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Reentry in survived subepicardium coupled to depolarized and inexcitable midmyocardium: Insights into arrhythmogenesis in ischemia phase 1B

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Abstract

BACKGROUND—Delayed ventricular arrhythmias during acute myocardial ischemia (1B arrhythmias) are associated with an increase in tissue impedance and are most likely sustained in a thin subepicardial layer.

OBJECTIVE—The goal of this study was to test the hypothesis that heterogeneous uncoupling between depolarized midmyocardium and surviving subepicardium results in heterogeneous refractoriness in the latter, providing the reentry substrate after a premature beat.

METHODS—A 3-dimensional bidomain slab was constructed comprising a normal subepicardial layer coupled to a slightly depolarized (−80 to −60 mV) but inexcitable midmyocardium. Experimentally measured tissue impedance served as input for the model. Four stages of heterogeneous uncoupling between the 2 layers were simulated, each corresponding to an experimental ischemic impedance value. Effective refractory periods (ERP), conduction velocities, and inducibility of reentry were examined.

RESULTS—Heterogeneous uncoupling resulted in subepicardial ERP dispersion, allowing reentry to occur. The minimum ERP dispersion needed to induce reentry was 28 ms. Reentry induction was only possible in this model at the 2 intermediate stages of uncoupling, and only when midmyocardial resting membrane potential was more negative than −60 mV. Complete uncoupling of the layers resulted in normal subepicardial conduction without arrhythmias. The minimum length of the reentrant pathway was 2.5 cm, comparable to 2.4 cm reported in previous experiments.

CONCLUSION—Heterogeneous uncoupling to a negative sink such as depressed inexcitable midmyocardium may be a substrate for ischemia 1B arrhythmias. Total uncoupling removes the arrhythmogenic substrate.

Keywords

Computer model; Cellular uncoupling; Reentry; Arrhythmias; Vulnerability

Introduction

Ventricular arrhythmias secondary to acute ischemia are a major cause of sudden cardiac death. Ischemia-induced ventricular arrhythmias occur in 2 phases: 1A (2 to 10 minutes after coronary occlusion) and 1B (15 to 45 minutes).^{1,2} Extensive experimental and theoretical research^{3–5} has focused mostly on investigating the mechanisms of phase 1A arrhythmias. Results show that arrhythmias in phase 1A are reentrant in 76% of the cases and that the substrate for arrhythmogenesis is provided by dispersions in conduction velocity (CV) and refractoriness resulting mostly from heterogeneities in metabolite and ionic concentrations in the ischemic region.^{3–6} Mechanisms of arrhythmogenesis in phase 1B are less well understood, even though this delayed phase shows more ventricular fibrillation episodes than phase 1A.⁷

Recent experiments² have shown that during ischemia phase 1B, the subepicardium survives functionally longer than the midmyocardium. The latter becomes inexcitable predominantly due to the increase in extracellular potassium concentration.⁸ In addition, increased intracellular calcium concentration and dephosphorylation of gap junction proteins⁹ during phase 1B result in gap junctional uncoupling, and thus in an increase in tissue resistance in the ischemic region. Experiments have shown that ischemia 1B ventricular fibrillation is based on reentry around fixed lines of block, which are thought to arise from heterogeneous subepicardial electrical uncoupling in the central ischemic zone (CIZ).^{2,10}

We hypothesize that heterogeneous uncoupling between depressed depolarized midmyocardium and the surviving subepicardium in ischemia phase 1B results in heterogeneous refractoriness in the subepicardium and that this dispersion in effective refractory period (ERP) contributes to the substrate for reentrant arrhythmias in ischemia phase 1B. Furthermore, we investigate how vulnerability to reentrant arrhythmias is affected by the degree of heterogeneous uncoupling between subepicardial and midmyocardial layers as well as by the degree of depolarization in the inactive midmyocardial layer.

The goals of the study are difficult to achieve by experimentation because of the complexity of ischemic changes and because the conditions outlined above do not occur in isolation. We therefore used a modeling approach with experimental data input to evaluate the contribution of heterogeneous subepicardial uncoupling to arrhythmogenesis. Varying degrees of homogeneous and heterogeneous uncoupling between subepicardium and viable but inexcitable midmyocardium were simulated and their effects on the inducibility of reentry tested.

