**Original Article**

**I_{Kr} Impact on Repolarization and Its Variability Assessed by Dynamic Clamp**

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**Background**—Repolarization and its stability are exquisitely sensitive to I_{Kr} features. Information on the relative importance of specific I_{Kr} abnormalities is missing and would assist in the evaluation of arrhythmogenic risk.

**Methods and Results**—In single guinea-pig myocytes, endogenous I_{Kr} was replaced by modeled I_{Kr} (mI_{Kr}) by dynamic clamp (DC) at a cycle length of 1 s. mI_{Kr} parameters were systematically modified, and the resulting changes in action potential duration (APD) and its short term variability (SD1) were measured. We observed that (1) I_{Kr} blockade increased SD1 more than expected by its dependency on APD; (2) mI_{Kr} completely reversed APD and SD1 changes caused by I_{Kr} blockade; (3) repolarization was most sensitive to inactivation shifts, which affected APD and SD1 concordantly; (4) activation shifts of the same magnitude had marginal impact on APD, but only when reducing mI_{Kr} they significantly increased SD1; (5) changes in maximal conductance resulted in a pattern similar to that of activation shifts.

**Conclusions**—The largest effect on repolarization and its stability are expected from changes in I_{Kr} inactivation. APD is less sensitive to changes in other I_{Kr} gating parameters, which are better revealed by SD1 changes. SD1 may be more sensitive than APD in detecting I_{Kr}-dependent repolarization abnormalities. *(Circ Arrhythm Electrophysiol. 2015;8:1265-1275. DOI: 10.1161/CIRCEP.114.002572.)*

**Key Words:** dynamic clamp • electrophysiology • HERG arrhythmia • I_{Kr} • modeling • repolarization

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Abnormalities of the rapid component of the delayed rectifier K⁺ current (I_{Kr}, HERG channel) are associated with remarkable prolongation and instability of repolarization. Both these conditions entail arrhythmic risk¹ as epitomized by the phenotype of HERG loss-of-function mutations (type 2 Long-QT syndrome).

One of the major challenges of translational electrophysiology remains prediction of the functional phenotype associated with a given channel abnormality. Even once specific functional defects have been identified in heterologously expressed channels, their impact on cell electric activity remains difficult to predict. This is because the action potential (AP) is determined by feedback interplay between membrane potential, many current components, intracellular Ca²⁺, and likely, further unknown factors. Dynamic clamp (DC) is a promising approach to this problem because it allows to test how the properties of a numerically modeled current affect the AP generated by a real myocyte.² An individual ionic current is orders of magnitude simpler to model than the whole AP; thus, DC is expectedly more reliable than whole AP modeling in predicting the outcome of a current abnormality.

This work exploits DC to systematically analyze the effect of changes in I_{Kr} conductance and gating properties on guinea-pig AP duration (APD) and its time-variability, an index of electric instability.¹ ³ ⁴ ⁵ The results provide information, potentially of general value, on the weight of individual I_{Kr} gating features in determining repolarization course and stability. Once clinically validated, such information might be instrumental to risk assessment and clinical management of I_{Kr} mutations; indeed, although reduced channel expression is the commonest mutation-induced derangement, gating abnormalities are also represented (examples given in Table I in the Data Supplement), and their consequences are more difficult to predict.

**Methods**

**Cell Isolation**

Dunkin–Hartley guinea pigs were euthanized by cervical dislocation under 800 mg/kg chloral hydrate intraperitoneal anesthesia. Ventricular myocytes were isolated by using a retrograde coronary perfusion method previously published,⁶ with minor modifications. Rod-shaped, Ca²⁺-tolerant myocytes were used within 12 h from dissociation. This investigation conforms to the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and to the guidelines for Animal Care endorsed by the University of Milano-Bicocca.

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**WHAT IS KNOWN**

- HERG mutations, resulting in abnormal $I_{Kr}$, affect repolarization and its stability and predispose to life threatening arrhythmias.
- HERG mutations may affect $I_{Kr}$ density and kinetic properties; the effect of mutations on electric stability is difficult to predict.

**WHAT THE STUDY ADDS**

- It demonstrates that an in-silico $I_{Kr}$ model can effectively replace native $I_{Kr}$ in its contribution to repolarization in a real myocyte.
- It provides systematic evaluation of how $I_{Kr}$ gating properties may affect repolarization and its stability.
- It identifies principles, of general applicability, concerning repolarization response to ionic current abnormalities.

$I_{Kr}$ Numeric Model

A deterministic, Hodgkin–Huxley-type, model of $I_{Kr}$ was developed by optimizing the Luo–Rudy model against $I_{Kr}$ profile recorded under AP clamp conditions. To this end, we developed a parameter optimization routine based on the least square difference between experimental and simulated $I_{Kr}$ time courses during AP clamp. Inactivation and its recovery were considered instantaneous and activation/deactivation monoeponential as in the Luo–Rudy model. Preliminary simulations (Figures I and II in the Data Supplement) indicated that these simplification slightly changed peak $I_{Kr}$ amplitude, but failed to impact on APD. Model code was implemented by using the Real-Time Experiment Interface (www.rtxi.org). Baseline model parameter values are listed in Table II in the Data Supplement, and their modifications are described in the relevant sections of Results.

Electrophysiology

Recordings were performed on single myocytes superfused with standard Tyrode’s solution (Table III in the Data Supplement) by patch clamp in the whole-cell configuration at 36.5°C. Ca$^{2+}$ and EGTA concentrations were calculated to achieve free pipette Ca$^{2+}$$=10^{-7}$ mol/L, with minimal buffering of systolic transients, as indicated by persistence of sharp myocyte contractions. $I_{Kr}$ was completely and selectively blocked by 5 μmol/L E4031 added to Tyrode solution.

APs were recorded (I-clamp mode) at pacing cycle length (CL) of 1 s and subsequently applied at the same CL as command potential in AP clamp. Inactivation/deactivation monoexponential as in the Luo–Rudy model. Inactivation and its recovery were considered instantaneous and activation/deactivation monoeponential as in the Luo–Rudy model. Preliminary simulations (Figures I and II in the Data Supplement) indicated that these simplification slightly changed peak $I_{Kr}$ amplitude, but failed to impact on APD. Model code was implemented by using the Real-Time Experiment Interface (www.rtxi.org). Baseline model parameter values are listed in Table II in the Data Supplement, and their modifications are described in the relevant sections of Results.

