Suppression of electrical alternans by overexpression of HERG in canine ventricular myocytes

Fei Hua, David C. Johns, and Robert F. Gilmour, Jr.

1Department of Biomedical Sciences, Cornell University, Ithaca, New York 14853; and 2Department of Neurosurgery, Johns Hopkins School of Medicine, Baltimore, Maryland 21205

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Hua, Fei, David C. Johns, and Robert F. Gilmour, Jr. Suppression of electrical alternans by overexpression of HERG in canine ventricular myocytes. Am J Physiol Heart Circ Physiol 286: H2342–H2352, 2004.—Suppression of electrical alternans may be antiarrhythmic. Our previous computer simulations have suggested that increasing the rapid component of the delayed rectifier K+ current (Ikr) suppresses alternans. To test this hypothesis, Ikr in isolated canine ventricular myocytes was increased by infection with an adenovirus containing the gene for the pore-forming domain of Ikr [human ether-a-go-go gene (HERG)]. With the use of the perforated or whole cell patch-clamp technique, action potentials recorded at different pacing cycle lengths (CLs) were applied to the myocytes as the command waveforms. HERG infection markedly increased peak Ikr during the action potential (from 0.54 ± 0.03 pA/pF in control to 3.60 ± 0.81 pA/pF). Rate-dependent alterations of peak Ikr were similar for freshly isolated myocytes and HERG-infected myocytes. In both cell types, Ikr increased when CL decreased from 1,000 to 500 ms and then decreased progressively as CL decreased further. During alternans at CL = 170 ms, peak Ikr was larger for the short than for the long action potential for both groups, but the difference in peak Ikr was larger for HERG-infected myocytes. The voltage at which peak Ikr occurred was significantly less negative in HERG-infected myocytes, in association with shifts of the steady-state voltage-dependent activation and inactivation curves to less negative potentials. Pacing at short CL induced stable alternans in freshly isolated myocytes and in cultured myocytes without HERG infection, but not in HERG-infected myocytes. These data support the idea that increasing Ikr may be a viable approach to suppressing electrical alternans.

APD alternans without adversely affecting contractility. The human ether-a-go-go gene (HERG) has been identified as the gene that encodes the pore-forming domain of the Ikr channel (5, 20, 27, 31). Because at present there are no Ikr agonists available to test the effects of increasing Ikr on alternans, we infected isolated ventricular myocytes with an adenovirus expressing HERG to increase the corresponding Ikr.

HERG overexpression not only increased overall Ikr as expected, but also changed the kinetics of the current. The increase in Ikr was associated with suppression of APD alternans during pacing at rapid rates. To determine whether the suppressant effect of HERG overexpression on APD alternans resulted from increased channel conductance, altered channel kinetics, or a combination of the two, computer simulations were conducted using an updated model of Ikr developed from our experimental measurements.

METHODS

This investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23). Experiments were approved by the Institutional Animal Care and Use Committee of the Center for Research Animal Resources at Cornell University.

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Adult beagle dogs of either sex were anesthetized with Fatal-Plus (390 mg/ml pentobarbital sodium; Vortex Pharmaceuticals; 86 mg/kg iv), and their hearts were rapidly excised and placed in cold, aerated (95% O2-5% CO2) Tyrode solution. Endocardial myocytes were isolated by enzymatic dissociation (10–12, 24). The myocytes either were suspended in incubation buffer containing 1 mmol/l CaCl2 for patch-clamp recording on the same day or in a culture medium consisting of Eagle’s minimum essential medium supplemented with nonessential amino acids (1×), vitamins (2×), Na pyruvate (1×), insulin-transferrin-selenium A (1×), 5% fetal bovine serum, and 100 U/ml penicillin-streptomycin (all from Gibco-BRL). On the day of electrophysiological recordings, the myocytes were washed once with sterilized PBS (Gibco-BRL) and then covered with a trypsin-EDTA solution (0.05%, Sigma) until the cells were free floating, after which culture medium with serum was added to stop the trypsin activity.

Adenovirus preparation. Inducible gene expression was accomplished using a hybrid ecdysone receptor system, as described previously (10–12, 24). The adenovirus shuttle vector pAdE1I contains an ecdysone-inducible promoter and the sequence for the enhanced green fluorescent protein with the S65T point mutation (excitation wavelength 475 nm and emission wavelength 510 nm). The full-length coding sequence of HERG (kindly supplied by M. Keating, University of Utah, Salt Lake City, UT) was cloned into the multiple cloning sites of pAdEGI to generate pAdEGI-HERG. The hybrid ecdysone receptor DB-EcR was cloned into pAdCGI to make vector pAECDBEcR. The

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Address for reprint requests and other correspondence: R. F. Gilmour, Jr., Dept. of Biomedical Sciences, T7 012 VRT, Cornell Univ., Ithaca, NY 14853-6401 (E-mail: rfg2@cornell.edu).

