Simulations of propagated mouse ventricular action potentials: effects of molecular heterogeneity

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Bondarenko VE, Rasmusson RL. Simulations of propagated mouse ventricular action potentials: effects of molecular heterogeneity. Am J Physiol Heart Circ Physiol 293: H1816–H1832, 2007. First published June 11, 2007; doi:10.1152/ajpheart.00471.2007.—The molecular heterogeneity of repolarizing currents produces significant spatial heterogeneity and/or dispersion of repolarization in many mammalian cardiac tissues. Transgenic mice are prominent experimental models for the study of the molecular basis of repolarization and arrhythmias. However, it is debated whether the small mouse heart can sustain physiologically relevant heterogeneity of repolarization. We used a comprehensive model of the mouse action potential (AP) to predict how small a region of the cardiac tissue can maintain spatial gradients of repolarization due to differential expression of channels. Our simulations of a one-dimensional multicellular ring or cable predict that substantial gradients in repolarization and intracellular Ca\(^{2+}\) concentration transients can be maintained through heterogeneity of expression of K\(^+\) channels in distances of \(<10\) cells that are sufficient to block propagation. The abruptness of expression gradients and the site of stimulation can cause Ca\(^{2+}\) transient oscillations and affect the stability of Ca\(^{2+}\) dynamics and AP propagation. Two different mechanisms of instability of AP propagation in one-dimensional cable occur at fast pacing rates. Transitions from periodic activity to alternans or to irregular behavior were observed. Abrupt gradients of channel expression can cause alternans at slower pacing rates than gradual changes. Our simulations demonstrate the importance of incorporating realistic Ca\(^{2+}\) dynamics and current densities into models of propagated AP. They also emphasize that microscopic aspects of tissue organization are important for predicting large-scale propagation phenomena. Finally, our results predict that the mouse heart should be able to sustain substantial molecularly based heterogeneity of repolarization.

In particular, the large-scale simulations of action potential (AP) propagation in an anatomically detailed model of the mouse heart presented by Sampson and Henriquez (59) have led to questions on the role of molecular heterogeneity in generating heterogeneity of repolarization in the mouse. On the basis of simulations of a model of mouse repolarization, modified from a rat cellular AP model by Pandit et al. (51), and a large number of grid points to simulate the whole mouse heart, Sampson and Henriquez concluded that “the major contribution of this work was to demonstrate that electrotonic modulation of AP duration (APD) tends to dominate the intrinsic difference in cell properties in the small mouse heart” (59). However, there are several experimental results from wild-type mouse hearts, transgenic mouse hearts, and mouse hearts that have been modified pharmacologically (6, 7, 40) that suggest that molecular heterogeneity of currents produces significant spatial heterogeneity and/or dispersion of repolarization.

Our group (12) recently published a cellular model of the mouse left ventricular apical and septal APs that can also be used to relate changes in ion channel expression with changes in AP shape and APD. Use of the mouse heart, with its small size and small number of cells, means that propagation through physiologically relevant numbers of cells can be accomplished without cellular model simplification or the use of continuum equation approximations. In the present study, we tested the ability of a model of mouse cardiac tissue with a complete cellular model to sustain physiologically relevant spatial gradients of repolarization. We used a comprehensive model of the mouse cardiac cell with discrete gap junctions and simplified geometries to examine the ability of molecular heterogeneity to change the spatial variations of repolarization and Ca\(^{2+}\) dynamics. We developed a one-dimensional (1D) model of the ventricular mouse cardiac tissue with propagation velocities and characteristics of AP repolarization matched to those measured by Gutstein et al. (35). Our simulations predict that substantial gradients in repolarization and intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transients can be maintained through heterogeneity of expression of ion channel currents in distances of \(<10\) cells at reduced and maximal conduction velocities. Although electrotonic interactions caused modifications of the AP, such modifications did not eliminate heterogeneity of repolarization. In addition, heterogeneity of repolarization was capable of causing transient block of AP propagation at rapid pacing rates. These results suggest that the mouse heart may be a suitable model for the study of the effects of heterogeneous channel expression on repolarization.

Molecular biological tools have helped to define the channels involved in cardiac electrical activity and various rhythm disturbances such as Brugada syndrome and long QT syndrome (2). The study of the relationship between channel defects and arrhythmias has also been augmented by the development of transgenic animals, primarily the mouse, in which the consequences of specific mutations can be examined in the context of a functional mammalian cardiac environment (46). However, recent computer simulations have raised questions about the appropriateness of the mouse heart for studying mechanisms of arrhythmias in larger animals and humans, particularly for understanding the consequences of spatial heterogeneity of repolarization (36, 59).

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Other important and unexpected aspects of the microscopic nature of conduction and repolarization heterogeneity were also observed. Abrupt and gradual changes of ion channel expression and the site of stimulation had distinct effects on the stability of AP propagation. Abrupt changes in cellular phenotype appear to be more potentially proarrhythmic. In particular, the electrotonic current spread at the junction between two repolarizing cell types could influence Ca\(^{2+}\)/H\(_{11001}\) handling not only in the area of the repolarization boundary but at distances far removed from the boundary. At pacing rates higher than the threshold for AP and [Ca\(^{2+}\)/H\(_{11001}\)]i alternans, the multicellular tissue models demonstrated alternans in AP repolarization. In some cases, the AP instability appeared as irregular activity, with a mechanism of instability that was different from that for alternans. We suggest that microscopic aspects of molecular heterogeneity are important in understanding dynamic instability and phenomena such as Ca\(^{2+}\) loading during tachycardia.

**METHODS**

We investigated 1D model tissues consisting of mouse ventricular myocytes using the complete set of cellular model equations from Bondarenko et al. (12). Two different types of cells were used to simulate AP propagation in inhomogeneous model tissue: the apex and the septum cells.

In model tissue, the myocytes were connected by gap junctions in a linear fiber (cable) (Fig. 1A) or in a ring (Fig. 1B) similar to that shown in Shaw and Rudy (62). The total number of cells in 1D tissue was \(N = 190\), which was chosen to match the circumference of the mouse heart surface, with a diameter of 6 mm, which approximately corresponds to the length of the mouse ventricle (59): \(\pi \times 6 \text{ mm} \approx 19 \text{ mm}\). Taking into account longitudinal myocyte length of 100 \(\mu\text{m}\) (21, 43, 49), we obtained 19 mm/0.1 mm \(\approx 190\) cells. Each model myocyte is described by the equations from Ref. 12 plus intercellular currents:

\[
\frac{dV_i}{dt} = -\frac{1}{C_{mi}}(I_{\text{mem},i} - I_{\text{stim},i} + I_{\text{gap},i}) \quad i = 1, \ldots, N
\]

