Optical measurements reveal nature of intercellular coupling across ventricular wall

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Poelzing, Steven, Bradley J. Roth, and David S. Rosenbaum. Optical measurements reveal nature of intercellular coupling across ventricular wall. Am J Physiol Heart Circ Physiol 289:H1428–H1435, 2005. First published April 29, 2005; doi:10.1152/ajpheart.01245.2004.—Previously, we showed that intercellular uncoupling through gap junctions is an important mechanism for maintaining transmural heterogeneities of repolarization that are responsible for ventricular arrhythmias in disease states such as heart failure. However, rotational anisotropy between transmural muscle layers also may influence coupling. To determine the effect of rotational anisotropy on transmural coupling, we developed a numerical three-dimensional model of passive cardiac tissue in which rotational anisotropy was varied in a controlled fashion. Simulations of optical mapping demonstrated that spatial averaging produced a voltage decay in space best fit by a single decaying exponential compared with the theoretically predicted decay. As fiber orientation varied by 90° with respect to the transmural surface, the effective transmural space constant (λTM) changed by only 0.31% in simulations. In contrast, reducing intercellular conductivity by 24% decreased λTM by 7.7%. In the canine wedge preparation (n = 5), λ measured by optical mapping of the epicardial and subepicardial surface was similar transverse (λTV = 0.73 ± 0.10 mm) and transmural (λTM = 0.70 ± 0.08 mm) to subepicardial fibers. We confirmed previous findings that λTM in subepicardial layers was significantly reduced by 14 ± 2% compared with deeper layers of myocardium, providing evidence for transmural uncoupling in the epicardial-midmyocardial interface. These data establish the theoretical and experimental basis for measuring intercellular coupling between muscle layers spanning the ventricular wall with optical mapping techniques. Furthermore, this study demonstrates that transmural uncoupling at the epicardial-midmyocardial interface may be attributable to heterogeneous expression of cardiac gap junctions and not rotational anisotropy.

gap junction; repolarization; voltage-sensitive dyes; space constant; optical mapping; modeling

CELL-TO-CELL COUPLING in the heart is mediated by cardiac gap junctions, which are intercellular channels that permit the transfer of electrical current and small molecules between neighboring myocytes. Intercellular coupling plays a critical role in propagation, repolarization, and arrhythmias (8). Despite the presence of significant heterogeneities in ion channel expression between cells that span the ventricular wall, Conrath et al. (5) suggested that transmural electrophysiological heterogeneities are essentially eradicated by high degrees of coupling between transmural muscle layers, implying that localized uncoupling is critical for maintenance of heterogeneous repolarization in the heart. Previously, we demonstrated that the principle ventricular gap junction protein connexin43 (Cx43) was heterogeneously distributed across the ventricular wall of the canine left ventricle. Specifically, Cx43 was reduced in the subepicardium compared with deeper myocardial layers (17, 18). Moreover, heterogeneous Cx43 expression patterns were paralleled by functional changes associated with coupling such as slowed conduction and increased action potential upstroke velocity in subepicardial cells compared with deeper myocardial layers. Furthermore, we demonstrated that such localized uncoupling produced transmural gradients of repolarization of sufficient magnitude to form a substrate for bidirectional block leading to reentrant arrhythmias (18). These findings suggest the hypothesis that electrophysiological heterogeneities across the ventricular wall are maintained by gap junction expression patterns.

Alternatively, it has been suggested that variations of fiber orientation between transmural muscle layers (i.e., rotational anisotropy) also may influence coupling. Previous studies demonstrated that sudden changes in fiber direction produce non-uniform propagation and repolarization across the ventricular wall (3, 4). These findings suggest that axial loading within a layer of myocardium may influence cell-to-cell coupling between transmural layers. Therefore, a direct measurement of cell-to-cell coupling between transmural muscle layers in the intact ventricle is important for understanding the substrate underlying arrhythmia mechanisms in the whole heart.

