a concentration-dependent manner, and diltiazem block of HERG tail current peak amplitude is shown in Figure 1F. Data were fit with the Hill equation to give an IC50 of 17.3 μmol/L that is ~100 times higher than that of verapamil. The Hill coefficient was 1.03, consistent with the binding of drug to a single site. In contrast to verapamil (see below), diltiazem block of HERG current was present immediately on depolarization, and no recovery of HERG current was observed after repolarization to –80 mV for up to 10 minutes; diltiazem block, however, was reversible with drug washout. The effect of nifedipine on HERG current was studied at concentrations up to 50 μmol/L using a protocol similar to that shown in Figure 1D. Despite the use of repetitive pulses and drug exposure periods of up to 30 minutes, nifedipine had no effect on HERG current amplitude (n=6 cells). These data are plotted in Figure 1F. A similar lack of effect of nifedipine was observed when the holding potential was –50 mV (data not shown), which accentuates dihydropyridine block of L-type Ca2+ channels.

**Intracellularly, but Not Extracellularly, Applied N-Methyl-Verapamil Blocks HERG Channels**

To test whether verapamil acts on HERG channels from the outside or inside of the cell membrane, we studied the effects of verapamil and N-methyl-verapamil, a permanently charged, membrane-impermeable analogue. HERG current was activated from a holding potential of –80 mV by a step to 20 mV for 4 seconds, followed by a step to –50 mV for 6 seconds to record tail current, with the protocol repeated at 15-second intervals. Figure 2A shows original current traces (left panel) and tail current peak amplitude plotted as a function of time (right panel). The extracellular application of 1 μmol/L verapamil reduced HERG tail current to 9.9±5.2% of the control value (n=6 cells, P<0.01), which was reversed with drug washout. Figure 2B shows original current traces (left panel) and tail current peak amplitude plotted versus time (right panel) with the addition to the bath of 20 μmol/L N-methyl-verapamil. It had virtually no effect on HERG tail current peak amplitude, which was 91.4±3.6% of the control value after 20 minutes of exposure (n=4 cells, P>0.05). Thus, the extracellular application of the membrane-impermeable N-methyl-verapamil analogue did not block HERG channels.

We then tested its effect by intracellular application. N-methyl-verapamil (2 μmol/L) was included in the pipette solution and immediately after obtaining whole-cell clamp HERG current was recorded using the same voltage protocol. Figure 2C shows original current traces (left panel) and tail current peak amplitude plotted versus time (right panel). HERG tail current peak amplitude decreased with time as N-methyl-verapamil diffused from the pipette into the cell, and this could be fit as a single-exponential decay with an average time constant of 2.3 minutes. By 10 minutes of whole-cell recording, the current had decreased to 35.8±1.9% (n=4 cells, P<0.05) of the initial value. In control experiments without N-methyl-verapamil in the pipette, HERG tail current peak amplitude decreased to 96.2±3.1% (n=4 cells, P>0.05) of the control value over the same time interval, which confirms that the decrease in HERG current was due to internally applied N-methyl-verapamil.

**Effects of pH** on Block of HERG Channels by Verapamil

Verapamil is a weak base, and the fractional distribution of drug between neutral (membrane permeable) and positively charged (membrane impermeable) forms can be controlled by
varying pH. We used this to evaluate further whether verapamil block of HERG channels required the neutral form of the drug. This approach has been previously used to help to elucidate the membrane sidedness of drug action on ion channels.21,22 The dissociation constant (pKa) of verapamil in solution has been estimated to be between 8.73 and 8.99.23 Assuming a pKa of 8.8, at pH 8.4, 28% of verapamil would be in the neutral form. As shown in Figure 3A, at this pH, extracellular application of 500 nmol/L verapamil reduced HERG tail current peak amplitude to 17.0 ± 3.4% of the control value (n = 4 cells). At a pH of 7.4, at which ≈4.0% of the drug is in the neutral form, 500 nmol/L verapamil reduced HERG tail current to 22.4 ± 2.4% (n = 9 cells), and at pH 6.4, at which ≈0.4% of verapamil is in the neutral form, 500 nmol/L verapamil reduced HERG tail current only to 81.9 ± 3.2% (n = 6 cells) of the control values. Thus, changes in pH0 that decrease the amount of drug in the neutral, membrane-permeable, form decreased drug block of HERG channels, which suggests that the neutral form of verapamil is required for HERG channel block. Figure 3A also shows that changing pH0 for control conditions had additional effects on HERG current. Particularly changing pH0 from 7.4 to 6.4 decreased HERG current amplitude and markedly accelerated the HERG tail current decay. The effects of pH0 on HERG current require further investigation and will be reported separately.