where \(i\) is the cell number, \(C_{mi}\) is the membrane capacitance of the \(i\)th cell, \(V_i\) is the membrane potential, \(I_{\text{mem},i}\) is the total membrane current (12), \(I_{\text{stim},i}\) is the stimulus current, \(I_{\text{gap},i} = g_{\text{gap}}(V_i - V_{i+1} + 2V_i - V_{i-1})\) is the gap current (16, 62), and \(g_{\text{gap}}\) is the gap conductance. All values of \(g_{\text{gap}}\) used in this paper are identical: \(g_{\text{gap},i} = g_{\text{gap}}\). We simulated AP propagation in the circular and linear fibers. Homogeneous (uniform) 1D tissues contain 190 septal or apex myocytes. Inhomogeneous 1D tissues consist of 95 septal and 95 apex cells, with abrupt inhomogeneity or with linear dependencies of conductivity for important K\(^{+}\) currents [rapidly inactivating transient outward K\(^{+}\) current (\(I_{\text{Kto},1}\)), slow-inactivating transient outward K\(^{+}\) current (\(I_{\text{Kto},s}\)), ultrarapidly activating K\(^{+}\) current (\(I_{\text{Kur}}\)), and nonin-
activating steady-state K⁺ current (I_{K_{ss}}) between a typical septal cell (cell 48) and a typical apex cell (cell 143) for the circular geometry of the tissue (referred to as a “smooth” or “gradual” ring). For the gradual inhomogeneous 1D fiber (referred to as a “linear,” “smooth,” or “gradual” cable), the conductivities for I_{K_{to,f}}, I_{K_{to,s}}, I_{K_{ur}}, and I_{K_{ss}} were linear functions between cell 1 (septal cell) and cell 190 (apex cell). We also used the approximation that all conduction delay occurs at the gap junctions. This approximation was validated for models of multicellular cardiac tissue in the detailed study of Shaw and Rudy (62).

The amplitude of I_{stim,i} was varied from 180 to 2,000 pA/pF to be equal to ~150% of the threshold value, depending on the intercellular conductance. For the isolated myocytes, I_{stim} = 60 pA/pF, which was ~120% of the threshold value. In all cases, the stimulus pulse duration (τ_{stim}) was equal to 0.5 ms. Details of the stimulation protocols are described in the text or figure legends. Model Eq. 1 was solved with the use of a fourth-order Runge-Kutta method with a time step of 0.0001 ms. We used the resting values of state variables of the model myocytes as the initial conditions in our simulations. In the inhomogeneous case, all cells were in equilibrium before stimulation. They have the same initial conditions because they are different only in voltage-dependent K⁺ currents, which are activated at potentials considerably more positive (greater than ~40 mV) than the resting potential (approximately ~82 mV). However, we should note that there is the possibility that different spatial distributions of initial conditions may lead to different patterns of alternans development (75). Simulations were performed with a single 3.2-GHz Intel Xeon “Irwindale” processor of a Dell Linux Cluster.

RESULTS

Electrotonic interactions and AP propagation in homogeneuous multicellular tissue models. The experimentally measured propagation velocity of AP in mouse heart tissues depends on the direction of propagation and intercellular coupling (5, 35, 68, 70). To simulate variations of AP propagation velocities in a 1D circular model with 190 myocytes (Fig. 1A), we used values for intercellular conductance (g_{gap}) that ranged from 1 to 80 nS/pF. Figure 1, C and D, shows AP propagation for two values of g_{gap} (5 and 80 nS/pF), which correspond to two propagation velocities, 0.28 and 1.14 m/s, respectively. Figure 1 shows substantial differences in times that the AP needs to propagate through the 1D tissue until collision.

We optimized model intercellular conductances to the experimental values of propagation velocities (35). The value of g_{gap} was chosen to be equal to 25 nS/pF to reproduce the maximal longitudinal experimental velocity of 0.62 m/s (35). Using the measured capacitance of mouse ventricular myocytes of 127 ± 7 pF (26), we can estimate the intercellular conductance between two model myocytes as 25 nS/pF × 127 pF = 3,175 nS. This value is in the range of experimentally measured conductances between guinea pig ventricular myocytes under normal pH conditions (90–3,900 nS) (47) and ensures a propagation velocity close to that measured in the mouse ventricle. Smaller intercellular conductances (558 ± 92 nS) have been obtained from mouse ventricular myocytes (80). However, these values, obtained by reduction of normal conductances through decreasing pH to 6.8, can be considered as a lower estimation limit. In cases where there is sustained Ca²⁺ loading, such as tachycardia, gap junctional conductance may be further reduced (48), thus enhancing spatial gradients of repolarization. We also investigated the dependence of the AP velocities on the stimulus number. For this purpose, we calculated propagation velocities for the APs initiated by the 1st and 10th stimuli and did not observe substantial differences (data not shown).

Longitudinal current flow slightly changes AP amplitude and repolarization. Figures 2 and 3 show the effects of electrotonic intercellular interactions in 1D multicellular tissue on the AP and important ionic currents in the apex cell compared with those from isolated myocyte for the largest g_{gap} = 80 nS/pF explored. Simulated data are shown for the 10th pulse at the pacing period 200 ms. As shown in Fig. 2A, APs in the tissue (dashed line) only change slightly compared with those of the isolated myocyte (solid line). There are two major changes: a decrease in AP overshoot and a minor increase in AP duration. Examination of the major K⁺ currents revealed substantial changes in two of them, I_{K_{to,f}} (Fig. 2C) and the much smaller I_{K_{ss}} (Fig. 2D). Decreases in these currents were responsible for the AP prolongation for the myocyte in the tissue. However, the changes in these ionic currents do not explain the decrease of AP overshoot. Decreased AP amplitude arises from longitudinal current flow that depolarizes neighboring cells (64, 65). To understand why this occurs, we can consider the electrotonic currents that enter the myocyte from the neighboring cell (I_{Ein}; magenta solid line in Fig. 3) and flows out of the myocyte to another cell (I_{Eout}) and their sum (I_{Ediff} = I_{Ein} + I_{Eout}). Note that I_{Ein} is inward to the cell during the initial phase of AP upstroke and has a negative sign. Figure 3 shows that I_{Ediff} changed from negative to positive during the AP upstroke, thereby effectively contributing to the decrease in AP overshoot. I_{K_{to,f}} became more important at the later stages of AP repolarization but cannot be clearly visualized on this scale. Similar small changes in AP shape were observed in the septal myocytes inside 1D tissue compared with those in the isolated myocyte (Fig. 2A, inset).

AP propagation in inhomogeneous multicellular tissue models. Experimental measurements have previously shown that the shape and duration of AP repolarization in the mouse heart depend on the heart region (3, 6, 7, 40, 42). This heterogeneity of repolarization is generally thought to be due to differential expression of the cellular ionic currents in cardiac myocytes from different heart regions (30, 34, 45, 79), although the ability of mouse heart to maintain such gradients has recently been questioned on the basis of simulations (59). We employed simulations to test the predicted behavior of mouse cardiac tissue to maintain heterogeneity of repolarization over relatively short distances. We used model parameters from two heart regions, apex and septum, for which the quantitative details of differences in repolarizing current densities are well measured in mouse (34, 79).

