Research Commentary

Influence of a Perfusing Bath on the Foot of the Cardiac Action Potential

Bradley J. Roth

Abstract—Recently, Spach et al (Circ Res. 1998;83:1144–1164) measured the transmembrane action potential 150 to 200 μm below the tissue surface during longitudinal and transverse propagation. They found that “during longitudinal propagation there was initial slowing of Vn [action potential] foot that resulted in deviations from a simple exponential...” (p 1144). They attributed this behavior to the effects of capillaries on propagation. The purpose of this commentary is to show that the perfusing bath plays an important role in determining the time course of the action potential foot, even when the transmembrane potential is measured 150 μm below the tissue surface. Using numerical simulations based on the bidomain model, we find that the action potential foot for transverse propagation is nearly exponential (τfoot = 314 μs). For longitudinal propagation, the action potential foot is not exponential because of an initial slowing (best-fit τfoot = 483 μs). We conclude that the perfusing bath must be taken into account when interpreting data showing differences in the shape of the action potential foot with propagation direction, even if the transmembrane potential is measured 150 μm below the tissue surface. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2000;86:e19-e22.)

Key Words: bidomain ■ action potential foot ■ perfusing bath ■ anisotropy

In 1981, Spach et al1 observed a smaller maximum rate of rise of the action potential, Vmax, and a larger time constant of the action potential foot, τfoot, during propagation parallel to the myocardial fibers (longitudinal) than during propagation perpendicular to the fibers (transverse). They attributed these differences to the discrete cellular structure of the myocardium. Their research has been cited widely and is often taken as evidence for discontinuous propagation in cardiac tissue.2

Several researchers3–11 have suggested that the observations of Spach et al may be caused by the bath perfusing the tissue rather than the discrete nature of the tissue itself. Recently, Spach et al12 presented additional evidence supporting their earlier data, but instead of measuring the transmembrane potential (Vn) at the tissue surface, as they did in 1981, they measured Vn 150 to 200 μm below the surface to eliminate bath perfusate effects. In their study, they emphasized the time course of the action potential foot. The purpose of this commentary is to model the experiment of Spach et al12 using a numerical simulation and to show that the perfusing bath plays an important role in determining the time course of the action potential foot, even when Vn is measured 150 μm below the tissue surface.

Materials and Methods

The simulation is similar to that described by Pollard et al13: a slab of cardiac tissue is superfused by a conductive bath (Figure 1). The bidomain model13 represents the anisotropic electrical properties of the cardiac tissue. This model is a continuum description that does not take into account the discrete nature of individual myocardial cells. The electrical potential in the isotropic perfusing bath obeys Laplace’s equation. At the interface between the tissue and the bath, the boundary conditions are continuity of the extracellular (bath and interstitial) potential, continuity of the normal component of the extracellular current density, and the vanishing of the normal component of the intracellular current density.14 All other boundaries are sealed.

A planar wavefront propagates in the x direction, and the z direction is perpendicular to the tissue-bath surface (Figure 1). Fibers are aligned in either the x direction (longitudinal propagation) or the y direction (transverse propagation). The tissue parameters are given in the Table. The common scale factor of the 4 bidomain conductivities15 is selected so that the resulting propagation speed of the action potential is typical of that observed in experiments.12

The ionic current through the membrane is described as a passive leak term plus an active sodium channel12,16 The sodium channel gates obey Ebihara-Johnson kinetics.17 We restrict our attention to the depolarization phase of the action potential.

We solve the bidomain equations for the tissue and Laplace’s equation for the bath by approximating the differential equations by finite differences.8 The time step is 2 μs. The space step in the z direction is 20 μm, and in the x direction is 50 μm for longitudinal propagation and 20 μm for transverse propagation. The boundary-value problem is solved iteratively using overrelaxation18; the iteration is terminated when the residual is <1 μV.

The membrane is at rest initially (Vn = −80 mV). At t = 0, Vn along the left edge (x = 0) is raised to 0 mV, initiating the action potential. Measurements of Vn, and its derivative are made at the midpoint of the slab, where the action potential waveform has reached a steady shape. The length of the slab is 15 mm for longitudinal propagation and 6 mm for transverse propagation (301 nodes in both cases). The slab is 0.5 mm thick, and its bottom surface is sealed. The transmembrane potential is measured at 3 depths: the...
Figure 1. Schematic diagram showing the geometry of the tissue slab and the perfusing bath.

The tissue-bath surface, 150 μm below the tissue-bath surface, and at the bottom of the tissue. The bath is 1 mm thick.

The time constant of the action potential foot is calculated by fitting a straight line to the phase-plane plot of dV_m/dt versus V_m over the range of V_m from –79 to –65 mV (approximately the first 15 mV of depolarization). The reciprocal of the slope of this line is τ_{foot}.