Methods

Model description

Simulations were conducted on a $30 \times 30 \times 0.7$ mm³ slab of myocardium (Figure 1A). Consistent with the goal of the study and the hypothesis tested, the model included representations of a surviving subepicardial layer (0.45-mm thickness), a coupling layer (0.10-mm thickness), and an inactive midmyocardial layer (0.15-mm thickness). The model was based on experimental findings reported by de Groot et al²; a cross-sectional view of the ventricular wall from that study is shown in Figure 1B as a reference. The inactive midmyocardium was assumed moderately depolarized (transmembrane potential there denoted by V_{mid}), from a normal resting membrane potential of -83 to -80 , -70 , and -60 mV,¹¹ thus functioning as a variable sink (it was implemented as a boundary condition). The electrical properties of the subepicardial and coupling layers were simulated using the bidomain approach. Electrophysiology in these 2 layers was assumed normal because there

are no experimental data on the spatial distribution of the electrophysiological properties in the layers during phase 1B, although experiments in phase 1A have suggested that the subepicardium was relatively unaffected by ischemia.^{12–14} Finally, membrane kinetics were represented by the Luo- Rudy dynamic model.¹⁵

Regional distribution of tissue conductivities in the model

Fibers were oriented along the x axis; fiber orientation did not vary transmurally (Figure 1A). Conductivities in the model were assigned in accordance with fiber orientation and taking into account the ischemic changes in tissue resistance. Because transmembrane potential in the inactive midmyocardium was fixed, there was no need to assign tissue conductivities in this layer. The normal subepicardium was given standard anisotropic bidomain conductivities¹⁶; the resulting ratio between CVs along and across fibers was 1.9, comparable to approximately 2.1 obtained in experiments.¹⁷ The thin coupling layer was assigned the same conductivity values as the subepicardium, with the exception of the intracellular conductivity in the transmural (z) direction, σ_{it} , which was decreased as a function of location within the layer to represent the uncoupling that takes place between the viable subepicardium and the inactive midmyocardium (Figure 1A).

In accordance with the hypothesis tested in this study, spatial heterogeneities were incorporated in the transmural intracellular conductivity in the thin coupling layer to represent heterogeneities in uncoupling between viable subepicardium and inactive midmyocardium within the CIZ. Without loss of generality, these were represented as a centrally located circular region in the coupling layer with an additional decrease in transmural intracellular conductivity (Figure 1A) as compared to the surrounding tissue. The circular shape of the region avoids sharp edge effects and allows easy assessment of the minimum size of heterogeneity in transmural uncoupling that could lead to arrhythmia induction. The radius of the central region of increased uncoupling was varied, 6.6, 7.6, 10.7, 13.1, and 14.0 mm, which translated in this region occupying 15%, 20%, 40%, 60%, and 68%, respectively, of the total volume of the coupling layer.

Experimental values of overall tissue impedance, R , as reported by de Groot et al.,² (mean $\pm$ SEM), were used as an input to the model. Figure 2 depicts the experimental data regarding the course of increase in R in CIZ, as compared to the nonischemic zone (NZ). Four stages of the change in tissue impedance were singled out from this plot, and at each stage R was related to the ischemic bidomain intracellular conductivity in the z direction, $\sigma_{it}^{ischemic}$, as follows. Tissue resistance R can be considered as arising from intracellular (G_i) and extracellular (G_e) conductances connected in series.⁸ Because G_e is believed to not change during phase 1B,¹⁰ then

$$\frac{G_i^{ischemic} + G_e^{normal}}{G_i^{normal} + G_e^{normal}} = \frac{R^{normal}}{R^{ischemic}}, \quad (1)$$

where G_i^{normal} and $G_i^{ischemic}$ are intracellular conductances of normal and ischemic tissue, respectively, with G_i^{normal} assumed equal to G_e^{normal} as per Kléber et al.¹⁸ In Eq. 1, R^{normal} and $R^{ischemic}$ are overall tissue resistance values in NZ and CIZ, respectively, for the 4 stages of ischemia shown in Figure 2. The ischemic change in intracellular conductance, $G_i^{ischemic} / G_i^{normal}$, is then calculated from Eq. 1.

The bidomain intracellular conductivity in the z direction ($\sigma_{it}^{ischemic}$) within the coupling layer was assumed to undergo the same degree of change (percentage-wise) as G_i under ischemia

1B, i.e., $\sigma_{it}^{ischemic} / \sigma_{it}^{normal} = G_i^{ischemic} / G_i^{normal}$. This allowed us to then calculate the corresponding values of $\sigma_{it}^{ischemic}$ at each stage of ischemia singled out in Figure 2.