Dynamic Clamp

In DC, APs, recorded from the myocytes, were acquired at a sampling rate of 5 kHz into the computer to drive the numeric $I_{Kr}$ model. Modeled $I_{Kr}$ (m$I_{Kr}$) was calculated in real-time (within one sampling interval) and injected into the myocyte during continued AP recording. Endogenous $I_{Kr}$, blocked by E4031, was thus replaced by m$I_{Kr}$, while leaving in place all the other determinants of myocyte electric activity. APs were recorded at a CL=1 s for at least 3 minutes after achieving a steady AP profile. 5 μmol/L E4031 was then applied to block endogenous $I_{Kr}$, m$I_{Kr}$ injection was then activated (under continuous E4031 superfusion) and the resulting AP changed recorded. After verifying that m$I_{Kr}$ injection restored the AP to control profile, the relevant model parameters were changed and the ensuing AP changes recorded within the same myocyte. APD variability measurements (SD1 and SD2, see subsequent subsection) were performed over at least 40 beats after AP profile had fully adapted under each condition.

Data Analysis and Statistics

APD was measured at the 90% repolarization level; beat-to-beat measurements were automatically performed by a custom software routine.

$$SD1 = \left| \left( APD_{n+1} - APD_n \right) \right| / n_{beats} \times \sqrt{2}$$

$$SD2 = \left\{ \left| \left( APD_{n+1} + APD_n \right) - 2APD_{mean} \right| \right\} / n_{beats} \times \sqrt{2}$$

According to a numeric analysis presented in Figure III in the Data Supplement, although both SD1 and SD2 are measures of variability, their ratio (SD1/SD2) is independent from variability magnitude, but proportional to its frequency content. Put in simple terms, although a high SD1/SD2 ratio indicates prevalence of beat-to-beat APD variability (SD1/SD2 = 0 in 1:1 alternans), a low one implies that a larger number of cycles is required for the same APD change (see Figure III in the Data Supplement for more detail). Whereas SD1 and SD2 are correlated in most circumstances, SD1/SD2 carries independent information; therefore, variability will be expressed in terms of SD1 and SD1/SD2 ratio.

To analyze $I_{Kr}$ distribution during the AP, repolarization was divided in 3 phases: Phase 1: the quasi-linear plateau phase; Phase 3: the quasi-linear terminal fast repolarization; and Phase 2: the transition between these 2 phases. Proximal and distal boundaries of each quasi-linear phase were determined with a custom-made recursive routine (ie, in an operator-independent manner) detecting divergence from linearity.

Boundaries of the interposed phase 2 were set accordingly. Mean current ($I_{mean}$) within each phase was calculated as

$$I_{mean} = \left[ \int_{t_2}^{t_1} I dt \right] / (t_2 - t_1)$$

where $t_1$ and $t_2$ are phase boundaries.

Means were compared by paired t test or ANOVA, as appropriate; Bonferroni’s method was used for adjusting significance cutoff in multiple post hoc comparisons. Correlation and regression coefficients were estimated by linear models (GraphPad Prism 5). P<0.05 was used to define significance. Data are presented as mean±SEM.

Results

$I_{E4031}$ Replacement by m$I_{Kr}$

APs were recorded at a CL of 1 s (Figure 1) in control conditions (ctr). E4031 superfusion prolonged APD (+10.4%±2.1%; P<0.05). As can be visually appreciated
from Poincaré plots, E4031 significantly increased SD1 only (+38.6%±5.6%; P<0.05), and SD1/SD2 became accordingly larger (+33.1%±10.4%; P<0.05). This indicates that \( I_{\text{Kr}} \) blockade preferentially augmented the faster (beat-to-beat) component of APD variability. DC was then activated in the continuing presence of E4031 and m\( I_{\text{Kr}} \) was injected. This shortened APD by 13.1%±1.5% and fully restored AP profile to make it precisely overlap control one (inset Figure 1A). m\( I_{\text{Kr}} \) injection reduced SD1 (−31.1%±3.2%; P<0.05 versus E4031) back to control (NS versus control) and SD2 below control (−10.6%±6.2%; P<0.05 versus control). Thus, m\( I_{\text{Kr}} \) failure to restore SD1/SD2 was only apparent, reflecting further elimination of a slower component of variability, rather than the persistence of the faster component. Only for this subtle aspect, m\( I_{\text{Kr}} \) diverged from native \( I_{\text{Kr}} \). Results in all aspects similar to those obtained at CL of 1000 ms were also obtained at CLs of 250 ms and 3000 ms, as reported in Figure IV in the Data Supplement.

### Correlation Between APD and Its Variability

Proportionality between SD1 and mean APD is an intrinsic system property (see introduction) and can thus be expected. To investigate whether such proportionality was perturbed by \( I_{\text{Kr}} \) blockade and restored to control by m\( I_{\text{Kr}} \) injection, single SD1 values recorded under the 3 conditions (control, E4031, m\( I_{\text{Kr}} \)) were pooled and plotted against the corresponding mean APD values (Figure 2A). Pooled data were then fitted by a linear function and raw residuals (ie, with their sign; rR) were summed separately for each condition. Bar graphs (Figure 2B) show that the sum of raw residuals (ΣrR) was negative for control and m\( I_{\text{Kr}} \) to become large and positive for E4031. This implies that, under \( I_{\text{Kr}} \) blockade, SD1 dependency on mean
APD deviated from the general pattern. SD1 versus APD relationships were then fitted separately for each condition. The regression intercept was similar between control and \( I_{Kr} \), but larger for E4031 (\( P<0.05 \) versus control, linear regression GraphPad Prism 5); although the regression slope tended to be larger for E4031, the difference did not achieve significance (Figure 2C). This indicates that, under \( I_{Kr} \) blockade, SD1 was larger than expected from its intrinsic dependency on mean APD. Nevertheless, \( I_{Kr} \) injection restored the control pattern; thus, the stabilizing effect of \( I_{Kr} \), unveiled by this analysis, was necessarily based on \( I_{Kr} \) properties represented in the numeric model.

**Impact of \( I_{Kr} \) Features on APD and Its Variability**

In this section, different \( I_{Kr} \) versions were prepared by changing gating parameters symmetrically around their value obtained from model optimization (control). The different models were applied under DC in the presence of E4031; APD and SD1 were measured from 40 APs in each condition; in all figures, control is represented in black, the negative parameter change in red, and the positive change in blue. Maximal conductance (\( g_{\text{max}} \)), midpotentials for steady-state activation (\( V_{0.5A} \)) and inactivation (\( V_{0.5I} \)), and activation/deactivation time-constant (\( \tau \)) were considered.

