Adenoviral Expression of $I_{Ks}$ Contributes to Wavebreak and Fibrillatory Conduction in Neonatal Rat Ventricular Cardiomyocyte Monolayers

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Abstract—Previous studies have shown that the gating kinetics of the slow component of the delayed rectifier $K^+$ current ($I_{Ks}$) contribute to postrepolarization refractoriness in isolated cardiomyocytes. However, the impact of such kinetics on arrhythmogenesis remains unknown. We surmised that expression of $I_{Ks}$ in rat cardiomyocyte monolayers contributes to wavebreak formation and facilitates fibrillatory conduction by promoting postrepolarization refractoriness. Optical mapping was performed in 44 rat ventricular myocyte monolayers infected with an adenovirus carrying the genomic sequences of KvLQT1 and minK (molecular correlates of $I_{Ks}$) and 41 littermate controls infected with a GFP adenovirus. Repetitive bipolar stimulation was applied at increasing frequencies, starting at 1 Hz until loss of 1:1 capture or initiation of reentry. Action potential duration (APD) was significantly shorter in $I_{Ks}$-infected monolayers than in controls at 1 to 3 Hz ($P<0.05$), whereas differences at higher pacing frequencies did not reach statistical significance. Stable rotors occurred in both groups, with significantly higher rotation frequencies, lower conduction velocities, and shorter action potentials in the $I_{Ks}$ group. Wavelengths in the latter were significantly shorter than in controls at all rotation frequencies. Wavebreaks leading to fibrillatory conduction occurred in 45% of the $I_{Ks}$ reentry episodes but in none of the controls. Moreover, the density of wavebreaks increased with time as long as a stable source sustained the fibrillatory activity. These results provide the first demonstration that $I_{Ks}$-mediated postrepolarization refractoriness can promote wavebreak formation and fibrillatory conduction during pacing and sustained reentry and may have important implications in tachyarrhythmias. (Circ Res. 2007;101:475-483.)

Key Words: $I_{Ks}$, postrepolarization refractoriness ■ wavebreak ■ gene expression

During ventricular and atrial fibrillation, conduction of the electrical wavefront is characterized by complex patterns of propagation, including reentry, wavefront fragmentation (wavebreak), and wavelet formation. A wavebreak occurs if the stimulatory efficacy of a wavefront does not suffice to excite all the tissue downstream. The free shoulder of a broken wave is then prone to curl and give rise to a rotor. To date, the molecular mechanisms of wavebreaks leading to fibrillatory conduction remain poorly understood.

Studies in single guinea pig myocytes showed that slow recovery of excitability during diastole was, in part, a consequence of the slow gating kinetics of the delayed rectifier potassium outward current $I_K$. Shortly after these studies were published, $I_K$ was found to be the result of the activation of 2 outward currents: $I_{Kr}$ and the $I_{Ks}$. Given the large amounts of $I_{Kr}$ present in guinea pig myocytes and the slow deactivation kinetics of this current, we surmise that $I_{Ks}$ is a likely candidate to have an important role in regulating excitability during the diastolic interval, ie, postrepolarization refractoriness.

It is our hypothesis that an increase in the density of $I_{Ks}$ may result in enhanced intermittent conduction block and wavebreak formation by means of postrepolarization refractoriness, which may explain fibrillatory conduction in conditions where the $I_K$ current is enhanced, such as during ischemic catecholaminergic release or in the case of genetic mutations such as SQT2.

In this study we used cultured neonatal rat ventricular myocyte monolayers infected with an adenovirus carrying the cDNA of the molecular correlate of $I_{Ks}$ as a model to investigate the phenomenon of fibrillatory conduction at the molecular level.

Materials and Methods

Myocyte Isolation and Culture

Ventricular myocytes from neonatal Sprague-Dawley rats (Charles River, Mass) were isolated and cultured according to Rohr et al. Briefly, the hearts from 1- and 2-day-old rats were aseptically removed and collected in calcium- and magnesium-free Hanks’ Balanced Salt Solution (HBSS). The ventricles were minced and...
monolayers and at low density in 22-mm coverslips for single cell experiments. Media changes were performed after 24 hours and every 48 hours thereafter with 5% FBS medium. Viral infection was performed at day 3 in culture and myocytes were incubated an additional 24 to 48 hours before experiments to allow for protein expression.

**Adenoviral Construct**

The I_{Ks} channel is formed by the coassembly of the pore forming subunit KvLQT1 (KCΝQ1) and the regulatory β subunit minK (KCΝE1). We generated an adenoviral construct containing a fused cDNA sequence of human KvLQT1 and minK (Ad_{I_{Ks}}) generously provided by Dr R. Kass (Columbia University). The cDNA was subcloned into the AdenoX Adenoviral Expression System (Clontech). Once purified (Virus Purification Kit, Clontech) and tittered (Rapid Titer Kit, Clontech), multicellular preparations were infected with varying multiplicities of infection (MOI) and immunostained using a specific anti-KvLQT1 antibody (US Biological). An MOI of 5 was found to be optimal in terms of uniformity and level of protein expression (Figure 1A). In addition, single neonatal rat ventricular myocytes were infected and standard patch-clamping was used to verify functional expression (Figure 1B and 1C). An adenovirus carrying the sequence of the Green Fluorescent Protein (Ad_GFP) at an MOI of 5 served as a control (see supplemental materials, available online at http://circres.ahajournals.org). No differences in cell appearance, uniformity of conduction, conduction velocity (CV), or APD were observed between Ad_GFP-infected and noninfected monolayers.

**Single Cell Electrophysiology**

**Myocytes**

Voltage clamp experiments were performed in single neonatal rat ventricular myocytes infected with Ad_{I_{Ks}} or Ad_GFP using a HEKA EPC 9/2 amplifier (HEKA Elektronik). 5-second depolarizing steps were applied in 10 mV increments from a holding potential of −50 mV while superfusing the cells with (in mmol/L): NaCl 130, KCl 5.4, MgCl 1.0, CaCl 1.0, HEPES 10, glucose 10, nifedipine (20 μmol/L); pH=7.4 (NaOH). The pipette filling solution contained (in mmol/L): KCl 30, potassium aspartate 90, K2ATP 5.0, Hepes 5.0, EGTA 10, MgCl 1.0, NaCl 15; pH=7.2 (KOH).