For each stage, we also defined a degree of uncoupling, DOU:

$$DOU = \left(1 - \frac{\sigma_{it}^{ischemic}}{\sigma_{it}^{normal}} \right) \times 100\%. \quad (2)$$

DOU was calculated as $42.4\% \pm 18.0\%$ at stage 1, $81.3\% \pm 11\%$ at stage 2, and $93.7\% \pm 12\%$ at stage 3. Further progression of ischemia results in DOU of 100% at stage 4 (end of ischemia phase 1B). Note that DOU of 100% does not correspond to the end of the increase in tissue resistance (as shown in Figure 2), because DOU is in reference to the transmural intracellular conductivity between midmyocardium and subepicardium only (i.e., in the coupling layer only), whereas the measured tissue resistance incorporates contributions for both intracellular and extracellular conductivities in all 3 directions.

Because of resolution limitations in impedance measurements and the heterogeneous nature of subepicardial uncoupling in the CIZ,² $R^{ischemic}$, and thus DOU, as calculated above, represent averages over CIZ tissue inside regions of stronger uncoupling and tissue outside these regions, where uncoupling is less. Therefore, in representing heterogeneous uncoupling in our model and basing on the experimental DOU values above, the degree of (strong) uncoupling within the central region (DOU_IN) and the degree of (lesser) uncoupling outside it (DOU_OUT), as shown in Figure 1A, were taken to be 50% and 0%, respectively, at stage 1; 100% and 0%, respectively, at stage 2; 100% and 50% at stage 3; and 100% and 100% at stage 4.

Simulation protocol

To evaluate the effects of degrees of uncoupling and midmyocardial depolarization on electrophysiological properties of the subepicardium, simulations were first conducted with spatially homogeneous DOU in the coupling layer. The results of these simulations were also used to facilitate understanding of mechanisms underlying inducibility of reentry in the model with heterogeneous uncoupling.

To ensure steady-state propagation (1% difference in action potential duration, APD, between the last 2 pacing beats), the model was paced for 5 beats from the $y = 0$ surface at a basic cycle length of 250 ms. Stimulus amplitude was $1.5 \times$ the normal diastolic threshold.

In the homogeneous model, APD was defined, after the 5th pacing stimulus, as the interval between time of maximum upstroke velocity and time of 90% repolarization. Average CV in the direction of the propagating wavefront was calculated by dividing the maximum distance traversed by the wavefront (30 mm) by the corresponding activation time. ERP was defined as the shortest coupling interval (CI) after the 6th pacing stimulus that allowed propagation through subepicardium. Postrepolarization refractory period (PRRP) was calculated as the difference between ERP and APD. These properties were evaluated for 3 degrees of homogeneous uncoupling, 0, 50% and 100% DOU, and the 3 values of V_{mid} .

In the heterogeneous uncoupling model, vulnerability to reentry was evaluated for the 4 stages of uncoupling described above and the 3 values of V_{mid} . In each case, after 5 pacing stimuli, a premature stimulus was applied at CIs in the range of 60 to 120 ms in steps of 1 ms to determine the vulnerable window (VW), defined as the range of CIs for which at least 1 reentrant beat was induced.

Computation

Bidomain equations were solved using a finite element method.¹⁹ Because the myocardium is characterized with a space constant along fibers that is 2.76 times larger than that across fibers,²⁰ spatial discretization in direction along fibers was 2.5 times that across fibers. The computational mesh consisted of 265,458 tetrahedral elements, with maximum element size of 0.035 mm³. A semi-implicit backward Euler integration scheme with 5- μ s time steps during the stimulus and 20 μ s after it was employed.²¹ Convergence tests were conducted by repeating simulations on a finer mesh (doubled element number) and with smaller time steps (half the original values). No differences were found in APD, and the changes in activation times were smaller than 3%.

Results

Effects of partial uncoupling from depolarized midmyocardium on subepicardial electrophysiological properties

Figure 3 illustrates the changes in APD (panel A), resting membrane potential (RMP, panel B), ERP and PRRP (panel C), and CV (panel D) for varying degrees of homogeneous partial uncoupling between subepicardial and midmyocardial layers (DOU = 0, 50%, and 100%), as well as varying levels of midmyocardial depolarization (V_{mid} = -80, -70, and -60 mV). APD and RMP shown are from a subepicardial site (x, y, z = 15.0, 15.0, 0.4 mm) as indicated by asterisk in Figure 1A.

