Experimental and theoretical ventricular electrograms and their relation to electrophysiological gradients in the adult rat heart

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The availability of many different genetically altered mouse models of known defects in the human cardiovascular system has created a strong demand for a more quantitative understanding of murine cardiovascular physiology (8, 15, 25, 36, 41–43, 57, 58). In addition, the recent completion of the rat genome (22) and improvements in methods for genetically modifying rat progeny (1) have provided a need for improved cardiovascular measurements in the rat. Within the past five years, significant advances have been made in adapting existing electrophysiological recording techniques for application to the mouse and rat heart. Important examples include recordings and analyses of the electrocardiogram (ECG) (4, 13, 18, 37, 61) and monophasic action potential (MAP) recordings (18, 32, 34).

However, a quantitative analysis and a mechanistic interpretation of the repolarization phase (or T wave) of the rodent ECG and surface electrograms remain very difficult, in part because of the high heart rate and very brief action potential (AP) duration (APD) in mice and rats compared with those of larger mammals including humans (27, 51, 53, 60). The ventricular APs in both mice and rats lack the distinct plateau phase that is characteristic of the mammalian ventricular AP. Instead, ventricular APs in mouse and rat have a triangular shape due to a very rapid phase of repolarization that almost immediately follows the initial depolarization. The lack of a plateau phase between depolarization and repolarization results in the absence of an isoelectric S-T segment, which separates the QRS complex from the T wave in the ECG of larger mammals. In the lead I ECG of the adult rat or mouse, the QRS complex is followed immediately by a positive deflection that is generated mainly by what in larger mammals would be termed early repolarization. Late or final repolarization of the rat or mouse AP gives rise to a smaller, longer-lasting, and usually negative deflection of the ECG in distinction to the positive T wave of the mammalian ECG. In this article, we have used the notation Tp for the early, positive deflection and Tn for the later, negative deflection to clearly identify these two separated deflections related to repolarization in the rat or mouse ECG.

Recently, the repolarization phase of the adult mouse ECG, and in particular its relationship to repolarization of the AP as measured by the MAP, has been the focus of a number of studies (13, 18, 27). Danik et al. (18) examined the relationship between MAP duration [at 50% (APD50) or 90% (APD90) repolarization] and the Q-T interval of the surface ECG. Their recordings revealed only a very weak correlation between any of these parameters; accordingly, they concluded that an accurate measurement of the repolarization of the murine ventricle could not be obtained from surface ECG recordings. In contrast, the study of Liu et al. (34), which was based on measurements of the epicardial and endocardial MAPs and included comparison to simultaneous recordings of limb lead ECGs, suggested that there may be a close correlation between the end of the T wave and the onset of the short plateau phase of the epicardial MAP. Liu et al. (34), in fact, concluded that the Tp of the ECG corresponded to the J wave of the ECG in larger mammals. Interestingly, in the Liu et al. (34) study, the MAP recordings exhibited a short but distinct plateau phase. The similarity between the J wave in mammals and early repolarization had been pointed out previously with respect to the rat ventricular AP and ECG by Gussak et al. (27). In agreement with Danik et al. (18), Liu et al. (34) concluded that the slow final phase of repolarization could not be detected in the adult mouse ECG.
A very significant contribution to the present understanding of the J and T wave of the mammalian ECG has been made by Antzelevitch and colleagues (26, 65, 66). They have developed a novel preparation consisting of a perfused “wedge” of the canine left ventricle (LV) and published extensive data based on microelectrode impalements and simultaneous transmural electrogram recordings. Their findings relate both the J and T waves of the ECG to the transmural gradient of AP waveforms and heterogeneous expression of underlying K⁺ and Ca²⁺ currents. Based, in part, on these findings, Gima and Rudy (23) developed a one-dimensional mathematical model to simulate many of the important features of the electrogram of this transmural wedge preparation. Recently, Bondarenko and Rasmussen (5) have also developed a similar one-dimensional mathematical model for the mouse (6) that reproduced in silico important mechanisms related to cardiac arrhythmia, such as AP alternans and Ca²⁺ concentration transients. In that work, the computer simulations have also suggested that mouse heart should be able to sustain substantial molecularly based heterogeneity of repolarization.

The present study extends the Gima and Rudy (23) work by developing a 2-D model of a significant fraction of the LV of the adult rat heart. Recent studies suggest that T-wave intervals do not correlate with a transmural dispersion of repolarization but rather with a whole heart dispersion of repolarization (47, 63). Accordingly, our computational model contributes to the understanding of how nontransmural gradients of repolarization, in particular, apex-to-base gradients, are related to the T wave of the rat ECG.

Many of the most important parameters of our model are based on our experimental recordings using voltage-sensitive dye methods (3, 21, 38, 44-46) of apex-to-base conduction times and gradients in epicardial APD. Optical data were combined with recordings of the extracellular electrogram and computations of the analogous signals in the extracellular space. We have focused on the rat heart because detailed models of the cellular electrophysiology of ventricular myocytes from the endocardium and epicardium are available (49) and because the Langendorff-perfused rat heart is used routinely for voltage-sensitive dye recordings in our laboratory (46). The availability of the rat genome and the very common use of the adult rat in Safety Pharmacology provided additional motivations for this study.