We studied the effect of pH0 on block by N-methyl-verapamil, to confirm that changing pH0 did not alter the extent of block when the permanently charged analogue was applied intracellularly. As shown in Figure 3B, when applied intracellularly through the pipette, 2 μmol/L N-methyl-verapamil blocked HERG current. At a pH0 of 7.4, HERG tail current peak amplitude was reduced to 33.0 ± 1.2% of the initial value, and at a pH0 of 6.4, HERG tail current peak amplitude was reduced to 27.0 ± 3.2% of the initial value (n = 4 cells, P > 0.05). As expected, altering pH0 did not affect block by N-methyl-verapamil. The results shown in Figures 2 and 3 suggest that verapamil permeates the cell membrane in a neutral form and that its binding site to the HERG channel is accessible from the internal side of the cell membrane.

Development of Drug Block by Verapamil

HERG block by verapamil was use and frequency dependent, as shown in Figure 4. In Figure 4A, 200 nmol/L verapamil, a concentration close to the IC50, was washed in for 10 minutes while the cell was held continuously at −80 mV to maintain HERG channels in a closed state. HERG channels were then rapidly activated by a depolarizing step to 60 mV for 200 milliseconds and then to −50 mV for 200 milliseconds to elicit tail current, before repolarizing to the holding potential.
Trains of these pulses were applied at intervals of 0.6, 2, or 20 seconds, with each cell studied only at 1 pulse frequency, and the development of drug block was plotted versus time. The time course of the development of HERG block was dependent on the frequency of HERG channel activation, which suggests activated (open or inactivated) state block by verapamil. At each pulse frequency, 10 to 15 pulses were required for a steady level of block to be reached. The extent of drug block was weaker at lower pulse frequencies, which suggests time-dependent drug unbinding at the holding potential between pulses.

We used a second voltage protocol to characterize further properties of verapamil block of activated channels, as shown in Figure 4B. From the holding potential, HERG channels were rapidly activated by a 100-millisecond step to 60 mV, which was followed by a 10-second step to 0 mV. Tail current was then elicited with a 6-second step to −50 mV. For control conditions, at 0 mV HERG current amplitude was nearly constant, and the subsequent repolarizing step to −50 mV caused a tail current that decayed gradually. The cell was then held continuously at −80 mV for 10 minutes to maintain channels in a closed state during verapamil (500 nmol/L) washin. With the first depolarization in the presence of verapamil, the initial HERG current amplitude was unchanged compared with the control current, indicating minimal rested (closed) state block. The current amplitude then declined during the maintained depolarization at 0 mV to reach a steady level of drug block. In 9 cells studied with this protocol, the current remaining at the end of the 10-second step to 0 mV declined to 28.6% of the initial value, and when fit as a single exponential it decayed with a time constant of 0.93 ± 0.08 seconds. Tail current peak amplitude was similarly reduced to 22.4 ± 2.4% of the control value. More importantly, the tail current recorded with verapamil exposure was slower to decay and it “crossed over” (Figure 4B, arrow) the control tail current trace to become larger in amplitude. When the step to −50 mV was increased to 60 seconds duration, the tail currents decayed to the same value (data not shown). These effects of verapamil were reversed with drug washout.