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recombinant products were plaque purified, expanded, and purified on CsCl gradients, yielding concentrations of the order of 10^{10} plaque-forming units (pfu/ml).

**Infection of myocytes with adenovirus vector.** Freshly isolated myocytes were plated onto 10 × 35-mm culture dishes and cultured with 0.5 ml of culture medium containing ~10^8 pfu/ml of AdC-DBEcR and either AdEGI or AdEGI-HERG for 2 to 3 h at 37°C with gentle shaking every 30 min. Fresh culture medium (1.5 ml) and ponasterone A, an analog of ecdysone (10 μmol/l), prepared in 1,000× stock solution), were added to each dish after infection. The culture medium was replaced on the next day, and new ponasterone A was added to each dish. Myocytes infected with virus were identified by green fluorescence. On the first day of cell culture, only a few cells exhibited fluorescence, whereas on the second day of cell culture almost all of the living cells were fluorescent, but with different densities. The fluorescence density continued to increase on the third day of primary culture.

**Patch-clamp recordings.** For these studies, the myocytes were continuously superfused with aerated (with 95% O_2 -5% CO_2 ) and buffered (pH 7.4). Only rod-shaped, relaxed myocytes with clear striations were used for the study.

Both whole cell patch-clamp and perforated patch-clamp techniques were used. There were no significant differences in the currents measured using either technique. Consequently, the data obtained using both methods were pooled. For the perforated patch clamp, the pipette solution containing (in mmol/l) 132 NaCl, 4 KCl, 1 MgCl_2, 2 CaCl_2, 10 glucose, and 20 HEPES (pH 7.4). Only rod-shaped, relaxed myocytes with clear striations were used for the study.

Amphotericin B (0.21 mg/ml) was used as the pore-forming agent. For the whole cell patch clamp, the pipette solution contained (in mmol/l) 130 aspartic acid, 15 KCl, 2 MgCl_2, 1 CaCl_2, and 5 Mg·ATP, adjusted to pH 7.3 with 2 mmol/l KOH. The pipette tip resistance was 1–3 MΩ when filled with pipette solution. An Axopatch-1D amplifier (Axon Instruments) was operated in voltage-clamp mode. Data acquisition and analysis were performed using pCLAMP 8 (Axon Instruments). Currents were low-pass filtered at 2 kHz and sampled at 5.5 kHz. Membrane capacitance was measured at ~60 mV using the built-in function of pCLAMP 8. The junction potential was zeroed before the pipette touched the cell and was adjusted by 5 mV in the negative direction after the membrane was broken. Series resistance was <10 MΩ and was left uncompensated.

Nisoldipine (2 μmol/l) or nifedipine (5 μmol/l) was present in the extracellular solutions to block L-type Ca^{2+} current (I_{CaL}) for all of the I_{Kr} recordings. I_{Kr} was defined as the drug-sensitive current blocked by the specific I_{Kr} blocking agent E-4031 (5 μmol/l, Wako). For AP-clamp recording of I_{Kr}, unless otherwise specified, APs recorded previously at a pacing CL = 1,000, 500, 320, 170, and 120 ms from a strip of canine endocardial muscle were applied to cells as the command potentials. Each AP was applied six times, or, in the case of alternans, six pairs of long and short APs were applied. Current typically reached steady state during the third AP. The subtraction current traces after reaching steady state were averaged. During alternans, current traces were averaged for long and short APs separately. Baseline I_{Kr} was defined as the subtraction current immediately preceding the upstroke of the AP. Peak I_{Kr} was defined as the absolute amplitude of the maximal subtraction current during repolarization. Conductance (g_{Kr}) was calculated according to $g = I/(V_m - V_K)$, where $V_m$ is the membrane potential at which the current recording was obtained and $V_K$ is the potas-

Fig. 1. Rapidly activating component of the delayed rectifier K⁺ current (I_{Kr}) during action potential (AP) clamp. A–C: representative current traces measured as E-4031 (5 μmol/l)-sensitive current during AP clamp at cycle length (CL) = 500 ms in control (A), cultured (B), and human ether-a-go-go gene (HERG)-infected myocytes (C). The AP waveform is shown as an inset in A. D: representative I_{Kr} when the AP recorded from the same cell was used as the command waveform in a HERG-infected myocyte. The inset shows the AP waveform. Cell capacitances for A, B, and C were 200, 198, and 171 pF, respectively.
sium equilibrium potential \((V_K = -95 \text{ mV})\). \(I_{Ca}\) was measured as the nifedipine (5 \(\mu\text{mol/l}\))-sensitive current and was elicited using APs recorded from the same cell with current clamp as the command potential.