The multicellular simulations used an inhomogeneous 1D model tissue with an abrupt transition region where 95 septal and 95 apical cells were connected in a ring with g_{gap} = 25 nS/pF and were stimulated from the septum region (cell 48) or from the apex region (cell 143). The simulation results for membrane potential plotted in Fig. 4, A and B, show an inhomogeneity of AP repolarization that depends on the cell type, except for a small transition region of ~10 myocytes at the boundaries between the septal and apical cells, thereby supporting experimental findings of molecularly based heterogeneity of repolarization (3, 6, 7, 40, 42). For comparison, the simulations for uniform 1D model tissues consisting of only
either septal or apical cells are shown in Fig. 4, C and D, respectively.

Figure 5 shows simulated AP durations at different levels of repolarization [25% (APD25), 50% (APD50), and 75% (APD75)] as functions of the distance in inhomogeneous and uniform 1D tissues ($g_{\text{gap}} = 25 \text{ nS/pF}$), which corresponds to a maximal propagation velocity (35). APD25 and APD50 showed clear heterogeneity of repolarization in inhomogeneous tissue for the stimulation both from the septum and the apex regions, with the shorter APDs in the apex region (Fig. 5, A and B). Although APD75 in inhomogeneous tissues is substantially influenced by electrotonic interactions, it also shows some differences from the uniform tissues. When stimulation was applied to the septal region of inhomogeneous tissue (cell 48), APD75 in this region was longer than in the apex region (Fig. 5C). In turn, when the tissue was stimulated from the apex region (cell 143), the APD75 in this region and in part of the septum region were approximately equal, except for the small region where the APs collided (Fig. 5C). This means that, although APD25 and APD50 are determined in major part by the cellular properties of the mouse ventricular myocytes and cellular ionic currents, APD75 is substantially determined by electrotonic currents. For comparison, Fig. 5 also includes APD25, APD50, and APD75 for uniform 1D tissues consisting of the septum or apex cells, respectively, and to show stimulation and AP collision artifacts.

As seen from Fig. 5, depending on the site of stimulation, apex or septum, an asymmetry in AP repolarization appears in 1D inhomogeneous model. The myocytes in the transition region (approximately from cell 75 to 110) have different APD50, depending on the point of the stimulus. When stimulation is applied to the septum region (cell 48), APD50 of cells from 75 to 110 is shorter than when stimulation is applied from the apex region (cell 143). To understand this asymmetry, we investigated corresponding APs and important electrotonic ($I_{\text{Ediff}}$) and ionic currents for cell 96 (apex cell) from the transition region. APs that were elicited by stimulation from the septum have slightly longer durations at the initial stage of repolarization than those obtained by stimulation from the apex region (dotted and solid lines in Fig. 5A). This longer AP duration is explained by the larger contribution of the inward currents, $I_{\text{Na}}$, at this stage of repolarization, which effectively slows repolarization. At the later stage, repolarization of the AP induced by the stimulation from the septum region (compared with that from the apex region) shows little change in the AP shape despite the large amplitudes of electrotonic currents (Fig. 3) and small decrease in $I_{K_{\text{to,f}}}$ and $I_{K_{\text{ss}}}$ that prolong AP duration (APD) at later stages of repolarization in 1D tissue. Inset, A: APs for the single septum myocyte (solid line) and myocyte 122 in 1D uniform multicellular tissue (dashed line) consisting of 190 septum cells.
region) is accelerated by increased $I_{K_{\text{to,f}}}$ and by reduced contribution from $I_{\text{Ediff}}$. Therefore, at this stage of repolarization, the AP becomes shorter (Fig. 5B). As described below, the asymmetry of repolarization, which is dependent on the site of stimulation, is also related to differential susceptibility to alternans.

The potentially important physiological effects of the tissue heterogeneity on AP propagation can be seen with rapid pacing in the 1D inhomogeneous cable from the apex region. Figure 6 shows simulation of AP propagation in inhomogeneous (abrupt inhomogeneity) and uniform (apex) cables stimulated from the apex with pacing period of 69 ms. As seen from Fig. 6A, AP propagation is blocked at the boundary of the two cell phenotypes. The cause of this block is a transiently increased APD in the septum region of the tissue model, which limits the diastolic interval and recovery time for major repolarizing currents. In contrast, the AP in the uniform apical tissue shows only periodic behavior (Fig. 6B), suggesting important roles of the tissue inhomogeneity in the AP propagation block for the mouse AP.

Inhomogeneous channel expression affects both repolarization and $[Ca^{2+}]_i$ transients. We were able to simulate $[Ca^{2+}]_i$ transients, with an abrupt transient region between the septal and apical cells. Such abrupt inhomogeneities are potentially proarrhythmic. In contrast, uniform 1D models consisting of only one type of cell did not show abrupt inhomogeneities in $[Ca^{2+}]_i$ transients (Fig. 7, C and D).

To understand how smooth gradients of cellular heterogeneity affect AP repolarization and $[Ca^{2+}]_i$ transients, the simulations were performed with an inhomogeneous 1D model, consisting of a linear spatial dependence of conductivities for important $K^+$ currents ($I_{K_{\text{to,f}}}, I_{K_{\text{to,s}}}, I_{K_{\text{ur}}},$ and $I_{K_{\text{ss}}}$) between a typical septum cell (cell 48) and a typical apex cell (cell 143). Model myocytes were connected in a ring, and stimuli were delivered to cells 48 and 143. Both stimulations resulted in smooth gradients of AP repolarization and $[Ca^{2+}]_i$ transients (data not shown). Values of APD$_{25}$ and APD$_{50}$ for both sites of stimulations show linear dependencies on the cell number, reflecting importance of cellular repolarization mechanisms. However, dependence of APD$_{75}$ on the cell number does not differ substantially from that in both the uniform 1D tissues and inhomogeneous 1D tissues with an abrupt transition region between the cell types.

Alternans in multicellular tissue models at rapid pacing. Experimentally, it has been observed that, in both normal and transgenic mouse hearts, alternans of APs and $[Ca^{2+}]_i$ transients appear at relatively fast pacing rates (6, 41, 42, 69). To examine the behavior of alternans in our model of multicellular
tissue, we used 1D cable of 190 cells (Fig. 1B) that was uniform (with the apical or septal myocytes), was inhomogeneous with 95 apical and 95 septal myocytes and abrupt transition region between them, and was inhomogeneous with linear change of the expression of four K$^+$ currents ($I_{K\text{to,s}}, I_{K\text{to,f}}, I_{K\text{ur}},$ and $I_{K\text{ss}}$) between a typical septal cell (cell 1) and a typical apical cell (cell 190). The cables were stimulated with relatively short periods (from 40 to 90 ms) from both the apex and septum regions. For comparison, we also stimulated isolated model myocytes in the same range of pacing periods, with $I_{\text{stim}} = 60 \, \text{pA/pF}$ and $\tau_{\text{stim}} = 0.5 \, \text{ms}$. Both 1D cables and isolated myocytes were stimulated for 4,000 ms to ensure a steady-state solution.