**HEK293 Cells**

Current clamp experiments were conducted in HEK293 cells (ATCC, CRL1573) using an Axopatch 1D amplifier (Axon Instruments). HEK293 cells were transfected (Effectene Transfection Kit, Qiagen) with guinea pig cDNA of Kir2.1 subcloned in a pcEP4 vector and cotransfected with the human cDNA of minK-KvLQT1 subcloned in a pcDNA3 vector. An S1-S2 pacing protocol was applied in the presence of Tyrode solution, in which each 300-ms S1 stimulus was followed by a 50-ms S2 stimulus of equal magnitude at progressively shorter intervals. The pipette solution consisted of (in mmol/L): KCl 20, K-aspurate 90, KH2PO4 10, EDTA 5.0, K2ATP 1.9, HEPES 5.0, and Mg2+ 7.9; pH 7.2 (KOH). All patch clamping experiments were performed at room temperature. Pipette DC resistances for both experiments ranged between 4 and 5MΩ.

**Optical Mapping**

Culture dishes were placed on a heating chamber and continuously superfused with HBSS without bicarbonate (Sigma) containing (in mmol/L): CaCl2 1.6, KCl 5.4, MgSO4 0.8, KH2PO4 0.4, NaHCO3 4.2, NaCl 136.9, NaHPO4 0.3, D-Glucose 5.5, and HEPES 10; pH 7.4 (NaOH). All experiments were performed at 35°C. Repetitive stimuli (duration, 5 ms; strength, twice diastolic threshold) were applied by a thin extracellular bipolar electrode at increasing frequencies, starting at 1 Hz, until loss of 1:1 capture or initiation of sustained reentry. Isoproterenol (200 μmol/L) was superfused when indicated. Electrical impulse propagation was recorded optically by staining the monolayers with the potentiometric dye di-8-ANEPPS (40 μmol/L; Molecular Probes) for 15 minutes. The changes in
fluorescence corresponding to transmembrane voltage were recorded by an 80×80 pixel CCD camera (SciMeasure Analytical Systems Inc, Decatur, Ga) in 2-second movies at 500 frames per second (LabWindows Acquisition). Illumination was restricted to the time between the upstroke and 80% of repolarization in each pixel. For CV measurements, activation times were calculated for each pixel, and local conduction vectors were determined as described.16,17

Wavelength (WL) was determined during sustained reentry using the phase-mapping technique.18 WL was defined as the expanse between the wavefront and the end of the repolarization tail1 and measured at 5 mm from the center of rotation (see the supplemental materials for details). Sites of wavefront breakup (wavebreak) marked by phase singularities (PSs) were also identified in phase maps.19,20 PSs are defined as sites where all phases of the action potential converge.

Statistical Analysis
Statistical analysis was performed with Origin software (version 7.0). One-way ANOVA was used for the analyses of CV, APD, WL, and frequency of reentrant activity. Regression analysis was performed on WL data. Values are expressed as mean±SE. A value of P<0.05 was considered significant.

Results
Of a total of 85 monolayers, 44 were infected with “Ad_-Ik,,, (Ik channel adenovirus), and 41 were infected with “Ad_GFP” (GFP adenovirus). Altogether, 584 optical mapping movies were analyzed.

Ik Overexpression
Spatially uniform protein expression was confirmed by immunohistochemistry in 10 multicellular preparations infected with Ad_Ik,, Figure 1A shows an image of a sparsely plated neonatal rat ventricular myocyte preparation immunostained using a specific anti-KvLQT1 antibody (US Biological) in green. An average of 94±4% of the cells showed positive staining. Functional protein expression of the Ik channel was confirmed by whole cell voltage clamp. Figure 1B illustrates representative examples of response currents obtained from single neonatal rat ventricular myocytes infected with Ad_Ik, (left) and Ad_GFP (right) by applying the voltage clamp protocol depicted in the inset and described in the methods section. Although both groups of cells yielded similar peak outward currents in the first 20 ms of recordings, only the Ik-infected myocytes exhibited slowly activating pulse currents and slowly deactivating tail currents. Figure 1C shows the activation curve constructed from measurements at the end of the 5-second pulses (when the Ik-like currents are maximal). Under these steady-state conditions current densities in the Ik group were 5.2±1.4 times larger than in the control group. Finally, when compared with control, cells infected with Ad_Ik showed no apparent changes in intrinsic transmembrane currents, including ICa,L, If, and IK. Further characterization of the infected myocytes can be found in the supplemental materials.

Action Potential Duration and Conduction Velocity
We then investigated the effects of Ik overexpression on APD in confluent electrically-coupled monolayers. Mean APD50 was determined from high-resolution APD maps during 1:1 activation in 32 control and 32 Ik monolayers. Ik decreased APD50 when compared with control but reached statistical significance only at the lower frequencies (1 Hz, P<0.0056; 2 Hz, P<0.0003; and 3 Hz, P<0.03) (see Figure 2). In addition, whereas in the control APD changed monotonically as a function of stimulation frequency, the Ik group had an initial bell-shaped profile demonstrating that Ik contributes more outward current to repolarization at 1 Hz (and at even slower frequencies) than at 3 Hz.21 Because Ik overexpression results in significant APD shortening only at relatively slow pacing frequencies in our monolayers, it is reasonable to hypothesize that the frequency of reentry in the Ik-overexpressing monolayers will be similar to the controls, unless the dynamics of reentry provide a substrate for further APD abbreviation. We therefore measured APD50 during reentry. As shown in Figure 3B, APD50 was shorter in Ik than control monolayers at all rotation frequencies (control slope: −7.31±0.89, Ik slope: −5.90±0.73; P=1.40×10−5).

During pacing, CV remained constant as the impulse traveled centrifugally from the stimulating electrode (see the supplemental materials). Under these conditions CV differences between control and Ik monolayers did not reach statistical significance. On the other hand, as expected,17,18,22 during reentry CV increased gradually when measured as a function of distance from the core in both groups as shown in Figure 3C and 3D. However, this increase was significantly less pronounced in the Ik monolayers, consistent with a less excitable preparation.17
**IKs Overexpression Reduces Wavelength and Increases Rotor Frequency**

The above results are in agreement with previous studies showing that, during sustained functional reentry, APD and CV are abbreviated in the vicinity of the center of rotation, which contributes to the significantly higher activation frequencies that are achievable during reentry compared with external pacing. We sought to address this issue by quantifying both WL and frequency during sustained reentry. Figure 3A shows snapshots of single spiral waves in control (8.3 Hz) and IKs (10.1 Hz) monolayers. Representations are available as movies in the supplemental materials. In Figure 3E we have plotted the WL measured for each rotor as a function of rotation frequency. Both groups showed similar frequency dependence; WL shortened in both as the rotation frequency increased (control slope: $-0.81\pm0.36$; IKs slope: $-0.76\pm0.20$). However, whereas control WLs ranged between 12.02 and 22.68 mm, IKs WLs were significantly shorter, ranging between 4.50 and 12.69 mm ($P=2.87\times10^{-9}$). Figure 4 compares the average rotation frequencies of 15 control monolayers with those of 20 littermate IKs monolayers. Individual rotor frequencies ranged between 4.9 and 13 Hz in both. On average, however, rotors in IKs monolayers were significantly faster ($P<0.0436$) than controls.