The purpose of this report is 1) to validate experimental measurements of the effective space constant transmurally by determining whether axial loading within layers of myocardium affects coupling between transmural layers and 2) to determine the role of cardiac fiber geometry (i.e., rotational anisotropy) in the maintenance of intercellular uncoupling and electrophysiological heterogeneities across the ventricular wall.

METHODS

Canine Wedge Preparation

To measure the effective space constant on the epicardial and transmural surface of the left ventricle, we developed a system for mapping with high resolution the spatial distribution of transmembrane potentials from the epicardial and transmural surface of the arterially perfused canine wedge preparation (11, 20, 32). Briefly, hearts were excised from five male mongrel dogs weighing 20–25 kg. Wedges of myocardium measuring ~3 × 1.5 × 1 cm were dissected from the anterior, anterolateral, or posterior wall of the left ventricle in proximity to secondary branches of the left anterior descending or circumflex coronary arteries, respectively. Wedges were perfused with oxygenated Tyrode solution (in mmol/l: 129 NaCl, 25 NaHCO3, 1.5 KCl, 5.4 glucose, 0.4 NaH2PO4, 1.2 MgSO4, 2.5 CaCl2, and 0.34 Na2HPO4).

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0.5 MgSO₄, 4 KCl, 5.5 dextrose, and 1.8 CaCl₂). Perfusion pressure was maintained at 50–60 mmHg. Wedges were discarded if collateral arteries shunted significant flow away from the preparation as evidenced by a coronary resistance <1.2 mmHg·m⁻¹·min⁻¹ (11). Preparations were completely immersed in temperature-controlled (36 ± 1°C) perfusate to prevent the formation of intramyocardial temperature gradients. Wedges were stabilized against a flat imaging window by applying a constant gentle pressure via a movable piston, obviating the need for pharmacological suppression of contraction. Preparations were determined to be stable for over 4 h of perfusion as judged by the stability (±5%) of coronary resistance, action potential duration, and QT interval.

**Transmural Optical Mapping System**

Previously, we developed an optical action potential mapping system (10) capable of resolving membrane potential changes as small as 0.5 mV with 1-ms temporal resolution from 256 sites simultaneously across the entire transmural surface of the wedge preparation (2, 11, 20). Briefly, after staining with the voltage-sensitive dye di-4-ANEPPS (15 μmol/l) by direct arterial perfusion for 10 min, dye was excited by 514 ± 5-nm light emitted by a 250-W tungsten-filament lamp. Fluoresced light was long-pass filtered at 610 nm and focused onto a 16 × 16-element photodiode array (C4675; Hamamatsu) through high numerical aperture photographic lenses in the tandem lens configuration (Nikon 85 mm F/1.4, 105 mm F/2.0) (10), yielding a spatial resolution of 0.33 mm between recording sites.

**Measurement of Effective Space Constant**

Previously, we developed a method for measuring the effective cardiac space constant with high-resolution optical mapping as a means for estimating intercellular resistivity in intact tissues (2). Wedges were stimulated through a 50-μm-diameter Teflon-coated platinum wire at a steady-state cycle length of 2,000 ms to obtain baseline action potential amplitudes. A subthreshold stimulus of 1.5-mA amplitude and 15-ms pulse width was delivered to achieve a baseline action potential amplitudes. A subthreshold stimulus of 1.5-mA amplitude and 15-ms pulse width was delivered to achieve a baseline action potential amplitude of 15.65 ± 3.9 mV. Previous studies demonstrated that elevating [K⁺], to this level results in a rightward shift of the inward rectifying potassium channel current-voltage curve (9, 13, 26). Therefore, we assumed a constant resting membrane resistivity. The transmembrane voltage response to the subthreshold stimulus (Vₘ) at each recording site was measured as the maximum change in Vₘ during the application of the ST stimulus relative to resting membrane potential normalized to the amplitude of the baseline action potential at each site. This procedure allowed comparison of Vₘ between recording sites, as described previously (14). The decay of Vₘ in the transmural direction was fit to an exponential to obtain the effective transmural space constant (λₜm). To assure reproducibility, we measured λₜm from three separate sites within each muscle layer and determined the average λₜm for each layer. This was repeated every 2 mm from epicardium to endocardium. The identical procedure was used to measure the effective longitudinal (λₜl) and transverse (λₜv) space constants on the epicardial surface.