The normal (control) values for APD, RMP, ERP, PRRP, and CV in this model are 113 ms, -83.0 mV, 128 ms, 15 ms, and 33.7 cm/s respectively. For fully uncoupled layers (DOU = 100%), the electrophysiological properties of subepicardial tissue return to normal values for all levels of V_{mid} , because there is no electrotonic influence from the depressed midmyocardium. Once the layers are partially uncoupled, CV remains mostly unaffected by both degree of uncoupling and level of midmyocardial depolarization, with changes smaller than 1.6 cm/s (5%) for all cases examined. Other electrophysiological parameters undergo, however, more noticeable changes. Specifically, for each level of V_{mid} , increasing DOU results in increases in subepicardial APD, RMP, and ERP, and in a slight decrease in PRRP. The maximum changes in APD and ERP are 48 ms (42%) and 41 ms (32%), respectively, and occur as DOU increases from 0 to 100% at V_{mid} of -80 mV. On the other hand, for each level of DOU, a less negative V_{mid} results in an increase in subepicardial APD and ERP and in a less negative subepicardial RMP. The corresponding maximum changes in APD and ERP are 12 ms (11%) and 16 ms (13%) as V_{mid} decreases from -80 to -60 mV with 0% DOU. Arrhythmias were not inducible with homogeneous uncoupling, neither partial nor complete, because midmyocardial electrotonic effects and thus electrophysiological properties were the same throughout the subepicardium.

Effects of heterogeneous uncoupling and midmyocardial depolarization on subepicardial electrophysiological properties

Because the degree of uncoupling between midmyocardium and subepicardium determines the subepicardial ERP, heterogeneities in the degree of uncoupling are expected to lead to subepicardial dispersion in ERP and vulnerability to reentry. Figure 4A illustrates an example of initiation of arrhythmia by a premature stimulus applied at CI = 104 ms in the 100%/50% heterogeneous uncoupling model as seen on the surface of the preparation; the pattern is figure-of-eight reentry. V_{mid} is -70 mV, and the radius of the central region is 14.0 mm. Premature stimulation elicits a wavefront, which propagates through subepicardial tissue partially coupled to the midmyocardium (50% uncoupling), but blocks in subepicardial tissue of extended refractoriness above the region of complete uncoupling, resulting in the establishment of a figure-of-eight reentrant circuit in the subepicardium. The

pattern of reentry is similar to patterns recorded subepicardially from isolated pig hearts during ventricular fibrillation episodes initiated by premature stimulation after 40 minutes of occlusion in the study by de Groot et al.² Figure 4B presents, as a reference, the experimental activation maps of the first 2 reentrant cycles in one such episode from the this study. Clearly, in both simulations and experiments, figure-of-eight reentry is established around fixed functional lines of block. de Groot et al.² suggested that these fixed functional lines of block might arise from heterogeneous subepicardial uncoupling in the CIZ; our simulations demonstrate that this is indeed the case for this model.

For the same V_{mid} and size of the region of increased uncoupling, but with a lesser degree of heterogeneous uncoupling (50%/0% case), the extension of refractoriness in the subepicardium above the central region is insufficient for the establishment of reentry; this behavior is illustrated in Figure 5 for $CI = 95$ ms. When the prematurely elicited wavefront reaches tissue above the partially uncoupled central region, it has already recovered and no reentrant circuit is established.

Similar to Figure 5A, Figure 6A presents the formation of a figure-of-eight reentry after premature stimulation at $CI = 94$ ms in the 100%/0% heterogeneous coupling model. V_{mid} is -70 mV, and the central region radius is 7.6 mm. No reentry is formed when the radius is reduced to 6.6 mm (Figure 6B), with other parameters unchanged. By the time the premature wavefront excites subepicardial tissue directly above the central region of increased uncoupling, subepicardial tissue above regions outside of it has not recovered from this excitation. Therefore, the premature beat dies within tissue above the central region and hence no reentry is induced.