**β-Adrenergic Stimulation**

IKs is enhanced by β-adrenergic stimulation via cAMP/protein kinase A-dependent pathways. We therefore administered...
isoproterenol (200 nmol/L) to 10 monolayers infected with Ad\_IKs and 10 control monolayers to assess its effect on both focal spontaneous activity, evident as typical target patterns with centrifugal propagation from the source, and self-sustained reentry (spiral waves). As shown in Figure 5, isoproterenol invariably increased the frequency of spontaneous focal discharges in both groups (A and B). However, it did not change the frequency of rotation during reentry in either group (C and D). A possible explanation for this finding could be that isoproterenol increases L-type calcium current which would tend to counteract the \( I_{\text{Ks}} \) activity.

**Wavebreaks and Fibrillatory Conduction**

Unlike control monolayers, the waves emanating from rotors in the \( I_{\text{Ks}} \) preparations often underwent wavebreak and formation of new, short-lived rotors in a time-dependent fashion. We will refer to this distinct pattern of electrical activity as fibrillatory conduction. As illustrated by the representative sequential phase maps presented in the top panel of Figure 6A, waves emanating from sustained rotors in control monolayers did not undergo wavebreak (0/15). In contrast, as illustrated in the bottom panel, stable rotors gave rise to multiple wavebreaks and fibrillatory conduction in 9 \( I_{\text{Ks}} \) preparations (see supplemental Movies). This corresponded to 45% of the \( I_{\text{Ks}} \) infected monolayers that sustained a stable rotor. The initial wavebreaks consistently occurred distally from the mother rotor and progressively “approached” it by impinging on incoming wavefronts resulting in more wavebreaks. The pattern eventually reached a steady state in which short-lived rotors coexisted with the main source, rotating at the same frequency. Figure 6B illustrates the patterns of evolution of wavebreaks with time. Each plot corresponds to a different monolayer and each point to an optical recording. Movies were sequential in time, as illustrated in panel A. In some instances the steady state fibrillatory conduction pattern was already advanced by the time of first recording. In the event that the mother rotor terminated, either another rotor took over as the source or all activity terminated. We did not find any episodes of fibrillatory conduction in which there was no driving source. As illustrated in Figure 7, both groups sustained rotors whose frequency overlapped over a range of
4.9 to 13 Hz. Yet only the $I_{Ks}$ monolayers underwent wavebreaks and fibrillatory conduction. Significantly, wavebreaks occurred at the highest frequency values in the latter group.

Because $\beta$-adrenergic stimulation enhances $I_{Ks}$, we performed an additional set of experiments in 5 Ad$_{I_{Ks}}$-infected and 5 Ad$_{GFP}$-infected preparations to determine whether the exposure to 200 nmol/L isoproterenol would increase wavebreak density (as shown in Figure 6). We observed that the number of singularity points indeed increased in both groups but as a result of collisions with spontaneous foci rather than of wavebreaks (data not shown). This is likely to be attributable to isoproterenol enhancement of spontaneous activity as shown in Figure 5A and 5B.

**Postrepolarization Refractoriness**

To gain insight into possible mechanisms underlying $I_{Ks}$-induced wavebreaks and fibrillatory conduction, we used a heterologous expression system. Our objective was to determine the role of $I_{Ks}$ on the threshold for excitation at diastolic potentials in the absence of other native cardiac-specific proteins that could confound the interpretation of the results. Using previously described methods, we group of HEK293 cells was stably transfected with Kir2.1, responsible for $I_{K1}$ to set the resting membrane potential at $\approx -70$ mV. A second group of HEK293 cells was cotransfected with Kir2.1 and KvLQT1-minK. Five cells in each group were subjected to a whole-cell current clamp S1-S2 stimulus protocol, as illustrated by the representative experiments in Figure 8. In Panel A, each square depolarizing pulse S1 (duration: 300 ms; frequency: 1–2 Hz) was followed by an S2 of equal magnitude, but briefer duration (50 ms), and the S1-S2 interval was varied, with the minimum S1-S2 duration adjusted so that S2 did not encroach on the S1 response. As shown in panel B, the amplitude and shape of the action potential-like response in cells expressing Kir2.1 alone was not affected by the timing

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**Figure 6.** Evolution of fibrillatory conduction

A, Top, Phase maps of a control monolayer; bottom, phase maps of littermate monolayers infected with Ad$_{I_{Ks}}$. Waves are generated by a single reentrant source rotating at 8.2 and 9.4 Hz. Scale bars=10 mm; numbers, time in seconds. B, The number of wavebreaks increased progressively in the 9 $I_{Ks}$ monolayers that developed fibrillatory conduction.

**Figure 7.** Occurrence of wavebreaks as a function of frequency.
of the S2 stimulus. In contrast, as shown in C, a clear time dependency was demonstrated in cells expressing both Kir2.1 and minK-KvLQT1 genes: progressively reducing the S1-S2 interval resulted in gradual changes in both the shape and amplitude of the S2 response, indicative of slow \( I_{Ks} \) deactivation.\(^2\)\(^4\) Similar results were obtained in all experiments surveyed in this manner. Taken together, these results support the idea that both \( I_{K1} \) and \( I_{Ks} \) are important in the recovery of the current requirements for excitation during the diastolic interval.