**Changes in Maximal Conductance (\( g_{\text{max}} \))**

To evaluate the impact of \( I_{Kr} \) conductance, \( g_{\text{max}} \) was changed by ±30% (±\( \Delta \)) from its control value (0.032 mS/\( \mu \)F).

A representative example of the effect of \( g_{\text{max}} \) changes in a single myocyte is shown in Figure 3A—3C. Figure 3A shows time series of APD values under control (0.032 mS/\( \mu \)F), −\( \Delta \) and +\( \Delta \). In this myocyte, −\( \Delta \) did not change APD appreciably, but increased its variability; +\( \Delta \) slightly reduced APD, but not its variability (average APD and SD1 values for this myocyte are shown under the traces in Figure 3B). Figure 3C illustrates average \( I_{Kr} \) profile of the myocyte under the 3 conditions; \( I_{Kr} \) is plotted as a function of time (\( I/t \) plot aligned with the corresponding average AP waveform) and as a function of \( V_m \) (\( I/V \) plot). The increase in \( I_{Kr} \) amplitude can be appreciated from both plots. The \( I/V \) plot shows that \( g_{\text{max}} \) changes simply scaled current amplitude (\( I_{\text{peak}} \)), without affecting current profile; indeed, the voltage at which \( I_{Kr} \) was maximal was unchanged (\( V_{\text{peak}} \); Table). Therefore, the apparent shift of \( I_{Kr} \) observed in the \( I/t \) plot was secondary to the slight AP shortening/prolongation occurring in this myocyte.

Statistics from 15 cells (Figure 3D) indicate that a 30% decrease of \( g_{\text{max}} \) did not affect APD significantly, but caused an appreciable increase of both SD1 (red versus black) and SD2, SD1/SD2 remaining unchanged (Table). Neither APD nor its variability was significantly changed by a \( g_{\text{max}} \) increment of the same magnitude (+\( \Delta \)).

**Changes in Mid-Activation Voltage (\( V_{0.5A} \))**

To evaluate the impact of changes in the \( V \)-dependency of steady-state activation, the midpoint of the \( I_{Kr} \) activation curve (\( V_{0.5A} \)) was shifted by ±15 mV (±\( \Delta \)) from its control value (−20.6 mV). According to simple reaction kinetics, shifts in the distribution of states at equilibrium may be associated to similar shifts in the velocity of transitions between states. Therefore, the curves defining \( V \)-dependency of activation time constants (\( \tau \)) were concomitantly shifted by ±15 mV (Figure 4A). The less likely alternative condition, in which shifts in steady-state activation occur without concomitant changes in \( V \)-dependency of \( \tau \), was tested in a separate series of experiments reported in Figure V in the Data Supplement.
An example of the effect of changes in $V_{0.5A}$ is shown in Figure 4B–4D. Figure 4B shows time series of APD values under control ($-20.6$ mV), $-\Delta$ and $+\Delta$. In this myocyte, $+\Delta$ slightly prolonged APD and measurably increased its variability; $-\Delta$ failed to affect APD and its variability appreciably. Figure 4D illustrates the average $m_{I_{Kr}}$ profile of the myocyte under the 3 conditions. The $I/V$ plot shows that $m_{I_{Kr}}$ profile was markedly changed by $+\Delta$ and, to a lesser extent, by $-\Delta$. Indeed, $+\Delta$, decreased peak $m_{I_{Kr}}$ and shifted $V_{peak}$ to less negative potentials ($P<0.05$); $-\Delta$ only slightly increased $m_{I_{Kr}}$ availability at potentials corresponding to repolarization phase 3 ($I_{peak}$ and $V_{peak}$ in Table). Because $m_{I_{Kr}}$ inactivation was instantaneous, no kinetic considerations apply to this section.

Statistics from 8 cells (Figure 4E) indicated that a $-15$ mV shift in $V_{0.5A}$ (accompanied by shifts in $r$ curves) failed to affect APD, SD1; and SD1/SD2 significantly. A positive shift in $V_{0.5A}$ also failed to affect APD, but increased SD1; SD1/SD2 also showed a trend to increase, close to statistical significance.

Changes in Mid-Inactivation Voltage ($V_{0.5I}$)

To evaluate the impact of changes in the $V$-dependency of steady-state inactivation, the midpoint of the $m_{I_{Kr}}$ inactivation curve ($V_{0.5I}$) was shifted by $\pm15$ mV ($\pm\Delta$) from its control value ($-30.7$ mV, Figure 5A, black line). Because $m_{I_{Kr}}$ inactivation was instantaneous, no kinetic considerations apply to this section.

An example of the effect of changes in $V_{0.5I}$ is shown in Figure 5B–5D. Figure 5B shows time series of APD values under control ($-30.7$ mV), $-\Delta$ and $+\Delta$. $-\Delta$ strongly prolonged APD and its variability; $+\Delta$ produced quantitatively similar changes but of opposite direction, that is, it shortened APD and decreased its variability. Figure 5D illustrates average $m_{I_{Kr}}$ profile under the 3 conditions; $m_{I_{Kr}}$ is plotted as function of time and as a function of $V_{m}$ ($I/V$ plot). The $I/V$ plot shows that, at variance with activation (above), positive and negative shifts of inactivation changed $m_{I_{Kr}}$ profile almost symmetrically. According to statistics from 10 myocytes, $+\Delta$ increased peak $m_{I_{Kr}}$ ($P<0.05$) and shifted $V_{peak}$ by $+10.2$ mV ($P<0.05$);
### Table. Effect of Changes in Model Parameters ($g_{\text{max}}$, $V_{0.5A}$, $r_{\text{a}}$, $V_{\text{peak}}$) on $m_{\text{Kr}}$ features, APD, and Its Variability

