A Major Role for hERG in Determining Frequency of Reentry in Neonatal Rat Ventricular Myocyte Monolayer

Luqia Hou,* Makarand Deo,* Philip Furspan, Sandeep V. Pandit, Sergey Mironov, David S. Auerbach, Qiuming Gong, Zhengfeng Zhou, Omer Berenfeld, José Jalife

Rationale: The rapid delayed rectifier potassium current, I_{Kr}, which flows through the human ether-a-go-go-related (hERG) channel, is a major determinant of the shape and duration of the human cardiac action potential (APD). However, it is unknown whether the time dependency of I_{Kr} enables it to control APD, conduction velocity (CV), and wavelength (WL) at the exceedingly high activation frequencies that are relevant to cardiac reentry and fibrillation.

Objective: To test the hypothesis that upregulation of hERG increases functional reentry frequency and contributes to its stability.

Methods and Results: Using optical mapping, we investigated the effects of I_{Kr} upregulation on reentry frequency, APD, CV, and WL in neonatal rat ventricular myocyte (NRVM) monolayers infected with GFP (control), hERG (I_{Kr}), or dominant negative mutant hERG G628S. Reentry frequency was higher in the I_{Kr}-infected monolayers (21.12±0.8 Hz; n=43 versus 9.21±0.58 Hz; n=16; \( P<0.001 \)) but slightly reduced in G628S-infected monolayers. APD_{80} in the I_{Kr}-infected monolayers was shorter (>50%) than control during pacing at 1 to 5 Hz. CV was similar in both groups at low frequency pacing. In contrast, during high-frequency reentry, the CV measured at varying distances from the center of rotation was significantly faster in I_{Kr}-infected monolayers than controls. Simulations using a modified NRVM model predicted that rotor acceleration was attributable, in part, to a transient hyperpolarization immediately following the AP. The transient hyperpolarization was confirmed experimentally.

Conclusions: hERG overexpression dramatically accelerates reentry frequency in NRVM monolayers. Both APD and WL shortening, together with transient hyperpolarization, underlies the increased rotor frequency and stability. (Circ Res. 2010;107:1503-1511.)

Key Words: hERG ■ delayed rectifier potassium channel ■ reentry ■ ventricular fibrillation ■ optical mapping

Ventricular fibrillation (VF) is a major cause of sudden cardiac death. Evidence suggests that, in mammalian species ranging in size from the mouse to human, VF may be maintained by highly periodic reentrant waves called rotors.\(^1,2\) The spiral waves that emanate from high-frequency rotors propagate through the ventricles and undergo intermittent, spatially distributed wavebreaks, resulting in complex patterns of conduction also known as fibrillatory conduction.\(^1,3\) Although the existence of rotors has been known for years, the ionic mechanisms responsible for their behavior remain incompletely understood. Recently, it was shown that the frequency and the stability of rotors are determined in great measure by the resting membrane potential (RMP) and the conduction velocity (CV), which are controlled by the dynamic interplay between the outward component of the inward rectifier potassium current (I_{K1}) and the rapid inward sodium current (I_{Na}).\(^4\) Other experiments have demonstrated that overexpression of the slow delayed rectifier current (I_{Ks}) does not affect reentry frequency but significantly increases the incidence of fibrillatory conduction in cardiomyocyte monolayers.\(^5\) However, the consequences of upregulation or gain of function in the rapid delayed rectifier current (I_{Kr}) on reentry frequency and dynamics has never been directly investigated either in the heart or any model system.

The human ether-a-go-go-related (hERG) potassium channel responsible for I_{Kr} is important in determining the shape and duration of the human cardiac action potential (AP).\(^6\) I_{Kr} suppression results in AP duration (APD) prolongation,
were plated in 35-mm tissue culture dishes at a density of 1×10^6 cells/dish for monolayers and at low density on 25-mm cover slips for single-cell patch-clamp experiments. To facilitate cardiomyocyte attachment, dishes were coated with human placental collagen type IV (Sigma). Cells were subsequently cultured at 37°C, 5% CO_2.

**Adenoviral Construct**

We generated adenoviral constructs containing wild-type (WT) or G628S cDNA sequence of hERG tagged with GFP, using the AdEasy vector system (Stratagene, La Jolla, Calif) as described previously^10^ and in the Online Data Supplement.

**Electrophysiology**

In whole-cell patch-clamp experiments, the bath solution was HBSS with Ca^{2+} and Mg^{2+} (Sigma); pH was adjusted to 7.4 with NaOH. Nifedipine (5 μmol/L) and 4-AP (2 mmol/L) were added to reduce extraneous ion currents. The pipette solution contained (in mmol/L): 1 MgCl_2, 5 EGTA, 150 KCl, 5 HEPES, 5 phosphocreatine, 4.5 K_2ATP, 2 mmol/L B-hydroxybutyric acid; pH was adjusted to 7.2 with KOH. Recordings were carried out at 37°C using a MultiClamp 700B amplifier (Axon Instruments, Forest City, Calif). After gigaseal formation and patch break, the tip potential was nullled and cell capacitive currents and series resistance were optimized (~80%) compensated. I_Kr currents were elicited with 0.5-second depolarizing steps applied in 10-mV increments from a holding potential of −60 to +50 mV. I_Kr was derived by subtracting currents recorded in the presence of E4031 (1 μmol/L) from control currents.

APs were recorded from individual myocytes using the current-clamp mode of the MultiClamp 700B amplifier after gigaseal formation and patch break. Stimulus pulses (1- to 2-ms duration) were generated using a World Precision Instruments DS8000 stimulator. The bath solution was HBSS with Ca^{2+} and Mg^{2+} (Sigma). The pipette solution contained (in mmol/L): 1 MgCl_2, 5 EGTA, 150 KCl, 5 HEPES, 5 phosphocreatine, 4.5 K_2ATP, 2 mmol/L B-hydroxybutyric acid. Recordings were carried out at 37°C.

**Optical Mapping**

High-resolution optical mapping was conducted using a charge-coupled device camera (80×80 pixels, SciMeasure). Cultured dishes were placed on a heating chamber connected and maintained at 37°C. Monolayers were superfused with HBSS (in mmol/L): 1.6 CaCl_2, 5.4 KCl, 0.8 MgSO_4, 0.4 KH_2PO_4, 4.2 NaHCO_3, 136.9 NaCl, 0.3 NaHPO_4, 5.5 d-glucose, and 10 HEPES; pH 7.4. Electric wave propagation was recorded by staining the cells with 40 μmol/L di-8-ANEPPS (Molecular Probes) for 15 minutes. We obtained 5-second movies at 200 frames per second and 2-second movies at 500 frames per second.