**Discussion**

We have generated a 2-dimensional biological excitable medium to investigate the consequences of the overexpression of \( I_{Ks} \) on excitation, propagation, and the dynamics of reentrant activation. The most important results are: (1) In agreement with previous work, \( I_{Ks} \) overexpression shortens APD during pacing reaching statistical significance only at low stimulation frequencies (1–3 Hz); (2) During sustained reentry, \( I_{Ks} \) over-expression decreases APD, CV, and WL at frequencies ranging between 4.9 and 13 Hz; (3) Despite a significant WL shortening, \( I_{Ks} \) overexpression results in "history dependent" sink/source mismatch during diastole that leads to wavebreak and fibrillatory conduction particularly at high frequencies of reentry. Computer simulations presented in the supplemental materials show that a spatially heterogeneous distribution of such mismatch could account for the findings of increased wavebreaks in the experiments; (4) Current clamp experiments on HEK293 cells cotransfected with \( KCNJ2 \) and \( KCNQ1-KCNE1 \) show that the slow deactivation kinetics of \( I_{Ks} \) alone may be sufficient to induce wavebreak formation. These results, together with those of computer simulations and patch clamp data presented in the supplemental materials, allow the prediction that spatially distributed differences in \( I_{Ks} \) deactivation kinetics during diastole underlie the mechanism of \( I_{Ks} \)-induced wavebreaks and fibrillatory conduction demonstrated in our monolayers. Overall, the results provide the first demonstration that \( I_{Ks} \) involvement in postrepolarization refractoriness can lead to wavebreak formation and fibrillatory conduction.

**Dynamics of Fibrillatory Conduction**

Studies in hearts from several species ranging in size from the mouse to the pig\(^1\)\(^8\)\(^,\)\(^2\)\(^2\)\(^,\)\(^2\)\(^5\) have demonstrated that even a single rotor can result in ECG patterns that are indistinguishable from VF, which suggests that at least some forms of fibrillation are highly organized and depend on the uninterrupted periodic activity of a small number of high-frequency reentrant sources. The rapidly succeeding wavefronts emanating form such sources propagate throughout the ventricles and interact with tissue heterogeneities, both functional and anatomical, leading to fragmentation and wavelet formation.\(^2\)\(^,\)\(^2\)\(^5\) the end result being fibrillatory conduction.\(^3\)\(^,\)\(^2\) Apparently chaotic waves often recorded optically in intact hearts and attributed to fibrillatory conduction may be generated by sources outside the field of view. In our monolayers, on the other hand, the area recorded corresponds to the entire preparation, allowing the strong conclusion that all the episodes of fibrillatory conduction were sustained by at least 1 stable rotor. Occasionally, rotor activity was abolished by spontaneous focal activity, and this resulted in the immediate termination of fibrillatory conduction.

**\( I_{Ks} \) Contribution to Repolarization**

The contribution of \( I_{Ks} \) to action potential repolarization has been a matter of debate.\(^2\)\(^6\)\(^,\)\(^2\)\(^7\) Even in humans, both loss of function\(^2\)\(^8\) and gain of function\(^2\)\(^9\) of \( I_{Ks} \) can result in APD (QT) interval prolongation. Therefore, how the interplay between \( I_{Ks} \) and other currents modulates APD and, perhaps more importantly, its accommodation to rate, remains unresolved. In our experiments on neonatal rat ventricular monolayers we found that \( I_{Ks} \) significantly blunts the APD response to frequency (Figure 2). Nevertheless, at increased stimulation frequencies (into the range of rotor frequencies) the shortening of the APD in the \( I_{Ks} \) preparations did not reach statistical significance when compared on a frequency by frequency basis. During reentry, however, overall APDs were significantly shorter in \( I_{Ks} \)-overexpressing monolayers (Figure 3B). This may suggest that \( I_{Ks} \) enhances the repolarizing influence exerted by the core of the rotor to further abbreviate the action potential allowing for the slightly faster frequencies of rotation observed in Figure 4. Our data are not conclusive in this respect, and more studies beyond the scope of this one would be required to elucidate the exact role of \( I_{Ks} \) on cardiac repolarization.

**Postrepolarization Refractoriness**

Recovery of excitability after an action potential can be a slow process that greatly outlasts the action potential duration.
Previous studies in isolated guinea pig ventricular myocytes have shown that activation failure can occur at diastolic potentials (even if the Na⁺ current, Iₔ, has had sufficient time to recover completely from inactivation) because of the slow gating kinetics of Iₖ. This phenomenon is called postrepolarization refractoriness. We surmised that Iₖ, the slow component of Iₖ, is an important determinant of postrepolarization refractoriness. Our experiments in HEK293 cells provide strong support to such an idea by revealing that Iₖ expression alone suffices to explain time dependent activation failure.

However, it was uncertain whether the results observed at the single cell level could be extrapolated to allow inferences related to the propagation of the electrical impulse where, in addition to cellular excitability, one has to consider electrical coupling and wavefront curvature, as well as structural heterogeneities, as potential contributors to the success or failure of propagation of the action potential. We found that Iₖ significantly shortens the wavelength while slightly but significantly increasing the frequency of reentry in the monolayers. Yet in this model, waves emanating from a stable source more readily encounter tissue that is repolarized but not yet excitable, leading to wave fragmentation. Such an apparent paradox can be readily explained by the results in the HEK293 cells showing that in the presence of full repolarization, the slow deactivation kinetics of Iₖ, significantly impair the recovery of excitability at early diastolic intervals. This is not to say that the Iₖ threshold, or the rate of recovery of Iₖ and Iᵣ, are not critical for cell excitability; however, under conditions in which these parameters are fully recovered at any given location, an increase in the number of Iₖ channels that remain open during diastole will act to oppose any depolarizing current. As such, the approach to threshold would be delayed with the possible occurrence of wavebreak.

Computer simulations (see supplemental materials) aided us in establishing that a spatial dispersion in the conformational state of the Iₖ channel, or spatially inhomogeneous recovery kinetics during diastole, may be sufficient to allow for wavebreak/reentry formation at specific locations. Therefore, the simplified nature of the monolayer, together with the results of simulations, allows us to postulate that the spatially distributed wavebreaks and intermittent block processes that are frequently observed during VF result in part from inherent spatial heterogeneities in Iₖ distribution and of its slow gating kinetics.

**Role of Iₖ Accumulation**

Iₖ activates in response to depolarization at potentials greater than −30 mV and reaches half-maximal activation at +20 mV. Iₖ activates more slowly than any other known K⁺ current and requires membrane depolarization in the order of seconds to reach a steady state, conditions that are never achieved in vivo. Although Iₖ activates slowly, because of its slow deactivation kinetics it will tend to accumulate at higher frequencies and therefore play an important role in VT/VF. Regardless of whether Iₖ accumulation occurs because of slow deactivation, β-adrenergic stimulation, rate-dependent conformational states, or all of the above, it is reasonable to expect that an increase in the magnitude of Iₖ during the action potential will have an effect on excitability. Provided that the previous history of depolarizations leaves a significant portion of repolarizing channels open, larger quantities of current will be needed for excitation immediately after the action potential has ended.