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>$\Delta$</th>
<th>DC</th>
<th>$+$A</th>
<th>No. of Cells</th>
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</thead>
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<tr>
<td>$g_{\text{max}}$, mS/µF</td>
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<td>0.032</td>
<td>$+30%$</td>
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</tr>
<tr>
<td>$V_{\text{peak}}$, mV</td>
<td>$-40.8\pm0.47$</td>
<td>$-41.7\pm0.24$</td>
<td>$-41.8\pm0.21$</td>
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<tr>
<td>$I_{\text{peak}}$, pA/µF</td>
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<td>0.91±0.04</td>
<td>1.24±0.03*</td>
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<tr>
<td>TTP, ms</td>
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<td>196.5±11.9</td>
<td>180.4±11.4*</td>
<td></td>
</tr>
<tr>
<td>$V_{\text{L}2}$, mV</td>
<td>$-15$</td>
<td>$-20.6$</td>
<td>$+15$ mV</td>
<td></td>
</tr>
<tr>
<td>$V_{\text{peak}}$, mV</td>
<td>$-41.8\pm1.4$</td>
<td>$-41.6\pm0.3$</td>
<td>$-33.5\pm0.26$*</td>
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<tr>
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<tr>
<td>$r_{\text{a}}$, 100 ms</td>
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<td>400 ms</td>
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<tr>
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<tr>
<td>TTP, ms</td>
<td>163.5±12</td>
<td>164.6±10.9</td>
<td>166±12.9</td>
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</tr>
</tbody>
</table>

$P<0.05$ vs DC.

### Discussion

The main findings of this study include the following: (1) injection of modeled $I_{\text{Kr}}$ ($m_{\text{Kr}}$) fully reversed the increments in APD and its variability induced by $I_{\text{Kr}}$ blockade; (2) repolarization was most sensitive to inactivation shifts, which concordantly affected APD and its variability; (3) similar changes in activation gating had marginal impact on APD but, when leading to $m_{\text{Kr}}$ decrement, significantly increased APD variability; (4) a similar pattern was observed for changes in maximal conductance; and (5) even if SD1 showed a clear-cut dependency on APD, $I_{\text{Kr}}$ blockade increased SD1 more than expected by APD prolongation alone (Figure 2); moreover, some among $I_{\text{Kr}}$ perturbations changed SD1 without affecting APD significantly.

A question addressed by the present study is whether some yet unidentified properties of HERG channels may account for the major impact of $I_{\text{Kr}}$ modulation on APD and its stability. Adequacy of $m_{\text{Kr}}$ injection in reversing the effects of endogenous $I_{\text{Kr}}$ blockade suggests that $I_{\text{Kr}}$ properties incorporated in the model are sufficient to account for $I_{\text{Kr}}$ functional contribution, except for what concerns the frequency content of APD variability ($m_{\text{Kr}}$ failed to restore SD1/SD2; Figure 1C). The present results also show that rather substantial changes in $I_{\text{Kr}}$ V-dependency, for example, those caused by activation shifts (Figure 4), may have a negligible impact on APD. Therefore, mean APD is a rather lax reporter of $I_{\text{Kr}}$ abnormalities, APD variability being remarkably more sensitive. Notably, a fully deterministic $I_{\text{Kr}}$ model, such as $m_{\text{Kr}}$, also reversed the effects of endogenous $I_{\text{Kr}}$ blockade on APD variability. This might suggest that the latter may not arise primarily from intrinsic (stochastic) $I_{\text{Kr}}$ variance; however, specifically designed experiments may be required to define this aspect.

As previously reported,13 $I_{\text{Kr}}$ blockade increased APD variability, mainly its beat-to-beat component (increased SD1/SD2). $m_{\text{Kr}}$ restored SD1 but not the SD1/SD2 ratio; this was because $m_{\text{Kr}}$ returned SD1 to control values, but further reduced SD2 ($P<0.05$, not shown). This observation may

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- $\Delta$ decreased peak $m_{\text{Kr}}$ ($P<0.05$) and shifted $V_{\text{peak}}$ by $-8.2$ mV ($P<0.05$), that is, to a value corresponding to the steeper portion of phase 3 repolarization ($I_{\text{peak}}$ and $V_{\text{peak}}$ in Table). As shown in Figure 5E, this resulted in significant concordant changes in both APD and SD1 (when APD prolonged SD1 increased and vice versa). SD1/SD2 was unchanged in the case of $-\Delta$ and showed only a trend to decrease for $+\Delta$.

### Changes in $m_{\text{Kr}}$ Distribution During Repolarization

Repolarization course was divided into 3 phases, as described in Methods and shown at the top of Figure 7: $m_{\text{Kr}}$ present within each phase was quantified as mean current ($I_{\text{mean}}$) in a representative subset of myocytes (n=5). Figure 7A reports the changes of $I_{\text{mean}}$ produced within each phase (1, 2, or 3) by changes in $m_{\text{Kr}}$ parameters; the corresponding changes in overall $I_{\text{mean}}$ ($I_{\text{mean tot}}$) are shown in the insets of each panel. Under baseline conditions (black), $I_{\text{mean}}$ increased from phase 1 to 3. Modulation of $g_{\text{max}}$ (left; $-\Delta$ red, $+\Delta$ blue) similarly affected $I_{\text{mean}}$ throughout the 3 phases; the effect was asymmetrical, that is, larger for $g_{\text{max}}$ reduction. Modulation of $V_{0.5A}$ (center) almost exclusively concerned $I_{\text{mean}}$ during Phase 3, with larger effect resulting from the positive shift (blue). Modulation of $V_{0.5A}$ affected to some extent $I_{\text{mean}}$ during all phases but, unlike $g_{\text{max}}$, sharply prevailed during Phase 1; negative (red) and positive (blue) $V_{0.5A}$ shifts exerted roughly symmetrical effects.

Comparison of Figure 7A with Figure 7B illustrates how the above $I_{\text{mean}}$ distribution patterns impact on APD and SD1 modulation (shown in Figures 3-6), represented for this purpose as % change from baseline values. For all parameters, the extent of APD changes correlated with changes in $I_{\text{mean}}$ during Phase 2. On the other hand, the extent of SD1 changes correlated with changes in $I_{\text{mean}}$ during Phase 3.

The format used in Figure 7B also highlights that, although significant changes in APD were achieved only by modulating $V_{0.5A}$, SD1 was affected by modulation of all parameters. Furthermore, the effects of symmetrical changes in $g_{\text{max}}$ or $V_{0.5A}$ produced strongly asymmetrical effects on SD1.

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$APD$ indicates action potential duration at 90% repolarization; $DC$, dynamic clamp; $SD1$, orthogonal APD variability; $SD2$, longitudinal APD variability (see methods); TTP, time to $I_{\text{peak}}$ and $V_{\text{peak}}$; $V_{\text{peak}}$ at peak $m_{\text{Kr}}$ ($I_{\text{peak}}$).
suggest failure of m$I_{Kr}$ to reproduce sensitivity of native $I_{Kr}$ to yet unidentified factors exerting their effect over multiple cycles (eg, changes in the intracellular milieu) and thus contributing mostly to SD2.