Table 1 shows the thresholds for instability of AP and $[\text{Ca}^{2+}]_i$ transients in different 1D fiber models and at different sites of stimulation as well as in isolated mouse model myocytes. The threshold was determined as the longest pacing cycle length that led to instability of the periodic AP. The instability appears in the form of AP and $[\text{Ca}^{2+}]_i$ transients alternans or their irregular activity. The isolated apex and septum myocytes change their activity from periodic to irregular at relatively shorter pacing periods (59 and 61 ms, respectively) than the myocytes in any of 1D tissues. This suggests a destabilizing role of electrotonic interactions in the multicellular tissues. In four of six cases in Table 1, the instability threshold is equal to pacing period of 79 ms. In four cases of the six, the instability appears in the form of AP and $[\text{Ca}^{2+}]_i$ transient alternans.

Abrupt spatial changes in repolarizing currents are more destabilizing than smooth transitions. The physical arrangement that was most susceptible to alternans was the 1D abruptly inhomogeneous tissue with 95 septal and 95 apical myocytes stimulated from the apical region. In this case, the threshold for alternans appearance was 82 ms (Table 1). An example of simulations of AP and $[\text{Ca}^{2+}]_i$ transients in this 1D tissue at pacing period 80 ms is shown in Fig. 8, A and B. Initially, the alternans starts from the abrupt transition region where septum myocytes adjoin the apex cells and then spreads mostly toward the region of the tissue containing apex cells (Fig. 8B). The alternans is discordant, with small and large $[\text{Ca}^{2+}]_i$ transients in different regions of the cable that oscillate in opposite phases. Pacing of uniform 1D tissues consisting of the apical (Fig. 8, C and D) or septal cells or abrupt inhomogeneous 1D tissue with 95 septal and 95 apical myocytes stimulated from the septum region (Fig. 8, E and F) with the same period of 80 ms does not produce alternans, suggesting a destabilizing role of the abrupt gradient of myocyte type together with the site of stimulation (the apex region).

A particularly interesting finding was that the relatively smooth gradient played a stabilizing role in inhomogeneous 1D tissue with linear change of the expression of four K$^+$ currents ($I_{K\text{to,s}}, I_{K\text{to,f}}, I_{K\text{ur}},$ and $I_{K\text{ss}}$) between a typical septal cell (cell 1) and a typical apical cell (cell 190) when stimulated from the septum region (79 ms). This simulation is consistent with the experimental observation that the relatively smooth gradient of repolarization from apex to base has a natural anti-arrhythmic protective role in the wild-type mouse heart (6, 40).
Mechanism of alternans in an inhomogeneous 1D model tissue. The mechanism of alternans development in the 1D model tissue with an abrupt inhomogeneity can be understood by comparing APs and Ca$^{2+}$/H$^{11001}$ dynamics for two stimulations of 1D linear fiber, from the septum (cell 1) and the apex (cell 190), at a site near the transition region (Figs. 8–10). Alternans in both [Ca$^{2+}$/H$^{11001}$] and AP appears during stimulation with basic cycle length of 80 ms only when stimuli are applied to the apex region (Fig. 8). [Ca$^{2+}$/H$^{11001}$] alternans demonstrates substantial variation in amplitude, where AP alternans is only in the microvolt range. However, the alternans are accompanied by variations in both ionic currents and gating Ca$^{2+}$/H$^{11001}$-release channels.

The dashed lines in Figs. 9 and 10 show the AP and Ca$^{2+}$/H$^{11001}$ dynamics in the absence of alternans when stimulation is applied to the septum. The AP upstroke activates voltage-gated currents, including $I_{Na}$ and L-type Ca$^{2+}$/H$^{11001}$ current ($I_{CaL}$). Ca$^{2+}$/H$^{11001}$ enters the cell (cell 96 in Figs. 9 and 10) through the L-type Ca$^{2+}$/H$^{11001}$ channels and triggers Ca$^{2+}$/H$^{11001}$ release through the ryanodine receptors (RyRs) into the subspace volume between RyRs and L-type Ca$^{2+}$/H$^{11001}$ channels rising subspace sarcoplasmic reticulum Ca$^{2+}$/H$^{11001}$ concentration ([Ca$^{2+}$/H$^{11001}$]$_{ss}$) and thereby causing positive feedback in which an increase of [Ca$^{2+}$/H$^{11001}$]$_{ss}$ leads to further increase in opening probability of RyRs and Ca$^{2+}$/H$^{11001}$ release (Fig. 9, C and D). The rate of RyR opening is determined by a balance between the transition rates from open and closed.
of RyRs slows down. That change in kinetic behavior of RyRs, in turn, decreases Ca$^{2+}$ release and [Ca$^{2+}$]$_{ss}$. As a result, the transition of RyRs back to the closed state becomes predominant (Fig. 9D). Consequently, the transition of Ca$^{2+}$ from the network sarcoplasmic reticulum to JSR exceeds Ca$^{2+}$ release flux, and [Ca$^{2+}$]$_{JSR}$ starts to refill (Fig. 9B). Increased [Ca$^{2+}$]$_{ss}$ also causes a rise in [Ca$^{2+}$]$_{i}$ that increases Ca$^{2+}$ uptake back to the sarcoplasmic reticulum and sarcolemmal Ca$^{2+}$ extrusion from the cell via Na$^+$/Ca$^{2+}$ exchanger (Figs. 9A and 10E). In the absence of alternans, all of the ionic and electrotonic currents and Ca$^{2+}$ fluxes are in balance after the cell adjusts to the increased stimulation rate.

This periodic activity can be unstable when the balance between the ionic and electrotonic currents and Ca$^{2+}$ fluxes in the cell is perturbed. The electrotonic currents can distort various phases of the AP in the transition region of the tissue and alter Ca$^{2+}$ homeostasis. This is observed when the inhomogeneous 1D model tissue is stimulated from the apex (Figs. 8–10). Figure 9A shows dynamics of [Ca$^{2+}$]$_{i}$ for cell 96 from the region where the alternans starts (solid line). The alternans in intracellular Ca$^{2+}$ appears along with the alternans in [Ca$^{2+}$]$_{JSR}$ and in [Ca$^{2+}$]$_{ss}$ (solid lines in Fig. 9B and C). [Ca$^{2+}$]$_{ss}$ determines the opening rate of the Ca$^{2+}$ release channels by alterations in the gating rates of RyRs (solid red and brown lines in Fig. 9D). As seen in Figs. 9 and

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<thead>
<tr>
<th>Object and Site of Stimulation</th>
<th>Instability Threshold, ms</th>
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<tbody>
<tr>
<td>Isolated apex myocyte</td>
<td>59 (irregular)</td>
</tr>
<tr>
<td>Isolated septum myocyte</td>
<td>61 (irregular)</td>
</tr>
<tr>
<td>1D homogeneous tissue, apex myocytes</td>
<td>79 (alternans)</td>
</tr>
<tr>
<td>1D homogeneous tissue, septum myocytes</td>
<td>79 (irregular)</td>
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<tr>
<td>1D abrupt inhomogeneous tissue, 95 septum and 95 apex myocytes, stimulation from the apex</td>
<td>82 (alternans)</td>
</tr>
<tr>
<td>1D abrupt inhomogeneous tissue, 95 septum and 95 apex myocytes, stimulation from the septum</td>
<td>79 (alternans)</td>
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<tr>
<td>1D inhomogeneous tissue, linear change from the septum to apex, stimulation from the apex</td>
<td>75 (irregular)</td>
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<tr>
<td>1D inhomogeneous tissue, linear change from the septum to apex, stimulation from the septum</td>
<td>79 (alternans)</td>
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AP, action potential; 1D, one-dimensional.