**Clinical Relevance**

Excessive APD prolongation secondary to Iₖ reduction leads to early afterdepolarization (EAD)-induced arrhythmias. On the other hand, our experiments show that increased Iₖ can lead to reentry by means of wavebreak and singularity point formation. In fact, this could well be the mechanism underlying arrhythmias in some short QT syndrome patients.

Most known class-III drugs block Iₖ and prolong APD. Unfortunately, Iₖ blockade typically causes excessive APD prolongation at slow heart rates. Blockade is much less effective at high rates. This so-called “reverse use-dependent action” can be pro-arrhythmic. Hence, selective Iₖ blockade may be an appropriate alternative with less pro-arrhythmic potential.

**Study Limitations**

It is generally accepted that the role of Iₖ, on excitability is species-dependent. Our neonatal rat ventricular myocyte model allowed us to express the human Iₖ channel and study its effects on conduction during pacing, sustained reentry, and fibrillatory conduction in a highly controlled environment. However it should be kept in mind that the channel is expressed in rodent myocytes with an ionic profile different from that of human myocytes. The presence of myofibroblasts in a monolayer is thought to be responsible for the low values of CV. Although 2-hour differential preplating and bromodeoxyuridine was used to reduce fibroblasts, total elimination cannot be achieved.

The conclusions that can be drawn from these results are conceptual and should be furthered in translational experiments before attempting to extrapolate them to the clinical setting.

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**Disclosures**

None.

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1. Expanded Methods

Animal Surgery

A total of 18 litters (10 pups each) of Sparague-Dawley neonatal rats (Charles River, MA) from both sexes were euthanized with the approval of the Department of Laboratory Animal Resources and the Committee for the Humane Use of Animals (CHUA) of SUNY Upstate Medical University. Prior to terminal surgery, the pups were anesthetized by isoflurane inhalation in an enclosed chamber containing a 10 cm x 10 cm gauze pad with roughly 2 mL of liquid isoflurane (Attane, Minrad Inc.) The animals were sacrificed by decapitation with minimal discomfort or pain. The thoracic cavity was then opened with sterile tools and the heart was quickly removed and placed in Ca\(^{2+}\) and Mg\(^{2+}\) free Hank’s Balanced Salt Solution (Cellgro).

Optical Mapping Setup

A schematic representation of the optical mapping setup is presented in Online Figure I. The excitation light, provided by two 450 W tungsten lamps, was filtered (absorbance infrared filters and 520AF80 excitation filters) before reaching the 35 mm-in-diameter cell preparation. Epifluorescence was also filtered (infrared filter and 645DF100 emission filter) prior to detection at 500 frames per second by an 80 x 80 pixel CCD camera (SciMeasure Analytical Systems, Inc., Decatur, GA). The camera lens used had a focal length of 12 mm with F=1:1.2 (Rainbow CCTV, Japan). The spatial resolution was ~0.44 mm per pixel (roughly 100 cells/pixel). Therefore, motion artifacts did not distort the optical signal significantly.

Wavelength Measurements

In the main manuscript “wavelength” was defined as the expanse between the wavefront and the end of the repolarization tail and measured at 5 mm from the center of rotation. The cartoon in Online Figure II, shows the site of such measurements in yellow. The yellow line is perpendicular to the tangent (green) at the site where the wavefront intersected a concentric circle with a 5 mm radius (red).
Online Figure I  Schematic representation of the optical mapping setup. TL: tungsten lamp, IRF: infrared filter, ExF: excitation filter, EmF: emission filter, NRVMM: neonatal rat ventricular myocyte monolayer.
Online Figure II  Cartoon illustrating method for wavelength measurements. See text for details.
2. Single Cell Electrophysiology

In the main manuscript monolayers of neonatal rat ventricular cardiomyocytes were used as two-dimensional biological excitable media to investigate the consequences of \( I_{Ks} \) overexpression on excitation, propagation and the dynamics of reentrant activation. In an effort to better understand the results obtained from the monolayer experiments, single cell electrophysiology experiments were performed in order to compare the ionic backgrounds of Ad_\( I_{Ks} \) and Ad_GFP infected neonatal rat ventricular myocytes. All experiments were conducted at room temperature using a HEKA EPC 9/2 amplifier (HEKA Elektronik).

**L-Type Calcium Current**

The L-type calcium current (\( I_{Ca-L} \)) is activated roughly at the same voltage range than \( I_{Ks} \) but with opposite conductance. For this reasons it was imperative to determine whether or not the magnitude of endogenous \( I_{Ca-L} \) was different between the \( I_{Ks} \) overexpressing and the control myocytes. We measured whole cell nifedipine (20 µM)-sensitive currents elicited by 500 ms-long voltage clamp pulses applied in 10 mV intervals from -50 mV to +50 mV. The holding potential was -40 mV to prevent the activation of \( I_{Na} \). A 400 ms hyperpolarization to -80 mV after each pulse was used to accelerate the recovery of \( I_{Ca-L} \) and enhance \( Ca^{2+} \) extrusion. The composition of the extracellular solution was (in mmol/L): NaCl 110, TEA-Cl 30, CsCl 5.4, MgCl\(_2\) 1.0, CaCl\(_2\) 1.8, HEPES 10, glucose 10; pH = 7.4 (CsOH) and that of the pipette solution was (in mmol/L): CsCl 140, MgCl\(_2\) 1, CaCl\(_2\) 1, EGTA 5, Mg-ATP 4, Hepes 5, TEA-Cl 10 and cAMP 0.1; pH = 7.2 (CsOH). As shown in the current-voltage relation plot in Online Figure III, the magnitude of \( I_{Ca-L} \) normalized to cell capacitance was not significantly different between the groups.

**Early Peak and Steady State Potassium Currents**

Whole cell peak currents elicited within the first 20 ms of voltage clamp steps ranging between -120 mV and +100 mV were measured in Ad_\( I_{Ks} \) and Ad_GFP infected myocytes. Steady state currents were measured at the end of 1 sec depolarizing pulses as illustrated in Online Figure IV. The holding potential was -80 mV but a 10 ms pre-pulse to -20 mV was applied right before the steps to inactivate \( I_{Na} \). The bath and pipette solutions were the same as reported for Figure 1 in the main manuscript, (in mmol/L): NaCl 130, KCl 5.4, MgCl\(_2\) 1.0, CaCl\(_2\) 1.0, HEPES 10, glucose 10, nifedipine (20 µmol/L); pH = 7.4 (NaOH) and KCl 30, potassium aspartate 90, K\(_2\)ATP 5.0,
Hepes 5.0, EGTA 10, MgCl$_2$ 1.0, NaCl 15; pH = 7.2 (KOH) respectively. The current-voltage relation plots constructed from these data are shown in Online Figure IV.