SD1 has been identified as a powerful predictor of Torsade de Pointes, leading to the suggestion that beat-to-beat APD variability may favor arrhythmogenesis. However, because of their strong correlation (see Figure III in the Data Supplement), SD1 and SD2 can hardly discriminate between beat-to-beat and slower forms of APD variability. Indeed, their ability to identify torsadogenic conditions is reported to be at least grossly similar by other studies. The numeric simulation reported in Figure III indicate that the SD1/SD2 ratio may provide a better chance to identify changes in beat-to-beat variability selectively; however, its prognostic value remains to be assessed.

The strong and concordant sensitivity of APD and its variability to inactivation shifts points to rectification as the $I_{Kr}$ gating property which, if altered, is most likely to result in overt repolarization abnormality in guinea-pig myocytes. Notably, APD shortening by positive inactivation shift was larger than APD prolongation resulting from the symmetrical negative shift (Figure 5B and 5E); this is consistent with incomplete rectification being the only mechanism thus far identified for Short-QT syndromes linked to HERG mutations. SD1 changes were more conspicuous for the negative inactivation shift instead (Figure 5B and 5E), thus reinforcing the view that effects on APD and on its variability are not necessarily concordant. Negative inactivation shifts are a common feature of the subset of type 2 Long-QT syndrome mutants in which loss of function is caused primarily by gating abnormalities, that is, those in which channel density is preserved. When including shifts in $\tau$ $V$-dependency, activation shifts had a clearly discernible effect on m$I_{Kr}$, but a surprisingly small one on APD (Figure 4E). Nevertheless, the positive shift, which resulted in a clear-cut reduction in peak m$I_{Kr}$, significantly increased SD1. Notably, when the $V$-dependency of $\tau$ remained unchanged, the effects of activation shifts on SD1 disappeared, thus suggesting the importance of parallel changes in steady-state and dynamical behavior. Overall, changes in activation/deactivation gating affected $I_{Kr}$ during Phase 3 only (Figure 7A); indeed, during Phases 1 and 2,
corresponding to the AP plateau, \( I_{Kr} \) was largely inactivated, thus making changes in activation/deactivation inconsequential. Although this is true in guinea-pig myocytes, \( I_{Kr} \) inactivation may be slightly weaker in the human AP, and this might partially unveil the effect of activation/deactivation gating.

Downregulation of channel expression or channel blockade (both represented by \( g_{\max} \) reduction) are the most common \( I_{Kr} \) abnormality underlying QT prolongation; therefore, the relatively small effect of \( g_{\max} \) changes on APD (Figure 3D) was unexpected. Possibly, the \( g_{\max} \) changes considered here (±30%) are small as compared with those occurring when QT prolongation is observed. Although \( g_{\max} \) reduction increased significantly SD1, the latter was not affected by \( g_{\max} \) increment (Figure 3D). Although the association of \( I_{Kr} \) deficiency with increased APD variability is a well-established notion, this asymmetry was unexpected and a further example of how symmetrical changes in channel properties may result in asymmetrical AP responses. SD1/SD2 ratio did not significantly increase in response to decrements in \( g_{\max} \); this contrasts with its increment after blockade of native \( I_{Kr} \). Again, this suggests that \( mI_{Kr} \) may not be fully representative of native \( I_{Kr} \) in terms of sensitivity to the type of perturbations reflected by SD2.

**Mechanistic Interpretation**

The AP profile is ultimately determined by a feedback loop linking membrane potential to the many (V-dependent) conductances contributing to total membrane current (\( I_m \)). Such a loop is preserved in DC mode; therefore, AP changes after modulation of an individual component of \( I_m \) (\( I_{Kr} \) in the present case) might be either buffered or boosted by the feedback response of other components. We will refer to this phenomenon as active response of AP to \( I_{Kr} \) perturbation. An additional factor to be considered in interpreting the observed effects is nonlinearity of the relationship between \( I_m \) (\( \propto dV/dr \)) and APD (\( \propto (dV/dr)^{-1} \)). Being merely numeric, this has been referred to as an intrinsic property of the system, of general value, and independent of the components underlying the active response. A relevant consequence of the intrinsic property is that a given change in \( I_m \) is expected to affect APD more if...
it occurs during a slow phase of repolarization (Figure 5 in Bányász). During Phase 1, current balance is dominated by the match between noninactivating $I_{CaL}$ and $I_{Na}$ components and $I_{Ks}$ onset, which yields a relatively small $I_{m}$, that is, slow repolarization. The fragility of this phase resides precisely in the fact that, being $I_{m}$ small (in the order of 0.3 pA/pF), it can be easily doubled, or halved, by even minor current changes. Because $dV/dt$ and $I_{m}$ are linearly related, the same applies to repolarization rate. Thus, high APD sensitivity to $I_{Kr}$ changes occurring in this phase is expected as the consequence of system’s intrinsic property.

Current theories on the genesis of early-afterdepolarizations points to Phase 2 as the one in which active responses are in a more delicate balance. Indeed, in this phase, the outward current surge supported by $I_{Kr}$ matches the inward resurgent components of $I_{CaL}$ and $I_{Na}$, which are prone to autoregenerative reactivation. Furthermore, rather dramatic changes in subsarcolemmal ionic (Ca$^{2+}$ and Na$^+$) concentrations occur in this phase and even subtle changes in their match with membrane potential may affect the sign and magnitude of the current carried by NCX (Na$^+$/Ca$^{2+}$ exchanger). Thus, we would expect Phase 2 to be a major source of SD1 through system’s active property.