Table 1. Threshold pacing periods for instability of AP propagation in the mouse model 1D cable and isolated ventricular myocytes

Fig. 8. AP propagation in a 1D model of the mouse inhomogeneous (A, B, E, and F) and uniform (C and D) multicellular cardiac tissues. A, C, and E show spatiotemporal pictures of the APs. B, D, and F show spatiotemporal pictures of the [Ca$^{2+}$]$_{i}$ transients; 190 model myocytes were connected in a cable. The uniform tissue contained apex cells is shown for comparison (C and D). The inhomogeneous tissues consisted of 95 septal (cells 1–95) and 95 apical (cells 96–190) myocytes. $I_{\text{stim}}$ (900 pA/pF, $\tau_{\text{stim}} = 0.5$ ms) was applied at a pacing period of 80 ms to cell 190 (A–D) or cell 1 (E and F). Arrows show time moments at which stimuli were applied. Intercellular connection strength ($g_{\text{gap}}$) was 25 nS/pF. Note the alternans of [Ca$^{2+}$]$_{i}$ transients in the apex region of 1D inhomogeneous cable that occurred when it was stimulated from the apex region (B).
10, development of alternans is associated with a decrease in the amplitude of $I_{CaL}$ ($\sim 20\%$, Fig. 10D), smaller $[Ca^{2+}]_{i}$ transients (Fig. 9A), smaller $[Ca^{2+}]_{ss}$ (Fig. 9C), larger JSR load (Fig. 9B), and decreased extrusion of $[Ca^{2+}]_{i}$ by the Na$^+$/Ca$^{2+}$ exchanger current (Fig. 10E). This reduction in $I_{CaL}$, opening probability of RyRs, and $Ca^{2+}$ release fluxes illustrates the important role of refractoriness of the major components of $Ca^{2+}$ handling system in the genesis of $Ca^{2+}$ alternans. In addition, the smaller AP amplitude and APD$_{25}$ (Figs. 5 and 10A), amplitude of inward part of $I_{Na}$ (Fig. 10B), and fast $I_{Na}$ ($\sim 5\%$, Fig. 10C) are observed during stimulation from the apex, the model parameters that are not directly involved in $Ca^{2+}$ handling.

Alternans development (solid lines in Figs. 9 and 10) occurs as a result of a complex interaction of the ionic and electrotonic currents and $Ca^{2+}$ fluxes in the cell with repeated stimulation. The major impact of this growing complexity is on the behavior of the $Ca^{2+}$-release mechanism. Clearly, substantial alterations in $[Ca^{2+}]_{i}$, $[Ca^{2+}]_{ss}$ (Fig. 9C), $[Ca^{2+}]_{JSR}$ (Fig. 9B), and gating of the RyRs (Fig. 9D) accumulate over many cycles. The electrotonic currents during stimulation from the apex have greater effect on AP amplitude and APD$_{25}$ compared with the stimulation from the septum (Figs. 10A and 5A) and subsequently on the amplitude of $I_{Na}$ leading to a decrease of $Ca^{2+}$ influx into the cell (Fig. 10D). The smaller $Ca^{2+}$ influx triggers smaller $Ca^{2+}$ release by RyRs and $[Ca^{2+}]_{ss}$ that in turn slows RyR opening (Fig. 9, C and D). As a result, this yields a smaller $[Ca^{2+}]_{ss}$, and increased $[Ca^{2+}]_{JSR}$. Smaller $[Ca^{2+}]_{ss}$ causes reduced $Ca^{2+}$-dependent inactivation of $I_{CaL}$, so that by the
next stimulus this current become larger. During the next stimulus, the larger $I_{CaL}$ triggers larger Ca\(^{2+}\) release from the JSR amplified by the larger [Ca\(^{2+}\)]\(_{JSR}\), which accelerates opening of RyRs (Fig. 9, C and D), yielding larger [Ca\(^{2+}\)]\(_{ss}\). The larger [Ca\(^{2+}\)]\(_{ss}\) causes increased Ca\(^{2+}\)-dependent inactivation of $I_{CaL}$ and so on.

We conclude that the alternans in an inhomogeneous mouse cardiac tissue appears at the rapid pacing through a loss of stability of the Ca\(^{2+}\)-release system, which includes complex interactions between electrotonic and ionic currents and Ca\(^{2+}\) fluxes in the cardiac myocytes. Alternans can be suppressed by the regulation of ionic currents or Ca\(^{2+}\) fluxes that drive cardiac cells back to the stable periodic behavior (data not shown).

**Mechanism of transition to irregular activity in a homogeneous 1D septal tissue.** Simulated 1D tissues demonstrated not only transition from periodic activity to alternans but also transitions from periodic to aperiodic behavior (Table

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**Fig. 10. Development of alternans during pacing 1D inhomogeneous cable with period of 80 ms.** Electrotonic and ionic currents for cell 96 only in the interval from 500 to 550 ms are shown. Solid and dashed lines correspond to the stimulation from the apex and septum regions, respectively. A–E show AP, $I_{Ediff}$, fast $I_{CaL}$, and Na\(^{+}/Ca^{2+}\) exchanger current ($I_{NaCa}$), respectively. Model parameters and stimulation protocols are the same as in Fig. 8.
Figure 11 shows examples of simulations of AP and $[\text{Ca}^{2+}]_i$ transients in inhomogeneous and uniform 1D tissues at a pacing period of 78 ms. Inhomogeneous tissue containing 95 septal and 95 apical cells shows alternans in $[\text{Ca}^{2+}]_i$ transients, with very little change in AP, when stimulated from the apex region (Fig. 11, A and B). The alternans was observed only in the apical region, whereas the septal cells demonstrate periodic $[\text{Ca}^{2+}]_i$ transients. In the uniform 1D tissue consisting of the apical cells, the $[\text{Ca}^{2+}]_i$ alternans develops in the whole cable, with very small changes in AP (data not shown). More complex (aperiodic) behaviors of both AP and $[\text{Ca}^{2+}]_i$ appear in the uniform 1D septal cable (Fig. 11, E and F). In this case, the AP progressively becomes more prolonged and ultimately its propagation is blocked (Fig. 11E). After a comparatively long refractory period due to this block, the AP can again propagate through the whole cable. Irregular activity in $[\text{Ca}^{2+}]_i$ transients is also observed during this AP propagation (Fig. 11F). More complex instability is obtained in inhomogeneous 1D cable consisting of the septal (cells 1–95) and apical (cells 96–190) myocytes when stimulated from the septum region (Fig. 11, C and D). The instability in this cable appears first in the form of $\text{Ca}^{2+}$ alternans that transfer with time to irregular activity and conduction block. The last scenario qualitatively resembles experimental observations when the alternans transfers into more complex aperiodic behavior (29, 52).