**Data Analysis**

Please refer to the Online Data Supplement for pacing protocols, patch-clamp data analysis, and optical mapping data processing and analysis.^11^,12

**Computer Simulations**

We have modified an existing mathematical model of the neonatal rat myocyte published by Korhonen et al.^13^ This model is unique in that the cytosolic Ca^{2+} is a function of temporal, as well as spatial coordinates. Because of the lack of transverse tubules,^14^ calcium ions entering into the cells via membrane channels must diffuse a small distance through the cytosol before reaching the sarcoplasmic reticulum and trigger the excitation-contraction coupling machinery. Some ionic current components were modified to reproduce the AP morphology and the restitution properties recorded from the experiments performed in our laboratory. (Details are given in the Online Data Supplement.)

**Statistical Analysis**

Data are expressed as means±SEM. Patch-clamp data were analyzed by 2-way ANOVA with Bonferroni post tests. Analyses of reentry
frequency were performed using 1-way ANOVA with Tukey’s multiple comparison test. A Student’s *t* test with Welch correction was used to analyze the average APD and CV, as well as the maximal diastolic potential (MDP) data. *P*<0.05 was considered to be significant.

### Results

**hERG Overexpression Upregulates *I*<sub>Kr</sub> in NRVMs**

At 200 or 30 multiplicities of infection of adenoviral expression, we observed maximal GFP-tagged WT or G628S mutant hERG channel expression in NRVM monolayers. Spatially uniform protein expression was consistently confirmed by fluorescent microscopy as illustrated in Figure 1A.

Figure 1B (top) illustrates the voltage-clamp protocol (see Methods) used to determine the voltage dependence of *I*<sub>Kr</sub> density. The same panel shows superimposed representative *I*<sub>Kr</sub> traces recorded from single NRVMs infected with Ad-GFP (middle) or Ad-hERG (bottom). The K<sup+</sup>-carried *I*<sub>Kr</sub> current was appreciably increased in the Ad-hERG–infected myocytes when compared to control. As summarized in Figure 1C, Ad-hERG–infected cells have a significantly steeper current density versus voltage relationship than Ad-GFP control. At 0 mV, outward current was 14.7±1.98 pA/pF in cells expressing hERG versus 2.8±0.53 pA/pF in cells expressing GFP (*P*<0.001). As shown in Figure 1D, the peak tail current also increased significantly in Ad-hERG myocytes. However, activation of the normalized tail current showed no kinetic difference between the 2 groups (Figure 1E).

**I*<sub>Kr</sub> Upregulation Accelerates Reentry Frequency**

Sustained functional reentry could be obtained from monolayers infected by Ad-GFP, Ad-hERG, and Ad-G628S. Figure 2A shows phase maps of single rotors generating spiral waves in Ad-GFP control (left), *I*<sub>Kr</sub>-overexpressing (middle),...
and G628S-overexpressing (right) monolayers. The corresponding color activation maps and time space plots demonstrating the stability and reproducibility of the respective reentry patterns are presented in Online Figures I and II. The graph in Figure 2B compares the ranges of individual rotation frequencies of control monolayers with that of I$_{Kr}$ overexpressing and G628S-overexpressing monolayers. The mean values are shown as horizontal bars for each experimental group. The average frequency is significantly higher in I$_{Kr}$-overexpressing monolayers than GFP controls (21.12±0.81, n=43; versus 9.21±0.58 Hz, n=16; P<0.001), whereas the frequency in the G628S monolayers is slightly lower (6.14±0.3 Hz, n=17; P=NS versus GFP).

To confirm the involvement of I$_{Kr}$ overexpression on the acceleration of rotor frequency, E4031, a specific I$_{Kr}$ blocker, was superfused at a concentration of 1 μmol/L in 23 monolayers infected with Ad-hERG. As shown in Figure 3, E4031 dramatically reduced the frequency of rotation (22.75±1.24 versus 9.82±0.74 Hz, P<0.05). This effect was partially reversed after a 5 minutes washout period (16.72±1.03 Hz, P<0.05 versus E4031).

WL Shortening Underlies I$_{Kr}$-Induced Rotor Acceleration

To elucidate the mechanism by which hERG accelerated reentry frequency, we first investigated the effect of I$_{Kr}$ overexpression on APD and CV during pacing at varying cycle lengths. From high-resolution APD maps, APD$_{80}$ was measured during 1:1 activation in 11 control and 8 I$_{Kr}$ overexpressing monolayers. Online Figure III shows examples of single pixel recordings at pacing cycle lengths between 1000 and 200 ms. The data demonstrates the excellent signal-to-noise ratio, which allowed accurate APD measurements. As shown in Figure 4A, I$_{Kr}$ expression reduced the APD$_{80}$ by more than 50% when compared with control during pacing at cycle length between 200 and 1000 ms (P<0.01). On the other hand, as shown in Figure 4B, the mean CV of Ad-hERG–expressing monolayers was similar to Ad-GFP monolayers at all pacing cycle lengths. Moreover, the WL (WL=APD×CV) was significantly shorter in the Ad-hERG monolayers than the control group at all pacing cycle length (Figure 4C).

We also plotted the rotation frequency in each monolayer as a function of the respective mean APD$_{80}$ in both control and I$_{Kr}$ overexpressing groups. As shown in Figure 5A, the relationship was very steep and highly significant for the Ad-hERG group. In some monolayers, hERG overexpression reduced APD$_{80}$ less than 30 ms, which enabled rotation frequencies of 25 Hz or higher. In contrast in the Ad-GFP group, although the slope of the relationship was significant, it was much less steep and the minimal APD$_{80}$ was 60 ms and the highest rotation frequency was 12.6 Hz.

As demonstrated previously, during functional reentry, the CV decreases as the curvature of the wavefront increases toward the center of rotation (core). We therefore measured the CV as a function of distance from the core to determine whether I$_{Kr}$ affected that relationship in the same manner as I$_{K1}$ overexpression does. In Figure 5B, we summarize data for 21 Ad-hERG monolayers and 12 Ad-GFP monolayers. As expected, CV increased gradually as a function of distance from the core in both groups. However, the Ad-hERG monolayers showed a larger CV at all distances from the core as compared with the Ad-GFP monolayers (P<0.05). Similarly, the curvature of the wavefront near its pivot point was determined and compared between groups (see Online Figure IV for details). Curvature was larger in the Ad-hERG monolayers than Ad-GFP controls. Taken together, this analysis showed that during reentry, excitability and CV are higher in the Ad-hERG monolayers than in the Ad-GFP control.

Figure 5C shows a comparison of WL measured during reentry as the expanse between the wavefront and the end of the repolarization wavetail (for details, see Online Figure XI and corresponding text in the Online Data Supplement). As expected, the WLs were significantly shorter in the Ad-hERG group. They ranged between 2.75 and 6.89 mm, whereas control WLs ranged between 7.88 and 22.22 mm (P<0.05). Altogether, the data presented thus far suggested that APD and WL shortening, together with increased CV, underlies rotor acceleration in I$_{Kr}$-upregulated monolayers.