Figure 7 portrays the duration of the VW for reentry induction (bars) as well as the maximum difference in ERP in the surviving subepicardium (ΔERP , crosses), for various levels of heterogeneous uncoupling and V_{mid} values (Figure 7A, the radius of the region of increased uncoupling is 14 mm), and several sizes of the region of increased uncoupling (Figure 7B). For each of the 3 V_{mid} values, ΔERP , an index of ERP dispersion, is determined from Figure 3C by calculating the difference between ERP obtained from a model with homogeneous DOU of value equal to that of the region of increased uncoupling (DOU_IN) and ERP from a model with homogeneous DOU equal to that outside of this region (DOU_OUT). ΔERP is a good estimate of the maximum dispersion in ERP for each case of heterogeneous uncoupling because APD and RMP in the center and at the edge of the slab in the heterogeneous model are nearly identical (<1% difference) to those in homogeneous models with DOU equal to that inside or outside the region of increased uncoupling (data not shown).

When the midmyocardium is fully coupled to the subepicardium everywhere, but partially uncoupled in the central region (heterogeneous model 50%/0%), no reentry can be induced for any value of V_{mid} ; ΔERP is then 5 to 8 ms. Reentry can only be induced with complete uncoupling (100%) inside the central region, not with partial uncoupling (50%), and thus only in intermediate stages of uncoupling (100%/0% and 100%/50%). Furthermore, no reentry can be induced for V_{mid} of -60 mV, although ΔERP reaches values as large as 25 ms. The minimum ΔERP in the subepicardium that results in the establishment of a reentrant circuit is 28 ms.

The longest VW is 24 ms, and occurs for V_{mid} of -80 mV and complete uncoupling inside the central region only (heterogeneous model 100%/0%); ΔERP is then also the largest, 41 ms. Furthermore, for each value of V_{mid} (-80 or -70 mV), increasing uncoupling outside the central region to 50% results in a decrease in VW duration, with concomitant decrease in ΔERP . Decrease in the VW duration results predominantly from an “elevation” of its lower

limit of vulnerability. The lower limit is determined by ERP in the subepicardium near the location of premature stimulation; local ERP increases as uncoupling increases for each level of V_{mid} (Figure 3C). On the other hand, for each of the 2 heterogeneous models in which reentry can be induced (100%/0% and 100%/50%), as V_{mid} changes from -80 to -70 mV, the VW duration decreases; so does ΔERP . The decrease in the VW duration is also predominantly due to the occurrence of its lower limit at a longer CI. The reason for this is that for each given level of uncoupling, ERP in the subepicardium near the site of premature stimulation increases as V_{mid} becomes less negative (Figure 3C). Clearly, subepicardial dispersion of refractoriness caused by heterogeneous uncoupling to the midmyocardium is the main reason for the existence of a VW for reentry under these conditions in this model after premature stimulation.

Figure 7B presents the effect of size (radius) of the region of increased uncoupling on VW duration in the 100%/0% model (i.e., full uncoupling inside the region and no uncoupling outside) and V_{mid} of -70 mV. Increasing the radius from 6.6 to 10.7 mm results in an increase in the VW duration from 0 to 12 ms. As the radius increases further, from 10.7 to 14.0 mm, the VW duration does not change. Similar changes in VW duration with increasing radius were obtained for V_{mid} of -80 mV (data not shown). Clearly, whereas ERP dispersion remains unaltered as the radius is varied, inducibility of reentry depends on the size of the uncoupling heterogeneities. Reentry can only be initiated for a central region radius larger than 7.6 mm, resulting in a minimum length of the reentrant pathway of 2.5 cm (CV of first reentrant beat is 30 cm/s with time of circulation 84 ms). This path length is similar to the minimum path length of 2.4 cm obtained in experiments with isolated pig hearts.²²

Discussion

Occurrence of spontaneous arrhythmias requires both a trigger (ectopic excitation) to initiate the arrhythmia, and a substrate (pre-existing proarrhythmic conditions) to sustain it. In this study, the trigger was provided by a single premature stimulus to exclusively investigate the proarrhythmic substrate supplied by the subepicardial electrophysiological heterogeneities, the latter caused by the heterogeneous electrotonic interactions with the depolarized midmyocardium (the latter acting as a variable sink). The main finding of this study is that heterogeneous uncoupling between surviving subepicardial tissue and the depolarized midmyocardium in the CIZ can provide the substrate for establishment of reentrant circuits in ischemia phase 1B. The overall mechanism is that heterogeneous uncoupling between subepicardium and the midmyocardium, as it occurs in the CIZ in the intermediate stages of ischemia 1B, results in increased subepicardial dispersion of refractoriness, which favors unidirectional block and establishment of reentrant activity. When uncoupling between surviving subepicardium and the midmyocardium becomes complete, as it occurs in the CIZ at the end of ischemia 1B, the arrhythmogenic substrate disappears. Our study also shows that the arrhythmogenic substrate depends on the degree of midmyocardial depolarization and the size of heterogeneities in uncoupling. The study found that a minimum subepicardial dispersion of refractoriness of 28 ms was required for initiation of reentry. It is important to note here that the mechanisms proposed in the present study do not rule out other possible mechanisms of arrhythmogenesis in ischemia 1B.