It was also important to demonstrate that the measurement of λₜm reflected spread of passive current and not active processes also manifested during intercellular conduction. Therefore, λₜm was measured during comparable conduction slowing caused by pharmacologically uncoupling (10 μmol/l carbenoxolone, n = 3) or reducing excitability (5 mmol/l flecainide, n = 3).

**Histological Analysis**

After each preparation was optically mapped, wedges were fixed in 10% buffered formalin for 24 h and embedded in paraffin. Sections were cut from the most superficial regions of the transmural surface (corresponding to the optically mapped region) and stained with hemotoxylin and eosin stain as described previously (32). Tissue sections were imaged with a Nikon Eclipse E600 microscope and digitized with a Sport RT Slider charge-coupled device camera for off-line assessment of the extracellular matrix. Invasion of inflammatory cells, fiber separation, and deposition of collagen were qualitatively assessed at positions where λₜm was measured.

The paraffinized blocks were then sectioned from epicardial to midmyocardial to endocardial layers every 2 mm, allowing for selective quantification of fiber orientation for each layer. The short axes of myocytes were measured from 50 myocytes within each layer of myocardium where λₜm and λₜv were obtained.

**Passive Numerical Model of Rotational Anisotropy**

Because of the difficulties of experimentally determining the effects of rotational anisotropy on passive electrophysiological properties of the heart, we developed a mathematical model of cardiac tissue to allow controlled manipulation of cardiac fiber rotation. The transmembrane potential, Vₘ, was calculated in a passive three-dimensional model of cardiac tissue as described previously (7, 15, 22). Briefly, the bidomain model represents the electrical properties of mammalian cardiac tissue (23). It consists of two coupled partial differential equations:

\[ \nabla \cdot (\vec{g}_m \nabla V_m) = -\nabla \cdot (\vec{g}_e \nabla V_e) \quad (1) \]

\[ \nabla \cdot (\vec{g}_e \nabla V_e) = -\beta \left( g_m V_m + C_m \frac{\partial V_m}{\partial t} \right) \quad (2) \]

where Vₑ is the interstitial potential, β is the ratio of membrane surface area to tissue volume (0.03 μm⁻¹), Gₑ is the membrane conductance (1.65 S/m²), and Cₑ is the membrane capacitance (0.01 F/m²). Because Gₑ is multiplied by β, uncertainties in β reflect uncertainties in Gₑ. We chose our value of β to match theory and experiments, as was done previously (2). Vₑ represents deviations of the transmembrane potential from rest. Except at the electrode surfaces, the boundaries of the tissue are sealed so that the perpendicular component of the current density is zero. When the fibers approach the tissue surface at an angle, this boundary condition is not equivalent to setting the derivative of the potential in the direction perpendicular to the surface equal to zero. The tissue is stimulated with a 1-mA amplitude and 30-ms-duration signal to approximate the conditions of steady state. Current is passed between circular electrodes located on the front and back transmural surfaces (Fig. 1).

The anisotropic electrical properties are specified by the intracellular (i) and interstitial (e) conductivities parallel to (L) and perpendicular to (T) myocardial fibers: gₑL = 0.01863 S/m, gₑT = 0.00186 S/m, gᵢL = 0.1863 S/m, and gᵢT = 0.0745 S/m. The fiber direction is constant and lies in the x-z plane at an angle θ with respect to the x-axis. The conductivity tensor gᵢ is then

\[ \begin{pmatrix} gᵢLcos²θ + gᵢTsin²θ & 0 & (gᵢL - gᵢT)cosθsinθ \\ 0 & gᵢT & 0 \\ (gᵢL - gᵢT)cosθsinθ & 0 & gᵢLsin²θ + gᵢTcos²θ \end{pmatrix} \quad (3) \]

with a similar equation for gₑ.