We studied inactivation properties of HERG current in control conditions and with verapamil present at a concentration (200 nmol/L) near the IC50. In 1 series of experiments, cells were held at 60 mV and hyperpolarized to −100 mV for 10 milliseconds, which is sufficient at room temperature to remove inactivation and maximize occupancy in the open state. A test step to 0 or 60 mV was then applied to elicit a large-amplitude outward current that rapidly decayed as HERG channels inactivated. The rate of inactivation was measured for each cell by fitting a monoexponential function to the decaying outward current. At test potentials of 0 and 60 mV, time constants in the absence of verapamil were 8.9 ± 0.6 and 3.0 ± 0.3 milliseconds (n = 9 cells), respectively. With 200 nmol/L verapamil in the extracellular perfusate, the time constants were 8.7 ± 0.6 and 2.9 ± 0.3 milliseconds (n = 9 cells), respectively, which are not different from the control values (P > 0.05). We also studied the effects of verapamil on recovery from inactivation. The cells were depolarized to 60 mV for 200 milliseconds to rapidly inactivate HERG channels, and the cells were then repolarized to −50 mV to elicit a tail current. The rising phase (“hook”) of the tail current represents the rapid recovery of HERG channels from inactivated to open states, whereas the slow decay phase of tail current represents HERG channel deactivation. After performing a double-exponential fit to the tail current trace, the time constant for the rising phase was used to measure recovery from inactivation. At −50 mV, the time constant of recovery from inactivation was 7.3 ± 0.3 milliseconds, and in the presence of 200 nmol/L verapamil it was 7.2 ± 0.3 milliseconds (n = 18 cells, P > 0.05). Thus, verapamil exposure did not affect the apparent rates of inactivation and of recovery from inactivation of HERG channels. The rate of deactivation, as shown in Figure 4B, was markedly slowed.

**Figure 5.** Time and voltage dependence of recovery of HERG current with repolarization during exposure to verapamil and N-methyl-verapamil. A, voltage protocol is shown above example current traces with recovery studied at −80 mV. B, Recovery in verapamil (500 nmol/L in the bath) at −50, −80, and −110 mV. C, Recovery in N-methyl-verapamil (2 μmol/L in the pipette) at −50, −80, and −110 mV. Fast (t1) and slow (t2) time constants of recovery and their amplitudes (A1, A2, calculated as relative current) are given at each recovery voltage.

**Verapamil and N-Methyl-Verapamil Unbind From HERG Channels During Repolarization**

HERG channels recover from verapamil or N-methyl-verapamil block on repolarization despite continued drug exposure. As shown in Figure 5A, a 100-millisecond step was applied to 60 mV to rapidly activate HERG current and was followed by
a 10-second conditioning step to 0 mV to obtain a steady level of drug block by verapamil (500 nmol/L). The membrane was then repolarized for a variable time to –50, –80, or –110 mV. A test pulse (step to 60 mV for 100 milliseconds and then to 0 mV for 200 milliseconds) was then applied to assess the recovery of HERG current from drug block. The peak amplitude of HERG current during the test pulse at 0 mV was normalized to that during the conditioning step at 0 mV, and peak amplitude is plotted as a function of the recovery time (∆t) in Figure 5B. At the onset of the recovery interval, the relative current was ∼25%, which represents the steady-state current remaining at the end of the preceding conditioning step (see Figure 5A). HERG current amplitude elicited with the test steps increased with longer recovery times as verapamil unbound from channels, and by 10 minutes of recovery ∼80% of HERG current could be elicited. The fraction of current that recovered was fit with 2 exponentials. At each recovery voltage, recovery from drug block contained a rapid component with similar time constants (τ1) of 0.9 to 1.1 seconds and amplitudes (A1) of 0.18 to 0.24. The slow-component time constant (τ2) showed prominent voltage dependence, increasing at more negative recovery voltages from 52 to 285 seconds with amplitudes (A2) of 0.28 to 0.39.

Data for the permanently charged N-methyl-verapamil (2 μmol/L in the pipette solution) analogue were obtained with the same voltage protocol and are shown in Figure 5C. Three to six cells were studied at each recovery voltage. Recovery of HERG current at –50 mV was fit by rapid (τ1) of 3.5 seconds) and slow (τ2 of 73.5 seconds) time constants with amplitudes of 0.19 and 0.43, respectively. At recovery voltages of –80 or –110 mV, the fitting procedure generated rapid time constants with very small amplitudes (A1 of 0.03), and recovery of HERG current occurred almost entirely through the slow time constant (A2 of 0.59 and 0.43, respectively). The slow-component time constant (τ2) also showed prominent voltage dependence, increasing at more negative recovery voltages from 74 to 338 seconds.