Electrophysiological properties of subepicardial tissue partially uncoupled from depolarized midmyocardium

Recent experimental data² documented the existence of a surviving subepicardium, 6 to 8 cell layers thick, overlying an inactive depolarized midmyocardium (Figure 1B) after 60 minutes of occlusion. This is surmised to stem from the existence of regional collateral blood flow in the surviving cell layers.¹² Correspondingly, our model incorporated a layer of

subepicardial cells overlying the inactive midmyocardial tissue (Figure 1A). The level of midmyocardial depolarization in the model was varied between -80 and -60 mV, consistent with experimental recordings during ischemia 1B.¹¹

Depolarization of the midmyocardium, in combination with remaining partial coupling between layers, resulted in shorter APD and ERP in the subepicardium and less negative RMP as compared to the normal values (Figure 3); this is consistent with results of experimental recordings in normal single cells connected to a simulated depolarized ischemic region through a varied conductance.^{23,24} Specifically, during the action potential plateau, electrotonic current flowing from subepicardium to midmyocardium causes abbreviation of local APD and ERP in the subepicardium. Our simulation results showed that coupling to depolarized midmyocardium causes up to a 32% decrease in subepicardial ERP. This suggests that the remaining partial coupling of the subepicardium to midmyocardium that is depressed could contribute to the shortening of subepicardial ERP in the CIZ during ischemia 1B.

At rest, injury current flows from the depolarized midmyocardium into the subepicardium, resulting in less negative subepicardial RMP. The levels of subepicardial resting depolarization obtained in our simulations are moderate; they are no less than -72 mV for all cases studied. Such moderate changes in RMP are consistent with sharp extracellular electrograms recorded on the subepicardium and with maintained subepicardial activation as observed experimentally during ischemia 1B⁷; a further decrease in subepicardial RMP would result in smaller and slower deflections in extracellular electrograms.²⁵

Decrease in subepicardial RMP (i.e., less negative RMP) arising from the remaining partial coupling between subepicardium and the depressed midmyocardium results in prolonged PRRP in the subepicardium (Figure 3); this is due to slow recovery from inactivation of the fast sodium current.^{26–28} Although ischemia-related increase in PRRP is typically attributed to hyperkalemia-induced depolarization,^{26–28} our results show that during phase 1B, prolonged subepicardial PRRP could also be due to subepicardial RMP decrease arising from the partial coupling of the subepicardium to the depolarized midmyocardium.

Changes in subepicardial CV, as obtained in the simulations, are smaller than 5% of the normal CV value for all cases considered. Thus, our model of uncoupling strongly suggests that the electrotonic load imposed by the depressed midmyocardium is not the main reason for the observed^{7,18} slow propagation on the subepicardium in ischemia 1B. This is an important insight that was uniquely obtained using the simulation approach here; it is difficult to prove experimentally because intercellular uncoupling occurs along with other ischemia-induced electrophysiologic changes. Reduced availability of sodium and calcium currents due to acidosis and hyperkalemia in the surviving subepicardium might be factors responsible for slow conduction in ischemia 1B^{18,29}; however, this remains to be demonstrated.

Vulnerability to reentry

In our simulations, heterogeneous uncoupling between subepicardial and midmyocardial layers results in significant subepicardial dispersion in ERP, which provides the main substrate for arrhythmia induction. With values of Δ ERP up to 8 ms, reentry was not inducible in the 50%/0% heterogeneous uncoupling case (stage 1 of uncoupling). However, figure-of-eight reentry was formed at more advanced stages of uncoupling (100%/0% and 100%/50%, stages 2 and 3, respectively), with a minimum Δ ERP of 28 ms for the cases tested (Figure 7A). Experiments in pigs² have similarly shown that with a single premature stimulus, ventricular fibrillation was inducible only between 30 and 50 minutes postocclusion, that is, at intermediate stages of ischemia 1B, which would correspond to

levels of uncoupling similar to the 100%/0% and 100%/50% heterogeneous uncoupling cases in our simulations. Further progression to stage 4 (100%/100% uncoupling) results in a decrease in vulnerability to reentry; this is consistent with experimental results.²