Results

Figure 2 shows the transmembrane potential as a function of x and z, for longitudinal and transverse propagation. In both cases, the wavefront is curved, with the action potential at the surface leading the action potential at the center. The spreading of the contours indicates that the rate of rise of the action potential is lower at the surface than in the bulk. The speed of longitudinal propagation is 0.552 m/s, and of transverse propagation is 0.203 m/s. The peak-to-peak interstitial potential, measured 150 μm below the surface is 23.0 mV for longitudinal propagation and 11.6 mV for transverse propagation.

Figure 3A contains a phase-plane plot of the action potential during longitudinal and transverse propagation, for V_m measured at the tissue surface. The rate of rise is 15% lower during longitudinal propagation (V_{max}=149 V/s) compared with transverse propagation (V_{max}=175 V/s). The inset shows a magnified view of the action potential foot. For propagation in either direction, the action potential foot is not exponential (an exponentially rising action potential foot would appear as a straight line in a phase-plane plot). The best-fit value of τ_{foot} is 706 μs for propagation in the longitudinal direction and 486 μs for propagation in the transverse direction.

The dotted curve in Figure 3A represents the action potential calculated when the bath is not present. In this case, the wavefront is not curved. The speed of longitudinal propagation is 0.505 m/s, and the speed of transverse propagation is 0.202 m/s. The time course of the action potential is independent of the direction of propagation. The action potential foot is exponential (τ_{foot}=294 μs), and V_{max} (201 V/s) is greater than when the bath is present.

Figure 3B contains similar data, but V_m is measured 150 μm below the tissue surface. As in Figure 3A, V_{max} is less for longitudinal propagation (196 V/s) than for transverse propagation (201 V/s), although the difference between the two (2.5%) is smaller than when V_m is measured at the surface. The action potential foot for transverse propagation is nearly exponential (τ_{foot}=314 μs), although it contains a slight “initial slur.” For longitudinal propagation, the action potential foot is clearly not exponential because of an initial slowing (best-fit τ_{foot}=483 μs).

At the bottom of the tissue (Figure 3C), V_{max} is larger, and τ_{foot} is smaller, for longitudinal propagation (V_{max}=214 V/s, τ_{foot}=272 μs) than for transverse propagation (V_{max}=203 V/s, τ_{foot}=292 μs). The action potential foot is nearly exponential, although there is a slight initial slur for propagation in the longitudinal direction. Note that V_{max} is greater than, and τ_{foot} is smaller than, if the bath were not present.

Tissue Parameters for the Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular, longitudinal bidomain conductivity</td>
<td>g_L = 0.45 S/m</td>
</tr>
<tr>
<td>Intracellular, transverse bidomain conductivity</td>
<td>g_T = 0.045 S/m</td>
</tr>
<tr>
<td>Interstitial, longitudinal bidomain conductivity</td>
<td>g_a = 0.45 S/m</td>
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<tr>
<td>Interstitial, transverse bidomain conductivity</td>
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<tr>
<td>Bath conductivity</td>
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<td>Surface-to-volume ratio</td>
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<td>Membrane capacitance</td>
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<tr>
<td>Membrane leak conductance</td>
<td>g_L = 0.5 S/m²</td>
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<tr>
<td>Leak reversal potential</td>
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<tr>
<td>Membrane sodium conductance</td>
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<tr>
<td>Sodium reversal potential</td>
<td>E_{Na} = 33.4 mV</td>
</tr>
</tbody>
</table>

Discussion

The data of Spach et al. are cited widely as evidence for discontinuous propagation in cardiac tissue. Their hypothesis...
of discontinuous propagation is supported by the following logic: (1) During 1-dimensional propagation in a tissue with continuous electrical properties, the time course of the action potential (including $V_{\text{max}}$ and $t_{\text{foot}}$) does not depend on the intracellular and interstitial conductivities; (2) experiments indicate that in cardiac tissue $V_{\text{max}}$ and $t_{\text{foot}}$ differ with the direction of propagation and therefore with conductivity; and (3) therefore, the conductivity of cardiac tissue is not continuous. A flaw exists in this line of reasoning: when a conductive bath perfuses the tissue, the propagation is not 1-dimensional. The extracellular conductivity is higher for the tissue near the surface (adjacent to the bath) than it is for the tissue far from the surface (deep within the bulk). Therefore, gradients in $V_m$ exist not only in the direction of propagation, but also in the direction perpendicular to the tissue surface. Reasoning based on the 1-dimensional cable model (such as used in the first premise of the syllogism above) is not applicable.

Several researchers have shown theoretically that the presence of the perfusing bath may account for the difference in the rate of rise with direction that was observed by Spach et al. The high-conductivity bath causes the wavefront to be curved (surface leading bulk) and the surface rate of rise to be slowed. This effect is more dramatic for longitudinal propagation than for transverse propagation because of the unequal anisotropy ratios of the tissue. For longitudinal propagation, the intracellular and interstitial conductivities are approximately the same, so large interstitial potentials exist in the bulk, although the potential in the high-conductivity bath is small. For transverse propagation, the interstitial conductivity is $\approx 4$ times greater than the intracellular conductivity, so the extracellular potentials are small both at the tissue surface and deep in the bulk. The smaller gradients of the extracellular potential result in smaller gradients in the transmembrane potential during transverse propagation compared with longitudinal propagation. Our calculated changes in propagation speed, $V_{\text{max}}$, and $t_{\text{foot}}$ measured at the tissue surface are qualitatively consistent with previous numerical models and with experimental data.