The inward component in both plots likely corresponds to the inwardly rectifying potassium current, $I_{K1}$. $I_{K1}$ is conductive during the diastolic interval and therefore can contribute to post-repolarization refractoriness.

Similarly, no significant differences were found regarding the magnitude of early peak outward currents measured within the initial 20 ms of depolarization as shown in the left plot. $I_{Ks}$ has not started to activate at this time interval, so the current measured (most likely the transient outward current $I_o$) is intrinsic.

Outward currents measured after 1 second (right plot) only reached steady state in the Ad_GFP infected myocytes, since in Ad_$I_{Ks}$ infected cells this current was summated to that of $I_{Ks}$.
Online Figure III  Nifedipine-sensitive current densities in Ad_GFP and Ad_I_{Ks} infected myocytes as a function of membrane voltage (p=NS). **Bottom left:** Voltage clamp protocol. **Bottom right:** Examples of current traces under control conditions and after the application of nifedipine (20 µM).
Online Figure IV  Current densities as a function of membrane voltage for **Left:** early peak currents measured within 20 ms of depolarization and **Right:** steady state currents measured after 1 second of depolarization in Ad_GFP and Ad_IKS infected myocytes (* p<0.05). **Bottom:** Voltage clamp protocol. Red ovals indicate the locations at which the pulse currents were measured for the construction of the plots above.
3. Additional Properties of the Cell Culture

**Uniformity**
Prior to the experiments all monolayers were thoroughly inspected to confirm the absence of structural heterogeneities (Online Figure V panels A and B). In addition, only monolayers that showed uniform impulse propagation during control pacing and that sustained conduction velocities >10 cm/s at all times were considered for analysis (See Online Figure V panels C and D).

**Density**
We performed systematic cell counts prior and 48 hours after viral infection in 5 monolayers infected with Ad_GFP and 5 monolayers infected with Ad_I\(_{KS}\). No significant differences in cell density were found neither before and after infection or between groups as shown in Online Figure VI panel A. Additionally, culture dishes were fixed and incubated with a primary antibody against myocyte marker sarcomeric α-actinin which revealed that 96 ± 3 % of the cells in the preparations corresponded to myocytes.

**Connectivity**
The Western immunoblot presented in Online Figure VI panel B shows that the levels of Cx43 protein expression are similar between preparations infected with Ad_GFP and Ad_I\(_{KS}\). Multicellular preparations infected with Ad_I\(_{KS}\) were fixed and stained with myocyte specific sarcomeric α-actinin antibody (red) and Cx43 antibody (green) to confirm Cx43 localization to the intercellular space (panel C).
Online Figure V  Neonatal rat ventricular myocyte monolayers. A. Fluorescent image of a preparation infected with Ad_GFP. B. Phase image of an isotropic culture. Scale bars = 100 μm. C and D. Activation maps for 2 Hz pacing in control (C) and I_{Ks} (D) monolayers. Scale bars = 500 μm. Isochronal lines are 2 ms apart.
**Online Figure VI**  
**A.** Cell densities before and after infection (p=NS). **B.** Western immunoblot shows that the levels of Cx43 protein expression are similar between preparations infected with Ad_GFP and Ad_IKS. **C.** Multicellular preparations infected with Ad_IKS fixed and stained with myocyte specific sarcomeric α-actinin antibody (red) and Cx43 antibody (green). Blue corresponds to DAPI nuclear stain.
4. Numerical Simulations

In the main manuscript we demonstrate that during sustained reentry, $I_{Ks}$ overexpression reduces excitability during diastole and leads to wavebreak despite significantly reducing the excitation wavelength. Here, we have carried out numerical simulations to test the hypothesis that, in the $I_{Ks}$-overexpressing monolayers, heterogeneous, spatially distributed differences in $I_{Ks}$ deactivation kinetics during diastole underlie the mechanism of $I_{Ks}$-induced wavebreaks and fibrillatory conduction.

Methods

Mathematical model

To our knowledge, no one has yet developed an ionic model of the action potential of the cultured neonatal rat ventricular cardiomyocyte. The model published in 2001 by Pandit et al.\(^3\) simulates the time course of the action potential of adult rat ventricular myocytes and features action potential duration (APD) increase with frequency, which is opposite to what occurs in neonatal preparations (see main manuscript). Therefore we decided to use a modified version of the well-established Luo-Rudy model\(^4\) for our simulations. We are fully aware that using such model imposes significant limitations to our ability to accurately simulate and/or make predictions about neonatal cardiomyocyte monolayer behavior. Nevertheless, we are confident that significant insight may be derived from our numerical results (see below).

We modeled the kinetics of the ionic currents using the Luo-Rudy 2000 model (LR2000)\(^4\) (http://www.case.edu/med/CBRTC/LRdOnline/LRdModel.c). A human Markovian model was used for $I_{Ks}$, as proposed by Silva and Rudy\(^5\) (http://rudylab.wustl.edu/research/cell/methodology/markov/IKHTML/IKsmodel.htm). The $I_{Ks}$ current was modeled using the equation in the form $I_{Ks} = G_{Ks} \cdot O_{Ks} \cdot (V_m - E_{Ks})$, where

$$G_{Ks} = G_{Ks} \cdot \left(1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{Ca^{2+}} \right)^{1.4}} \right), \quad O_{Ks} \text{ is the fraction of channels in the open state(s), } E_{Ks} \text{ is the equilibrium potential for } I_{Ks} \text{ and } G_{Ks} \text{ is a measurement of the } I_{Ks} \text{ expression level, originally not named in reference}^5.$$

In addition, the $I_{Kr}$ current was removed from the model, since no E-4031 sensitive currents were present in our neonatal rat ventricular myocytes. The temperature was
set at 37° C. Since the original LR2000 model gives a resting membrane potential of ~ -90 mV (which is more negative than the RMP reported in the literature$^6$ and that obtained in our measurements), we adjusted the model by shifting the $I_{K1}$ I-V curve by 10 mV to the right. The simulation code was based on previous work$^5$. The differential equations were integrated using a modified Euler method (improvements included the use of an analytical expression to update the gating variables and calcium concentrations, as well as forcing and scaling $I_{Ks}$ state occupancies to the meaningful values). Since the $G_{Ks}$ conductance value taken from Silva$^5$ yielded an $I_{Ks}$ level approximately two times greater than that measured in our experiments, we left it unscaled, unless otherwise noted.