APs were recorded from isolated myocytes during perforated patch clamp using the Axopatch-1D amplifier in current-clamp mode. The myocytes were stimulated using trains of 15–40 stimuli of 2-ms duration and 2-nA intensity. The pacing CL was decreased progressively until 2:1 block occurred.

Data interpretation and analysis. Myocytes studied on the day of isolation are referred to as control myocytes. As reported in previous studies \((11, 14, 24)\), infection with AdEGI and AdC-DBEcR had no detectable effects on the measured currents (data not shown). Therefore, the data obtained from myocytes infected with AdEGI and AdC-DBEcR were pooled with the data obtained from myocytes cultured for 2–3 days without viral infection and are referred to as cultured myocytes. Myocytes infected with AdEGI-HERG and AdC-DBEcR were studied on the second or third day of culture, after the inducible HERG-mediated current had increased to maximal levels, and are referred to as HERG-infected myocytes. Because there were no significant differences in the results obtained from myocytes studied after 2 days of culture versus 3 days of culture (data not shown), data from the two groups of myocytes were pooled for analysis.

Data were fit using linear regression (Minitab, student version 12), exponential equations, or the Boltzmann equation \((\text{the Levenberg-Marquardt method; Clampfit 8, Axon Instruments})\). Statistical analysis of the data was performed using paired or unpaired \(t\)-tests or ANOVA coupled with Scheffe’s \(F\)-test, as appropriate. Data are presented as means \(\pm SE\). \(P < 0.05\) was considered significant.

### RESULTS

**Effects of HERG overexpression on \(I_{Kr}\).** Peak \(I_{Kr}\) density during AP clamp for CL = 500 ms was 0.54 \(\pm\) 0.03 pA/pF in control \((n = 26)\) and decreased to 0.17 \(\pm\) 0.02 pA/pF \((n = 8)\) after 2–3 days in culture in the absence of adenovirus expressing HERG. After infection with the adenovirus expressing HERG, peak \(I_{Kr}\) density increased markedly to 3.60 \(\pm\) 0.81 pA/pF \((n = 16)\). Examples of \(I_{Kr}\) from the three groups of myocytes during an AP at CL = 500 ms are shown in Fig. 1, A–C. In addition, APs recorded from HERG-infected myocytes were applied to the same cell as the command waveform. \(I_{Kr}\) during one of these APs is shown in Fig. 1D.

Figure 2A shows representative \(I_{Kr}\) tracings during APs at CL = 500, 170, and 120 ms in control and HERG-infected myocytes. In both types of myocytes, \(I_{Kr}\) increased progressively throughout the AP, reaching a peak during terminal repolarization. However, the voltage at which peak \(I_{Kr}\) occurred was significantly less negative for HERG-infected myocytes than for control myocytes \((-34.8 \pm 1.4 \text{ vs. } -55.0 \pm 0.9 \text{ mV at CL = 500 ms; } n = 15 \text{ and 26, respectively})\).

Peak conductance for \(I_{Kr}\) calculated at the voltage at which peak \(I_{Kr}\) occurred, increased significantly from a mean value of 0.0148 \(\pm\) 0.0009 ms/\(\mu\text{F} in control myocytes to 0.0576 \(\pm\) 0.011 ms/\(\mu\text{F in HERG-infected myocytes at CL = 500 ms.}

\(I_{Kr}\) densities at different CLs were normalized by the peak current at CL = 1,000 ms for each myocyte. The relationships

![Graphs and diagrams showing data for HERG overexpression effects on \(I_{Kr}\)](image_url)
between normalized peak $I_{Kr}$, normalized baseline $I_{Kr}$, and CL in HERG-infected myocytes were compared with control myocytes (Fig. 2, A and C). In HERG-infected myocytes, changes in both peak $I_{Kr}$ and baseline $I_{Kr}$ were similar to those in control myocytes. Decreasing the CL from 1,000 to 500 ms produced a small but significant increase of peak $I_{Kr}$ from 1.00 to 1.02 ± 0.01. As CL was decreased further, peak $I_{Kr}$ continuously decreased, until at CL = 120 ms peak $I_{Kr}$ was ~70% of the peak $I_{Kr}$ at CL = 1,000 ms. Baseline $I_{Kr}$ was not significantly different at CL = 1,000, 500, and 320 ms but increased at CL = 170 and 120 ms. At CL = 120 ms, baseline $I_{Kr}$ increased to ~17% of peak $I_{Kr}$ at CL = 1,000 ms.