With its large $dV/dt$, resulting from auto-regenerative recovery of inward-rectifying K$^+$ currents ($I_{Kr}$ and $I_{K1}$), Phase 3 would be expected to be relatively immune to current perturbations. Analysis of the patterns shown in Figure 7 is largely, but not entirely, coincident with such theoretical expectations. The only intervention changing APD substantially was modulation of inactivation midpoint ($V_{0.5_{I}}$), that is, the one having a sizable impact on $m_{I_{Kr}}$ during Phase 1. This fulfills the expectation that current changes during slow repolarization may have a larger impact on APD (intrinsic property; see above). The pattern was somewhat different for SD1, which was sensitive also to $m_{I_{Kr}}$ changes during Phases 2 and 3. Notably, APD stabilization (decreased SD1) required $m_{I_{Kr}}$ to increase during Phase 2. Changes in activation/deactivation gating had a negligible effect on APD (Figures 4, 6, and 7B). This is conceivably
Figure 7. Relationship between $m_{I_K}$ distribution and repolarization changes. Quantification of $m_{I_K}$ ($I_{\text{mean}}$) during 3 AP phases (1, 2, or 3 as shown at the top). A. Effect of changes ($\Delta$) in $m_{I_K}$ parameters ($g_{\text{max}}$, $V_{0.5A}$, $V_{0.5I}$) on $I_{\text{mean}}$ within each AP phase (black, baseline; red, $-\Delta$, and blue, $+\Delta$); changes in overall $I_{\text{mean}}$ ($I_{\text{mean tot}}$) are shown in the inset of each panel (n=5). B. Response of APD and SD1 (as $\Delta\%$ vs control) to changes in $m_{I_K}$ parameters (as above). *P<0.05 vs baseline. AP indicates action potential; APD, action potential duration; SD1, orthogonal APD variability; and SD2, longitudinal APD variability.

This approach is more stringent than parameter estimation from traditional V-clamp protocols because the current profile during AP-clamp closely depends on gating kinetic properties that might be otherwise incorrectly estimated. $I_{E4031}$ could be adequately reproduced even if $m_{I_K}$ included simplifications, performed in the sake of computational speed. Whereas native $I_{Kr}$ inactivates with a time constant in the 1 to 4 ms range, $m_{I_K}$ inactivation was made instantaneous. Furthermore, $m_{I_K}$ activation/deactivation kinetics was described by a single exponential. Numeric simulations (Figures I and II in the Data Supplement) showed that these simplifications were indeed inconsequential on repolarization because they affected $I_{Kr}$ during Phase 3 only, when repolarization rate is high.$^{18,19}$ This interpretation implies that activation/deactivation gating might be more relevant to APD modulation if $I_{Kr}$ inactivation during Phase 1 were less complete, as may be the case in canine and human myocytes.$^{16}$

Limitations

The whole experimental approach used in this study relies on the assumption that $I_{E4031}$, on which $m_{I_K}$ was optimized, accurately reflects $I_{Kr}$. This requires E4031 selectivity for $I_{Kr}$ and constancy of E4031 block throughout the electric cycle. Although at the concentration used, E4031 is indeed selective,$^{20}$ block voltage-dependency might theoretically distort the current profile during the AP. Nevertheless, the kinetics of V-dependent E4031 binding and unbinding are slow,$^{21}$ thus ensuring that, once block has achieved steady-state during repetitive depolarization (at physiological CLs), it will remain constant throughout the electric cycle.$^{22}$ $I_{E4031}$ recordings under AP-clamp were obtained under conditions, allowing achievement of steady-state E4031 blockade. Thus, under the present conditions, close similarity between $I_{E4031}$ and $I_{Kr}$ can be reasonably assumed. A further issue is how accurately $m_{I_K}$ reproduces $I_{E4031}$ properties. In this study, $m_{I_K}$ parameters were optimized on $I_{E4031}$ recorded during AP-clamp.

Generalization of results to other species should consider species-specific aspects of guinea-pig electrophysiology; these include pronounced $I_{Kr}$ inactivation during the plateau phase (discussed above) and strong $I_{Kr}$ expression, which might reduce the impact of $I_{Kr}$ abnormalities.

Implications

Identification of the relative impact of $I_{Kr}$ gating properties may provide a new framework for predicting the consequences of $I_{Kr}$ mutations on APD and its variability, 2 quantities strongly

because they affected $I_{Kr}$ during Phase 3 only, when repolarization rate is high.$^{18,19}$ This interpretation implies that activation/deactivation gating might be more relevant to APD modulation if $I_{Kr}$ inactivation during Phase 1 were less complete, as may be the case in canine and human myocytes.$^{16}$

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Implications

Identification of the relative impact of $I_{Kr}$ gating properties may provide a new framework for predicting the consequences of $I_{Kr}$ mutations on APD and its variability, 2 quantities strongly
associated with arrhythmogenic risk. Although the relative weight of gating components (inactivation versus activation) might be at least partially species-specific, the concept that the impact of a given defect may strictly depend on how it alters $I_{Ko}$ during repolarization (Figure 7) is likely of general value.

The present results point to a surprising lack of APD sensitivity in detecting $I_{Ko}$ abnormalities and identify SD1 as a more sensitive index. This may be relevant to the choice of risk identifiers to be applied in mutation or drug screening. Although the prognostic significance of SD1/SD2 awaits to be tested, its suitability to report on the frequency content of risk identifiers to be applied in mutation or drug screening.

Being tested in the context of a real guinea-pig myocyte, the outcome of $I_{Ko}$ modulation may represent a reference for validation of guinea-pig AP numeric models; detection of possible discrepancies would likely provide valuable information for overall model optimization.

Finally, by confirming/challenging theoretical expectations, the present results have refined our interpretation of the complex relation between current and repolalrization abnormalities.

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**Disclosures**
None.

**References**
Impact on Repolarization and Its Variability Assessed by Dynamic Clamp

Claudia Altomare, Chiara Bartolucci, Luca Sala, Joyce Bernardi, Gaspare Mostacciolo, Marcella Rocchetti, Stefano Severi and Antonio Zaza

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Supplement to Introduction

Clinical KCNH2 mutations with gating abnormalities

The prevailing I_{Kr} abnormality underlying QT prolongation is reduced membrane channel density, modelled in this study by changes in \( g_{\text{max}} \) (see manuscript Fig 3). However, mutations leading to changes in I_{Kr} gating have also been reported and are the rule in short QT syndromes (Table S1). As shown in Table S1, in many cases the gating abnormality, identified as potentially responsible for QT prolongation, concerns “activation” gating. This is in apparent contrast with the findings of the present study, in which “activation” abnormalities are shown to have minor effects on APD. On one hand, a larger weight of activation/deactivation abnormalities in humans might depend on weaker I_{Kr} inactivation during the AP plateau (see manuscript for discussion). On the other, it should be considered that 1) functional characterization of mutations in heterologous systems at room temperature has obvious limitations; 2) attribution of the phenotype to faster deactivation was based in the best case \(^1\) on an entirely numerical AP model and in the others \(^2,^3\) largely conjectural.