**Numeric Simulations**

To gain further mechanistic insight into the effects of hERG overexpression on rotor frequency and stability, we con-
ducted computer simulations at the single-cell level, as well as in 2D using a modified model of the NRVMs (for details see Online Figures V through IX and corresponding text in the Online Data Supplement). Figure 6A shows APs elicited in the single cell model by pacing at 1 Hz in control, and hERG increase ($I_{Kr} \times 2.1X$). As expected, APD abbreviated on $I_{Kr}$ upregulation. Interestingly, in the $I_{Kr} \times 2.1X$ model the AP was followed by a highly reproducible transient hyperpolarization (see also Figure 7A).

We investigated the consequences of $I_{Kr}$ upregulation on rotor dynamics in a 2D monolayer model. As shown in Figure 6B, the reentry frequency was significantly increased from 12.8 Hz in control to 18.5 Hz in $I_{Kr} \times 2.1X$, which was consistent with the experimental result shown in Figure 2. Similar to experiments, overexpressing $I_{Kr}$ (5.21X) in the simulations increased the CV during reentry at all distances from the core compared with the controls (Figure 6C).

To determine whether the changes seen in Figure 6A had any role in $I_{Kr}$ overexpression-induced rotor acceleration, we subjected the single cell model to pacing at varying frequencies (from 1 to 20 Hz). We surmised that at the highest frequencies the ensuing APD shortening and transient hyperpolarization should increase $I_{Na}$ availability and therefore excitability. To test that hypothesis, the fast inward sodium current ($I_{Na}$) was recorded in two sets of simulations (control, and $I_{Kr} \times 2.1X$, respectively), and peak $I_{Na}$ was plotted against pacing frequency for each case. As a negative control, in yet another set of simulations we scaled $I_{Ks}$ ($H_{100}$), whereas $I_{Kr}$ was kept unchanged. Differences in cell excitability were investigated in both sets in terms of peak $I_{Na}$ amplitude. As shown in Figure 7A, whereas $I_{Kr}$ upregulation produced a concomitant transient hyperpolarization after the abbreviated AP (left), $I_{Ks}$ upregulation did not result in membrane
hyperpolarization (top right). Therefore, as shown in Figure 7B, the effects of $I_{Kr}$ upregulation on $I_{Na}$ availability were substantially less at any given activation frequency, compared with the effects of $I_{Kr}$ upregulation. The difference was most significant at the highest frequencies between 12 and 20 Hz. This could explain the effect of $I_{Kr}$ upregulation on CV increase during high frequency reentry (Figure 5B) but not low frequency pacing (Figure 4B).

As discussed above in Figure 5A, the slope of the line defining the relationship between rotation frequency and APD was steeper in the case of hERG overexpression than in control. We surmised that such an increase in the sensitivity of rotation frequency to APD abbreviation was the result of the transient hyperpolarization. Therefore, we studied the effects of premature stimuli (S1-S2 protocol) in single cell, 1D cable and 2D monolayer models (see Online Figure X). In Figure 7C, a single $I_{Kr}$ 5.21X cell was paced at 2 Hz (S1) for 10 seconds followed by a premature stimulus (S2) at varying intervals. It is clear from that graph that at the shorter S2 intervals, more $I_{Na}$ was activated in $I_{Kr}$ 5.21X model compared to control. This increase in peak $I_{Na}$ disappeared when the transient membrane hyperpolarization was suppressed by clamping the MDP to $-70$ mV. As illustrated in Online Figure X (A), in the 1D cable of 10 mm in length, this increase in $I_{Na}$ translated into increased CV which again disappeared when the MDP was clamped to $-70$ mV. We further verified the role of the transient hyperpolarization during reentry in 2D monolayer models by clamping the MDP to various voltages. As expected, the frequency of reentry was progressively reduced with increasing the value of MDP (see Online Figure X, B). The clamped voltages in these simulations covered the range of our experimentally observed MDPs and thus clearly underscored the role of the transient hyperpolarization in addition to the APD abbreviation in increasing the rotor frequency and CV during reentry in $I_{Kr}$ upregulation.

$I_{Kr}$-Induced Transient Hyperpolarization in NRVMs

To determine whether a transient hyperpolarization predicted by simulations occurred experimentally in the in NRVMs, we obtained single cell AP recordings using current clamp. In Figure 8A, we present 2 representative AP recordings at a pacing cycle length of 2000 ms. Consistent with the simulation results, APD shortening and a transient hyperpolarization were demonstrated in the myocytes infected with Ad-hERG when compared to Ad-GFP. In Figure 8B, we quantified the MDP after each AP in Ad-hERG ($-67.6 \pm 0.98$ mV; $n=8$) and Ad-GFP cells ($-63.9 \pm 1.11$ mV; $n=6$; $P=0.03$). As shown in Figure 8C, APD was measured at three different levels (APD$_{30}$, APD$_{50}$ and APD$_{80}$) and showed a significant reduction when hERG was overexpressed. Taken together, these results demonstrated that APD shortening and transient hyperpolarization are both important consequences of $I_{Kr}$ overexpression.

Discussion

We have investigated the consequences of $I_{Kr}$ upregulation on electric impulse propagation and reentry dynamics in 2D biological and numeric models. The most important results are as follows. (1) $I_{Kr}$ overexpression significantly increased the frequency of reentry. (2) During pacing at 1 to 5 Hz, $I_{Kr}$ overexpression shortened APD and WL more than 50%, but did not affect the CV. (3) During sustained reentry, rotation frequency demonstrated a steep and highly significant dependence on $I_{Kr}$ upregulation–induced shortening of APD. The CV was significantly larger in the hERG group versus the control group at all distances from the core. In addition, the WL was significantly shorter in Ad-hERG monolayers than in control. (4) Computer simulations and single cell AP recordings demonstrated that APD shortening and the tran-
sient hyperpolarization after each AP in hERG-overexpressing NRVMs increased the \( I_{Kr} \) availability, which contribute to the increased rotor frequency.