At the CL where APD alternans was maximal (CL = 170 ms), normalized peak $I_{Kr}$ was significantly smaller during the long AP than during the short AP in both HERG-infected myocytes (0.73 ± 0.02 vs. 0.85 ± 0.02) and control myocytes (0.81 ± 0.02 vs. 0.86 ± 0.01). However, normalized peak $I_{Kr}$ during the long AP was smaller for HERG-infected myocytes than for control myocytes, whereas there was no difference during the short AP, resulting in a greater difference in peak $I_{Kr}$ during APD alternans for HERG-infected myocytes. At CL = 120 ms, where APD alternans was small, normalized peak $I_{Kr}$ still was significantly different between the long and short APs in HERG-infected myocytes (0.70 ± 0.02 vs. 0.71 ± 0.02), whereas no significant difference in peak $I_{Kr}$ was detectable in the control myocytes, possibly because the amplitude of the difference was too small.

**Altersations of $I_{Kr}$ kinetic properties in HERG-infected myocytes.** In addition to a larger magnitude, $I_{Kr}$ recorded from HERG-infected myocytes appeared to exhibit altered kinetics compared with $I_{Kr}$ recorded from control myocytes (Fig. 2A). Therefore, additional experiments were conducted to characterize selected aspects of $I_{Kr}$ kinetics, including the steady-state voltage dependence of activation and inactivation and the time constants of activation and deactivation. The reversal potential also was determined.

The voltage dependence of steady-state $I_{Kr}$ activation was measured using tail currents elicited by an “activation protocol,” which consisted of a depolarizing step from −85-mV holding potential to −40 mV to inactivate sodium current and a second 500-ms duration test pulse from −40 mV to voltages ranging from −30 to +40 mV in 10-mV steps, followed by repolarization to −40 mV, where tail currents were elicited (Fig. 3A). As expected from the results obtained using AP clamp, $I_{Kr}$ tail currents elicited by +40-mV test voltages were significantly increased in HERG-infected myocytes compared with control myocytes (7.3 ± 1.5 vs. 0.72 ± 0.04 pA/pF). Similarly, $I_{Kr}$ tail currents were reduced significantly in cultured myocytes without HERG infection compared with control myocytes (0.37 ± 0.05 vs. 0.72 ± 0.04 pA/pF). Figure 3A illustrates typical currents in a HERG-infected myocyte. All of the tail currents were normalized by the tail current elicited by a +40-mV test pulse for each cell. The steady-state activation curves were plotted for both control myocytes and HERG-infected myocytes (Fig. 3B), where the data were fit to the Boltzmann equation. The steady-state activation curve for HERG-infected myocytes was shifted to less negative voltages [half-activation potential ($V_{1/2}$) = −3.4 ± 0.8 mV, open circle] compared with control myocytes ($V_{1/2}$ = −7.5 ± 1.8 mV, filled circle). The slope factors for both groups were not significantly different (6.9 ± 0.2 mV for HERG-infected myocytes and 6.3 ± 1.0 mV for control myocytes, $P = 0.43$).

The reversal potential for $I_{Kr}$ was determined by measuring tail currents elicited by repolarization to a range of test potentials from −20 to −100 mV after a 250-ms duration pulse to +20 mV in the “deactivation protocol.” Figure 4A shows typical current traces from a HERG-infected myocyte. Data were normalized using the tail current obtained with a +40-mV test pulse in the activation protocol (Fig. 3A). There was no significant difference between the reversal potential of control myocytes (−86.1 ± 1.7 mV) and HERG-infected myocytes (−86.2 ± 0.8 mV).

Figure 4B shows the fully activated current-voltage relation for $I_{Kr}$ in control and HERG-infected myocytes. For potentials less than or equal to −20 mV, $I_{Kr}$ was determined as the tail current elicited by the deactivation protocol (Fig. 4A). For potentials greater than or equal to −10 mV, $I_{Kr}$ was determined as the current at the end of a depolarizing test pulse during the activation protocol (Fig. 3A). All currents were normalized by the tail current elicited by a +40-mV depolarization in the activation protocol for each cell. The current-voltage relationship was linear at voltages more negative than −60 mV for control myocytes and at voltages more negative than −50 mV for HERG-infected myocytes.
The voltage dependence of $I_{Kr}$ inactivation was determined from the fully activated current-voltage relationship. A line was fit to the linear part of the relationship, which represented the fully activated $I_{Kr}$ ($I_{Kr^*}$) predicted to occur in the absence of inactivation. The ratio of fully activated $I_{Kr}$ with and without inactivation ($I_{Kr}/I_{Kr^*}$) at any given voltage represented the voltage-dependent inactivation. The relationship between $I_{Kr}/I_{Kr^*}$ and voltage was fit using the Boltzmann equation (Fig. 4C). Compared with control myocytes, HERG overexpression shifted the $V_{1/2}$ to a less negative voltage ($-10.3 \pm 1.3$ vs. $-24.4 \pm 3.8$ mV). The slope factors were not significantly different for the two types of myocytes ($-14.0 \pm 0.6$ vs. $-17.2 \pm 4.3$ mV for HERG-infected and control myocytes, respectively).