**Antagonism of Dofetilide Block of HERG Channels by Verapamil**

The methanesulfonanilide antiarrhythmic drug dofetilide blocks I_{Kr} and HERG channels in nanomolar concentrations, and it is thought to bind to the internal pore of the channel at a site that involves the serine residue at position 620.24 If verapamil and dofetilide compete for a common receptor site, then binding to the receptor by one drug should interfere with binding of the other drug. We tested whether verapamil could antagonize dofetilide binding. In these experiments, HERG channels were activated from the holding potential of –80 mV by a depolarizing step to 20 mV for 4 seconds, which was followed by a repolarizing step to –50 mV for 6 seconds to elicit tail current, and the protocol was repeated every 15 seconds. HERG tail current peak amplitude (normalized to the initial value) was plotted versus time, and the period of drug application is indicated by the horizontal line(s). In Figure 6A, the application of 10 μmol/L verapamil resulted in the complete block of HERG current. With 15 minutes of washout the tail current amplitude recovered to 89.6±2.4% (n=4 cells; see Figure 6D) of control. In Figure 6B, the application of 100 nmol/L dofetilide also resulted in the complete block of HERG current. In contrast to verapamil, recovery of HERG current after dofetilide washout was minimal, and at 15 minutes of washout only 7.4±2.9% (n=5 cells; see Figure 6D) of tail current amplitude had recovered. We then tested for antagonism of dofetilide binding by verapamil. As shown in Figure 6C, HERG current was first blocked by 10 μmol/L verapamil, and 100 nmol/L dofetilide was applied, as in Figure 6B. Dofetilide was then washed from the chamber, followed 2.5 minutes later by verapamil with the gradual recovery of HERG current. With 15 minutes of washout, the HERG tail current amplitude recovered to 51.9±7.8% (n=5 cells; see Figure 6D) of control (P<0.01 compared with dofetilide alone). Thus, pretreatment with verapamil prevented dofetilide binding and permitted the recovery of HERG current with drug washout. These results suggest that verapamil and dofetilide may compete for a common receptor site, although an allosteric interaction...
of block. In general, phenylalkylamines block native and use- and voltage-dependent properties, and wide ranges of IC₅₀ values have been reported for the concentration dependence of block. In general, phenylalkylamines block native and use- and voltage-dependent properties, and wide ranges of IC₅₀ values have been reported for the concentration dependence of block. In general, phenylalkylamines block native and use- and voltage-dependent properties, and wide ranges of IC₅₀ values have been reported for the concentration dependence of block. In general, phenylalkylamines block native and use- and voltage-dependent properties, and wide ranges of IC₅₀ values have been reported for the concentration dependence of block.

Ser631Ala (B) mutations. Data were fit with the Hill equation and give IC₅₀ values of 0.16 μmol/L for wild type, 3.46 μmol/L for Ser631Ala, and 71.09 μmol/L for Ser620Thr.

Discussion

Calcium Channel Antagonist Effects on Cardiac Ion Channels

Calcium channel antagonists block L-type Ca²⁺ channels with use- and voltage-dependent properties, and wide ranges of IC₅₀ values have been reported for the concentration dependence of block. In general, phenylalkylamines block native and cloned L-type Ca²⁺ channels with IC₅₀ values in a range of 250 nmol/L to at least 15.5 μmol/L. Benzothiazepines produce block in a similar concentration range, whereas dihydropyridines can produce more potent Ca²⁺ channel block. Our experiments with HERG K⁺ channels showed a different drug sensitivity pattern than that found for block of L-type Ca²⁺ channels and suggest that verapamil blocks of HERG K⁺ channels in a concentration range similar to that required for block of L-type Ca²⁺ channels.