**METHODS**

**Experimental Preparation**

The experimental protocol used in the study was reviewed and approved by the University of Calgary Health Sciences Animal Care Committee to ensure that all experiments followed the guidelines of the Canadian Council for Animal Care. Rat hearts were isolated using a procedure similar to one published previously (46). Male Sprague-Dawley rats (250–300 g) were injected with 300 units of heparin (ip) 15 min before the heart was isolated. The animals were anesthetized with pentobarbital sodium and euthanized by cervical dislocation, after which the heart was immediately excised and placed in ice-cold Krebs-Henseleit solution. Following the removal of extraneous tissues, the aorta was cannulated and perfusion with Krebs-Henseleit solution (37°C) was started at a constant flow rate of 13 ml/min. The Krebs-Henseleit buffer consisted of (in mM) 118.0 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.0 CaCl₂, 25.0 NaHCO₃, and 11.1 glucose. It was bubbled with 95% O₂-5% CO₂ in a 37°C water bath for a pH of 7.4. This solution passed through a glass heating coil (Radnoti Glass Technology, Monrovia, CA) immediately before entering the aorta. The heart was immersed in solution in a water-jacketed chamber throughout these recordings. The heating coil and water-jacketed chamber were warmed using a thermocirculator (Harvard Apparatus, Saint Laurent, QC, Canada). The temperature in the bath near the heart was monitored using a thermocouple device (Harvard Apparatus) and recorded throughout each experiment. With this setup, the heart temperature could be controlled to within 1°C of the desired recording temperature (37°C). 4-Aminopyridine (4-AP) was prepared as a 1 M stock solution in distilled water. The pH of the 4-AP stock solution was adjusted to 7 by titration with HCl.

**Optical Mapping**

After perfusion was begun and the pacing and electrogram electrodes were attached, the heart was immersed in the water-jacketed recording chamber and maintained at 37°C. A 30-min stabilization period followed, during which the pressure and electrogram were monitored and recorded. The solution was then switched to one containing 1 μM 4-([2-(di-n-butylamino)-6-naphthyl]vinyl)pyridinium (di-4-ANEPPS; Molecular Probes, Portland, OR) for a period of 5 min. Di-4-ANEPPS was prepared as an 10 nM stock solution in DMSO (stored frozen) and added to the standard Krebs-Henseleit solution (0.01% DMSO in the final solution). After the dye-loading procedure had been completed, the heart was perfused with the standard Krebs-Henseleit solution for the remainder of the experiment unless otherwise noted. To reduce the influence of motion artifacts on our recordings, the heart was positioned and stabilized in the chamber using adjustable supports on either side. In addition, for short periods of time (~10 s) during each recording, the heart was gently pressed against the front glass of the chamber using a remotely operated paddle behind the heart to immobilize it and to obtain a relatively flat surface for imaging. The motion blocker cytochalasin D (3 μM; Sigma-Aldrich, Oakville, ON, Canada) was added to the perfusate to reduce the contractile force sufficiently that APDs could be measured reliably (29). The imaging system has been described in detail elsewhere (46).

As explained in the next section, the optical maps were processed to produce activation and APD maps of the epicardial surface. Recordings of the activation sequence were carried out in sinus rhythm. However, recordings aimed at measuring APDs were carried out using pacing from the base to ensure that the rate of activation was consistent across preparations.

**Data Processing**

Data were processed off-line using software developed in the IDL development environment (Research Systems, Boulder, CO) with external routines written in C++ (Visual C++, Microsoft, Redmond, WA) for the most computationally demanding tasks. The data recorded for each individual pixel were processed as follows: 1) the background fluorescence level was subtracted from the signal, 2) any linear trend in the data was removed, and 3) the sign of the data was changed so that a depolarization corresponded to a positive change in the signal. Areas of the field of view not covering the preparation were manually masked and excluded from further analysis. The activation time for each pixel was detected as the time of the maximum rate of rise of the fluorescence signal. Activation maps were computed for individual cycles with activation times referenced to the stimulus pulse (paced rhythms) or the R (or S) wave of the electrogram (sinus rhythm). Signal-averaged activation maps were then computed by averaging the activation times for individual pixels over all cycles. The activation maps used in this study are signal averaged over 5 s of recording (20–25 cycles).

Repolarization times were detected as the time of crossing a level corresponding to 80% repolarization. This detection was done using lowpass filtered (5-point moving average smoothing) and signal-
Langendorff-perfused rat heart preparations were used for electrogram recordings. The hearts were immersed in a perfusing solution. Our simulations are based on the bidomain equations to account for both the intracellular and extracellular domains of this cardiac tissue. The functional coupling of these two domains is accomplished using nonlinear sets of equations that describe the transmembrane ionic currents, which are expressed in the sarcolemma of the rat ventricular myocyte as described in detail by Pandit et al. (49). In fact, two distinct ventricular myocyte models were used in these calculations: the first depicts myocytes within the epicardium, and the second simulates electrophysiological processes in endocardial myocytes. The initial conditions used were those obtained after pacing the myocyte models at 1 Hz until steady state was reached.

The numerical solution of the large nonlinear partial differential system yields spatial distributions and temporal characteristics of the extracellular potential (V_e), intracellular potential (V_i), and transmembrane potential. All bidomain parameters were based on those reported in previous experimental work (39). A three-dimensional (3-D) orthotropic conductivity tensor was used to replicate the laminar fiber structure of the heart: higher conduction velocity occurs along fibers, medium conduction velocity across fibers in the same sheet, and lowest conduction velocity is across sheets in the tissue. The cardiac tissue conductivity values from the literature (39) have been uniformly rescaled to match the reported apex-to-base (70 cm/s) and transmural conduction velocities (45 cm/s) in the mammalian ventricle. The bath conductivity was set to 20 mS/cm. The capacitance per unit area and the surface area-to-volume ratio are set to 1 μF/cm² and 2,000/cm², respectively. Table 1 summarizes the parameters used for the bidomain equations.

The interface between the cardiac tissue and the bath is modeled as described previously (33). All the other boundaries are assumed to be electrically insulated. The spatial and temporal discretization steps of the numerical model are set to 50 μm and 5 μs, respectively. All simulations were carried out for a minimum of 500 ms after a single current stimulus was introduced at a selected endocardial site. The fiber-laminar structure of the heart can be represented by a local tensor model of a passive and isotropic conductor that interfaces with cardiac tissue, as to capture the experimental preparations where the hearts were immersed in a perfusing solution.