Equation 1 is solved using successive overrelaxation, and Eq. 2 is solved using Euler’s explicit method. The space step within the tissue is Δx = 0.1 mm. The number of grid points, Nᵢ, in each direction is 101, resulting in a total tissue size of 10 × 10 × 10 mm.

To simulate optical mapping conditions where signal can come from layers of tissue below the tissue surface (7), a weighted average from layers of tissue below the tissue surface (7), a weighted average
voltage \( (V_m) \) decay in space due to a subthreshold stimulus is fit by (16)

\[
e^{-\frac{x}{\lambda_{\text{effective}}}}
\]

where \( x \) is the distance from the electrode and \( \lambda \) is the space constant. This equation is valid for anisotropic tissue if \( \lambda \) is interpreted as the space constant in the \( x \) direction. However, it is not valid for cardiac tissue with unequal anisotropy ratios in the intracellular and extracellular spaces. In that case, it should be thought of as the first term in a perturbative expansion for the transmembrane potential (21). Therefore, Eq. 5 should be a reasonable approximation for the transmembrane potential induced near the electrode.

Shown in Fig. 2A is the simulated \( V_m \) response as a function of distance from the stimulating electrode. Fitting \( V_m \) to Eq. 5 yields an actual space constant \( (\lambda_{\text{actual}}) \) equal to 0.59 mm \((R^2 = 0.99)\). Each pixel in optical mapping measures a spatial average of \( V_m \) from a small volume of myocardium located under each recording pixel. We included this spatial averaging in our numerical simulation. As a result, the volume-averaged \( V_m \) (Fig. 2A) significantly deviates from \( V_m \) of individual myocytes within a space constant without averaging. Therefore, Eq. 5 poorly fits volume-averaged \( V_m \) resulting in a space constant \( (\lambda = 1.57 \text{ mm}) \) significantly greater than \( \lambda_{\text{actual}} \). However, a simple decaying exponential (Eq. 6) is a better fit for volume-averaged \( V_m \), resulting in an effective space constant \( (\lambda_{\text{effective}}) \) equal to 0.34 mm (Fig. 2B):

\[
e^{-\frac{x}{\lambda_{\text{effective}}}}
\]

One reason Eq. 6 may provide a better fit to the calculated \( V_m \) than Eq. 5 is that the spatial averaging has a larger effect near the electrode, where the distribution of \( V_m \) is very localized, than far from the electrode, where the distribution is diffuse. Thus Eq. 5 overestimates the rapid falloff near the electrode.

To validate the use of Eq. 6 to measure \( \lambda \), it was necessary to demonstrate that \( \lambda_{\text{effective}} \) correlates to \( \lambda_{\text{actual}} \) over the range of space constants measured in intact myocardium \((0 \text{ to } 2 \text{ mm})\). It has been previously demonstrated that \( \lambda \) varies as one divided by the square root of the cell surface-to-volume ratio of individual myocytes \( (\beta) \) (24), and \( \lambda_{\text{effective}} \) closely followed this relationship \((R^2 = 0.98)\). When values of \( \beta \) varied over a physiological range, \( \lambda_{\text{actual}} \) correlated well with \( \lambda_{\text{effective}} \) \((R^2 = 0.96)\). This finding confirms that a single decaying exponential fit to volume-averaged data accurately reflects changes in \( \lambda_{\text{actual}} \).

As the interpixel distance increases, which simulates decreasing optical magnification (Fig. 2C), \( \lambda_{\text{effective}} \) increases as expected. Importantly, the percent change in \( \lambda_{\text{effective}} \) is unaffected by changes in \( \beta \). However, as interpixel distance increases, the ability to distinguish small \( V_m \) changes decreases as one measures farther from the stimulating electrode. Therefore, higher optical magnifications will decrease the ability to resolve very small changes in \( V_m \). An optical magnification of 0.33 mm is used in simulation and experimental data. Henceforth, \( \lambda_{\text{effective}} \) is referred to as the effective space constant \( (\lambda) \) and volume-averaged \( V_m \) is simply referred to as \( V_m \).