**Pathophysiological Implications**

The contribution of \( I_{Kr} \) to repolarization has been recognized for decades. Since hERG was identified from a human hippocampal cDNA library in 1994, more than 300 mutations have been found to be associated with LQTS. These mutations result in suppression of hERG channel function, either by impaired trafficking or dominant negative effect of channel subunits on channel function. Gain-of-function mutations have been found as well. Recently, a hERG-linked short-QT syndrome was identified with an inactivation-attenuating hERG channel mutation.9,19,20

A Dual Mechanism for \( I_{Kr} \) Overexpression–Induced Rotor Acceleration

Although the clinical phenotypes associated with alterations in potassium channels have been characterized for years, it was unknown how those alterations affected reentry frequency and dynamics. The results of our study suggest that the consequences of \( I_{Kr} \) overexpression are somewhat similar to those recently demonstrated for the ventricles of transgenic mice overexpressing the inward rectifier potassium channel (\( I_{K1} \)), which resulted in a shorter WL, sustained membrane hyperpolarization, and exceedingly fast and stable reentry. In the same study, computer simulations established that during sustained reentry the diastolic membrane potential was 4 mV more negative (≈−94 mV) in the transgenic when compared to the WT case (≈−90 mV). Such hyperpolarization was enough to increase the \( Na^+ \) channel availability by ≈30%, which contributed to reducing the core size and stabilizing the reentry. Similar to the \( I_{K1} \)-overexpressing mouse, our \( I_{Kr} \)-overexpressing monolayers also resulted in APD and WL shortening. However, unlike the transgenic \( I_{K1} \) overexpressing mouse, the hyperpolarization associated with \( I_{Kr} \) overexpression was transient and the membrane potential returned to the resting level within 100 ms or less (Figures 6 and 8). Therefore, a relative increase in CV was manifest during high frequency reentry, but was undetectable at the lower pacing frequencies.

Nevertheless, as demonstrated by additional computer simulations presented in Figure 7, increase in \( Na^+ \) channel availability at the time of maximal hyperpolarization would certainly contribute to increase the CV (also see Online Figure X), hence the frequency and stability of the rotors and to reduce the incidence of wavebreak and fibrillatory conduc-
tion. Initially, this was quite unexpected because upregulation of the other component of the delayed rectifier \( K^+ \) current, \( I_{Ks} \), has been shown to contribute to postrepolarization refractoriness, and to increase the incidence of wavebreaks and fibrillatory conduction, with only a modest increase in rotor frequency. Yet, as demonstrated in the computer simulation (Figure 7), whereas \( I_{Kr} \) upregulation produced a transient hyperpolarization after the abbreviated AP, \( I_{Ks} \) upregulation did not result in membrane hyperpolarization and therefore its effects on \( I_{Na} \) availability were substantially blunted at any given activation frequency. The difference is most significant at the highest frequencies which are relevant to those usually observed during reentry in the monolayers. Our additional computer simulations presented in Online Figure X with MDPs clamped to less negative potentials in single cell, 1D cable and 2D monolayer models further confirm that the transient hyperpolarization contributes to the acceleration of reentry in \( I_{Kr} \) upregulation. Note that the effects of increased \( I_{Na} \) availability and CV disappeared when the transient hyperpolarization was artificially suppressed.

**Different \( I_{Kr} \) Versus \( I_{Ks} \) Kinetics, Different Consequences During Reentry**

Although counterintuitive, the above results add up when one considers the different kinetics of \( I_{Kr} \) and \( I_{Ks} \), as well as their different contributions to repolarization. \( I_{Kr} \) activates rapidly and displays marked inward rectification because of the fact that, at positive membrane potentials, the rate of \( I_{Kr} \) inactivation is faster than the rate of \( I_{Ks} \) activation. Consequently, \( I_{Kr} \) has a relatively low conductance during the AP plateau (phase 2) but continues to increase throughout a substantial

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**Figure 8.** \( I_{Kr} \) overexpression produces transient hyperpolarization after each AP in isolated NRVMs. A, Representative current clamp recordings. Left, AP in myocyte infected with Ad-GFP. Right, AP in myocyte infected with Ad-hERG. B, MDP in Ad-hERG and Ad-GFP cells. C, Frequency dependence of APD50 (squares), APD50 (triangles), and APD80 (circles) in myocytes infected with Ad-GFP (filled symbols) or Ad-hERG (open symbols). Student’s paired t test.
portion of the repolarization phase (Figure 7A), which explains the marked APD shortening and transient hyperpolarizing effects of \( I_{Kr} \) overexpression in our NRVMs. In contrast, although \( I_{Kr} \) increases somewhat more rapidly during the plateau, it already begins to decline before phase-3 repolarization takes place (Figure 7B). As such \( I_{Kr} \) contributes relatively less than \( I_{Kr} \) to the repolarization of the ventricular APs and thus its APD shortening effect is relatively less than \( I_{Kr} \) at any given level of upregulation (data not shown). This, together with the fact that \( I_{Kr} \) upregulation does not lead to transient hyperpolarization after each AP, helps explain the substantially lesser effect of \( I_{Kr} \) upregulation on rotor frequency in the monolayers than \( I_{Kr} \) upregulation. In addition, as demonstrated previously,2,22,23 because of its slow deactivation kinetics, \( I_{Kr} \) will tend to accumulate at high frequencies and therefore play an important role in postrepolarization refactoriness22,23 and fibrillatory conduction.4 In contrast, during reentry, the shorter APD produced by \( I_{Kr} \) overexpression gives Na\(^{+}\) channels enough time to recover, without impinging on the excitability during the diastolic interval before the next wavefront invades the tissue, thus avoiding wavefront–wavetail interactions that could destabilize the rotor. In addition, the transient hyperpolarization induced by \( I_{Kr} \) upregulation further increases Na\(^{+}\) channel availability and excitability, both of which are very sensitive to the membrane potential. Finally, \( I_{Kr} \) is not known to accumulate, which would make it unlikely to play any important role in postrepolarization refactoriness and would explain why, in the \( I_{Kr} \)-overexpressing monolayers, the spiral waves are able to rotate at very high frequencies without breaking.

Study Limitations

The effect of hERG was examined in the NRVM monolayer model, which allowed us to overexpress hERG channel and study its effect on reentry dynamics in a highly controlled environment. However, by overexpressing the hERG channel alone, we have not considered the importance that the regulatory \( \beta \)-subunits have in reconstituting native \( I_{Kr} \)24 or their role in controlling reentry frequency. Furthermore, the consequences of channel expression in neonatal rodent myocytes are likely to be different from those in human adult myocytes because the ionic profile is different. Also, the closeness of the RMP to the K\(^{+}\) equilibrium potential in normally polarized adult cardiomyocytes would substantially diminish the contribution of the transient hyperpolarization to the overall effect observed in NRVMs. However, reentry typically occurs in association with ischemia/reperfusion injury (a situation in which external K\(^{+}\) is usually elevated); therefore, depolarization of the RMP may increase the relevance of the latter mechanism in the adult heart. Finally, the conclusions that have been drawn from this study are only conceptual and mechanistic in relation to the cardiac fibrillatory process. In no way do we attempt to relate them to the clinical setting. Further studies in translational models would be necessary before any extrapolation to humans can be made.

Conclusion

This is the first study to systematically evaluate the role of \( I_{Kr} \) in the frequency and stability of reentry. We demonstrate that hERG overexpression dramatically accelerates reentry frequency in NRVM monolayers. APD shortening and transient hyperpolarization-induced increase in excitability are the mechanisms underlying reentry acceleration.