Electrogram Recordings

Langendorff-perfused rat heart preparations used for electrogram recordings were prepared in the same way as for optical mapping, except that no dye (di-4-ANEPPS) or motion blocker (cytochalasin D) was added to the perfusate. Electrograms were recorded using Ag-AgCl electrodes in the form of small loops sutured onto the surface of the heart for epicardial recordings. Endocardial recordings were obtained using an Ag-AgCl “hook” electrode (Teflon insulated, except at the tip), which was inserted through the wall using a needle and then pulled back to make contact with the endocardial surface. Figure 1A shows the electrode arrangement. Note that recordings were made with the heart intact and in sinus rhythm. The preparation shown in Fig. 1A has been cut open after the completion of the study to examine the position of the endocardial electrode. Electrograms were recorded using an alternating current-coupled differential amplifier (model 1700, A-M Systems, Carlsborg, WA) with a highpass cutoff of 0.1 Hz. Signals were lowpass filtered at 500 Hz and acquired at the same sampling rate as the optical mapping data (950 Hz). Electrogram signals shown in this article were signal averaged over 20 s of recordings.

Mathematical Simulations

We have developed a mathematical model for the purpose of simulating electrophysiological phenomena arising from a segment of rat LV that is assumed to be in a perfusion medium or bath (a passive and isotropic conductor). The size of this ventricular tissue was chosen to be ~4 mm (endo- to epicardium) by 12 mm (apex to base). As shown in Fig. 1B, both endocardium and epicardium surfaces interface with a 4 × 12-mm bath, yielding an overall tissue-bath dimension of 12 × 12 mm. The mathematical model includes the formulation of a passive and isotropic conductor that interfaces with cardiac tissue, as to capture the experimental preparations where the hearts were immersed in a perfusing solution.

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In our mathematical model, for every anatomical point on the ventricular wall, the corresponding fiber and sheet angles were used to compute the 2-D projection of the 3-D conductivity tensor of the bidomain mathematical formulation.

Signals that would be sensed by transmural electrogram leads were calculated by taking the difference of the simulated $\phi_e$ at the endocardial and epicardial boundary points. This is illustrated in Fig. 1. Lead I equals $\phi_e$ at the epicardial base minus $\phi_e$ at the endocardium. APD_{90} was calculated for every discrete point of the LV tissue, using the difference between the activation time and the repolarization time to 90% relaxation. Activation times were obtained as the time of the maximum rate of rise of the simulated transmembrane potentials. Repolarization times were calculated as the time of crossing a level corresponding to 90% of repolarization to the transmembrane resting potential.

RESULTS

Optical Mapping of Activation and Measurement of APD

Optical mapping experiments were carried out on seven Langendorff-perfused rat hearts. Motion artifacts were minimized by using cytochalasin D (3 μM) in combination with a mechanical restraint of the preparation. Isochronal activation maps were constructed from data collected in sinus rhythm, as described in METHODS. Figure 2A shows a typical pattern of the activation sequence. Note that the first detectable activation (epicardial breakthrough) occurred at a site near the apex. The activation wave was then moved approximately uniformly toward the base of the LV, and activation was completed in 2 to 3 ms.

APDs were measured at sites across the epicardium from these recordings. To ensure that the heart rate was consistent across all preparations (to avoid any influence of rate dependence of the APD), APDs were measured from recordings made during fixed rate pacing. The pacing electrode was positioned at the base of the LV of each preparation. The average APDs at the base, midregion, and apex of the LV were measured. Figure 2B shows a summary of these results. Note

### Table 1. Parameters of the bidomain model

<table>
<thead>
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<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Capacitance per unit area, μF/cm²</td>
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</tr>
<tr>
<td>Surface area-to-volume ratio, cm²</td>
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</tr>
<tr>
<td>Bath conductivity, mS/cm</td>
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<td>Intracellular conductivity along fibers, mS/cm</td>
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</tr>
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<td>2.1</td>
</tr>
<tr>
<td>Bath conductivity, mS/cm</td>
<td>20</td>
</tr>
</tbody>
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Parameter values of the bidomain equations.
that there is a distinct APD gradient in the rat LV: the epicardial APD is shorter at the apex than at the base (Fig. 2C).

Recording of Surface Electrograms

Surface electrograms were recorded from five Langendorff-perfused rat heart preparations. Each of these hearts was prepared in the same way as for the optical mapping studies. However, no dye (di-4-ANEPPS) was added, and the perfusate did not contain cytochalasin D. The recording electrodes were positioned with one epicardial electrode located near the base of the LV and an endocardial electrode located near the base of the LV. The experimental results for lead I configuration were consistent across all five preparations, as shown in Fig. 3A.

To verify that the QT interval in these recordings reflected the APDs in this preparation, additional measurements were carried out in three hearts in the presence of 5 mM 4-AP. This large concentration of 4-AP produces a substantial block of the transient outward K+ current (Ito) and therefore a large prolongation of the AP. As seen in Fig. 3B, 4-AP application prolonged the QT interval and began to separate the Tp wave longation of the AP. As seen in Fig. 3B, 4-AP application even resulted in a brief isoelectric ST segment, similar to that observed in larger mammals. These results clearly show that the QT interval (Q-to-Tp interval) in these recordings is directly related to APD.

Modeling of the LV Wedge Preparation

Requirements for a positive T wave. Recorded LV electrograms and the average distributions of the activation sequences and APD data from the optical maps obtained on the epicardial surface were used to guide our computer simulations. However, some other important information necessary to develop a realistic computational LV wedge is not available in the literature. In particular, although it is known that the AP shapes of endo- and epicardial myocytes in the rat heart are different (49), little information is available about the relative amount of endo- and epicardial myocytes across the ventricular wall. Accordingly, we made use of the information provided by the experiments as a basis for a plausible computational model of the LV wedge preparation from the adult rat. After validating the basic properties of this model, we used it to quantify the influence of some important variables we did not have control of during the experiments. Our main goals were to evaluate the influence of the distribution of endo- and epicardial myocytes, the transmural activation sequence, the transmural APD distribution, and the Ito distribution.