**Statistical Analysis**

Statistical analysis of the data was performed using a Student’s \( t \)-test for paired data or a single-factor ANOVA. A \( P < 0.05 \) was considered statistically significant. All values are reported as means \( \pm \) SD unless otherwise noted.

**RESULTS**

**Numerical Validation of Optical Space Constant**

With the use of a three-dimensional passive model of cardiac tissue, it has been previously demonstrated that the membrane...
Experimental Validation of the Effective Transmural Space Constant

\( \lambda_{TM} \) measures coupling. If \( \lambda_{TM} \) is an index of axial resistivity, one would expect that \( \lambda_{TM} \) is related to conduction velocity as predicted by theory (29). The effects of gap junction blockade on \( \lambda_{TM} \) are demonstrated for a representative canine wedge experiment in Fig. 3A. As expected, \( V_m \) was significantly reduced at progressive distances from the point of stimulation during the administration of carbenoxolone compared with control, consistent with an effect of uncoupling. For all experiments, \( \lambda_{TM} \) was significantly reduced from 0.73 \pm 0.13 mm in control to 0.50 \pm 0.06 mm during carbenoxolone administration, as shown in Fig. 3B. Importantly, a 22% reduction in \( \lambda_{TM} \) was paralleled by a significant reduction in transmural conduction velocity (\( \theta_{TM} \)) by 30% relative to control (Fig. 3C). When conduction was reduced to a similar extent by reducing excitability with the sodium channel blocker flecainide (by 26% relative to control), \( \theta_{TM} \) was not paralleled by a change in \( \lambda_{TM} \) (0.68 \pm 0.08 mm, Fig. 3B), confirming that \( \lambda_{TM} \) measures changes in coupling.

Fig. 2. Effect of spatial averaging of the transmembrane potential (\( V_m \)) on the space constant \( \lambda \). A: volume-averaged \( V_m \) represents the 0.3 \times 0.3-mm average of myocyte \( V_m \). Volume-averaged \( V_m \) is altered for distances less than 1 mm from the point of stimulation. B: myocyte \( V_m \) is better fit by \( e^{-x/\lambda} \) than by \((e^{-x/\lambda})/x \) (dashed line). C: increasing the area of tissue imaged increases \( \lambda \) but does not affect the fit of Eq. 6.

Fig. 3. Alterations in \( \lambda_{TM} \) are attributable to altered coupling, not excitability. A: \( V_m \) measured by optical mapping was significantly lower at progressive distances from the point of stimulation in the presence of the gap junction blocker carbenoxolone. \( \lambda_{TM} \) was 0.68 mm in control and 0.47 mm during carbenoxolone administration for this representative example. B: \( \lambda_{TM} \) was significantly reduced only in the presence of carbenoxolone and not flecainide (a sodium channel blocker). C: transmural conduction velocity (\( \theta_{TM} \)) was reduced in the presence of carbenoxolone and flecainide.
Coupling within and between transmural muscle layers. Representative optical measurements of $V_m$ in response to a subthreshold stimulus are shown in Fig. 4. The $V_m$ response on the epicardial surface is anisotropic where voltage decay in space is much greater transverse compared with longitudinal to fibers as expected from the anisotropic nature of axial resistivities (2). Fitting the decay of $V_m$ longitudinal and transverse (Fig. 4, left) to an exponential yields effective longitudinal and transverse space constants ($\lambda_L$ and $\lambda_{TV}$) of 1.45 and 0.73 mm, respectively. Importantly, the effective transmural space constant ($\lambda_{TM}$) within the subepicardium was similar in magnitude to $\lambda_{TV}$ within the epicardial muscle layer, strongly suggesting that transverse coupling is similar to transmural coupling. Summary data (Fig. 5A) indicate that $\lambda_L$ was significantly larger than $\lambda_{TV}$ and subepicardial $\lambda_{TM}$ ($1.58 \pm 0.29$, $0.73 \pm 0.10$, and $0.72 \pm 0.08$ mm, respectively), whereas there were no significant differences between $\lambda_{TV}$ and subepicardial $\lambda_{TM}$.