The time constants for deactivation at $-40$ and $-85$ mV were determined using two different types of protocols. The time constant for deactivation at $-40$ mV was determined by fitting the decay of the tail current shown in Fig. 3A to a biexponential function. For control myocytes, the slow time constant of deactivation was 413 $\pm$ 33 ms and the fast time constant was 47 $\pm$ 4 ms ($n = 10$). Deactivation time constants for HERG-infected myocytes tended to be smaller ($381 \pm 10$ ms and $40 \pm 2$ ms, $n = 14$), but the difference was not statistically significant.

Because $I_{Kr}$ was very small at $-85$ mV, secondary to the small electrical driving force, the time constant for deactivation at $-85$ mV was determined using a two-pulse protocol (Fig. 5A). The myocyte initially was depolarized from a holding potential of $-85$ to $+20$ mV for 100 ms to activate $I_{Kr}$, after which the membrane potential returned to $-85$ mV. After a time interval ranging from 2 to 162 ms (in 20-ms increments), a second pulse was given. During the second pulse, the myocyte was depolarized to $+20$ mV for 15 ms, followed by a repolarizing ramp with a slope of $-1.5$ mV/ms. For short intervals between the two pulses, deactivation was incomplete, resulting in more accumulation of $I_{Kr}$ and hence a larger current during the repolarizing ramp of the second pulse. The decay of the peak current was fit to single-exponential function. The time constant for deactivation at $-85$ mV was significantly larger in control myocytes than in HERG-infected myocytes ($34 \pm 6$ ms, $n = 7$, vs. $19 \pm 2$ ms, $n = 13$).

The activation time constant at $+20$ mV was measured using the protocol shown in Fig. 5B. From a holding potential of $-85$ mV, a depolarizing step to $+20$ mV ranging in duration from 20 to 180 ms (in 20-ms increments) was followed by a repolarizing ramp to the holding potential with a slope of $-1.5$ mV/ms. The peak current during the ramp increased with longer depolarizing pulses and was fit using a single-exponential function. The resulting time constants were similar for freshly isolated myocytes ($45 \pm 9$ ms, $n = 5$) and HERG-infected myocytes ($48 \pm 5$ ms, $n = 7$).

**Computer model of $I_{Kr}$**. Ordinary differential equation computer models of $I_{Kr}$ were created for both control and HERG-infected myocytes based on the experimentally determined kinetics (see the Appendix). Specifically, the steady-state voltage dependence of activation and inactivation for HERG-infected myocytes were shifted to less negative voltages compared with control myocytes, whereas the activation and inactivation time constants were not changed (Fig. 6, A–D). Driving the models with the same APs used in the experiments (as shown in Fig. 2) produced similar differences in current...
traces for control and HERG-infected myocytes (Fig. 6, E and F).

A computer model of the AP for a single canine myocyte (7) subsequently was used to study the effects of changing $I_{Kr}$ in HERG-infected myocytes on APD alternans. The model using $I_{Kr}$ from control myocytes exhibited stable APD alternans at short CLs (Fig. 6G). After incorporation of $I_{Kr}$ from HERG-infected myocytes, APD alternans was absent at all CLs. Similar results were obtained experimentally, as described in the next section.

**Effects of HERG overexpression on APD alternans.** To determine the effects of HERG overexpression on APD alternans, isolated myocytes were stimulated at decreasing CLs until 2:1 block was observed. Figure 7 shows examples of APs at different CLs and the relationship between APD and CL. In the control myocyte (Fig. 7A), APD alternans at CL = 230 ms occurred at positive diastolic intervals (DIs) for both the long and short APs. Decreasing CL to 150 ms increased the amplitude of APD alternans. In addition, the DI for the long AP became negative. Overall, in freshly isolated myocytes, transient APD alternans at the beginning of each new CL occurred in all myocytes. Stable negative DI alternans was observed in 4 of 17 myocytes and stable positive DI alternans was observed in 9 of 17 myocytes.