The mechanism of transition from periodic to irregular activity can be understood by comparing AP propagation in a 1D homogeneous cable consisting of septal cells when stimulated with two different basic cycle lengths (78 or 80 ms) (Fig. 12). When the cable was stimulated at 80 ms, the AP is a periodic function of time (Fig. 12A). Similar periodic behavior was demonstrated by the ionic currents (Fig. 12, B–E) and $[\text{Ca}^{2+}]_i$ transients (data not shown). At the shorter basic cycle length (78 ms), the periodic AP became irregular, with progressively reduced amplitude and increased duration. For example, APD$_{50}$ during the irregular activity varies over a wide range, from 10.1 to 73.1 ms (Fig. 12A). This transformation of AP was determined by the changes in ionic currents, which showed progressively smaller amplitudes due to smaller recovery time at the shorter pacing cycle length of 78 ms (Fig. 12, B–E). Smaller amplitudes of $I_{\text{Na}}$ (data not shown) reduced AP upstroke and amplitude. This led to a slower activation of the other ionic currents and reduction of amplitudes of outward $K^+$ currents (Fig. 12, C–E). The reduction of $K^+$ currents prolonged the APD, during which they continued to inactivate and further decrease in amplitude, thereby closing the positive feedback by causing additional AP prolongation. This process is opposed in part by a reduction in $I_{\text{CaL}}$; however, this reduction does not completely compensate for AP prolongation caused by a reduction of $K^+$ currents. The AP prolongation continued until a relatively large reduction of $I_{\text{Na}}$ occurred, which failed to trigger full-scale APs and caused block of AP propagation in inhomogeneous and uniform 1D tissues at a pacing period of 78 ms. Inhomogeneous tissue containing 95 septal and 95 apical cells shows alternans in $[\text{Ca}^{2+}]_i$ transients, with very little change in AP, when stimulated from the apex region (Fig. 11, A and B). The alternans was observed only in the apical region, whereas the septal cells demonstrate periodic $[\text{Ca}^{2+}]_i$ transients. In the uniform 1D tissue consisting of the apical cells, the $[\text{Ca}^{2+}]_i$ alternans develops in the whole cable, with very small changes in AP (data not shown). More complex (aperiodic) behaviors of both AP and $[\text{Ca}^{2+}]_i$ appear in the uniform 1D septal cable (Fig. 11, E and F). In this case, the AP progressively becomes more prolonged and ultimately its propagation is blocked (Fig. 11E). After a comparatively long refractory period due to this block, the AP can again propagate through the whole cable. Irregular activity in $[\text{Ca}^{2+}]_i$ transients is also observed during this AP propagation (Fig. 11F). More complex instability is obtained in inhomogeneous 1D cable consisting of the septal (cells 1–95) and apical (cells 96–190) myocytes when stimulated from the septum region (Fig. 11, C and D). The instability in this cable appears first in the form of $\text{Ca}^{2+}$ alternans that transfer with time to irregular activity and conduction block. The last scenario qualitatively resembles experimental observations when the alternans transfers into more complex aperiodic behavior (29, 52).
propagation (Figs. 11E and 12A). During the relatively long time interval between the two APs due to block, most of the ionic currents are able to recover from inactivation and to generate regular AP, however, with increased amplitude. The process continues until further block of AP propagation (Figs. 11E and 12A). Thus the threshold basic cycling length for onset of irregular APs was determined by the refractoriness (recovery time) of the major inactivating K\textsuperscript{+} currents. The irregular nature of this process is determined by noncommensurate time scales of the relatively slow AP prolongation and the relatively fast discrete stimulation cycle length.

In summary, our simulations suggest that the mouse heart is capable of sustaining substantial gradients of repolarization due to differences in the expression of repolarizing K\textsuperscript{+} channels. This spatial gradient of repolarization is dominant at more positive potentials; nevertheless, it can occur even in a well-coupled system with near maximal propagation velocities. When an abrupt transition between cells with different intrinsic repolarizations is encountered, electrotonic current flow can influence cellular behavior, resulting in frequency-dependent instabilities in AP repolarization and Ca\textsuperscript{2+} handling. These instabilities appear in the form of conduction block, alternans, or irregular activity, have different mechanisms of excitation, and spread beyond the intrinsic border zone. Our results strongly suggest that microscopic aspects of channel heterogeneity may be critical in understanding triggered and reentrant arrhythmias and their degeneration into fibrillation.

Fig. 12. Development of irregular activity during pacing 1D homogeneous cable consisting of septal cells with period of 78 ms (solid lines) and 80 ms (dashed lines). Ionic currents for cell 187 in the interval from 1,500 to 3,200 ms are shown. A–E: APs, I\textsubscript{CaL}, I\textsubscript{Kto,f}, slow-inactivating transient outward K\textsuperscript{+} currents (I\textsubscript{Kto,s}), and ultrarapidly activating K\textsuperscript{+} currents (I\textsubscript{Kur}), respectively. Model parameters and stimulation protocols are the same as in Figs. 8 and 11 for period of 78 and 80 ms, respectively.
DISCUSSION

Cardiac arrhythmias have been a frequent subject of mathematical investigation (for review, see, Refs. 11, 38, 76). The generation of spiral waves and their degeneration into chaotic behavior have been accepted as a mechanism of pathophysiological conditions such as tachycardia and fibrillation (31, 32, 77, 78). Recently, the species-specific models have been used in large-scale repolarization and arrhythmia studies (see for example, Refs. 15, 22, 23, 39, 50, 55, 59). Several of these simulations have neglected or greatly simplified Ca\(^{2+}\) handling to address issues of scale. This can be valid but carries the risk of overlooking the potential importance of Ca\(^{2+}\) homeostasis in arrhythmogenesis (1, 67, 72, 77). As the field progresses, one important issue that will need to be addressed is what aspects of cellular and molecular behavior must be preserved and integrated on moving from cellular to organ function.

The mouse will be an important tool for developing an understanding of how to integrate cellular-to-organ function. The small size of the mouse heart means that it will soon be possible to model every individual cell in the heart and their electrical connections without resorting to continuum approximations of propagated phenomena or to overly simplified cellular models. Because it can be readily manipulated genetically to test different model predictions, the mouse heart will be crucial for developing and testing the computational tools for understanding the much larger human heart.

The mouse heart as a model of mammalian cardiac activity. The small size of the mouse heart has raised concerns about its appropriateness as a model of conducted arrhythmias. It has been noted that it is extremely difficult to generate fibrillation in this heart without significant slowing of conduction, such as that which occurs in late heart failure and hypertrophy (6, 17, 42, 69). Reentrant arrhythmias can also be difficult to trigger with electrical stimulation in a normal mouse heart. Our own simulations also suggest that reentry would be difficult to sustain without some slowing of propagation (10). On the other hand, the small heart size may not be the limiting factor in mouse, as fibrillation in the mouse atrium, which is much smaller than the ventricle, has been observed experimentally (17, 56, 66, 71).