There is no complete set of experimental data that specifies in required detail the numbers or the relative distributions of the two different types of myocytes (epi- and endocardial) that comprise the rat LV wall. We therefore simulated a number of different fractions of epicardial and endocardial myocytes in our model. Activation was initiated with a current stimulus on the endocardial surface, therefore initiating a strictly transmural AP propagation. Figure 4A illustrates how different ratios of epicardial to endocardial myocytes affect the computed transmural electrogram. A consistent finding was that wedges having a high percentage of epicardial myocytes generated electrograms with positive T waves. Moreover, as the ratio of epicardial to endocardial myocytes decreased progressively, the amplitude of the computed T waves gradually decreased, flattened, and finally reversed polarity to become weakly negative. This dependency of T-wave amplitude (and polarity) on the percentage of epicardial myocytes is mainly due to electrotonic effects and can be better understood by transmembrane potential maps taken at 30 ms and presented in Figs. 4, B–D. As presented by Fig. 4B, with no epicardial myocytes at all, there is a weak repolarization gradient from right to left (i.e., the endocardium is more repolarized than the epicardium). This weak reverse gradient, caused by the propagation delay through the wall, gives rise to the weak negative T wave seen in Fig. 3. Lead I electrogram records from 5 Langendorff-perfused rat hearts. Note that during repolarization, lead I shows a prominent early positive T-wave deflection (Tp) followed by a smaller negative T-wave deflection (Tn). These recordings were obtained in separate studies from the optical mapping experiments. These preparations were not perfused with either 4-[(2R)-2-(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS) or cytochalasin D (see METHODS). A: records under control conditions. B: lead I electrogram records of 3 rat hearts after perfusion with 4-aminopyridine (4-AP, 5 mM). The Tp shifts to the right, i.e., QT interval lengthens as expected in these hearts. The amplitude of the Tp wave increases for 2 rat hearts and decreases for the third one. Note that a brief isoelectric ST segment appears in the record for rat 3 as the repolarization wave separates from the QRS complex.
all subsequent simulation results that are presented in this section are based on computational wedges with a transmural phenotype distribution of 75% of endocardial and 25% of epicardial myocytes.

**Influence of the activation sequence on the T-wave morphology.** The activation sequence obtained from the epicardial surface (Fig. 2) started near the apex and proceeded to the base of the LV over a total of 2 to 3 ms. However, these experiments provide no information about the transmural activation sequence. We therefore used our model to evaluate the influence of the activation sequence on the morphology of the T-simulated wave. Three different activation sequences were considered: one in which activation proceeds entirely from apex to base with no transmural component (act 1), one in which activation proceeds entirely from endocardium to epicardium with no apex-base component (act 2), and one in which activation proceeds in both directions with the earliest epicardial breakthrough at the apex (act 3). As illustrated in Fig. 5A, these activation sequences were produced by simultaneously stimulating either a small area at the apex across the entire wall (act 1), a thin endocardial layer from apex to base (act 2), or an endocardial layer that reached further through the wall near the apex (act 3). Figure 5B shows the simulated epicardial activation sequence resulting from stimulating the model according to act 3. Initiating the computer simulation with the act 3 stimulus resulted in a simulated epicardial activation sequence that is similar to experimental observations, i.e., epicardial activation starts at the apex and is complete in 2 to 3 ms. (see Fig. 2). The other two stimulation protocols resulted in highly unrealistic epicardial activation sequences: epicardial activation in response to act 1 proceeded from apex to base over a time period of 20 ms, whereas epicardial breakthrough in response to act 2 occurred simultaneously across the entire preparation. Figure 5C shows how the different activation sequences affect the transmural electrogram. Note that there is very little difference between the electrograms resulting from the strictly transmural activation (act 2) and the more realistic activation sequence obtained with the act 3 stimulation protocol. Thus, as long as activation proceeds mainly in the transmural direction, our model produces a relatively consistent T-wave morphology. Based on this observation, we made no further attempts to exactly match experimental data for epicardial activation. In the simulations that follow, act 3 was adopted as the standard stimulation protocol for activating our model.

**Adjustment of Q-Tp interval duration.** Xia et al. (63) have shown experimentally that the peak of the T wave and the end of the T wave coincide with the shortest end of repolarization (EOR) on the epicardial surface and the longest EOR on the endocardial surface, respectively. In our experimentally recorded electrograms, the peak of the Tp wave and the end of the Tn wave occur around 20 and 60 ms after the onset of the QRS complex, respectively. This is not in agreement with the APDs of epicardial and endocardial myocyte models of Pandit et al. (49), which are considerably longer. This discrepancy is primarily due to differences in temperature between the model and experiments. The mathematical formulations of Pandit et al. (49) were based on patch-clamp experiments mainly recorded at 23°C. In contrast, the experimental measurements in this article are based on the Langendorff setup and were made at 37°C. In this study we have chosen to adjust our model to

**Figure 4.** A: simulated transmural electrograms (lead I) for in silico rat LV wedges for systematic changes in the relative amounts of endocardial and epicardial (epi) myocytes. Note that wedges having a small percentage of epicardial myocytes generate electrograms with low-amplitude T waves. As the relative amount of epicardial myocytes increases, the T-wave amplitude increases. B: transmembrane potential map taken at 30 ms for the case of a model consisting exclusively of endocardial myocytes. Note the weak reverse gradient of repolarization, with the epicardium less repolarized than the endocardium (see text). C: transmembrane potential map taken at 30 ms for the case of 25% of epicardial myocytes. Note the normal gradient of repolarization, with the epicardium more repolarized than the endocardium. D: transmembrane potential map taken at 30 ms for the case of 50% of epicardial myocyte. Note that the transmural gradient of transmembrane potential during repolarization is very substantial for this phenotype distribution.

in Fig. 4A (0% epi). On the other hand, in a case with 50% of epicardial myocytes (Fig. 4D), the two halves of the tissue have significantly different repolarization time courses, which give rise to a strong repolarization gradient from left to right (i.e., epicardium is more repolarized than endocardium) and to a substantial positive T-wave amplitude (Fig. 4A). Thus propagation delays through the wall can, in principle, result in a negative T-wave deflection, but not in the presence of a significant endo-epi APD gradient. Since the existence of the endocardial-epicardial gradient is well established (49), the negative deflection in the biphasic rat T wave cannot be explained by transmural propagation delays.