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Disclosures

None.

References


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Novelty and Significance

What Is Known?

- The human ether-a-go-go-related (hERG) potassium channel responsible for the rapid component (I(Kr)) of the delayed rectifier current I(K) is important in determining the shape and duration of the human cardiac action potential.
- Alterations in the density of I(Kr) change the action potential duration (APD) and may result in cardiac arrhythmias and sudden cardiac death.
- Gain-of-function mutation in hERG (N588K) leads to short QT syndrome, indicating that an increase in I(Kr) could be arrhythmogenic.

What New Information Does This Article Contribute?

- Overexpression of I(Kr) significantly alters the frequency and stability of functional reentry: the mechanism underlying ventricular tachyarrhythmias including fibrillation, the most dangerous type of cardiac arrhythmia.
- The increase in reentry frequency depends on APD abbreviation and wavelength shortening, as well as on transient hyperpolarization of the resting membrane potential.
- Transient hyperpolarization increases the sodium channel availability and cellular excitability and therefore contributes significantly to an increase in reentry frequency.

Both loss- and gain-of-function mutations in hERG have been associated with cardiac arrhythmias and sudden death. The data presented in this article demonstrate, for the first time, a direct link between I(Kr) density and reentry dynamics. The increase in frequency of reentry was achieved not only by a shortening of APD and wavelength, but also by transient hyperpolarization of phase 4 of the action potential. These findings extend our understanding of the molecular mechanisms of ventricular fibrillation and complement previous studies that strongly suggest that repolarizing potassium currents contribute to fibrillation.
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Luqia Hou, MS*1,2, Makarand Deo, PhD*1, Philip Furspan, PhD1, Sandeep V. Pandit, PhD1, Sergey Mironov, PhD1, David S Auerbach, MS1,4, Qiuming Gong, MD, PhD3, Zhengfeng Zhou, MD, PhD3, Omer Berenfeld PhD1, José Jalife, MD1,2

Online Supplement

Methods

Adenoviral Construct

We generated adenoviral constructs containing WT or G628S cDNA sequence of hERG tagged with GFP, using the AdEasy vector system (Stratagene, La Jolla, CA). Viruses were purified using a kit (Virus Purification Kit, Clonetech) and the titer calculated (Rapid Titer Kit, Clontech) before multicellular preparations were infected with varying multiplicities of infection (MOI). MOIs of 200 and 30 were found to be optimal for uniform expression of WT and mutant hERG channels, respectively, based on the GFP level across the monolayer. An adenovirus carrying the sequence of GFP (Ad-GFP) at an MOI=10 served as control. All viral infections were performed after 48 hours in culture. After infection, the myocytes were cultured for an additional 48 hours to allow sufficient protein expression before mapping or patch-clamping.

Pacing Protocol

Monolayers were paced using 5-ms pulses at 2X threshold through a thin bipolar electrode. Incremental pacing started at 1 Hz, until loss of 1:1 capture or induction of sustained reentry. The Ikr blocker, E4031 (1 µM) was superfused when indicated.

Data Analysis

Single Cell Electrophysiology: We used commercially available software (pCLAMP, version 10.2; Axon Instrument, Forster City, CA). Ikr peak pulse currents were measured using the Clampfit subroutine of the pCLAMP software, normalized to cell capacitance, and plotted against voltage.

Optical Movies: Ensemble averaging at each pixel was performed over 5 or more propagating wavefronts synchronized with stimuli. Background fluorescence was subtracted from each frame, and spatial (3x3 pixels) and temporal (7 pixels) conical convolution filters were applied. Dominant frequency (DF) maps and phase maps were constructed in monolayers showing sustained reentry as described. Optical APDs were measured at 80% repolarization. The local CV was measured as described. Briefly, the distributions of activation times (50% of upstroke) for the spatial regions of 5x5 pixels were fitted with the plane, and gradients of activation times gx and gy were calculated for each plane along the x and y axes, respectively. The magnitude of the local CV was calculated for each pixel as $\sqrt{g_x^2 + g_y^2}$. Mean values and standard deviations for CV and APD were calculated for the entire visible surfaces and for sequential activations. During pacing, wavelength (WL) was calculated as $WL = APD \times CV$. 
However, it is well known that during functional reentry both APD and CV vary greatly as a function of distance from the center of rotation (core). Therefore, during sustained reentry, the WL was measured from the phase maps as the expanse between the wavefront and the end of the repolarization wavetail, at a fixed distance of 10 mm from the core.

**Supplemental Experimental Data:**

The methods for the construction of phase maps, activation maps and time-space plots have been described in great detail previously.3-7

Figure I presents color activation maps of sustained reentry corresponding to the phase maps shown in Figure 2 of the main article. Neonatal rat ventricular monolayers were infected with Ad-GFP, Ad-hERG, or Ad-G628S. In Figure II, we illustrate the original optical signals and corresponding time-space plots for the same experiments. Each time-space plot (bottom) was constructed along the horizontal white line drawn on the equivalent reentry snapshot (top). Please note that Ad-hERG significantly abbreviated the spatial extension of the excited state (white) and greatly accelerated reentry frequency, as reflected by the large increase in the number of rotations in the respective time-space plot. In contrast Ad-G628S appreciably increased the spatial extension of the excited state and reduced the number of rotations in the time-space plot.

Figure III shows examples of single pixel recordings at pacing cycle lengths between 1000 and 200 ms. The excellent signal-to-noise ratio shown by all examples provides evidence of our ability to accurately measure APD at three different levels (30, 50 and 80%) of repolarization.

We determined the role of wavefront curvature in determining the changes of reentry frequency and dynamics secondary to hERG modification, as illustrated in panel A of Figure IV: 1) A snapshot of the phase movie showing a rotor with a singularity point (SP) at its pivoting tip was randomly selected for each of a total of n=9 monolayers in each group. 2) In each snapshot we determined by visual inspection the orientation of the wavefront tip in the immediate vicinity (i.e., within 3 pixels) of the SP. 3) We then drew a line in that orientation across the SP. 4) Subsequently, we drew a second line perpendicular (90 degree angle) to the SP crossing line and extended it to approximate the first ½ winding of the wavefront; i.e., to the nearest transition between phases blue and purple in the phase map. 5) We measured the length of the second line. 6) We took that length being equivalent to the diameter of a hemi-circle approximately delineating the wavefront in its first ½ winding and therefore as being inversely proportional to its curvature at that location.