The small size of the mouse heart has also raised concerns about its ability to function as a model of molecularly based heterogeneous repolarization. Sampson and Henriquez (59) have published a simulation study of the whole mouse heart, incorporating regional heterogeneity of repolarizing currents based on a modified model of the rat ventricular AP (51). This model was structurally accurate for the whole mouse heart; however, it has some limitations, such as longer APDs than those observed experimentally. This may reflect the observation that major repolarization currents are different between species. For example, \(I_{Kur}\) may be absent in a modified rat model, but such a current is present in mouse ventricular myocytes and is encoded by the Kv1.5 channel. Another important difference is the absence of detailed Ca\(^{2+}\) dynamics. Nevertheless, a modified rat model was used to evaluate the ability of changes in channel expression to modify the spatial distribution of repolarization. Sampson and Henriquez (59) concluded that the high-resistance plateau of the cardiac AP resulted in a large space constant and ensured a relatively uniform repolarization. Although both their simulations and our simulations show a significant contribution of electrotonic interactions at late stages of repolarization (APD\(_{75}\)), there is a large difference in the predicted distance over which these interactions are important at earlier stage of repolarization (APD\(_{50}\)).

In our studies, we used a comprehensive mouse myocyte model with current densities and Ca\(^{2+}\) dynamics closely estimated from voltage-clamp data of the mouse ventricular myocytes (12). The mouse AP is very short and reflects different levels of expression of key repolarizing currents that were found in larger mammalian ventricles. In particular, the channels contributing to the transient outward current (Kv4.2, Kv4.3, and Kv1.4) and to the ultrarapidly activating currents (e.g., Kv1.5) dominate repolarization (13, 14, 34, 79). This expression of currents and the shape of the mouse ventricular AP more closely resemble the human atrium rather than the human ventricle (74). It also means that the mouse AP has no extended high-resistance plateau phase. The resistance of the plateau, therefore, is in proportion to the rapid dV/dt of repolarization and the small spatial wavelength of the AP. The long delayed portion of repolarization is at very negative potentials and has a relatively high resistance due to the deactivation of the Kv1.5-, Kv4.2-, and Kv1.4-mediated currents, leaving only the relatively flat portion of time-independent inwardly rectifying K\(^{+}\) current (\(I_{K1}\)) active during this phase of repolarization. Consequently, our results show what less heterogeneity of voltage in this range (as demonstrated by the APD\(_{75}\) results in Fig. 5) under normal propagation conditions, consistent with the experimental findings of Knollmann et al. (40) at APD\(_{70}\) and APD\(_{90}\), although large measurement variability of durations in these voltage regions means that functionally significant differences cannot be ruled out (see Fig. 6 from Ref. 82). However, at more depolarized potentials, more repolarizing K\(^{+}\) currents are active, and longitudinal current flow has had less time to disperse the intrinsic cellular gradients. At such potentials, there is a noticeable transition region spanning ~10 cells where it changes from the apex to septal AP phenotype, as shown in our APD\(_{25}\) and APD\(_{50}\) results (Figs. 4 and 5). This is consistent with the experimental data of Knollmann et al. (40) who observed significant transmural and apex-to-septum gradients in APD\(_{30}\) and APD\(_{50}\) in mice.

How well do the events during the mouse AP serve as a model of human heart? The question is a broad one, with answers that probably depend on the exact situation and aspect of physiology to be studied. However, there are some broad similarities between mouse ventricular and human atrial APs: they both have \(I_{K10, f}\) as one of the major repolarization currents and they both possess triangular shapes. These facts suggest that heterogeneity of repolarization in mouse ventricular tissue and human atrial tissue due to differential expression of \(I_{K10, f}\) can lead to conduction block at rapid pacing rates when stimulated from the region with higher expression of \(I_{K10, f}\). In addition, the AP propagation in human atrial tissue can be unstable at fast heart rates due to incomplete recovery of \(I_{K10, f}\) in a way similar to that shown in irregularly behaving mouse ventricular tissue.

The case for Ca\(^{2+}\) loading has some parallels with the repolarization of the mouse ventricular AP and the early phase (notch) of the AP in human ventricular tissue. This rapid early repolarization stage (notch), which has been implicated in
regulation of Ca\textsuperscript{2+} release (57), involves overlap of similar currents (\(I_{\text{K}\text{to,f}}\) and \(I_{\text{CaL}}\)). However, human atrial and ventricular APs are substantially longer than the mouse ventricular APs (up to 100 times for APD\textsubscript{50}) and differ in the set of repolarization currents that show different gating properties. Larger mammals also have larger hearts, although the myocyte size is approximately the same. This suggests different time scales and mechanisms of arrhythmia generation in human heart that are at least quantitatively distinct from those for mice. For example, arrhythmia in the human heart can be generated by reentrant mechanism using programmed stimulation; however, it is very difficult to create fibrillation in the normal undiseased mouse heart using similar techniques. Whether the qualitative properties of arrhythmogenesis in the mouse model are the same as in larger mammals is obviously an area for considerable extra investigation.

**Heterogeneity of repolarization and electrotonic interactions.** Electrotonic interactions in cardiac tissues affect AP repolarization and cause a change in the AP amplitude and duration. Such changes were observed experimentally with rabbit atrioventricular nodal myocyte coupled either to atrial or to ventricular myocytes (65). The cell coupling markedly increased APD of the atrial cell and decreased APD of the ventricular cell compared with that observed in the isolated myocyte, so that APD of the atrial and ventricular myocytes become close to that of the atrioventricular nodal myocyte (65).

The electrotonic effects on the dynamics of cardiac alternans were studied previously both analytically (27) and numerically (4, 18, 75). It was shown that the electrotonic effects play a crucial role in alternans stability (75) and determine spatial scale of discordant alternans (27). Watanabe et al. (75) showed that the dispersion of repolarization can lead to an asymmetry of the electrotonic currents, which is similar to our results (Fig. 5), and this asymmetry affects alternans stability. Cherry and Fenton (18) demonstrated that electrotonic effects can both suppress and promote alternans, and this behavior depends on the cellular model of repolarization.

The simulations of Sampson and Henriquez (59) suggest that electrotonic interactions can suppress heterogeneity of repolarization in a small mouse heart. Indeed, decreased heterogeneity was observed experimentally by Knollmann et al. (40) at late stages of repolarization, where no or small differences in APD\textsubscript{70} and APD\textsubscript{90} were observed in different heart regions. However, at earlier stages of repolarization, both experimental data (40) and our simulations (Figs. 4 and 5) demonstrate a likelihood that substantial heterogeneity of repolarization can be sustained over most of the AP duration in normal cases. For degrees of gap junctional uncoupling, similar to those associated with disease states such as heart failure, gradients in repolarization were also maintained for APD\textsubscript{70} and APD\textsubscript{90} over a very short distance, approximately an order of magnitude less than those predicted in the simulations of Sampson and Henriquez (59). In the small transition region at the boundary between apical and septal cells, APDs tend to be strongly altered by electrotonic effects, as seen in experiments with coupled single myocytes (65). This means that both electrotonic intercellular currents and ionic cellular currents are important for AP repolarization in mouse cardiac tissue.