The ratio of maximum QRS amplitude to maximum T-wave amplitude in the experimentally recorded transmural electrograms (Fig. 3) was around 10%. To simulate this waveform characteristic, our model required that at least 25% of the transmural wall consisted of epicardial myocytes. Therefore,
match the APD in the experiments by adjusting the maximal conductivity of $I_{to}$ of the Pandit et al. (49) model. Our rationale for this choice is presented in DISCUSSION.

Figure 6 presents simulated transmural electrograms (lead I) following homogeneous changes in the maximum conductance of the $I_{to}$ current in both endocardial and epicardial regions. The solid line shows the simulated results under control conditions with $I_{to}$ as reported in the original Pandit et al. (49) article and assuming that 25% of the transmural wall consists of epicardial myocytes. Note that following an increase of $I_{to}$, the QT interval shortens. However, the dependence of the T-wave amplitude on the density of $I_{to}$ is more complex. Following small changes in $I_{to}$ density, the amplitude of the T wave increases. However, further increasing the amount of $I_{to}$ beyond 150% results in a reduced T-wave amplitude. An increase in $I_{to}$ density of approximately 50% compared with the original Pandit model parameters is necessary to reproduce the mean of the experimentally recorded Q-Tp interval of 19 ms. This homogeneous 50% increase in $I_{to}$ density was used as a baseline for all subsequent simulations presented in this article. However, it is important to note that this simulated waveform does not reproduce the biphasic (Tp/Tn) shape of the repolarization waveform in the experimental records (Fig. 3).

**Requirements for a biphasic T wave.** The optical mapping data (Fig. 2) show that the APDs in adult rat LV epicardium are heterogeneously distributed, with the APD being longest near the base of the LV. This is in agreement with the recent findings of Brunet et al. (9) in the LV of mouse hearts. In this preparation, rapidly inactivating transient outward K$^+$ current densities were found to be significantly higher in apex than in base myocytes, whereas rapidly activating, slowly inactivating outward K$^+$ current and steady-state outward K$^+$ current ($I_{ss}$) densities in the apex and base cells were similar. In an attempt to simulate this pattern of heterogeneous distributions of APD, the following simplified approach was used. The maximum conductance of $I_{to}$ was reduced in the basal half of the epicardial layer (see Fig. 7A) to increase epicardial APD in this area. Figure 7, B and C, shows the resulting transmural electrograms

**Fig. 5.** Simulations illustrating the effects of initiating action potential propagation (activation) from different endocardial regions. A: areas of the model preparation stimulated to produce the 3 different activation sequences considered (see text). B: epicardial activation sequences resulting from the act 3 activation sequence. Note that activation proceeds from apex to base over ~2 ms, consistent with our experimental observations (Fig. 2). C: lead I electrograms resulting from the 3 activation sequences considered. Note that the 2 transmural activation sequences (acts 2 and 3) produce very similar repolarization waves.
(lead I) and epicardial APD gradients for selected degrees of \( I_{to} \) density reduction in this area. A reduction of \( I_{to} \) density in the basal epicardium region decreases the positive T-wave amplitude and results in a biphasic T wave.

In addition, this reduction of \( I_{to} \) in the basal epicardium region results in an apex-to-base APD gradient that is similar to that observed experimentally. The average APD\(_{80} \) distribution obtained from our optical mapping data is also shown in Fig. 7C. Note the different scales for the simulations and experimental values. The calculated APD\(_{80} \) values from the optical mapping are longer than those of the simulations. This is a known artifact of using cytochalasin D to reduce motion artifacts (44). At 55% \( I_{to} \) reduction in the basal epicardium region, the relative APD gradient in the simulation is similar to the relative average APD gradient observed in the experiments (Fig. 7C). Furthermore, the simulation generates a biphasic repolarization wave (Fig. 7B), which is characteristic of the lead I ECG from adult mice and rats (27). This biphasic T wave is characterized by a \( T_p \), followed by a \( T_n \).

Gima and Rudy (23) have demonstrated that the T-wave/repolarization complex in mammalian ventricles can be strongly modulated by interactions between two or three different transmembrane ionic currents, particularly when they are expressed in a spatially heterogeneous fashion across the transmural aspect of the LV. In part, this is due to distinct types of myocytes within the ventricular wall of mammals: the so-called M cell, as well as the endocardial and epicardial myocytes. At present, there is little information concerning the presence or functional role of M cells in the rat ventricle. However, our results consistently demonstrate significant apex-to-base APD gradients on the epicardium of the rat LV. Thus the functional interactions between these regions of the LV were explored in our simulations. Figure 8 illustrates how the transmural gradient caused by the epicardial region having short APD (near the apex) contributes significantly to the generation of the \( T_p \) of the simulated ventricular electrogram during early repolarization. Moreover, Fig. 8 also demonstrates that the transmural gradient caused by epicardial regions having longer APD (near the base) contributes to the \( T_n \). In summary, therefore, this combination of two different electrophysiological “gradients” (transmural and apex to base) can give rise to the observed biphasic rat ventricular repolarization wave or the \( T_p + T_n \) complex.