**Mathematical Modeling**

We have modified an existing mathematical model of neonatal rat myocyte by Korhonen et al.8 This model is unique in that cytosolic Ca\(^{2+}\) is a function of temporal as well as spatial coordinates. Due to lack of t-tubules,9 calcium ions entering the cells via membrane channels have to diffuse a small distance through the cytosol before reaching the sarcoplasmic reticulum (SR) and trigger the excitation-contraction coupling (ECC) machinery. The following ionic current components were modified in order to reproduce the AP morphology and restitution
properties recorded from the experiments performed in our laboratory. Other formulations in the Korhonen model remain unchanged.

**Fast Na\(^+\) current (I_{Na})**

The formulation for the fast Na\(^+\) current was based on a mammalian ventricular myocyte model (LRd 1999). The maximum conductance of the current (\(G_{Na}\)) was adjusted to obtain a maximum \(\frac{dV}{dt}\) (135 mV/ms) and AP amplitude (91 mV) in the range of our experimentally recorded values. The voltage dependence of \(I_{Na}\) steady-state inactivation and activation were modified to fit our experimental data in neonatal rat ventricular myocytes (see Figure V; \(n=16\), measured at 22 °C). The inactivation curves (\(h\) and \(j\) gates) were shifted by +2 mV to compensate for the temperature-dependent changes in the \(I_{Na}\) kinetics (Q\(_{10}\) compensation). An additional +10 mV shift was introduced in the steady-state inactivation to compensate for the liquid junction potentials and extrapolation to the physiological [Na]\(_o\) concentration (154 mM). Thus, the fast Na\(^+\) current was modeled as:

\[
I_{Na} = G_{Na}m^3hj(V - E_{Na})
\]

\[
\tau_x = \frac{1}{(\alpha_x + \beta_x)}
\]

where \(x\) in the subscript can be \(m\), \(h\) or \(j\). \(E_{Na}\) is the reversal potential for sodium.

\[
\alpha_m = 0.32 \frac{V + 47}{1 - e^{-0.1(V+47)}}
\]

\[
\beta_m = 0.056e^{-V/11}
\]

If \(V \leq -40\) mV,

\[
\alpha_h = 0.135e^{(70+V)/-6.8}
\]

\[
\beta_h = 3.56e^{0.079V} + 310000e^{0.35V}
\]

\[
\alpha_j = 2.0 \times (-127140e^{0.2444V} - 0.00003474e^{-0.04391V}) - \frac{(V + 37.78)}{(1 + e^{0.311(V+79.23)})}
\]

\[
\beta_j = \frac{0.1212e^{-0.01052V}}{1 + e^{-0.1378(V+40.14)}}
\]

And if \(V \geq -40\) mV,

\[
\alpha_h = \alpha_j = 0
\]

\[
\beta_h = \frac{1}{0.13(1 + e^{(V+10.66)/11.1})}
\]

\[
\beta_j = \frac{0.3e^{-0.0000002535V}}{(1 + e^{-0.1(V+32)})}
\]
Time-independent $K^+$ current

The formulation for the time-independent $K^+$ current ($I_{K1}$) was modified in accordance to our experimental data ($n=5$). Figure VI shows the I-V plots for $I_{K1}$ from experiments and the modified NRVM model. Note that the experimental values have been shifted by -10mV to compensate for the liquid junction potential errors. The resulting $I_{K1}$ formulation is as follows:

$$I_{K1} = k_{Ik1} \times 0.0515 \times \frac{[K^+]_o}{[K^+]_o + 210} \times \frac{V - E_K - 6.1373}{0.1653 + e^{0.0319(V-E_K-6.1373)}}$$

The value of $k_{Ik1}$ was set to 2.0 to match the recorded peak inward $I_{K1}$ density in our experiments.

Cytosolic Calcium transients

The cytosolic calcium transients in Korhonen model vary between 200 nM (diastolic) and 700 nM (systolic). We reduced the absolute amplitudes to lie between 250 nM and 500 nM during diastole and systole, respectively (see below). This was achieved by reducing the maximum $Ca^{2+}$ release from ryanodine receptors (RyR) ($k_{RyR}$) by 50%.

L-type calcium current ($I_{CaL}$)

$I_{CaL}$ formulation involves a voltage-dependent activation gate ($\alpha$), a voltage-dependent inactivation gate ($\beta$) and one $[Ca^{2+}]$-dependent inactivation gate ($\gamma_{Ca}$). In order to compensate for the reduced $[Ca^{2+}]$ distributions, the steady-state curve of $Ca^{2+}$-dependent inactivation ($f_{Ca\infty}$) was reduced to match the original formulation by ten-Tusscher et al. Thus the $f_{Ca}$ gate was formulated as follows:

$$f_{Ca\infty} = \frac{\alpha_{fCa} + \beta_{fCa} + \gamma_{fCa} + 0.23}{1.46}$$

where,

$$\alpha_{fCa} = \frac{1}{1 + ([Ca^{2+}]_{subSL}/0.000325)^8}$$

$$\beta_{fCa} = 0.1 \times \frac{1}{1 + e^{([Ca^{2+}]_{subSL}-0.00005)/0.0001}}$$

$$\gamma_{fCa} = 0.2 \times \frac{1}{1 + e^{([Ca^{2+}]_{subSL}-0.000075)/0.0008}}$$

$[Ca^{2+}]_{subSL}$ is the subsarcolemmal calcium concentration.

Delayed rectifier $K^+$ Currents, $I_{Kr}$ and $I_{Ks}$

Rapid component of the delayed rectifier $K^+$ current, $I_{Kr}$, exhibits rapid activation and prominent inward rectification. We replaced the $I_{Kr}$ formulation in the Korhonen model by a formulation...
based on Luo-Rudy dynamic (LRd 1995) model of ventricular myocyte. It consists of a time-dependent activation gate, $X_r$, and a time-independent inactivation gate, $R$, to approximate very rapid inactivation process of this channel. $I_{Kr}$ is thus expressed as:

$$I_{Kr} = g_{Kr} \cdot X_r \cdot R \cdot (V - E_K)$$

where, $E_K$ is the reversal potential of potassium and $g_{Kr}$ is the maximum conductance of $I_{Kr}$ which exhibits a square root dependence on extracellular potassium concentration ($[K^+]_o$) and is expressed as

$$g_{Kr} = 0.0005228\sqrt{[K^+]_o/5.4}$$

Figure VII shows normalized $I_{Kr}$ densities recorded experimentally and reproduced by the computer model for control and for $I_{Kr}$ upregulation. The $g_{Kr}$ was scaled by a factor of 5.21 (obtained from our experimental recordings) to reproduce the $I_{Kr}$ densities in hERG overexpressed myocytes.