The reason why reentry could be induced in the 100%/50% model but not in the 50%/0% model for the same size of the region of increased uncoupling and the same V_{mid} value is that ERP changes nonmonotonically as DOU increases. Specifically, for a given V_{mid} , changes in subepicardial ERP are smaller when DOU increases from 0% to 50% than from 50% to 100% (Figure 3). Consequently, with V_{mid} of -70 mV, the maximum dispersion in ERP in the 100%/50% model is 28 ms, whereas it is only 7 ms in the 50%/0% model. As shown in the present study, only when the level of uncoupling is large and the load exerted by the midmyocardium on the subepicardium is significantly reduced can the properties of subepicardial tissue be close to normal.

Limitations

The model used here does not represent the complexity of the real organ, with its intricate geometry and fiber orientation. In the present study, we focused on the heterogeneous uncoupling between depressed depolarized midmyocardium and the surviving subepicardium, as it occurs in ischemia phase 1B, to demonstrate how it can contribute to the substrate for reentrant arrhythmias. Thus, ischemia-related alterations in ion currents and concentrations in the surviving subepicardial and coupling layers were not intended to be represented in the model; moreover, that as indicated in the Methods section, experimental data regarding the electrophysiological properties of these 2 layers during phase 1B are currently not available. The model, however, faithfully represents the electrotonic interactions between viable subepicardium and depolarized midmyocardium, allowing the simulation results to provide important insights into the development of the arrhythmogenic substrate during ischemic phase 1B. Simulations with this model demonstrate that even in the absence of ischemia-induced changes in the subepicardium, a substrate for reentry may be present, resulting from electrotonic interactions alone, and explain how the substrate resolves itself even when ischemia-induced electrophysiological changes persist.

The ionic model used in this study is the dynamic Luo-Rudy model largely derived from experiments on guinea pigs. The most appropriate membrane model to use in our study would have been a pig model because the experimental recordings we use in the study for comparison were in pigs. However, there are no pig membrane models available; thus we used a trusted and well-characterized model, the dynamic Luo-Rudy model. Variations in CV and APD within the subepicardium due to species-related differences³⁰ could quantitatively change the results. However, the mechanisms uncovered in this article would remain the same. In view of the validity of the mechanisms proposed here, the dynamic Luo-Rudy membrane model is particularly advantageous. Indeed, because the midmyocardial electrotonic effect is mainly to shorten the subepicardial APD, shorter action potentials, such as in the guinea pig, would result in smaller APD dispersion and therefore smaller inducibility of reentry, which would be the worst-case scenario as far as the proposed mechanisms are concerned. If APD is longer, as in pigs and humans, this will result in a better manifestation of the proposed mechanisms (larger APD dispersion and larger inducibility of reentry).

Furthermore, because the subepicardial and coupling layers are very thin (0.55 mm for both layers in our study), possible transmural heterogeneity of ion channel expression was not modeled within these layers. In both layers, we used a potassium current density ratio IK_r/IK_s that is the same as in the epicardium.³¹ Because the midmyocardium is reported to have smaller IK_s ,³¹ it is possible that the IK_s in the subepicardial and coupling layers would also be smaller. This would result in extended APD in these layers, which, as discussed

previously, would not change the mechanisms proposed in this study but would only result in larger inducibility of reentry.

For computational simplicity, midmyocardial tissue was modeled as inexcitable. This may not be physiologically realistic because lack of excitability is only expected to take place when a cell has a resting potential lower than (less negative than) -60 mV,¹⁸ contrary to our simulations. However, whether the midmyocardium is modeled as active or inactive does not affect the conclusions of the study because a resting potential of -70 mV, even if the tissue remained excitable, would cause the same decrease in subepicardial ERP and reentry initiation as observed in the present study.

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Figure 1.