Table S1: KCNH2 mutations including channel gating abnormalities (reduced channel expression also present in some of them) described in patients with Long- or Short- QT syndromes. The functional abnormality identified as potentially responsible for the electrical phenotype is also listed.

<table>
<thead>
<tr>
<th>KCNH2 mutation</th>
<th>Clinical Phenotype</th>
<th>Putative pathogenetic gating defect</th>
<th>reference</th>
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<tr>
<td>R531Q</td>
<td>LQT</td>
<td>Positive shift and acceleration of deactivation</td>
<td>(^1)</td>
</tr>
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<td>R531W</td>
<td>LQT</td>
<td>Positive shift and acceleration of deactivation</td>
<td>(^1)</td>
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<tr>
<td>V535M</td>
<td>LQT</td>
<td>Negative inactivation shift with slowed recovery</td>
<td>(^4)</td>
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<td>K28E</td>
<td>LQT</td>
<td>Negative inactivation shift</td>
<td>(^5)</td>
</tr>
<tr>
<td>F29L</td>
<td>LQT</td>
<td>Faster deactivation</td>
<td>(^2)</td>
</tr>
<tr>
<td>N33T</td>
<td>LQT</td>
<td>Faster deactivation</td>
<td>(^2)</td>
</tr>
<tr>
<td>R56Q</td>
<td>LQT</td>
<td>Faster deactivation</td>
<td>(^2)</td>
</tr>
<tr>
<td>M124R</td>
<td>LQT</td>
<td>Faster deactivation</td>
<td>(^3)</td>
</tr>
<tr>
<td>H70R</td>
<td>LQT</td>
<td>Altered trafficking + faster deactivation</td>
<td>(^2)</td>
</tr>
<tr>
<td>L86R</td>
<td>LQT</td>
<td>Faster deactivation</td>
<td>(^2)</td>
</tr>
<tr>
<td>N588K</td>
<td>SQT</td>
<td>Positive inactivation shift</td>
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Supplement to Methods

Table S2: mIKr parameters: 

Table S2: mIKr parameters

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<th>Parameter</th>
<th>Value</th>
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<td>$g_{max}$</td>
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<td>$V_{0.5A}$</td>
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<td>$sA$</td>
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<tr>
<td>$V_{0.5I}$</td>
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<td>$sI$</td>
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<td>$\tau_{act/deac}$</td>
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Table S3: Recording solutions

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<th>Intracellular pipette solution</th>
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<tr>
<td>KCl (mM)</td>
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<td>23</td>
</tr>
<tr>
<td>K+ aspartate (mM)</td>
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<td>110</td>
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<td>CaCl$_2$ (mM)</td>
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<td>0.2</td>
</tr>
<tr>
<td>MgCl$_2$ (mM)</td>
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<td>3</td>
</tr>
<tr>
<td>HEPES-NaOH (mM)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HEPES-KOH (mM)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>EGTA-KOH (mM)</td>
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</tr>
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<td>D-glucose (mM)</td>
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<tr>
<td>GTP-Na⁺ salt (mM)</td>
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<tr>
<td>ATP-Na⁺ salt (mM)</td>
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<td>5</td>
</tr>
<tr>
<td>Creatine-P-Na⁺ salt (mM)</td>
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</tr>
<tr>
<td>pH</td>
<td>7.35</td>
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</table>

Effect of gating kinetics on modelled $I_{Kr}$

The impact of inactivation time-dependency on mIKr was tested by numerical simulations. To this end, inactivation kinetics was introduced in mIKr; dependency of inactivation time constant ($\tau$) on membrane potential, shown in Fig S1A, was simulated according to previous estimates supported by experimental results, obtained in the guinea pig at physiological temperature $^7$. Fig S1B compares mIKr profiles with instantaneous and time-dependent inactivation during an AP. The difference between the two models is limited to a small difference in peak current. Since this difference occurred during AP Phase 3, its impact on APD is expectedly small (see manuscript). This expectation was tested by adding either of the two mIKr versions (with instantaneous or time-dependent inactivation) to “total membrane current” density ($I_m$), obtained by differentiation of an AP waveform. New AP profiles were then calculated by numerical integration of $I_m$ traces containing the two mIKr versions respectively. Figure S1C shows that mIKr inactivation time-dependency had no visible effect on $I_m$ profile, and caused only a minor (3 ms) prolongation of APD at 90% repolarization (visible as slight “thickening” of the AP trace). A similar approach was used to test for
the effect of inactivation time-dependency on the outcome of changes in activation rate. The results (not shown) were again suggestive of a minor impact of inactivation time-dependency.

Overall, these simulations indicate that omission of inactivation time-dependency from the mI<sub>Kr</sub> formulation should not impact significantly on the outcome of DC studies. Nevertheless, it should be considered that, whereas voltage and current interact in close-loop during a true AP, the present simulations tested only the primary effect of current changes on potential course (open-loop condition). Therefore, they do not rule out the importance of inactivation time-dependency, particularly if slowed by gating abnormalities.

![Figure S1: Effect of inactivation time-dependency on mI<sub>Kr</sub>.](image)

A) voltage dependency of the inactivation time constant (τ) incorporated into the model; B) comparison between mI<sub>Kr</sub> (right scale) with instantaneous (inst) or time-dependent (t-dep) inactivation during the AP course (left scale); C) total membrane current (I<sub>m</sub>) and AP tracings containing mI<sub>Kr</sub> with either inst or t-dep inactivation.

Whereas I<sub>Kr</sub> deactivation is reportedly biexponential, in mI<sub>Kr</sub> activation/deactivation kinetics was described by a single exponential. To test the impact of a slow deactivation component we performed the simulations shown in Figure S2. Adding the slow kinetic component slightly increased peak I<sub>Kr</sub>, but had negligible effect on APD. Persistence of a fraction of activated channels during diastole would result in “accumulation” of open state at short diastolic interval; however, also this would be irrelevant because, as shown on the plot of gating variables, 100% of channels activate very early during repolarization even in the absence of the slow component.
The simulation was performed with the Luo-Rudy AP model in which the original Ik, formulation was replaced by mlKr (the formulation used in our work). mlKr was used in two versions: 1) the original one, with activation/deactivation represented as a single exponential (act/deact fast); 2) with activation/deactivation represented as a sum of 2 exponentials as in the human O’Hara-Rudy model (act/deact slow).