Calcium channel antagonists are also known to block several K⁺ channels; however, block generally has required relatively high drug concentrations. For example, methoxyverapamil (D600) at concentrations of 2 to 10 μmol/L suppressed delayed rectifier K⁺ current (Iᵦ) in calf Purkinje fibers and cat ventricular cells, whereas in frog atrial cells Iᵦ was blocked by D600 but only at very high concentrations (IC₅₀=820 μmol/L). Verapamil blocked hKv1.5 K⁺ channels expressed in HEK 293 cells with an estimated Kᵦ of 21.1 μmol/L. Limited data are available for Iᵦ and HERG channels. In guinea pig ventricular myocytes, verapamil (1 μmol/L) was recently reported to suppress Iᵦ tail current by 49%, and in transfected COS cells some Ca²⁺ channel antagonists, including verapamil (EC₅₀=0.83 μmol/L), recently have been reported to suppress HERG current, although in these reports the mechanism of drug block was not examined. Our findings agree with these recent reports, although the IC₅₀ that we found for verapamil block of HERG tail current (143 nmol/L) is slightly lower, and diltiazem in higher drug concentrations completely blocked HERG channels in our experiments. These results provide a cellular mechanism for previous reports of action potential prolongation with low verapamil concentrations.

Characteristics of HERG Channel Block and Unblock by Verapamil

Our experiments showed that block of HERG channels by verapamil required channel activation, as drug binding did not occur to the rested, or closed, state. Verapamil also unbinds from HERG channels on repolarization to voltages close to the normal cardiac cell resting potential. As shown in Figure 4B, the tail current decay at –50 mV in verapamil was slower and crossed over the control tail current decay. A similar crossover occurred with N-methyl-verapamil suppression of HERG current (see Figure 2C). The crossover can be explained by time-dependent verapamil unbinding from...
HERG channels, with the drug unbound channels then opening before deactivating. Although the crossover has been ascribed to drug unbinding from open channels, drug unbinding from inactivated HERG channels that then recover through the open state also could cause a crossover. Our results do not directly distinguish between these possibilities. The finding that the rates of inactivation and recovery from inactivation are not altered by verapamil suggests that the crossover does not occur from changes in the kinetics of channel inactivation properties.

The recovery of HERG current from verapamil block was multiexponential, as shown in Figure 5. Because at physiological pH verapamil (pKa ≈ 8.8) exists in both neutral and charged forms, one possibility for this is that the rapidly recovering component represents the unbinding from the channel protein of the membrane-permeable neutral form of verapamil, whereas the slowly recovering component represents the charged moiety of verapamil trapped in the channel pore. This is supported by the observation that recovery of HERG current at −80 and −110 mV from block by the membrane-impermeable N-methyl-verapamil analogue is dominated by a slow time constant similar to that found with the slow recovery process obtained with verapamil. The presence of a rapid component of recovery at −50 mV with N-methyl-verapamil is consistent with rapid drug unbinding due to the increased probability of HERG channel gating at this voltage. The voltage dependence of the slow component of recovery can be explained by activation trapping of the charged moiety of verapamil and of N-methyl-verapamil in HERG channels after channel closure, with hyperpolarization favoring closure of the activation “gate.” In the trapping hypothesis, the activation gate must open before the charged drug can unbind, and less negative recovery potentials increase the likelihood that the activation gate will open transiently and release the charged drug. At more negative voltages the probability of channel opening is reduced, and the likelihood of drug remaining in the channel pore is increased. Comparable findings have been observed with tetraethylammonium and its derivatives, which block K⁺ channels, and with analogues of lidocaine and disopyramide, which block Na⁺ channels.

Verapamil unblocking after repolarization permits HERG channels to become available for opening. This process would be expected to be faster at more physiological temperatures and may be an important pharmacological property. Most other drugs that block HERG channels, including class III antiarrhythmic agents, show minimal unbinding even with drug washout. Thus, the frequency-dependent effects of verapamil on HERG current result from use-dependent drug binding during cell depolarization as well as from time- and voltage-dependent drug unbinding with repolarization.