Parameter sensitivity analysis. The computational model developed here has nearly one hundred parameters. Some of them are related to the tissue model, whereas others are part of the myocyte mathematical models of the three different regions identified in this work: endocardium, epicardium base, and epicardium apex. It is well known that with enough free parameters, one can have a model fit almost any data. For this reason we have chosen to adjust very few parameters and have taken all the other values from the previous works where the original models were proposed and validated. The tissue model

Fig. 7. Transmural electrograms (lead I) and APD gradient on the epicardium generated by simulated rat LV wedges following changes in the density of \( I_{to} \) in epicardial myocytes near the LV base. \( I_{to} \) was reduced in the basal epicardium (region marked “Epicardium Base”; A) to reproduce the apex-base gradient in APD observed with optical mapping and the apex-base gradient in \( I_{to} \) density reported in the literature. B: transmural electrograms obtained from these simulations. Reduction of \( I_{to} \) in the basal region decreased the \( T_p \) wave amplitude and results in a biphasic repolarization wave. C: epicardial APD\(_{80} \) distributions calculated from these simulations. Reduction of \( I_{to} \) in the basal region increased the apex-to-base APD gradient. The average APD\(_{80} \) values obtained from optical mapping data are also shown for comparison. (Note the different scales for the simulations and experimental values.) The experimentally measured APD\(_{80} \) values are longer than those of the simulations primarily due to the use of cytochalasin D (see text). At 45% \( I_{to} \) reduction in the basal epicardium, the relative APD gradient in the simulation is similar to the average relative APD gradient observed experimentally. In addition, under these conditions, our model generates a biphasic T wave similar to our experimental records.
was based on the bidomain formulation with parameters (see Table 1) taken from Muzikant and Henriquez (39), and conductivities were adjusted to reproduce the conduction velocities as reported previously (34). The relative size of each region that composes the computational wedge was adjusted with one single parameter, the ratio of epicardial to endocardial myocytes. As presented by Fig. 7A, the final model has 25% of epicardial myocytes. This value was chosen to reproduce the relative T-wave peak to QRS peak obtained in the experiments. Propagation was initiated by stimulating different sites of the tissue. The stimulus site named act 3 in Fig. 5A was chosen since it better reproduced the activation sequence observed in the experiments. Finally, rat ventricular myocytes were modeled as described in detail by Pandit et al. (49). In fact, the original model proposes two distinct ventricular myocyte models: the first depicts myocytes within the epicardium, and the second simulates electrophysiological processes in endocardial myocytes. Two modifications were performed to the original models: we have chosen to increase the maximum conductance of $I_{to}$ by 50% in both epicardial and endocardial models to reproduce the QT interval observed in the experiments, and we generated a third phenotype, epicardium base, by reducing $I_{to}$ expression to 55% of that of the epicardium apex. With the three phenotypes described in Table 2, we were able to reproduce the apex-to-base APD gradient observed in the experiments and the $I_{to}$ density gradient reported previously (9).

The parameter values of the final model may not be optimal in the sense that different sets of parameters may exist that better reproduce the experiments. Nevertheless, a more important question is how sensitive the final model and results are to each of the adjusted parameters. Figure 9A presents the sensitivity of the waveform of the transmural electrogram to the percentage of epicardial myocytes. The T-wave amplitude decreases with small percentages of epicardial myocytes. Nevertheless, even with only 10% of epicardial myocytes, the T wave is clearly biphasic and not flat as in the case of Fig. 4.

Figure 9B presents the sensitivity of the transmural electrogram to the activation sequence. As before there is very little difference between the electrograms resulting from the strictly transmural activation (act 2) and the more realistic activation sequence obtained with the act 3 stimulation protocol (see Fig. 5). Nevertheless, T waves are clearly biphasic for all three activation sequences.

Figure 9C presents the sensitivity of the waveform of the transmural electrogram to the adjustment performed on $I_{to}$

![Diagram](image-url)
maximum conductance as a simplified mechanism to shorten the QT interval duration to reproduce the values observed by the experiments. As before, following an increase of \( I_o \) conductance, QT interval shortens. Nevertheless, T waves are still biphasic for any scaling factor introduced in \( I_o \) maximum conductance. In particular, this is also true when \( I_o \) is not altered (control case in the figure), i.e., when the values for \( I_o \) maximum conductance are the original ones presented previously (49).

The sensitivity analysis presented by Fig. 9 suggests that from all the parameters we have chosen to adjust, only one really contributes to the biphasic waveform of the simulated T wave: the \( I_o \), maximum conductance of the epicardium base.

Figure 7B presents the sensitivity of the waveform of the transmural electrogram to the adjustment performed on \( I_o \) in the region we called the epicardium base. This is the only parameter that, if altered, can eliminate the biphasic nature of the T wave of the final computational model. Therefore, the parameter sensitivity analysis performed here confirms that the apex-to-base repolarization gradient is the key element for the generation of a biphasic T wave in our computational model.

**DISCUSSION**

**Activation and Repolarization of the Rat LV**

The breakthrough, or first activation on the epicardium of the rat LV free wall, occurs at a site (or sites) near the apex, as has been reported previously (46). The activation wavefront then spreads toward the base of the LV. The mathematical model that we have developed represents a significant fraction of the LV free wall. To begin to investigate the basis of the LV electrogram, we have adjusted the parameters in this model so that the sequence of epicardial activation matches our voltage-sensitive dye results. It is interesting to note, however, that although a clear pattern of apex-to-base activation is observed on the epicardial surface, the spread of the activation is primarily transmural, i.e., from endocardial to epicardial, as suggested by the simulation results presented in Fig. 5.

Optical mapping measurements in the guinea pig (31) and mouse (3) LV epicardium have shown that the APD in these species exhibits a distinct gradient: it is shortest at the apex and longest at the base. A different (sometimes opposite) APD gradient has been reported in the LV epicardium of large mammals (11, 28, 40, 59, 67). Figure 2B, which consists of an optical map of the rat LV epicardium, reveals a somewhat similar pattern of APD heterogeneity to those found in guinea pig and mouse. The APDs observed in our optical mapping recordings are significantly longer than those used in our model. Note, however, that the motion blocker cytochalasin D prolongs the AP in the mouse and rat ventricles (3, 44). In addition, optical mapping methods are known to reduce the rate of rise of the AP due to spatial averaging (24). For this reason the very sharp peak of the rat ventricular AP is blunted somewhat in optical records. Any chosen or defined repolarization level (percentage of the AP peak amplitude) of an optical AP will therefore correspond to a less depolarized level of the “true” AP (e.g., a microelectrode recording or, in this case, the model-generated AP). Since we could not fit the absolute APD values obtained from the simulations to the observed APD values of the optical maps, we have focused on fitting the relative apex-to-base gradient of the APD values (the APD difference from base to apex), as shown in Fig. 7. This ensures that our simulations exhibit an apex-to-base APD gradient similar to that derived from our mapping data.