The gating variables $X_r$ and $R$ are governed by,

$$X_{r\infty} = \frac{1}{1 + e^{-(V+21.5)/7.5}}$$

$$\tau_{Kr} = \frac{1}{\left(\frac{0.00138(V + 14.2)}{1 - e^{-0.123(V+14.2)}} + \frac{0.00061(V + 38.9)}{e^{0.145(V+38.9)-1}}\right)}$$

$$R = \frac{1}{1 + e^{(V+9)/22.4}}$$

The slow component, $I_{ks}$, was adapted from LRd 1999 model which consists of two activation processes, represented by a fast gate, $X_{s1}$ and a slow gate, $X_{s2}$.

$$I_{Ks} = g_{Ks} \cdot X_{s1} \cdot X_{s2} \cdot (V - E_{Ks})$$

Where $g_{Ks}$ is the maximum conductance of $I_{Ks}$, which is cytosolic calcium-dependent. We replaced the intracellular calcium concentration in the original formulation with the subsarcolemmal concentration, $[Ca^{2+}]_{subSL}$. Thus the $g_{Ks}$ is expressed as

$$g_{Ks} = 0.0866 \times (1.0 + \frac{0.6}{1 + \left(\frac{0.000038}{[Ca^{2+}]_{subSL}}\right)^{1.4}})$$

The gating variables $X_{s1}$ and $X_{s2}$ are governed by,
\begin{equation}
X_{s_{1,\infty}} = X_{s_{2,\infty}} = \frac{1}{1 + e^{-(V-1.5)/16.7}}
\end{equation}

\begin{equation}
\tau_{Xs1} = \frac{1}{\left[ \frac{7.19 \times 10^{-5} \times (V + 30)}{1 - e^{-0.148(V+30)}} + \frac{1.31 \times 10^{-4} \times (V + 30)}{e^{0.0687(V+30)} - 1} \right]}
\end{equation}

\begin{equation}
\tau_{Xs2} = 4 \times \tau_{Xs1}
\end{equation}

\begin{equation}
E_{Ks} \text{ in the } I_{Ks} \text{ expression is the reversal potential computed by the following expression}
\end{equation}

\begin{equation}
E_{Ks} = \frac{RT}{F} \times \ln \left( \frac{[K^+]_o + P_{Na,K} \times [Na^+]_o}{[K^+]_i + P_{Na,K} \times [Na^+]_i} \right)
\end{equation}

where \( P_{Na,K} = 0.01833 \) is the Na/K permeability ratio.

Table I lists the modified parameters used in our NRVM model. Initial conditions for the modified parameters are given in Table II.

**Computer Simulations:**

The modified NRVM computer model was used to study the effects of \( I_{Kr} \) overexpression in single cell and 2D monolayer simulations. The single-cell model was first paced for 50 sec to attain steady state and the values of all state variables were saved. These steady state values were used as initial conditions for all the simulations. For single cell simulations, the model was paced at varying frequencies (from 1 to 20 Hz). After attaining steady state for 20 sec, the APs and fast inward sodium current \( (I_{Na}) \) were recorded in the last 3 sec. hERG overexpression was implemented by scaling \( I_{Kr} \) by a factor of 5.21 (5.21X), which was derived from our experiments (see Figure VII). Peak \( I_{Na} \) was plotted against pacing frequency for each case. In yet another set we scaled \( I_{Ks} \) (25X) with normal \( I_{Kr} \). Differences in cell excitability in terms of peak \( I_{Na} \) amplitude were investigated in both sets. The cell excitability was also studied by pacing the single cell model (Control and \( I_{Kr} \) 5.21X) at 2 Hz for 10 seconds (S1) followed by a premature stimulus (S2). The timing of S2 was varied between 10 ms and 100 ms after the repolarization due to the last S1 and the peak \( I_{Na} \) was recorded. A similar protocol was used to study the effects of cell excitability on the conduction velocity along a 10 mm long cable for both the cases. We also used a disk-shaped 2D geometry, 35 mm in diameter, to simulate a NRVM monolayer. The disc was constructed with a resolution of 100 \( \mu \)m per computational cell (total = 95,093 cells). Reentry was induced via cross-field stimulation and the DF was determined as in the experiments. We used a similar protocol to induce reentry in the \( I_{Kr} \) 5.21X monolayer. A subset of simulations was performed by clamping the minimum diastolic potential at less negative values during reentry \( I_{Kr} \) 5.21X monolayer models to study the effects of transient hyperpolarization on the conduction velocity and the frequency of reentry.
Simulated action potentials:

Action potential (AP) parameters recorded from experiments using patch-clamp techniques and those obtained from the modified NRVM computer model are listed in Table III. The simulated single-cell APs were elicited by injecting a stimulus current of -100 pA/pF (duration 0.5 ms) at 1 Hz pacing frequency. The amplitude of the simulated APs was approximately 91 mV, which is in line with our experiments. Our model reproduced the neonatal rat ventricular myocyte AP morphology in agreement with the experiments and with previous studies.8,14

Figure VIII shows the experimental and simulated AP traces and the intracellular calcium transients in the model for 1Hz pacing. The Ca^{2+} ions enter the cell via sarcolemmal (SL) channels (L- and T-type Ca^{2+} channels) and diffuse through the cytosol to reach the SR which is an important feature of the Korhonen model.8 Therefore, the cytosolic Ca^{2+} concentrations at SL and SR differ slightly as seen in the figure. Figure IX shows the APD restitution curves for the model and experimental data, measured at 80% repolarization (APD_{80}). The protocol for restitution included pacing the modified NRVM model for 20 sec for each pacing frequency before measuring APD_{80}.

In summary, our modified model is able to closely mimic our experimental single cells action potentials. This model was then used to explore the consequences of overexpression of I_{Kr} in the monolayers, as reported in the main article.

Role of the transient hyperpolarization

Our experimental data suggest that in addition to the APD abbreviation, hERG overexpression results in a transient hyperpolarization after each action potential which contributes to the observed increased rotation frequency. To provide further support to that hypothesis, we conducted additional simulations. In panel A of Figure X, we present results using S1-S2 protocol in a 1D cable (10 mm) to measure changes in conduction velocity (CV). Similar to what we demonstrated experimentally (see Fig. 7C), upregulating I_{Kr} 5.21X increased CV at all S1-S2 intervals. However, CV did not increase when the resting membrane potential was clamped to -70 mV. As shown in panel B, IKr upregulation increased the frequency of reentry in the monolayer model. As expected, the frequency of reentry was progressively reduced when the level of the clamped voltage was progressively depolarized. Altogether, the results strongly support the role of the transient hyperpolarization in addition to the APD abbreviation in increasing the rotor frequency and CV during reentry in I_{Kr} upregulation.