Heterogeneities of coupling across the ventricular wall. To determine the transmural profile of cell-to-cell coupling across the ventricular wall, we measured $\lambda_{TM}$ from multiple layers from epicardium to endocardium. Interestingly, the spatial extent of $V_m$ in the midmyocardium, as demonstrated in Fig. 4, is greater than the spatial extent of $V_m$ in the subepicardium, suggesting that coupling is reduced closer to the epicardium. In general, $\lambda_{TM}$ (Figs. 4 and 5B) is reduced in the subepicardium compared with the midmyocardium (0.72 ± 0.08 and 0.82 ± 0.06 mm, respectively). There were no significant differences between $\lambda_{TM}$ in the midmyocardium and endocardium.

Effect of rotational anisotropy on $\lambda_{TM}$. The apparent difference between $\lambda_{TM}$ in the subepicardium and midmyocardium could be due to reduced expression of Cx43 protein in the subepicardial layer (17) or rotational anisotropy. Histological analysis demonstrated that fiber angle changed by $\sim 10^\circ$/mm (Fig. 6A) consistent with previous findings in canine myocardium (12). Furthermore, fiber orientation in canine left ventricle changed by up to $180^\circ$ from epicardial layers to endocardial layers. Therefore, the model used in this study accounts for the maximum change in fiber orientation. The effect of rotational anisotropy on $\lambda_{TM}$ was determined using the aforementioned three-dimensional passive model of cardiac tissue and Eq. 6. The effects of rotational anisotropy on $V_m$ are demonstrated in Fig. 6B. Fibers intersected the transmural surface at 0, 45, and $90^\circ$. As fiber angle increased, $\lambda_{TM}$ changed minimally from 0.322 to 0.322 to 0.323 mm, respectively. The maximum change in $\lambda_{TM}$ for varying fiber orientation was 0.31%. When intercellular gap junction conductance ($g_i$) was decreased in simulations by 24% to model the difference in gap junction expression in epicardial compared with other myocardial layers, $\lambda_{TM}$ changed by 7.7%. Therefore, gap junction conductance, not rotational anisotropy, most likely accounts for observed transmural heterogeneities in coupling, and specifically coupling within the epicardial-midmyocardial interface.

DISCUSSION

Previously, we have shown that transmural gradients of repolarization may underlie the substrate for ventricular arrhythmias in a variety of experimental conditions such as the long QT syndrome and heart failure (1, 11). While it is well established that ionic heterogeneities exist between myocytes that span the ventricular wall (28), the resulting transmural gradients of repolarization can be largely eradicated in multicellular preparations with high degrees of cell-to-cell coupling, implying that intercellular uncoupling is critical to the maintenance of these repolarization gradients (5). Moreover, we recently demonstrated that reduced subepicardial gap junction expression may be responsible for localized uncoupling between epicardial and midmyocardial layers, suggesting the hypothesis that gap junction expression patterns underlie arrhythmia substrates dependent on transmural dispersion of repolarization (17, 18). However, others have suggested that variations in fiber direction between muscle layers (i.e., rota-
Intercellular coupling is determined by axial resistivity, which is governed not only by junctional resistance (i.e., the number and function of gap junctions) but also by the number of gap junctions encountered per unit length. Therefore, fiber orientation influences axial resistivity, because more gap junctions are encountered transverse to fibers compared with the longitudinal direction, resulting in greater transverse resistivity. In this report we demonstrated that the values for effective \( \lambda \) in canine was also similar to space constants in three-dimensional tissue is best approximated by the relationship \( \exp(-x/\lambda)/x \), or even more complicated relationships in the case of unequal anisotropy ratios (16, 21). We simulated the effects of optical mapping by volume averaging \( V_m \) across multiple neighboring cells, because in contrast to \( V_m \) measured from microelectrodes, voltage-sensitive dyes register and average \( V_m \) from myocytes within a recording pixel (6). These simulations showed that volume averaging by optical mapping preferentially affected the measurement of \( V_m \) in close proximity to the stimulus electrode and specifically within a space constant (Fig. 2). Importantly, volume-averaged \( V_m \) was best fit by a single exponential (Fig. 2) yielding a \( \lambda_{\text{effective}} \) that correlated well with \( \lambda_{\text{actual}} \) predicted by bidomain theory over a physiological range of space constant values. Differences between theoretical values of \( \lambda_{\text{effective}} \) obtained from simulations of volume-averaged \( V_m \) and \( \lambda_{\text{actual}} \) experimentally measured by optical mapping were most likely due to the choice of parameters for the simulations. Furthermore, the passive bidomain model was used for the numerical simulations, because in general, the myocardial membrane is active, but for the weak, subthreshold stimuli used in this study, the passive model should provide a good first approximation. Tissue has unequal anisotropy ratios, and all the well-known features of virtual anodes and the “dog-bone” transmembrane potential distribution are present in the calculation (27) and the experiment (2) (Fig. 4). However, they are negligibly small for the weak stimuli considered in this study. The tissue surfaces are assumed to be insulated. When fibers approach an insulated boundary, a transmembrane potential can be induced (19). These effects occur in our model but are generally small for weak stimuli. In summary, these simulations indicate that \( V_m \) decay in space due to a subthreshold stimulus obtained by optical mapping should be fit to a single exponential to more accurately reflect changes in cell-to-cell coupling.