We ran 0D (single cell), 1D (cable) and 2D simulations, using an integration step of 10 µs for 0D and 1D simulations and 100 µs for 2D simulation. For 1D and 2D we used a monodomain model, with a capacitance of 153.4 pF at each node. The internodal conductance was set to 0.24 µS and the grid size was 0.1 mm. The 1D simulations were performed on a 1D cable (400 nodes in length corresponding to ~40 mm) and the 2D simulations were performed on a square sheet (300×300 nodes corresponding to approx. 30×30 mm). Both 1D and 2D domains were modeled to be structurally homogeneous (with the same kinetics and current densities in all nodes) except for one 2D simulation, as noted in the Results section. In all simulations we used square pacing stimuli 0.5 ms in width and 12 nA in amplitude (per 153.4 pF node capacitance which corresponds to about 80 pA/pF). Initial conditions for all simulations were set as presented in Online Table I. Extracellular concentrations were fixed to the following values (in mmol/L): $[Na^+]_o = 140$, $[K^+]_o = 4.5$, $[Ca^{2+}]_o = 1.8$.

Initial conditions for $I_{Ks}$ were different in different simulations. Silva and Rudy$^5$ presented a markovian state model for $I_{Ks}$, shown in Online Figure VII panel A, which is reproduced from their original work. We will use their presentation scheme (color scheme and geometrical location on the diagram) to describe in the Results section the initial $I_{Ks}$ conditions we have created. For instance, Online Figure VII panel B shows the initial state occupancies calculated as steady state values for initial $V_m$ in the single cell model shown in Online Figure VIII.
Results and Discussion

**Single cell simulations (0D)**

To verify the numerical stability in our models we ran single cell simulations with integration steps of 1, 10 and 100 µs. As shown in Online Figure VIII, there is no difference between 10 and 1 µs steps, confirming that the widely used 10 µs step is appropriate. The slight difference between 10 and 100 µs step we arbitrary deemed as acceptable.

In Online Figure IX we present the results of single cell simulations carried out with different levels of $I_{Ks}$ expression. Interestingly, the results show that a large (over 25-fold) increase of the $G_{Ks}$ (from 0.75× to 20×) conductance causes a relatively small (less than 3-fold) increase in the $I_{Ks}$ amplitude (from 400 to 1200 pA), proving that the cell model is not very sensitive to the $I_{Ks}$ current expression level.

**Simulations of propagation along a (1D) cable**

We simulated propagation along a 1D homogeneous cable, as described in the Methods section, with repetitive stimulation applied to the leftmost 4 mm of the cable at a frequency of 10 Hz. At the beginning of the simulation we applied inhomogeneous initial conditions of $I_{Ks}$ state occupancy, creating a gradient of zone 1/zone 2 occupancy, as shown in Online Figure X panel A, where on the left side of the cable ~50% of channels are in zone 1 and ~50% of channels are in zone 2. On the right side only ~10% of channels are accumulated in zone 1 and ~90% of channels are in zone 2. Once we assumed overall occupancies for closed zone 1, closed zone 2 and open zone, we calculated the occupancies of each particular state within the zones, using transition rates corresponding to the initial $V_m$ value. Online Figure X panels B, C and D show initial state occupancies obtained with this procedure for three different spots in the cable. Since it is known that $I_{Ks}$ accumulates in zone 1 states, this procedure allowed us to model spatial dispersion of $I_{Ks}$ accumulation with post-repolarization refractoriness. Online Figure XI shows the propagation patterns originating from such conditions. After four beats in which activation was 1:1 throughout the entire cable (and one stimulus which was not captured), the fifth beat failed and 2:1 block began at X ~ 17 mm. Subsequently, the block drifted towards the electrode and finally disappeared. A simulation in which the initial conditions were the same in all nodes demonstrated initial block followed by 1:1 capture without conduction block (data not shown).
The above simulation demonstrates that spatial dispersion in the conformational state of the channel responsible for $I_{Ks}$, with the current overexpressed to a level similar to that measured in our experiments, is sufficient to create intermittent propagation block in a 1D cable.

**2D simulations with inhomogeneous initial conditions**

We carried out additional simulations to determine whether establishing initial conditions of spatial dispersion in the conformational state of the $I_{Ks}$ channel would be sufficient to allow for the formation of wavebreak/reentry in a 2D sheet. Movie 1 shows the result of a simulation of a structurally homogeneous monolayer with inhomogeneous initial $I_{Ks}$ state occupancy. The domain was paced by stimuli applied to the 4 mm width stripe adjacent to left edge at a frequency of 7 Hz.

The initial closed zone 2 occupancy was calculated as follows:

$$zone_2 = 0.5 + 0.4 \cdot \frac{1}{1+\exp\left(\frac{x-150}{-10}\right)} \cdot \frac{1}{1+\exp\left(\frac{y-150}{-10}\right)} ,$$

where $x$ and $y$ are geometrical coordinates in nodes. Closed zone 1 occupancy was expressed as $zone_1 = 0.999 - zone_2$. Initial open state occupancy was uniformly assigned as 0.001. Subsequently, we calculated detailed state occupancies within zones as described above for the 1D case. Such a formulation created initial conditions in the form of $I_{Ks}$ de-accumulation in the upper right-hand quadrant and accumulation in the rest of the domain with a smooth transition, as shown in Online Figure XII panel A.

In Movie 1 we can observe a train of planar waveforms propagating from left to right at 143 ms intervals, commencing from 50 ms (only 1 stimulus delivered at 193 ms was not captured). At about 910 ms a wavefront-wavetail interactions resulted in the formation of a singularity point. The initiated rotor subsequently collided with oncoming wavefronts and finally at ~1100 ms a train of planar wavefronts began permanently breaking at the just established secondary rotor. Finally, from 3300 ms, the interaction between the rotor and pacing electrode leads also to intermittent fibrillatory conduction.

Movie 2 shows data from a second simulation, achieved using the same pacing protocol. Here inhomogeneous initial closed zone 1/2 occupancy of de-accumulation (recovery) was modeled as
\[ zone_2 = 0.5 + 0.49 \cdot \exp\left( -\frac{(x - 200)^2}{60^2} - \frac{(y - 150)^2}{90^2} \right), \]

where \( x \) and \( y \) are geometrical coordinates in nodes. Closed zone 1 and open states occupancy, as well as detailed state occupancies within the zones, were calculated as above.