APD at 90% of repolarization (APD$_{90}$) was prolonged at CL = 1,000 ms in myocytes cultured for 2–3 days [from 273 ± 12 ms in control myocytes ($n = 17$) to 675 ± 65 ms in cultured myocytes ($n = 7$)]. The cultured myocytes still exhibited APD alternans at short CLs (Fig. 7B). Overexpressing HERG dramatically shortened APD$_{90}$ to 77 ± 9 ms at CL = 1,000 (APD ranged from 36 to 130 ms; $n = 16$; Fig. 7C). During pacing at decreasing CLs, HERG-infected myocytes did not exhibit stable APD alternans at any CL except for one myocyte in which APD alternans with very negative DI occurred.

$I_{Ca}$ in myocytes overexpressing HERG. Alteration of $I_{Ca}$ has been reported in cultured myocytes. To determine whether $I_{Ca}$ might be reduced during APs in cultured myocytes and whether such a reduction might account, at least in part, for suppression of APD alternans in HERG-infected myocytes, $I_{Ca}$ alternans was studied in control, cultured, and HERG-infected myocytes. Examples of $I_{Ca}$ in control, cultured, and HERG-infected myocytes are shown in Fig. 8. Compared with control myocytes, in which $I_{Ca}$ was 1.12 ± 0.1 pA/pF ($n = 10$), HERG-infected myocytes ($n = 5$) and cultured myocytes ($n = 7$) had reduced magnitudes of $I_{Ca}$ (0.88 ± 0.05 and 0.74 ± 0.08 pA/pF, respectively).
Although HERG-infected myocytes tended to have a larger $I_{Ca}$ than cultured myocytes, the difference was not statistically significant.

**DISCUSSION**

The results of this study indicate that 1) after 2–3 days in primary culture, $I_{Kr}$ density in isolated canine ventricular myocytes (CVMs) decreased significantly; 2) infecting isolated myocytes with adenovirus encoding the HERG gene markedly increased peak $I_{Kr}$ density; 3) the rate dependency of peak $I_{Kr}$ in HERG-infected myocytes was similar to that in control myocytes except that the difference in peak $I_{Kr}$ during APD alternans was larger; 4) overexpression of HERG shifted voltage-dependent activation and inactivation to less negative membrane potentials without altering the slopes of these functions and the time constants for activation and inactivation; 5) the changes in $I_{Kr}$ morphology during the AP in HERG-infected myocytes could be explained by changes in $I_{Kr}$ kinetics; 6) increasing $I_{Kr}$ in myocytes suppressed APD alternans; and 7) $I_{Ca}$ was reduced with time in culture, but increasing $I_{Kr}$ did not decrease $I_{Ca}$ further.

Alterations of $I_{Kr}$ in HERG-infected myocytes. On the basis of studies using molecular, genetic, and physiological techniques, HERG has been identified as the gene encoding the pore-forming domain of $I_{Kr}$ (5, 31). Although currents resulting from expression of HERG in various expression systems are similar to endogenous $I_{Kr}$, they differ in their gating, single channel conductance, and sensitivity to specific $I_{Kr}$ blockers (1, 3, 21, 22, 35, 38, 39), which suggests that coassembly of HERG with additional subunit(s) (e.g., KCNE1 and KCNE2) may be required to form the native channel (1, 6, 22). In our experiments, several properties of $I_{Kr}$, in addition to current magnitude, were altered in HERG-infected myocytes compared with freshly isolated myocytes. If coassembly of a β-subunit with the HERG-encoded α-subunit is necessary to recapitulate native $I_{Kr}$, selected overexpression of HERG might have altered the stoichiometry between α- and β-subunits and thereby altered the voltage-dependent properties of $I_{Kr}$.

The differences between the electrical properties of $I_{Kr}$ in HERG-infected and freshly isolated myocytes also may have resulted from factors other than the absence of a β-subunit. For example, because we expressed the $I_{Kr}$ gene for the human heart in canine myocytes, alterations of the expressed $I_{Kr}$ might reflect a species-dependent variation in channel properties. However, the canine $I_{Kr}$ gene (cERG) reportedly has a very high homology to the human gene (97%), and the currents recorded in *Xenopus* oocytes expressing cERG channels had functional properties very similar to HERG (36). Changes in current kinetics also may have resulted from coassembly of ERG with alternative splice variants of ERG1 (18–20), although recent biochemical studies have suggested that only the full-length ERG1 is expressed in the adult mouse, rat, or human heart (27). Finally, posttranslational processing mechanisms and phosphorylation cascades that regulate $I_{Kr}$ (2, 4, 16) may be abnormal in these cultured myocytes, leading to altered properties of the expressed HERG channel.