**Distribution of Endocardial and Epicardial Myocytes**

A previous one-dimensional model of the dog ventricle (23) that was developed to mathematically reconstruct important features of the mammalian ECG assigned 35% of the transmural ventricular volume to epicardial myocytes. Measurements obtained from human ventricles (20) have suggested that epicardial cells constitute only \( \sim 10\% \) of the transmural thickness. In rat ventricle, the volume or percentage of the transmural
wall, which has the biophysical characteristics of epicardial myocytes (as opposed to endocardial myocytes), has not been determined. In our model, the average APD distribution was reproduced most accurately by assuming that this epicardial region consisted of \sim 25\% of the transmural thickness. Electrophysiological studies of single myocytes from rodent and mammalian ventricles have consistently identified apex-to-base and epicardial-to-endocardial gradients in the densities of $K^+$ currents (14, 27, 48, 62, 64, 68). Work from the Backx’s laboratory (62) has drawn attention to the heterogeneous distribution of ionic current densities within the LV of the rat heart under control conditions and in the setting of altered hormone metabolism (hypothyroidism) or heart failure (54). The use of only two different phenotypes/models for the murine LV (or 3 phenotypes for the case of larger mammals) may be too simplistic and may not reproduce important features of cardiac electrophysiology. We have found that it is necessary to introduce a third phenotype/model in our computational wedge of the rat LV to reproduce the APD gradients observed in the rat LV epicardium. In particular, we created a third phenotype model for the epicardial region near the base of the LV (see Fig. 7A). In the absence of experimental data regarding differences in ionic current densities between apical and basal epicardium, we have modeled the APD differences between these areas by modifying a single parameter. Myocytes in the epicardial-base region are modeled as having lower density of $I_{\text{to}}$ than myocytes in the epicardial-apex model. As additional experimental data become available, this aspect of our model may have to be refined. The inclusion of this third simple phenotype/model allowed us to reproduce important features observed in the experiments: apex-to-base APD gradients on the rat LV epicardium and electrograms with biphasic T waveforms.

Electrotetric Effects

In formulating our model, the discrete, stepwise APD distribution suggested from isolated myocyte recordings was adopted and used as a basis for a 2-D mathematical model. Therefore, the models presented in this article consider abrupt transitions from a phenotype region to another, i.e., a myocyte in the endocardial region can have as a neighbor an epicardial myocyte. One might expect a model that considers smooth transitions between different phenotype regions to be more realistic. However, our simulations show that a model with abrupt transitions behaves, in terms of APD distribution and electrogram waveforms, like one with smooth transitions. Despite the use of models with abrupt transitions, smooth transmural (Fig. 4) and apex-to-base APD gradients (Fig. 7C) were observed. To further investigate this issue, we have performed simulations (not shown in this study) considering the smooth transitions between phenotypes. The transition was modeled as sigmoidal changes in $I_{\text{to}}$, magnitude over the tissue and considered both transmural and apex-base gradients. The case with gradual transitions yields very similar T waves to the case with abrupt transitions. Therefore, the exact modeling of the transition between endocardial and epicardial, as well as apical and basal, myocyte models is of limited importance.

This phenomenon is due to cell-cell electrotetric interactions. These interactions may reduce APD dispersion and thus prevent the occurrence or maintenance of arrhythmias (10, 52). Our results also agree with recent findings that demonstrate the transmural gradient in LV can be markedly reduced by electrotetic interactions in both human and canine LV (16, 56). In addition, our simulations demonstrate that this marked reduction in transmural APD gradient can have the effect of emphasizing other anatomical and/or electrophysiological gradients. In particular, we have shown that apex-to-base gradients can play an important role in the generation of biphasic T waveforms.

Relationship of the Repolarization Wave of the Rat Transmural Electrogram to Underlying Ionic Mechanisms

The positive repolarization wave. As expected, after the administration of a high dose (5 mM) of 4-AP, the peak of the $T_p$ wave was shifted to the right in all our experiments. However, the amplitude of the T wave increased for two of the rats and decreased for the other one (see Fig. 3). Similar experiments using the same $K^+$ channel blocker have been performed in mouse ventricle by Danik et al. (18). A similar pattern of results was obtained. It should be noted that in the rat ventricle, there is a highly 4-AP-sensitive component of the sustained outward $K^+$ current, denoted $I_{\text{to}}$ in the Pandit model (2). The administration of 5 mM 4-AP would completely block this component of $I_{\text{to}}$ as well as a large fraction of $I_{\text{to}}$ current. However, sensitivity analysis (results not shown) with respect to the partial block of $I_{\text{to}}$ indicated that the T-wave shape in our model is much more sensitive to $I_{\text{to}}$ size than it is to $I_{\text{ss}}$.

We have therefore focused on computations that allowed us to better understand the role of $I_{\text{to}}$ in the repolarization wave. Xia et al. (63) have shown experimentally that the peak of the T wave coincides with the shortest EOR on the epicardial surface. Therefore, the right shift of the T-wave peak agrees with the fact that the blocking of $I_{\text{to}}$ significantly increases the epicardial APD. As in the experiments, $I_{\text{to}}$ blocking invariably shifts the peak of T wave to the right. In addition, depending on the amount of $I_{\text{to}}$ reduction, the T-wave amplitude may increase as well as decrease (see Fig. 6), which is also consistent with our experimental observations (Fig. 3).