In Movie 2 we can observe a train of planar waveforms passing from left to right at 143 ms intervals, beginning at 50 ms (only 1 stimulus delivered at 193 ms was not captured). At 480 ms an incomplete wavebreak initiates right of the center of the domain leading to an incomplete figure-of-8 reentry. Later, the wavefronts of both rotors merge on the right hand side and travel to the left, finally colliding with and breaking the subsequently oncoming wavefronts. Eventually both rotors mutually annihilated.

**2D simulation with structural heterogeneity**

Movie 3 shows the result of a simulation with structural heterogeneity. Here we multiplied the transition rate \( \delta \) (see Online Figure VII panel A) by a scaling factor

\[ k_\delta = 1 + 6 \cdot \exp\left( -\left( \frac{x - 150}{90} \right)^2 + \left( \frac{y - 150}{135} \right)^2 \right), \]

(where \( x \) and \( y \) are geometrical coordinates in nodes), ranging from 1 in the periphery to 7 in the center (as shown in Online Figure XIII). All other parameters (including kinetics, current densities and initial conditions) were homogeneous and \( G_{Ks} \) was increased 10 times. The \( I_{Ks} \) states occupancies were initiated with steady state values corresponding to the initial \( V_m \). Since the transition rate varied across the domain, the initial \( I_{Ks} \) state also varied implicitly. The domain was paced at 7 Hz, with stimuli applied to the 4 mm width stripe adjacent to left edge.

After \( \sim 1000 \text{ ms} \) of the simulation we can clearly see in Movie 3 the first completely developed wavebreak with wavelets that then merge into a wavefront moving to the left. Subsequently those waves collide with the incoming wavefront. Thereafter, no more wavefront managed to enter and cross the central area from the left, due to collisions with the pair of rotors.
Conclusions

The 1D and 2D simulations presented above showed the possibility of $I_{Ks}$-related wavebreak occurrence (or a conduction block as its 1D equivalent).

Interestingly, in all 1D and 2D simulations the block or wavebreak does not occur immediately at the onset of stimulation but after some latency lasting several beats. We attribute the lag time to the large time constants governing the $I_{Ks}$ channel and to the fact that transitions between its 15 closed states may not immediately affect the ionic currents.\(^5\)

It is also worth stressing here that whereas in the 1D simulation shown above the initial site of block moves towards the electrode location, in the 2D simulations (Movie 2 and 3) retrograde wavefront propagation can be seen. This is equivalent to the movement of the wavebreak site toward the mother rotor in our experiments (see Figures 6 and 7 of the main manuscript).

Although only the simulation presented in Movie 1 showed the fibrillatory conduction due to interference at the pacing site, it is important to keep in mind that Movies 2 and 3 demonstrated the possible scenario of singularity point(s) creation from a planar wavefront due to an $I_{Ks}$-related mechanism even in idealized symmetric conditions. Analysis of the details of the later fate of those singularity points (drifting, meandering, mutual annihilation etc) is beyond the scope of this work.

As we see it, both hypothetical mechanisms investigated above for the observed wavebreaks seem reasonable. For example, the spatial dispersion of the zone 1/2 occupancy could occur in the so-called “cross-field stimulation” scenario.\(^7\) However, the experimental verification of the dispersion in the kinetics would be extremely difficult to achieve. Because of the elaborate protocols that would be needed to accurately assess the kinetics parameters, verification would require a significant number of long lasting patch clamp experiments conducted at different locations of the same monolayer.

In conclusion, our simulations demonstrate that a spatial dispersion in the conformational state of the $I_{Ks}$ channel or spatially inhomogeneous $I_{Ks}$ recovery kinetics during diastole may be sufficient to allow for the formation of wavebreak leading to reentry at specific locations in the monolayer.
**Online Table I. Initial conditions common for all simulations**

<table>
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<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
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</thead>
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<td>Transmembrane potential</td>
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<td>Intracellular potassium concentration</td>
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<tr>
<td>NSR calcium concentration</td>
<td>$[\text{Ca}^{2+}]_{\text{NSR}}$</td>
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<td></td>
</tr>
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</table>

Gating variables were set to steady state values corresponding to initial $V_m$. 
Online Figure VII  A. Markovian state model for IKS, reproduced from published reference\textsuperscript{3}. B. State occupancies calculated as steady state values for initial $V_m$ in a single cell model.
Online Figure VIII  Results from single cell simulation confirm numerical stability. Diagrams show A. transmembrane potential $V_m$ and B. $I_{ks}$ calculated for a beat evoked during the 20th second of pacing at 7 Hz, the last of 200 beats evoked by pacing at 10 Hz at integration steps of 1, 10 and 100 µs (red, green and blue, respectively).
Online Figure IX  A. Transmembrane potential $V_m$ and B. $I_{Ks}$ calculated for the a beat evoked during the 20th second of pacing at 7 Hz for different levels of $I_{Ks}$ expression (1× corresponds to $G_{Ks} = 0.779$ mS/µF). Cell capacitance 153.4 pF.
Online Figure X  Simulation using a 1D cable with spatially inhomogeneous initial $I_{ks}$ state. **A.** Spatial dispersion of zone 1 and zone 2 occupancies, with the left region corresponding to cells with $I_{ks}$ accumulated in zone 1 (as if after fast/recent pacing), right region corresponding to cells with $I_{ks}$ not accumulated (as if after certain time of resting) and transition region in between. Both occupancies sum 0.999; 0.001 is assigned to the open states occupancy. **B. C. D.** Initial occupancy of particular $I_{ks}$ states at three different locations: $X = 5, 15, 25$ mm, respectively.
**Online Figure XI**  Evolution of the 1D cable. The horizontal axis denotes geometrical coordinate along the cable and the vertical axis denotes time. Colors denote transmembrane voltage $V_m$ in mV, as shown by the color bar.
Online Figure XII  Distribution of $I_{Ks}$ closed zone 2 occupancy at the beginning of simulation. Both axes are scaled in nodes. **A.** De-accumulation (recovery) region in the upper right-hand quadrant. **B.** Island of $I_{Ks}$ de-accumulation (recovery) in the center of the sheet.
**Online Figure XIII**  Distribution of scaling factor $k_\delta$ for the closing transition rate of the second voltage sensor transition (parameter $\delta$ in reference$^3$). Both axes are scaled in nodes.
References