**Ionic mechanism for APD alternans.** Studies using an ionic model for CVMs have indicated that the development of APD alternans is caused primarily by incomplete recovery of $I_{Ca}$ from inactivation at short DIs (7). In the present study, $I_{Ca}$ was
measured during each myocytes’ own AP rather than during a standard square-wave pulse protocol to account for differences in $I_{Ca}$ that might result from the marked differences in AP morphology between freshly isolated myocytes, cultured myocytes, and HERG-infected myocytes. $I_{Ca}$ was reduced by ~20% in HERG-infected myocytes, which potentially could account for the suppression of alternans in those cells independent of increased $I_{Kr}$. However, $I_{Ca}$ was reduced to the same extent in noninfected myocytes after 2–3 days in culture, yet they still displayed APD alternans, most likely because the decrease in $I_{Ca}$ was accompanied by a reduction of outward current, $I_{Kr}$ in particular. The observation that APD alternans persisted in noninfected myocytes, despite a reduction of $I_{Ca}$, indicates that suppression of APD alternans in HERG-infected myocytes could not be attributed to reduced $I_{Ca}$.

Studies using the CVM computer model also have shown that increasing outward repolarizing currents such as $I_{Kr}$, the slowly activating component of the delayed rectifier K+ current, and the inward rectifier K+ current $I_{K1}$ shortens APD, which prolongs the DI and allows $I_{Ca}$ to recover more fully before the onset of the next AP (7). As a result, the magnitude of APD alternans is reduced. This observation suggests that increasing outward current might be a viable approach to suppressing APD alternans. The present study is the first to test and verify this idea experimentally.

**Effects of altering $I_{Kr}$ on APD alternans.** Overexpression of HERG was accompanied by a shortening of APD, as expected from previous studies by Johns and coworkers (11, 24). In those studies, however, the refractory period was prolonged, despite the reduction of APD. This observation likely resulted from the use of relatively low stimulus current strengths to determine the refractory period, in that increased outward current during terminal repolarization, secondary to HERG overexpression, apparently offset the depolarizing stimulus current and the membrane potential failed to reach threshold. An inability to generate APs at short DIs would be expected, in and of itself, to suppress APD alternans, given that the steep portion of the APD restitution relation occurs at such intervals. In the present study, larger stimulus current strengths were used, which resulted in the generation of APs at short DIs. Even at these short DIs, APD alternans did not occur and the slope of the APD restitution relation did not exceed 1.

The increase in peak $I_{Kr}$ in HERG-infected myocytes may have resulted from an increase in conductance, a change in current kinetics, or both. With respect to the kinetics of $I_{Kr}$, the shift of the voltage-dependent activation curve to less negative
voltages would be expected to decrease peak $I_{Kr}$, whereas the shift of the voltage-dependent inactivation curve to less negative voltages should increase peak $I_{Kr}$. On the basis of our computer simulations, the net effect of the kinetic changes in HERG-infected myocytes was an increase in peak $I_{Kr}$ by 33% (from 0.54 to 0.72 pA/pF). However, achieving the same increase in peak $I_{Kr}$ in the computer simulations as in the experiments required an increase in the conductance of $I_{Kr}$ from 0.0355 to 0.1779 mS/μF. This nearly fourfold increase in the conductance of $I_{Kr}$ was similar to the fourfold increase in peak $I_{Kr}$ conductance observed experimentally, indicating that the increase in $I_{Kr}$ in HERG-infected myocytes was caused primarily by an increase in the number of channels.

The computer simulations also indicated that APD alternans was especially sensitive to increased $I_{Kr}$ resulting from changes in voltage-dependent inactivation (13). Shifting voltage-dependent inactivation to less negative membrane potentials by as little as 5 mV abolished APD alternans, with only a modest shortening of APD. Given that voltage-dependent inactivation in the HERG-infected myocytes was shifted by $\sim$14 mV, changes in peak $I_{Kr}$ resulting from this change in kinetics would be sufficient to suppress APD alternans, independent of augmented conductance. If the change in kinetics in HERG-infected myocytes was caused by alterations of the stoichiometry between α- and β-subunits, it might be possible to achieve a similar change of kinetics by transfecting antisense oligonucleotides against one or more β-subunits to suppress the expression of those β-subunits. The change of $I_{Kr}$ resulting from suppressing β-subunit expression alone might then be sufficient to suppress APD alternans, without producing marked changes in APD at long CLs.

Limitations. The primary limitation of the study is that the effects of HERG overexpression were determined in cultured, as opposed to freshly isolated, myocytes. Because we used the ecdysone expression system, the ecdysone receptor needed to be expressed first by the DBEeCR gene to stimulate expression of the HERG gene. As a result, increased levels of $I_{Kr}$ were not detected until at least the second day of cell culture, by which time the magnitudes of $I_{Ca}$, $I_{K1}$ had declined. It has been well established that these and other currents change over time in cultured adult cardiac myocytes (23, 25, 32, 37). Because we did not monitor changes in every current, it
remains possible (albeit unlikely) that alterations of currents other than \(I_{Kr}\) contributed to the suppression of APD alternans in HERG-infected myocytes. In addition, altered regulation of ion channel gene expression induced by the conditions of cell culture may have affected HERG expression, particularly with respect to the coexpression of \(I_{Kr}\) channel subunits. Resolving this issue would require measuring HERG and candidate \(\beta\)-subunit levels in the cultured myocytes, which was beyond the scope of the present study.