To demonstrate that the optical measurement of \( \lambda \) was indeed a reliable index of intercellular coupling by gap junctions, we used the gap junction inhibitor carbenoxolone to uncouple myocytes. Decreases in \( \lambda_{\text{TM}} \) by pharmacological uncoupling were closely paralleled by expected decreases in transmural conduction (Fig. 3) (25). However, because conduction is also in part dependent on sodium channel availability, we slowed conduction to the same extent with the sodium channel blocker flecainide, but we observed no change in \( \lambda_{\text{TM}} \). These data confirm that the changes observed in \( \lambda_{\text{TM}} \) reflected changes in gap junction coupling (passive intercellular properties) rather than active cellular properties.

Previously, we developed a method for measuring the effective space constant as an index of cell-to-cell coupling using a high-resolution optical mapping system capable of measuring \( V_m \) with sufficient fidelity to calculate the effective space constant within a single layer of ventricular myocardium (2). In those studies, the effective space constant was measured using a single decaying exponential, whereas bidomain theory predicts that the amplitude of the electrotonic spread of \( V_m \) in three-dimensional tissue is best approximated by the relationship \( \exp(-x/\lambda)/x \), or even more complicated relationships in the case of unequal anisotropy ratios (16, 21). We simulated the effects of optical mapping by volume averaging \( V_m \) across multiple neighboring cells, because in contrast to \( V_m \) measured from microelectrodes, voltage-sensitive dyes register and average \( V_m \) from myocytes within a recording pixel (6). These simulations showed that volume averaging by optical mapping preferentially affected the measurement of \( V_m \) in close proximity to the stimulus electrode and specifically within a space constant (Fig. 2). Importantly, volume-averaged \( V_m \) was best fit by a single exponential (Fig. 2) yielding a \( \lambda_{\text{effective}} \) that correlated well with \( \lambda_{\text{actual}} \) predicted by bidomain theory over a physiological range of space constant values. Differences between theoretical values of \( \lambda_{\text{effective}} \) obtained from simulations of volume-averaged \( V_m \) and \( \lambda_{\text{actual}} \) experimentally measured by optical mapping were most likely due to the choice of parameters for the simulations. Furthermore, the passive bidomain model was used for the numerical simulations, because in general, the myocardial membrane is active, but for the weak, subthreshold stimuli used in this study, the passive model should provide a good first approximation. Tissue has unequal anisotropy ratios, and all the well-known features of virtual anodes and the “dog-bone” transmembrane potential distribution are present in the calculation (27) and the experiment (2) (Fig. 4). However, they are negligibly small for the weak stimuli considered in this study. The tissue surfaces are assumed to be insulated. When fibers approach an insulated boundary, a transmembrane potential can be induced (19). These effects occur in our model but are generally small for weak stimuli. In summary, these simulations indicate that \( V_m \) decay in space due to a subthreshold stimulus obtained by optical mapping should be fit to a single exponential to more accurately reflect changes in cell-to-cell coupling.