A: Ventricular slab model of uncoupling in ischemia phase 1B, including a subepicardium, a coupling layer, and an inactive midmyocardium with dimensions as indicated. A centrally located circular region in the coupling layer with an additional decrease in transmural intracellular conductivity was incorporated to represent heterogeneities in uncoupling between viable subepicardium and inactive midmyocardium within the central ischemic zone (CIZ). Fiber direction is denoted by arrow on the slab surface. Stimuli were applied on the transmural side of the subepicardial and coupling layers only, as indicated by dark gray color, because the midmyocardium was assumed inactive. The asterisk marks the subepicardial site from which action potential traces were taken. Note that the scaling in the thickness direction has been greatly expanded. **B:** Experimental evidence used to construct

the model. Shown is a slice of a pig heart stained with 2,3,5-triphenyltetrazolium chloride 60 minutes postischemia. Viable Lactate dehydrogenase (LDH) containing tissue appears in red (surviving subepicardium). The inset shows periodic acid Schiff staining of a microsection from the slice, with dark purple staining corresponding to the presence of glycogen and indicating subepicardium viability (up to 8 cell layers). Adapted with permission from de Groot et al.²

Figure 2.

Time course of tissue resistance within the central ischemic zone (CIZ) (open circles) and the normal zone (NZ, closed triangles), as obtained from measurements in isolated perfused pig hearts 90 minutes after occlusion.² Circled numbers denote stages of ischemia. Stage 1 represents uncoupling at the onset of ischemia phase 1B. Stages 2 and 3 are of intermediate uncoupling, whereas stage 4 corresponds to uncoupling at the end of ischemia phase 1B.

Figure 3.

Changes in subepicardial electrophysiological parameters: action potential duration (APD) (A), resting membrane potential (RMP) (B), effective refractory period (ERP) (dashed line), postrepolarization refractory period (PRRP) (solid line) (C), and conduction velocity (CV) (D) for different degrees of homogeneous uncoupling (DOU, with values of 0, 50% and 100%). Midmyocardial transmembrane potential is -80 (triangles), -70 (stars), and -60 mV (circles).

Figure 4.

A: Progression of the spatial distribution of transmembrane potential on the surface of the preparation for an episode of figure-of-eight reentry induction. The sequences of snapshots are labeled by the instant of time measured from the premature stimulus application. Arrows indicate activation sequence. DOU_ IN/DOU_ OUT is 100%/50% and premature stimulation is at CI = 104 ms. The radius of the central region of uncoupling is 14 mm, and the midmyocardium is depolarized at -70 mV. **B:** Isochronal activation maps for the first 2 reentrant cycles during ventricular fibrillation after 40 minutes of ischemia used as a comparison to the simulation results (adapted with permission from de Groot et al2). Areas with dV/dt_{min} less negative than -2.5 V/s are highlighted with dashed lines. Arrows indicate gross activation sequence. Numbers and lines indicate activation times and 20 ms isochrones, respectively. CI = coupling interval; DOU_ IN/DOU_ OUT = degree of uncoupling within/outside the central region.

Figure 5.

Progression of the spatial distribution of transmembrane potential on the surface of the preparation for an episode of no reentry induction. The sequences of snapshots are labeled by the instant of time measured from the premature stimulus application. DOU_IN/DOU_OUT is 50%/0%, and premature stimulation is at CI = 95 ms. The radius of the central region of uncoupling is 14 mm, and the midmyocardium is depolarized at -70 mV. Abbreviations as in Figure 4.

Figure 6. Progression of the spatial distribution of subepicardial transmembrane potential in an episode of figure-of-eight reentry induction (**A**) and of no induction (**B**) for 2 values of the radius of the central region of increased uncoupling, 7.6 mm (**A**) and 6.6 mm (**B**). The sequences of snapshots are labeled by the instant of time from the premature stimulus application. Arrows indicate activation sequence. Premature stimulus CI = 94 ms for both **A** and **B**. In both cases DOU_IN/DOU_OUT is 100%/0% and the midmyocardium is depolarized at -70 mV. Abbreviations as in Figure 4.

Figure 7.

A: Vulnerable windows for different cases of heterogeneous uncoupling, DOU_IN/DOU_OUT, and different degrees of midmyocardial depolarization, V_{mid} (black, gray, and white). Radius of central region of uncoupling is 14.0 mm. Vulnerable window duration is zero for V_{mid} of -60 mV. **B:** Vulnerable window for different radii of the central region. DOU_IN/DOU_OUT is 100%/0%, and V_{mid} is -70 mV. ΔERP is an estimate of the maximum dispersion in effective refractory period in the subepicardium (see text for more detail). Abbreviations as in Figure 4.