Intracellular Location of Phenylalkylamine Binding Domain of HERG Channels

Several lines of evidence suggest that the verapamil binding domain is located at a site accessible from the inside of the cell. First, N-methyl-verapamil blocked HERG current only when applied intracellularly. Second, varying pH (6.4 to 8.4), which changes the proportion of drug in the membrane-permeable neutral form, altered block by extracellularly applied verapamil. Third, verapamil competes with the methanesulfonanilide antiarrhythmic drug dofetilide for block of HERG channels. Dofetilide is thought to bind to HERG channels near the internal mouth of the channel pore. In addition, the Ser620Thr mutation, which is near the internal mouth of the pore, decreased verapamil affinity 20-fold more than occurred with the Ser631Ala mutation, which is near the external mouth of the pore.

Our experiments show that verapamil binds with little or no affinity to the closed state, but with high affinity to the channel during depolarization when the channel is either open or inactivated. The Ser620Thr and Ser631Ala mutations interfere with C-type inactivation. The finding of reduced block by verapamil in these mutants supports the concept that C-type inactivation plays an important role in high-affinity drug binding to HERG channels. Previous reports, however, have suggested that the extent of C-type inactivation may not parallel drug sensitivity. Wang et al. reported that interfering with the development of C-type inactivation in HERG channels using high extracellular K⁺ did not have the same effect on drug affinity as did mutations that removed C-type inactivation. Ficker et al. showed that mutation to a serine residue in bovine EAG channels in a position equivalent to HERG Ser620 enhanced dofetilide binding but did not produce inactivation. Our data with the Ser620Thr and Ser631Ala mutations show that the Ser620Thr mutation decreased verapamil affinity for HERG channels 20-fold more than occurred with the Ser631Ala mutation. One explanation for our results is that the decrease in high-affinity drug binding found with the mutated channels may be caused indirectly by interfering with the channel protein conformation associated with intact C-type inactivation, which may then alter drug binding affinity or restrict drug access to its binding domain. In addition, the mutation to the serine in position 620 may decrease further verapamil binding by altering a residue directly involved in its binding site similar to that proposed for dofetilide. An alternative explanation is that incomplete inactivation, which has been reported for the Ser631Ala mutation, might account at least partially for its increased verapamil sensitivity. Clearly, structural information about the HERG channel and drug binding to it would help to resolve these questions. Finally, it should be recognized that the mechanism of HERG channel block by verapamil may not be identical for both the charged and uncharged forms of the drug molecule.

Clinical Significance

Verapamil exerts effects on cardiac arrhythmias that can be distinguished from those of other calcium channel antagonist drugs. Some forms of ventricular tachycardia are sensitive to verapamil, it prolongs human atrial refractoriness, and it may modify electrical remodeling in atrial fibrillation or rapid pacing. Although the antiarrhythmic efficacy of verapamil has usually been ascribed solely to its suppression of L-type Ca²⁺ channels, our findings suggest that block of HERG
channels also may contribute to the cardiac effects of verapamil.

Suppression of $I_{Kr}$ or HERG channels, prolongs action potential duration and increases the refractory period and thereby may terminate reentrant circuits.14 This also can lead to the development of long-QT syndrome.14 Verapamil, which does not cause long-QT syndrome, may counteract the potential of HERG channel block–induced QT prolongation and early afterdepolarization generation through its block of L-type Ca$^{2+}$ channels.42 Verapamil also unbinds from HERG channels at voltages near the resting potential, which may contribute to diminished HERG channel block. Finally, verapamil can cause bradycardia. Although this has been ascribed to block of inward Ca$^{2+}$ current, suppression of $I_{Kr}$ has also been reported to depress pacemaking.43

**Conclusion**

Verapamil potently blocks HERG K$^+$ channels expressed in HEK 293 cells. Diltiazem weakly blocks HERG current, whereas nifedipine does not exert an antagonist effect. Verapamil block was use and frequency dependent. Verapamil appears to enter the cell in its neutral form to bind to the channel protein at a site accessible from the inside of the cell. Although intact C-type inactivation seems important for highest-affinity verapamil block, block still can occur in the absence of apparent C-type inactivation, and the serine residue at position 620 may participate directly in verapamil binding. These findings suggest that verapamil shares HERG channel blocking properties with some newer class III antiarrhythmic drugs, and these findings provide new insights to explain some of the diverse effects exerted by Ca$^{2+}$ channel antagonist drugs on cardiac arrhythmias.

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