Recently, Spach et al. measured $V_m \approx 150 \mu m$ below the tissue surface, where they claim “there should be minimal effects of the superfusate solution.” Although Spach et al. recorded the action potential rate of rise, their main goal was to present “a detailed experimental analysis of the time course of the foot of the cardiac action potential ($V_{\text{m foot}}$) during propagation in different directions in anisotropic cardiac muscle.” They observed that “during longitudinal propagation there was initial slowing of $V_{\text{m foot}}$ that resulted in deviations from a simple exponential; corollary changes occurred at numerous sites during transverse propagation.” They attributed these results to an effect of capillaries on conduction.

The results in Figure 3B show that the influence of the perfusing bath extends at least $150 \mu m$ below the tissue surface. Furthermore, the bath causes the action potential foot to rise more slowly than exponentially, and this slowing is greater for longitudinal propagation than for transverse propagation. These results agree qualitatively with the recent experimental data of Spach et al. The action potential foot is particularly sensitive to the perfusing bath, more so than other features of the action potential. Quantitatively, the biggest discrepancy between our calculations and the data of Spach et al. lies not in the action potential foot, but instead in $V_{\text{max}}$. Our calculations indicate that $V_{\text{max}}$ $150 \mu m$ below the tissue surface is only 2.5% less for longitudinal propagation than for transverse propagation, whereas the experimental data show an average difference of 22%. The source of this discrepancy is unclear. It may arise from the discrete nature of the tissue, from capillary effects, from incorrect parameter values in the simulation, or from the presence of dead tissue $200$ to $300 \mu m$ below the tissue surface. Our model does not incorporate a dead core of tissue. According to Spach et al., the dead core has an enlarged interstitial space, which might increase the intersti-
tial conductivity and cause the core to function approximately in the same manner as the perfusing bath.

Spach et al.\textsuperscript{12} supported their theory of capillary effects by comparing their data with that measured by Fast and Kléber\textsuperscript{20} in monolayers of neonatal cardiac myocytes. They suggested that because such monolayers are devoid of capillaries, the action potential foot should be exponential. The action potentials measured by Fast and Kléber\textsuperscript{20} do indeed have an exponential foot. However, the monolayers of Fast and Kléber\textsuperscript{20} are also devoid of “deep” tissue far from the perfusing bath, so there can be no gradients of $V_m$ with depth. Therefore, the data of Fast and Kléber\textsuperscript{20} are also consistent with the hypothesis that the perfusing bath determines the shape of the action potential foot. Thus, data from monolayers does not distinguish between the capillary mechanism and the perfusing bath mechanism for slowing the action potential foot.

One way to distinguish between the 2 mechanisms (capillaries versus perfusing bath) would be to repeat the experiments of Spach et al.\textsuperscript{1,12} with and without a perfusing bath present. The tissue would have to be kept alive when the perfusing bath was absent, perhaps by arterial perfusion. The results in Figure 3A indicate that when the bath is eliminated, the action potential foot should become exponential, with no differences between longitudinal and transverse propagation. Furthermore, the maximum rate of rise of the action potential should increase and become independent of propagation direction. Although this experiment is easy to conceive, it would be susceptible to several sources of error. If $V_m$ were measured optically, the data would represent an average over a depth of a few hundred microns. Because the model predicts that $V_m$ changes dramatically over such distances, the data would be difficult to interpret. Microelectrode measurements, on the other hand, are sensitive to capacitative coupling to the perfusing bath, and the degree of such coupling depends on the bath depth. The rapid depolarization phase of the action potential is particularly sensitive to electrode capacitance. Although it is possible to correct the data for the influence of electrode capacitance, these corrections would be crucial when comparing data measured at different bath depths.

We cannot conclude from our study that capillaries are not important during action potential propagation. Nor can we conclude that discontinuous propagation does not occur (particularly in diseased tissue). These factors may well play a role in propagation. We can conclude, however, that the influence of a perfusing bath must be taken into account when interpreting data showing differences in the shape of the action potential foot with propagation direction, even if $V_m$ is measured 150 $\mu$m below the tissue surface. Therefore, differences in action potential shape with direction\textsuperscript{1,12} cannot be taken as definitive evidence supporting discontinuous propagation or capillary effects if a perfusing bath is present. Finally, without additional experiments, we cannot exclude the possibility that in healthy tissue the difference in the shape of the action potential upstroke with propagation direction is simply an artifact of the way the tissue was perfused.

**Acknowledgments**

This research was supported by NIH Grant RO1HL57207. We thank the School of Engineering and Computer Science at Oakland University for their computational support.

**References**

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Circ Res. 2000;86:e19-e22
doi: 10.1161/01.RES.86.2.e19

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