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measured in canine Purkinje (1.9 mm) and trabecular (1.0 mm) preparations (29, 30), providing further validation for our approach to estimating intercellular coupling in multicellular preparations. Interestingly, this is the first report to our knowledge demonstrating that coupling transverse to epicardial fibers (within a layer) was comparable to coupling between myocardial layers (Figs. 4 and 5).

A major advantage of our experimental approach for estimating \( \lambda \) is the ability to simultaneously assess intercellular coupling from multiple locations across the ventricular wall. Serial measurements of \( \lambda_{TM} \) within the subepicardium were similar, indicating that the extent to which cells are coupled within a muscle layer is fairly homogeneous. In sharp contrast, there was considerable heterogeneity of \( \lambda_{TM} \) between muscle layers that depended on a muscle layer’s proximity to the epicardial surface. Specifically, \( \lambda_{TM} \) in subepicardium was significantly less (by 14 ± 2%, Fig. 5) than \( \lambda_{TM} \) in deeper transmural muscle layers, consistent with previous findings of localized increased transmural resistivity in subepicardium (32).

One of the major goals of this study was to rule out the potential contributions of rotational anisotropy to the development of heterogeneous transmural coupling. The computer simulations in this study demonstrated that the maximal effect of rotational anisotropy on the measurement of \( \lambda_{TM} \) was on the order of only 0.31% (Fig. 6). In contrast, we measured significantly greater changes in \( \lambda_{TM} \) across the ventricular wall, indicating that reduced coupling at the epicardial midmyocardial interface cannot be explained by rotational anisotropy. Given recent findings of selective underexpression of the principle ventricular gap junction protein (Cx43) in subepicardial layers (17, 18, 31), it is most likely that heterogeneities of coupling across the ventricular wall are due to heterogeneous gap junction distribution and not rotational anisotropy. Importantly, this report establishes the theoretical and experimental basis for measuring intercellular coupling between muscle layers spanning the ventricular wall, which will be important for elucidating the functional effects of reduced gap junction expression in diseases such as ischemia and heart failure.

**Limitations**

The effective intercellular resistance of cardiac tissue primarily arises from two sources: the resistance of the myoplasm and the intercellular junctions. We attribute changes in the effective space constant tojunctional resistance changes, but in
principle, the myoplasmic resistance also could be responsible for part of these effects. We previously demonstrated that cell size is not significantly different between transmural muscle layers. We cannot rule out the possibility that there were regional differences in extracellular resistances. In addition, the values of $g_{IL}$ and $g_{IT}$ were reduced to better model experimental results.

Furthermore, optical mapping cannot measure the absolute amplitude of action potentials without a calibrated ratio system. Discrepancies between findings in this study and previous studies could be due to differing normalization methods.

The canine wedge preparation offers the unique opportunity to optically measure electrical activity from deep within the myocardium. However, the method of exposing the transmural surface for optical mapping could contribute to heretofore uncharacterized electrophysiological changes from normally coupled ventricular myocardium, whether due to boundary conditions or damage.

Unfortunately, experimental systems are not available to directly assess the effects of rotational anisotropy by varying fiber angle in a controlled fashion. Perhaps tissue engineering approaches will permit this in the future. Consequently, we employed computer modeling that allowed us to vary fiber angle with precision while maintaining all other relevant parameters constant. Also, we would emphasize that the effective space constant measured by optical imaging should not be considered mathematically or theoretically equivalent to the actual cardiac space constant as originally defined from one-dimensional muscle strips (29). However, the results of a previous study (2) and the present study do strongly support the notion that the effective space constant is an index of intercellular coupling that can be derived from intact two- and three-dimensional tissues.

REFERENCES

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GRANTS