The biphasic repolarization wave. The majority of lead I and lead II ECG records from adult rats (17) and mice (18) exhibit a biphasic shape. Following the QRS complex, there is a brief, positive deflection and this is followed by a later and much smaller negative-going component. Our simulations provide new insights into how these two distinct deflections can be related to the transmural and/or apex-to-base gradients of repolarization in the adult rat LV. In brief, the apex-to-base APD gradient can effectively divide the LV wall in two regions that have opposite sequences of repolarization. Specifically, in the region of the LV near the apex, the repolarization sequence can be opposite to that of depolarization. In this region the epicardium repolarizes before the endocardium. This sequence of electrophysiological changes can give rise to the $T_p$, which in both rat and mouse ECGs is often denoted as the T wave but, as noted above, has also been referred to as a J wave (26, 34). At the peak of $T_p$, note that the difference in AP at the apex of the epicardium compared with the endocardium is greater than the AP difference between epicardium at the base and endocardium (see arrows in Fig. 8). In contrast in the LV region near the base, longer APDs reverse the sequence of repolarization. Thus, in the basal region, the endocardium repolarizes...
before the epicardium and this sequence of repolarization can generate a small slow \( T_n \). At the peak of the \( T_n \) wave the AP differences between the epicardium near the base and the endocardium are greater than the corresponding differences between the epicardium at apex and endocardium. Time-dependent or dynamic interactions between these two distinct APD patterns of heterogeneity underlie the biphasic transmural electrograms waves corresponding to repolarization in the rat heart. Figure 8C shows that, in principle, the close similarity between the weighted algebraic sums of these two APD differences (apical epicardium AP – endocardial AP and basal epicardial AP – endocardial AP) can account for the biphase lead I transmural electrogram record obtained from our experimental preparation.

**Interpretation of the rat T wave.** Figure 8 also illustrates why the end of T wave in the rat ventricular electrogram is poorly defined. The APs in all three locations shown in Fig. 8B have very long gradual “tails” of final repolarization, typical of rat APs. As a result, although there are significant voltage gradients in early repolarization (down to about \(-50 \) mV), these gradients are less significant in late repolarization. As our simulations show, this results in large T-wave deflections corresponding to early repolarization but minimal ones corresponding to late repolarization. Our model predicts that the \( T_n \) corresponds roughly to the end of early repolarization at the epicardial apex, whereas the \( T_n \) corresponds to the end of early repolarization at the endocardium and/or epicardial base. The end of the T wave is difficult to define because of its very gradual decline but indicates the onset of slow, final repolarization. In summary, the rat T wave provides useful information about the dispersion of APDs and repolarization during the early repolarization phase (APD\(_{50}\) or APD\(_{60}\)). However, it is difficult to obtain reliable information about final repolarization (APD\(_{90}\)).

**Limitations of this Study**

Although our combination of voltage-sensitive dye mapping and bidomain theory contributes significantly to an improved understanding of the rat ventricular electrogram, we acknowledge that some aspects of our work remain incomplete. For example, we have chosen a 2-D rectangular geometrical configuration of the LV free wall on which to simulate the most important aspects of the electrophysiological phenomena of rat LV. In future work, it will be important to carry out a similar computational analysis based on more realistic 3-D geometry. Second, our mathematical modeling does not take explicit account of either the LV Purkinje fiber system or the possibility that populations of M cells are present in the midmyocardium of LV. Since the emphasis of this study is on repolarization, the omission of the Purkinje conduction system is unlikely to be a significant limitation. Very little information is available about M cells in the rat heart, making it difficult to predict their importance to the electrogram wave shape at this time. The fundamental theoretical basis for this study is the single myocyte models of rat endo- and epicardium developed by Pandit et al. (49, 50). These mathematical formulations were based on patch-clamp experiments mainly recorded at 23°C. In contrast, the experimental measurements in this article are based on the Langendorff setup and were made at 37°C. As a result, the APD of the model does not agree quantitatively with the experimental measurements. This problem is not unique to this particular modeling study, as most computational models of cellular electrophysiology are based on data recorded at reduced temperature. Our approach to compensating for this discrepancy by increasing the maximum conductance parameter for the \( I_{to} \) current by 50% is a simplification, but one for which there is significant experimental basis. For example, Brouillet et al. (7) have shown that an increase in temperature increases the maximum conductance of \( I_{to} \) in mouse LV myocytes and shortens APD. Shimoni and Banno (55) studied \( I_{to} \) current in rabbit ventricular myocytes and found that the amplitude of \( I_{to} \) increased by a factor of 6.14 for a 10°C increase in temperature, compared with only a 2.7-fold increase for L-type Ca\(^{2+}\) current. In addition, Pandit et al. (49) have demonstrated that \( I_{to} \) is the most important ionic current in determining the rat ventricular APD. Taken together, these observations clearly indicate that a substantial increase in \( I_{to} \) amplitude is likely to be the most significant consequence (in terms of APD) of an increase in temperature. However, a detailed analysis would of course also have to take into account changes in other ionic currents, as well as changes in rate constants for ion channel gating.

The results of Shimoni and Banno (55) illustrate the difficulty of compensating for temperature differences in an ionic model, since the temperature sensitivity of different ion channels may vary quite widely. An adjustment of an ionic model to physiological temperature is therefore much more involved than a simple scaling of rate constants and is perhaps best done as part of the original model development process, as has indeed been done in some models in the literature (35). A thorough adjustment of the Pandit model to physiological temperature is thus well beyond the scope of this article and would likely require substantial new patch-clamp data.

It is also worth noting that there is substantial variability in \( I_{to} \) density even in the absence of a change in temperature. Our 50% adjustment of \( I_{to} \) amplitude is in fact well within the 70% range of variability in \( I_{to} \) amplitude observed in the LV of mouse hearts by Brunet et al. (9).

Finally, our simulations and experiments suggested the existence of two different epicardial regions (apex and base) giving rise to opposite transmural repolarization sequences in the ventricular wall. It is possible, however, that other gradients arising from different regions of the myocardium may contribute to the modulation of the rat T wave. Notwithstanding these limitations, our simulations provide an important proof of principle: nontransmural gradients can influence and modulate the repolarization wave of the electrogram.

In summary, a combination of voltage-sensitive dye recordings and mathematical modeling has yielded new insights into the basis for the well-known biphase repolarization waveforms of the rat ventricular electrocardiogram. Electrical recordings of the rat and mouse are frequently used in basic medical research, Safety Pharmacology studies, and in the multidisciplinary studies of the genetic basis of cardiovascular disease. The insights resulting from our experimental and theoretical analysis are of significant interest and potential importance in these endeavors.

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