Our simulations also show that electrotonic interactions can play a destabilizing role in AP propagation. We found that the threshold period for AP instability during pacing of isolated ventricular myocytes is shorter than that for the myocytes in the tissue, suggesting destabilizing role of electrotonic currents in multicellular tissue (Table 1). This conclusion is supported by the experimental data of Baker et al. (7) who studied vulnerability to arrhythmia of perfused mouse hearts treated with chemical uncouplers, diacetyl monoxime and cytochalasin D. They found that, in the control hearts, one or several bursts were sufficient to elicit a monomorphic ventricular tachycardia. In contrast, burst pacing of the hearts perfused with diacetyl monoxime or cytochalasin D triggered the APs, but the hearts become quiescent immediately after the end of the burst (7). A similar destabilizing role of electrotonic interactions was implicated in experiments with guinea pig hearts where threshold pacing frequency for alternans was smaller in cardiac tissue than in isolated myocytes (53, 73).

However, there is experimental evidence that the electrotonic interactions can also stabilize AP generation. For example, electrotonic interactions between rabbit Purkinje and ventricular myocytes suppressed early afterdepolarizations in Purkinje myocytes (37). Beat-to-beat repolarization variability was also suppressed experimentally by electrical coupling of two guinea pig ventricular myocytes (81). Cell-to-cell coupling is a complex process, which can serve to either stabilize or destabilize regular cardiac rhythm and may depend on the nature of the cells that are coupled and the degree of heterogeneity of repolarization.

**Heterogeneity of repolarization and Ca\textsuperscript{2+} dynamics.** There are at least two important general classes of behavior during repolarization that have a strong link to arrhythmogenic behavior in all mammalian hearts. Broadly, these include altered Ca\textsuperscript{2+} homeostasis and changes in dispersion, or heterogeneity, of repolarization (2, 7, 44, 54, 60, 72). Recently, several well-developed and computationally efficient Ca\textsuperscript{2+} handling models have been published (see, for example, Refs. 28, 33, 61). These models are based on different biophysical mechanisms and utilize different modes of simplification. It is unclear at present what the differences in predictive value these models have and whether they might influence some of the observations made in this study. Problems in Ca\textsuperscript{2+} handling are usually associated with triggering events, such as early and delayed afterdepolarizations. On the other hand, dispersion of repolarization is often thought of as a separate issue involving changes in repolarization duration. However, because Ca\textsuperscript{2+} entry and extrusion involve currents that are active during repolarization, it has long been known that Ca\textsuperscript{2+} can play a role in dispersion of repolarization. Similarly, the interaction between repolarizing K\textsuperscript{+} currents and the Ca\textsuperscript{2+} current during repolarization has suggested that altered or prolonged depolarization can change Ca\textsuperscript{2+} loading.

Our simulation results demonstrate the effect of spatial organization of heterogeneous repolarizing currents. The non-uniform repolarization alters the Ca\textsuperscript{2+} entry into the cell, which is determined predominantly by earlier phases of repolarization, during activation of I_{\text{CaL}}. It also alters Ca\textsuperscript{2+}-handling stability, particularly where an abrupt change in repolarization is encountered (see Figs. 8 and 9 and Table 1). Such instability includes development of AP and [Ca\textsuperscript{2+}], alternans at rapid pacing rates or appearance of irregular activity (Figs. 8 and 11). The threshold pacing periods for onset of AP instability are affected by refractoriness of the Ca\textsuperscript{2+}-handling system (in case of Ca\textsuperscript{2+} alternans) or by the influence of major
repetitive pacing rates, and the mechanism of this instability differs of alternans, which may be similar to our simulations (Fig. 8. This instability appears in the tissue region with smaller APD50 and can be regulated by the variations of ionic current amplitudes (both directly and indirectly involved in Ca^2+ handling) or rates of RyR gating. The importance of RyR gating and JSR load in the mechanism of alternans was emphasized in experimental investigations of the rat cardiac myocytes, which have similar Ca^2+ handling (24, 25). They reported that pharmacologically depressed RyRs increased susceptibility to alternans and the alternans are accompanied by alterations of sarcoplasmic reticulum content. Similar depression of RyRs (smaller opening probability) was observed in our simulations during rapid pacing. As result of this depression, Ca^2+ release is decreased, and this leads to alternans development (Fig. 9). Discordant Ca^2+ alternans were observed experimentally in rat cardiac tissue at rapid pacing rates (1). These discordant alternans developed because of heterogeneity of Ca^2+ cycling in neighboring cells, suggesting that a role for heterogeneity of repolarization in the genesis of alternans, which may be similar to our simulations (Fig. 8B). Even uniform mouse cardiac tissue becomes unstable at rapid pacing rates, and the mechanism of this instability differs from that for Ca^2+ alternans. In the former case, the instability of AP propagation appears in the form of irregular activity due to reduction of the recovery time interval for major repolarization currents that leads to smaller availability of these currents and prolongation of APD. This mechanism is similar in both isolated myocytes and myocytes in 1D tissue. The difference is in longer threshold pacing basic cycle length due to destabilizing effects of electrotonic interaction. Although the instability in the form of Ca^2+ alternans does not change AP substantially, the transition from periodic to irregular behavior affects both AP and Ca^2+ dynamics and causes propagation block. For example, APD50 during irregular activity varies over a wide range from 10.1 to 73.1 ms (Fig. 12A). Similar irregular behaviors of APD and amplitude were observed experimentally and predicted based on model simulations in different species (8, 9, 11, 18, 19). A more complex scenario of transition to irregular behavior was obtained in simulated 1D inhomogeneous cable when stimulated from the septum region (Fig. 11, C and D). In this case, the periodic AP and [Ca^{2+}]_i transients sequentially change to alternans and further to irregular activity and conduction block, qualitatively resembling experimental observations of when the alternans changes into more complex aperiodic behavior and arrhythmia (29, 52).

Presently, there is no consensus about what is the primary cause of instability and alternans in the heart (58, 63). The interdependence between perturbations of membrane potential, electrotonic and ionic currents, and [Ca^{2+}]_i transients, even with the most sophisticated experiments, has not revealed the initiator of this instability. One of the arguments of the Ca^{2+}-driven hypothesis relies on the experiments when Ca^{2+} alternans were observed under periodic potential-clamp conditions (20, 73). On the other hand, there is voltage-driven, APD restitution hypothesis that claims that the appearance of instability and alternans in the heart occurs as a result of incomplete recovery of AP-forming currents after preceding repolarization cycle at rapid pacing rates (58). Recently, a hypothesis about coupling of membrane voltage and Ca^{2+} cycling in genesis of cardiac alternans was presented (63). Our simulation data are in line with this last hypothesis, as we found the complex changes of electrotonic interactions, ionic currents, AP, and Ca^{2+}-handling system, which are the results of cardiac tissue inhomogeneity, can lead to instability and alternans.

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