A more general limitation of the study is that pronounced shortening of APD, as occurred after overexpression of HERG, and the attendant shortening of the refractory period could predispose to the development of tachyarrhythmias. In addition, the variability of HERG expression observed in the isolated myocytes might increase heterogeneity of refractoriness in the intact heart and promote the development of arrhythmias by that mechanism. It seems possible, however, based on our previous computer modeling studies (7) that a more modest increase of \(I_{Kr}\) in an intact heart (where \(I_{Ca}\) would be maintained at normal levels) might suppress alternans without producing marked changes in APD. Furthermore, increased intrinsic heterogeneity, secondary to variability in the expression levels of HERG, might be ameliorated by the electrotonic interactions that occur in an intact syncitium. Nevertheless, the potential drawbacks of increasing \(I_{Kr}\) as a mechanism of suppressing ventricular tachyarrhythmias will need to be critically evaluated in future studies of multicellular preparations and intact hearts before such an approach can be tested clinically.

APPENDIX

\(I_{Kr}\) was modified from the CVM model (7). The equations for steady-state voltage-dependent open probabilities of the activation and inactivation gates were taken from the experimentally measured data fit to the Boltzmann equation. The maximum conductances were adjusted so that the peak \(I_{Kr}\) during the action potential at \(CL = 500\) ms was consistent with the experimental data.

For freshly isolated myocytes, steady-state voltage-dependent open probabilities of the activation and inactivation gates (\(X_{Kr}^{oc}\) and \(Y_{Kr}^{oc}\)) and the maximum conductance (\(G_{Kr}\)) were

\[
X_{Kr}^{oc} = \frac{1}{1 + e^{-\frac{v - d_{Kr}^{oc}}{1.5}}}
\]

\[
Y_{Kr}^{oc} = \frac{1}{1 + e^{\frac{v - d_{Kr}^{oc}}{1.5}}}
\]

\[
\tilde{G}_{Kr} = 0.0355 \text{ mS/\mu F}
\]

where \(v\) is voltage.

For HERG-infected myocytes, steady-state voltage-dependent open probabilities of the activation and inactivation gates and the maximum conductance (\(\tilde{G}_{Kr}\)) were

\[
X_{Kr}^{oc} = \frac{1}{1 + e^{-\frac{v - d_{Kr}^{oc}}{1.2}}}
\]

\[
Y_{Kr}^{oc} = \frac{1}{1 + e^{\frac{v - d_{Kr}^{oc}}{1.2}}}
\]

\[
\tilde{G}_{Kr} = 0.1779 \text{ mS/\mu F}
\]

The time constants for activation and inactivation (\(\tau_{x_{Kr}}\) and \(\tau_{y_{Kr}}\)) were the same for two groups of cells.

\[
\tau_{x_{Kr}} = 20.0 + \frac{1}{e^{-\frac{v - 4.1}{0.05}} + e^{-\frac{v - 4.3}{0.05}}}
\]

\[
\tau_{y_{Kr}} = \frac{1}{e^{-\frac{v - 0.1}{0.05}} + e^{-\frac{v - 0.02}{0.05}}}
\]

where \(V\) is voltage.

\(I_{Kr}\) was calculated using the following equations

\[
i_{Kr} = \tilde{G}_{Kr}X_{Kr}Y_{Kr} \left( \left[ K^{+} \right] / [4(V - E_{Kr})] \right)
\]

\[
\frac{dX_{Kr}}{dt} = \frac{X_{Kr}^{oc} - X_{Kr}}{\tau_{x_{Kr}}}
\]

\[
\frac{dY_{Kr}}{dt} = \frac{Y_{Kr}^{oc} - Y_{Kr}}{\tau_{y_{Kr}}}
\]

where \([K^{+}]_{o}\) is extracellular \(K^{+}\) concentration, \(E_{Kr}\) is the reversal potential for \(I_{Kr}\), and \(X_{Kr}\) and \(Y_{Kr}\) are the open probabilities of the activation and inactivation gates.

To obtain the same amplitude of APD alternans as in the original CVM model, \(P_{Ca}\), the maximum \(Ca^{2+}\) permeability was increased to 0.000028 cm/ms. All of the other equations and parameters were unchanged from the original CVM model.

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