Interpretation of SD1, SD2 and SD1/SD2 indexes of variability
SD1 (a.k.a. “Short Term Variability”) and SD2 (a.k.a “Long Term Variability”) are indexes of temporal variability, initially applied to the analysis of heart rate and more recently applied to repolarization. In previous work, whereas SD1 was used to quantify “beat-to-beat” variability, SD2 was assumed to reflect variability occurring over a larger number of beats; however, the specificity of these terms was never addressed. In order to clarify the meaning of SD1 and SD2 in terms of variability dynamics, we have tested their performance in a numerical model of variability. The model consisted in a series of 300 values (Y, with mean = 300) in which variability (V) was introduced by a sinus function of adjustable amplitude and frequency. Y was generated to oscillate symmetrically around mean Y. In V, the number of beats (Nbeats) over which Y changes monotonically (i.e. the sinus value goes from 0 to 1) is inversely proportional to the frequency (f) of the sinus wave. Figure S3A shows the results of a simulation in which was set to f = 0.3 degr/beats, to analyze the case of a fully monotonic variation of Y over the sampled interval (Nbeats = 300). While SD1 was much smaller than SD2 in this case (see below), both indexes showed the same dependency on perturbation amplitude, as demonstrated by constancy of SD1/SD2 (multiplied by 10 to make it compatible with figure scales). While f = 0.3 is an extreme case, independency of SD1/SD2 from V amplitude held true at all f values (not shown). These simulations lead to the conclusion that 1) being as affected as SD2 by variations over a large Nbeats, SD1 does not selectively detect changes in short-term variability (“STV” and “LTV” are actually misnomers for SD1 and SD2 respectively); 2) the SD1/SD2 ratio is instead insensitive to the amplitude of variability. Figure S3B shows that SD1/SD2 is instead a function of f, becoming larger as the latter is increased. Therefore, SD1/SD2 specifically reflects the “frequency content” of V, being large when short-term variability prevails over long-term one (it becomes infinite in the extreme case of true alternans). Figure S3C shows Poincaré plots for
three specific cases: a) with $f = 0.3$ ($N_{\text{beats}} = 300$) data points almost overlap the identity line and $SD1/SD2$ approaches $0$; b) with $f = 90$, $N_{\text{beats}} = 4$ (e.g. in the cycle 0, 1, 0, -1...) and Poincaré plot forms a square, yielding $SD1/SD2 = 1$; c) In the special case of pure “alternans” ($f=180$), $N_{\text{beats}} = 2$ (e.g. -1, 1...), points cluster in 2 positions only, Poincaré plot forms a line orthogonal to the identity one, $SD2$ is null and $SD1/SD2 = \infty$. This analysis leads to the conclusion that 1) $SD1$ and $SD2$ report on changes of variability amplitude, irrespective of its frequency content; 2) the $SD1/SD2$ ratio is insensitive to variability amplitude, but specifically reflects its frequency content, i.e. it is suitable to discriminate between short-term and long-term variability.

Figure S3: Variability indexes vs variability properties. A) dependency of $SD2$ (left scale), $SD1$ and $SD1/SD2$ (right scale, $SD1/SD2$ multiplied by 10) on variability amplitude; B) dependency of $SD1/SD2$ on variability frequency ($f$); C) Poincaré plots at 3 frequency points, corresponding to arrows in panel B. Note that in the upper panel, while visually overlapping the identity line, data points are slightly above it.
Supplement to Results

\textbf{I}_{E4031} \textit{replacement by mI}_{Kr} \textit{at various cycle lengths}

Adequacy of mI\textsubscript{Kr} in replacing the native current was also tested at CLs of 250 ms and 3000 ms within the same myocyte. As shown in Fig S4, APD and SD1 increments induced by E4031 were maximal at CL 1000 ms, which represents a condition of marked bradycardia in the guinea-pig. On the other hand, mI\textsubscript{Kr} ability to revert E4031-induced changes was similar at all CLs, thus indicating adequacy of the current model under different AP profiles. We also evaluated the time course of APD adaptation when switching from the shorter to the longer CL. An initial APD shortening in the first several cycles, the ensuing APD prolongation was well fitted by a single exponential. E4031 tended to emphasize the initial shortening and to prolong the time constant (\(\tau\)) of adaptation, but the changes did not achieve statistical significance. All E4031-induced changes were reversed by mI\textsubscript{Kr} injection.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure_s4.png}
\caption{\textbf{I}\textsubscript{E4031} \textit{replacement by mI}_{Kr} \textit{at various cycle lengths}. A) Time course of APD adaptation when switching from the shorter (250 ms) to the longer (3000 ms) CL. APD values were normalized to the steady-state values recorded at CL3000 for clarity. B) Statistics of the time constant (\(\tau\)) of adaptation (\(n \geq 6\)). C) Statistics of APD, SD1 and SD1/SD2 in all experimental conditions (* = \(p<0.05\) vs ctr; \(n \geq 5\)).}
\end{figure}

\textbf{Effect of V\textsubscript{0.5A} changes without concomitant shifts in \(\tau\) curve}

According to simple reaction kinetics, shifts in the distribution of states at equilibrium may be associated to similar shifts in the velocity of transitions between states. Therefore, the data concerning V\textsubscript{0.5A} changes reported in the manuscript (Fig 4) were generated by concomitantly shifting V-dependency of activation time constants (\(\tau\)A). The less likely alternative condition, in which shifts in steady-state activation occur without concomitant changes in V-dependency of \(\tau\)A, was tested in a separate series of experiments and is presented in Figure S5. Shifts in V\textsubscript{0.5A} alone slightly modified mI\textsubscript{Kr} profile and V-dependency (Fig S5d) during late repolarization (Phase 3), but failed to affect APD and its variability (Fig S5e).
**Figure S5: Effect of V_{0.5A} changes without shifts in τ curve.** Recordings performed at V_{0.5A} = -20.6 mV and with Δ ± 15 mV are shown as: **a)** Steady-state activation (P_o) computed by the model in three conditions (colour) and superimposed inactivation curve (dotted line); below τ_{act/deact} curve in black line; **b)** representative time course of APD recorded at three values of V_{0.5A}; **c)** example of 10 APs for each conditions with relative APD and SD1 values and Poincaré plot at the right of panel; ctr APD is evidenced as dot line; **d)** m_{IKr} profile aligned with the corresponding average AP waveform represented as I/t and I/V_m plots; **e)** statistics of APD, SD1 and SD1/SD2 in all experimental conditions (NS; n = 7).
SUPPLEMENTAL REFERENCES