Method of measuring the wavelength (WL)

As shown in Figure XI, WL during reentry was defined by the length of the black line drawn on the phase map 10 mm from the center of rotation (white circle) and perpendicular to the tangent at the wavefront (blue).6 In both experiments (panel A) and simulations (panel B) I_{Kr} upregulation reduced WL significantly during sustained reentry.
Figures and Tables

**Table I.** Modified parameters and their values in our NRVM model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Na}$</td>
<td>Maximum $I_{Na}$ conductance</td>
<td>6.3 mS/µF</td>
</tr>
<tr>
<td>$g_{CaL}$</td>
<td>Maximum $I_{CaL}$ conductance</td>
<td>$5.67 \times 10^{-5}$ dm$^3/(F \text{ ms})$</td>
</tr>
<tr>
<td>$k_{RyR}$</td>
<td>Scaling factor for $J_{RyR}$</td>
<td>0.005 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{IK1}$</td>
<td>Scaling factor for $I_{K1}$</td>
<td>2.0</td>
</tr>
<tr>
<td>$k_{NCX}$</td>
<td>Scaling factor for $I_{NCX}$</td>
<td>$1.1340 \times 10^{-16}$ pA/(pF (µm)$^4$)</td>
</tr>
<tr>
<td>$I_{NaK}^{max}$</td>
<td>Maximum NaK-ATPase current</td>
<td>3.1995 pA/pF</td>
</tr>
<tr>
<td>$g_{Nab}$</td>
<td>INab conductance</td>
<td>0.0039 mS/µF</td>
</tr>
</tbody>
</table>

**Table II.** Initial conditions for the modified formulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$</td>
<td>Membrane potential</td>
<td>-72.79684 mV</td>
</tr>
<tr>
<td>$m$</td>
<td>$I_{Na}$ activation gating variable</td>
<td>0.015898145552</td>
</tr>
<tr>
<td>$\hat{h}$</td>
<td>$I_{Na}$ fast inactivation gating variable</td>
<td>0.947273505079</td>
</tr>
<tr>
<td>$j$</td>
<td>$I_{Na}$ slow inactivation gating variable</td>
<td>0.908486044987</td>
</tr>
<tr>
<td>$X_r$</td>
<td>$I_{Kr}$ time-dependent activation gating variable</td>
<td>0.025742210977</td>
</tr>
<tr>
<td>$X_{s1}$</td>
<td>$I_{Ks}$ fast activation gating variable</td>
<td>0.012668791315</td>
</tr>
<tr>
<td>$X_{s2}$</td>
<td>$I_{Ks}$ slow activation gating variable</td>
<td>0.028399873909</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>Intracellular Na$^+$ concentration</td>
<td>13818.5982638 µM</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>Intracellular K$^+$ concentration</td>
<td>150953.3914836 µM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{subSL}$</td>
<td>Subsarcolemmal Ca$^{2+}$ concentration</td>
<td>0.2626943 µM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{subSR}$</td>
<td>Cytosolic Ca$^{2+}$ concentration near SR</td>
<td>0.2630095 µM</td>
</tr>
</tbody>
</table>

**Table III.** Comparison of the AP characteristics of the NRVM model with the experimental values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiments</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDP (mV)</td>
<td>-72.09±2.5</td>
<td>-72.7968</td>
</tr>
<tr>
<td>AP amplitude (mV)</td>
<td>98.82±11.75</td>
<td>91.65</td>
</tr>
<tr>
<td>APD30 (ms)</td>
<td>80±26.99</td>
<td>95.0</td>
</tr>
<tr>
<td>APD50 (ms)</td>
<td>113.98±44.59</td>
<td>112.5</td>
</tr>
<tr>
<td>APD80 (ms)</td>
<td>166.88±34.43</td>
<td>129.7</td>
</tr>
</tbody>
</table>
**Figure I.** Activation maps of the NRVM monolayers infected with Ad-GFP, Ad-hERG, or Ad-G628S. Scale bar on the right of each map indicates different time point as shown in different color. Scale bars in white: 10mm.

**Figure II.** Optical signals and time-space plot (TSP) show the singularity points in monolayers infected with Ad-GFP, Ad-hERG, or Ad-G628S. TSPs were constructed for activity recorded along the horizontal white lines in the optical recordings. Scale bars in white: 10mm.
Figure III. Single pixel recordings from Ad-GFP and Ad-hERG infected monolayers under pacing cycle length of 1000ms, 500ms, 333ms, 250ms, and 200ms.
**Figure IV.** Rotor curvature in monolayers infected with Ad-GFP and Ad-hERG. Panel A, Snapshots of the phase movies in Ad-GFP and Ad-hERG infected monolayers. White lines indicated the distance between the tip of rotating spiral wave (Singularity point at center of core) to the wavefront at ½ of the spiral wave full winding (the boundary between blue and purple bands – see text). Scale bar: 10 mm; Panel B, quantification of the distance between the core and the wavefront. *: p<0.05. Long white lines: The orientation of the wavefront at the tip. Short white lines: Orientation perpendicular to the wavefront at the tip; used to measure the tip-to-½ winding distance.
Figure V. (A) Voltage dependence of $I_{\text{Na}}$ inactivation and (B) activation kinetics of the model were adjusted to our experimental recordings ($n=16$). The error bars represent ±SEM.

Figure VI. The inward rectifier potassium current ($I_{\text{K1}}$) in our NRVM model was modified according to the experimentally recorded I-V relationship ($n=6$). The error bars represent ±SEM.
Figure VII. Normalized \( I_{Kr} \) current density in the modified NRVM model was adjusted according to experimental recordings in control and hERG overexpressed (\( I_{Kr} \, 5.21X \)) myocytes.

Figure VIII. (A) Action potentials recorded in our experiments during 1 Hz pacing. (B) Action potentials elicited by 1 Hz pacing stimulus in our NRVM computer model. (C) Temporal distribution of cytosolic calcium at subsarcolemmal (SL) and near sarcoplasmic reticulum (SR) during pacing.
Figure IX. The computer model reproduced experimentally observed APD restitution properties. The error bars represent standard error.
Figure X. Effects of transient hyperpolarization in the $I_{Kr}$ 5.21X model. **A.** The same S1-S2 protocol as in Fig. 7C was used in a 1D cable of 10 mm length. The increase in $I_{Na}$ was translated into proportional increase in CV for all S2 intervals. Note that the increase in CV was not observed when the MDP was clamped to -70 mV. **B.** The frequency of reentry in 2D $I_{Kr}$ 5.21X monolayer model decreased progressively when the MDP was clamped to more positive voltages, thus indicating the role of transient hyperpolarization in accelerating reentry.
Figure XI. Wavelength measurement in experiments (A) and simulations (B). A, Snapshots of the phase movies in Ad-GFP and Ad-hERG infected monolayers. White circles indicated the distance of 10 mm from the center of the reentry. In each panel, WL is given by the length of the black line extending between the wavefront (blue) and the end of repolarization wavetail (yellow). Scale bar: 10 mm; B, Snapshots of the voltage map in control and $I_Kr$ 5.21X monolayers in simulation.
References:


