Extracellular PotentialsRelated to Intracellular Action Potentials during Impulse Conduction in Anisotropic Canine Cardiac Muscle

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SUMMARY This paper considers a quantitative description of intracellular and transmembrane currents in anisotropic muscle, with emphasis on the factors that determine the extracellular potentials. Although \( V_{\text{m}} \), of the intracellular action potential had no relation to changes in conduction velocity in anisotropic tissue with constant membrane properties, the extracellular waveforms were quite sensitive to velocity changes. Large amplitude biphasic deflection occurred in the fast areas, and in the slow areas the waveforms were of lower amplitude and triphasic in shape; i.e., negative potentials preceded the biphasic positive-negative deflection. The extracellular potentials were simulated on the basis of a model of intracellular currents, and the theoretical and measured results showed good agreement. In tissue with anisotropic conductivity, the relationship between the spatial intracellular potential gradient and the magnitude of the extracellular potential of the excitation wave was opposite to the classical relationship in isotropic tissue. Due to the influence of the effective intracellular conductivity on the spread of intracellular currents and on conduction velocity, in anisotropic tissue the extracellular potential decreased as the intracellular potential gradient increased. The peak values of the positive and negative potentials and the spatial distribution of the potential gradients varied considerably along the activation front. These findings were accounted for by differences in the distribution and spatial extent of the transmembrane currents, which were determined by the intracellular currents.

The theoretical analysis showed that intracellular and transmembrane currents were proportional to the local conduction velocities of the wavefront. Thereby, it was not possible to have a "uniform layer" of current when there were differences in conduction velocity along the length of the excitation wave. The implications of the analysis are considerable, since the gratifying agreement between the theoretical and measured results indicates that the details of the extracellular waveforms can be explained on the basis of the distribution of intracellular currents; i.e., extracellular potentials provide a sensitive index of intracellular current flow.

THE GENERAL physical principles underlying the generation of extracellular potentials from intracellular potentials are well known (Lorente de Nó, 1947; Rosenfalck, 1969; Plonsey, 1969). However, detailed analysis of this relationship has been performed only for structures of simplified geometry representing one-dimensional isotropic cylinders, such as nerves (Plonsey, 1964), muscle fibers (Håkansson, 1956), and Purkinje strands (Spach et al., 1972). These analyses used cable theory with the assumption that the intracellular potential varied only along the axis of the cylinder and that the intracellular conductivity was the same throughout the cylinder. For this special case, the intracellular potential gradients primarily determine the magnitude of the extracellular potentials during depolarization (Rall, 1969) and repolarization (Spach and Barr, 1976). However, in multidimensional anisotropic tissue, the relationships that exist between intracellular and extracellular potentials are considerably more complex, and no analysis exists.

The application of standard equations of cable theory to the core conductor model has provided most of the information that explains the spread of intracellular currents (Jack et al., 1975). If this model could be extended to explain the origin of extracellular potentials in multidimensional anisotropic tissue, it would provide a link between the two for detailed in vitro extracellular studies of propagation, as well as for studies of electrocardiographic generators (Plonsey, 1974; Corbin and Scher, 1977). A major problem is that the transmembrane currents that produce the potentials in extracellular space cannot be measured. The concept of using intracellular currents to derive the transmembrane currents, which is an integral part of the core conductor model, has been applied to cardiac muscle in the analysis of extracellular potentials (Spach and Barr, 1976; Miller and Geselowitz, 1978). Such an approach is advantageous since...
it provides a way to describe in detail the spread of intracellular currents which, in turn, determine the currents that flow between intracellular and extracellular space. Thereby, it provides all of the information necessary to develop, evaluate, and compare various models of bioelectric sources.

To obtain a detailed explanation of how the extracellular potentials of excitation waves arise from intracellular currents in anisotropic muscle, we conducted the following experimental-theoretical procedure. First, we measured the waveforms and potential distributions in vitro at the surface of a two-dimensional sheet of ventricular muscle within an area in which all of the sources of current could be accounted for in detail. Second, the measured activation sequences were used to calculate the intracellular potential distribution based on the assumption that all cells had the same action potential shape. Third, to account for the intracellular current flow in all directions, we analyzed the theoretical relationships that exist between conduction velocity, the effective intracellular conductivity, and the intracellular current. This analysis provided a way to use the experimentally measured conduction velocities to calculate the effective intracellular conductivities along the major and minor axes of the preparation. Fourth, the intracellular currents were used to calculate the transmembrane currents. Finally, extracellular waveforms and potential distributions were computed, and we compared these results with the corresponding experimental measurements.

The predictions of the theory were verified for a variety of measured extracellular waveforms and potential distributions. We found that, in tissue with anisotropic conductivity, the relationship that exists between the intracellular potential gradient produced by activation wavefronts and the magnitude of the extracellular potential was the opposite of that found for cases in which the conductivity was constant throughout. That is, due to the influence of the effective intracellular conductivity on the spread of intracellular currents and on conduction velocity, anisotropic tissue the extracellular potential decreased as the intracellular potential gradient increased in the direction of the propagating action potential. Also, in anisotropic tissue, the potential gradient in the volume conductor reversed in direction near the surface of the tissue, which never occurred in isotropic tissue in the classical experiments of Lorente de Nó (1947). The pattern of current spread in the volume conductor was studied further by similar analysis of the computed and measured potentials at varying distances from the surface of the tissue.

**Methods**

**Electrical Measurements**

We studied in vitro preparations from the hearts of 27 dogs (weight 15–22 kg), and successful experiments were completed in nine preparations. Each dog was anesthetized with pentobarbital sodium (30 mg/kg, iv). The heart was excised, and 30-mm × 20-mm sections of ventricular muscle of 12-mm thickness were pinned to the floor of a circular tissue bath. The diameter of the bath was 15 cm, and it was high enough to maintain the perfusate level 4.5 cm above the tissue surface. The millimolar composition of the perfusate was: NaCl, 128; KCl, 4.69; MgSO₄, 1.18; NaH₂PO₄, 0.41; NaHCO₃, 20.1; CaCl₂, 2.23; and dextrose, 11.1. The solutions were gassed in a reservoir with a gas mixture of 95% O₂-5% CO₂ and perfused through the 800-ml volume tissue bath at a rate of 100 ml/min. Heating units that surrounded the bath maintained the temperature constant at 36°C.

The extracellular and intracellular electrodes, the accompanying AC and DC amplifiers, and the calibration procedure have been described in detail previously (Spach et al., 1972). The extracellular electrodes were made of flexible tungsten wire, 50 µm in diameter, which was insulated except at the tip. The reference electrode was positioned 7 cm away. Each exploring electrode was connected via a grounded shielded cable to one input of an AC coupled differential amplifier with a frequency response flat between 0.1 and 30,000 Hz. The overall rise time (10–90%) for both the extracellular and intracellular recording systems was less than 20 µsec.

A pacemaker stimulus of 1.5 times threshold was delivered via a unipolar electrode to the surface of the preparation at a rate of 1/sec. A PDP-11/20 computer system (Barr et al., 1976) controlled the rate and synchronized the pacing stimuli with the data recording. The outputs of the amplifiers were sampled at a rate varying from 5,000 to 16,000 samples/sec. The computer stored the data and displayed the waveforms immediately on a Tektronix 4002 display unit. After the waveforms were found to be free of artifact, they were recorded on digital tape. The output of each amplifier was also displayed on a Tektronix 565 oscilloscope with 3A3 dual-trace differential amplifiers. Continuous monitoring of both display units ensured that the digitizing rates were sufficient for accurate digital reproduction of the waveforms (Barr and Spach, 1977). To record each excitation map, two exploring electrodes were used to measure the waveforms at 95–130 sites, located within an area of 12 mm × 20 mm. Care was taken to position the electrode gently against the surface to avoid injury. Each recording was made simultaneously with a time reference electrode, and 40–90 minutes were required to record each map. A dissecting microscope equipped with a Nikon F250 35-mm camera was used to document each recording position. Measurements to determine changes in conduction velocity while maintaining temperature constant (36°C) showed a decrease of 0–4% over 2 hours, changes similar to those of atrial muscle (Spach et al., 1971). Any
change in the time of the reference electrode waveform greater than this resulted in our discarding the data and repeating the entire recording procedure.

To measure the changes in the extracellular waveforms as a function of distance from the surface of the preparation, the extracellular electrode was moved away from the surface in a sequence of 25- to 50-μm steps as determined by a micrometer of the micromanipulator. Photographs made after each recording confirmed that the position of the tip remained along a line perpendicular to its position at the surface.

Data Analysis

The digitally recorded waveforms were redisplayed and photographed for detailed inspection. A second computer program was used to convert the waveforms to potential distributions, and the iso-potential maps were printed for each millisecond following the onset of excitation. To compare the measured and computed extracellular potential distributions, the voltage values were transcribed to drawings of the preparation, and the final equipotential lines were drawn by hand. The time of the peak negative derivative (intrinsic deflection) of the extracellular waveforms was used to construct isochrone activation maps. The isochrones were interpolated to measure the time of excitation and the conduction velocity at each site 300 μm apart. Conduction velocity was measured as the distance traveled, normal to the orientation of the isochrone, per unit of time. The relationship between the peak-to-peak voltage of the extracellular waveforms and the local velocity was analyzed statistically using multiple linear regression and Student’s t-test for paired observations (Sterling and Pollack, 1968). The level of significance was taken as 0.01%.

Theory

The general theoretical problem is to determine a mathematical expression for finding the extracellular from the intracellular potentials. The main difficulty is giving a detailed description of the current that flows between intracellular and extracellular space. If one knew the transmembrane current distribution throughout the preparation at each time instant, the extracellular potential at any given point in space could be computed and the results compared with the temporal and spatial potential measurements. As noted by Plonsey (1964), such a fit would allow quantitative interpretation of the biological properties of the bioelectrical sources. For our initial considerations, the overall electrical properties of anisotropic tissue (i.e., tissue with properties that have different values when measured along axes in different directions) have been ascribed to the orientation of uniform parallel fibers (Hermann, 1879; Rosenfalck, 1969; Clerc, 1976; Corbin and Scher, 1977).

As we were unable to find previous specific treatment of the origin of extracellular potentials based on intracellular potentials in anisotropic multidimensional tissue, several theoretical and experimental analyses of the multidimensional spread of intracellular currents should be noted. For a point source in a single cell, Eisenberg and Johnson (1970) showed that three-dimensional intracellular current spread produced intracellular potentials near the source that were different from those predicted by one-dimensional cable theory. Barr and Jakobsson (1976) used a vector formalism that was well suited for the analysis of intracellular current flow in three-dimensional anisotropic tissue simulating smooth muscle. The effects of changing geometry, e.g., terminating boundaries, on the spread of intracellular currents has been analyzed by Rall (1969) and presented in detail by Jack et al. (1975).

Experimental justification for extending these theoretical approaches to the analysis of cell-to-cell current flow (intracellular current) in cardiac muscle is provided by the experimental results of Woodbury and Crill (1961) and by Sakson et al. (1974). They showed that electrotonic current spread occurs not only along cardiac fibers but in the transverse direction, the space constant for voltage decrement being greater parallel to the fiber orientation than perpendicular to fiber direction. Joyner et al. (1975) used cable theory and active equivalent circuits to model action potential propagation in two-dimensional cardiac muscle. They demonstrated theoretically that, in tissue composed of cells with the same membrane properties (Hodgkin and Huxley, 1952), the shapes of the isochrones were dependent upon the coupling resistance between cells along the major and minor axes. Finally, Clerc (1976) showed that the disparity in conduction velocities measured along the longitudinal and transverse fiber orientation of ventricular trabeculae can be explained by the difference in the resistivity of the intracellular and extracellular paths for current flow in the two directions, confirming theoretical predictions.

The Model

The primary purpose of the model was to provide a detailed description of the currents flowing between intracellular and extracellular space; i.e., the transmembrane currents throughout the tissue. In a one-dimensional cylinder, the transmembrane current is given by the spatial change in intracellular current (Plonsey, 1969). For multidimensional tissue, the total transmembrane current per unit volume, \( I_m \), that enters extracellular space (Miller and Geselowitz, 1978) can be expressed as

\[
I_m = - \left( \frac{\partial I_u}{\partial x} + \frac{\partial I_u}{\partial y} + \frac{\partial I_u}{\partial z} \right),
\]

where \( I \) is the intracellular current density along the three axes. In Equation 1, it is assumed that the
cell-to-cell current flow, $I_n$, can occur in any direction and that the sum of the components along each axis accounts for the total cell-to-cell current flow.

Since the intracellular current cannot be measured directly, it must be inferred from other measurements. Assume, for the moment, that changes in the intracellular potential, $\phi$, occur only along one axis. This is analogous to a uniform cylinder with internal resistivity, $R_i$, for which the longitudinal current density, $I_n$, is given by

$$I_n = -\frac{1}{R_i} \frac{\partial \phi}{\partial x}, \quad (2)$$

where $x$ is the distance along the longitudinal axis of the cylinder.

For cardiac muscle, the measurements of Weidmann (1970) and Clerc (1976) of the intracellular resistivity, $R_i$, can be used for the numerical solution of Equation 2. The values of $R_i$ are determined as the specific resistance in $\Omega \cdot$ cm from measurements in whole bundles, where the effective area providing the circuit for current flow can be approximated accurately by the use of small bundles (Weidmann, 1952). The total intracellular resistivity is affected by the resistivity of the intracellular myoplasm, the resistance and distribution of the cell-to-cell connections, and the relative volume of the intracellular and extracellular space. We use the term effective intracellular resistivity, $R_e$, to include the composite effect of all of these. Thus, the effective intracellular conductivity, $\tilde{\sigma}$, in mho/cm is

$$\tilde{\sigma} = \frac{1}{R_e}. \quad (3)$$

By combining Equations 2 and 3 we have

$$I_n = -\tilde{\sigma} \frac{\partial \phi}{\partial x}. \quad (4)$$

From Equations 1 and 4, the transmembrane current per unit volume of tissue, $I_m$, can be expressed as

$$I_m = \frac{\partial}{\partial x} \left[ \tilde{\sigma} \frac{\partial \phi}{\partial x} + \frac{\partial}{\partial y} \left( \tilde{\sigma} \frac{\partial \phi}{\partial y} \right) \right]. \quad (5)$$

The extracellular potential, $\phi_e$, in a volume conductor of homogeneous conductivity, $\sigma_e$, is given by (Plonsey, 1969)

$$\phi_e(P, t_0) = \frac{1}{4\pi \sigma_e} \int \frac{I_m}{r} dV, \quad (6)$$

where $I_m$ is the transmembrane current per unit volume of tissue at time instant $t_0$ and $r$ is the distance from the point of observation, P, to the source (Fig. 1). This equation ignores the effects of extracellular anisotropy.

In terms of Figure 1, the integral in Equation 6 can be approximated by a discrete summation as follows

$$\phi_e(P, t_0) = \frac{1}{4\pi \sigma_e} \sum \left[ \frac{\partial}{\partial x} \left( \tilde{\sigma} \frac{\partial \phi}{\partial x} \right) + \frac{\partial}{\partial y} \left( \tilde{\sigma} \frac{\partial \phi}{\partial y} \right) \right] \frac{(a + b)^2 + d^2}{2a dx dy}, \quad (7)$$

where the numerator represents Equation 5 for the case of the two-dimensional tissue as shown in Figure 1, and the descriptors $a$, $b$, and $d$ shown in Figure 1 give the distance $r$ from each source element to the observation point, P. The total transmembrane current in each block represented in Figure 1 is determined by using Equation 5 to evaluate the transmembrane current per unit volume at the center of each block and multiplying that transmembrane current by the volume of the block.

To evaluate Equation 7, it is necessary to know the values of the effective conductivities in the $x$ and $y$ directions at each site. For uniform cylinders, the effective intracellular resistivity, $R_e$, is related to the velocity of the propagating action potential by

$$R_e = K \frac{1}{\theta^2}. \quad (8)$$

where $\theta$ is the conduction velocity, and $K$ represents
the passive membrane properties and the radius of the cylinder. In this equation it is assumed that the conduction velocity is altered by changes in the intracellular current distribution that do not affect the membrane properties (Hodgkin, 1954). Equation 8 can be derived from numerous formulations of the relation between the passive properties of the tissue and active propagation (Håkansson, 1956; Jack et al., 1975). Clerc (1976) has shown that this can be applied to cardiac muscle where \( R_a \) is the effective intracellular resistivity of the tissue. Equation 8 can be used to derive the values of \( R_a \) in all of the blocks represented in Figure 1 from measurements of the conduction velocity. Thereby, the intracellular current density, \( I_n \), can be determined from Equation 4 and the associated extracellular potential, \( \phi_e \), computed from Equation 7. In Equation 8, we assume that the extracellular resistivity is small compared to the effective intracellular resistivity at any location in the tissue. We consider that, although this assumption may result in small errors in the calculated value of \( R_a \), the basic relationship between effective intracellular resistivity and conduction velocity is preserved.

Procedure for Computing Extracellular Potentials

Equation 7 was the basis of our calculations of the extracellular waveforms at 90-130 positions on the surface of the preparation, as well as at varying distances from the surface. The resistivity of the extracellular fluid was constant throughout the volume conductor (150 \( \Omega \cdot \text{cm} \)). The width, \( dx \) or \( dy \), of each block was 25 \( \mu \text{m} \) (Fig. 1). We assumed that the thickness, \( 2a \), of the active tissue was 300 \( \mu \text{m} \), as will be described in the Results.

We assigned the same measured action potential to all sites (the action potential measurements are described in the Results). The action potential was divided into 12-\( \mu \text{sec} \) time divisions. In the digitized waveform the duration of the upstroke was 2.4 msec and \( V_{\text{max}} \) was 150 V/sec. Following the upstroke there was a typical negative notch preceding the plateau phase (Hoffman and Cranefield, 1960). The action potential used in the calculations is shown in Figure 2. The intracellular potential distribution was derived as follows: Activation times were assigned to positions 300 \( \mu \text{m} \) apart on the basis of the measured isochronous time maps. The computer program interpolated these activation times to each site 25 \( \mu \text{m} \) apart. The value of the intracellular potential, \( \phi_i \), at each site was computed for any instant throughout the excitation sequence by knowing the time of activation of each site and the time instant being considered, as done previously for ventricular repolarization (Spach and Barr, 1976).

The effective intracellular resistivity, \( R_a \), varied from site to site to account for the anisotropic properties of the tissue. We found it impossible from our tissue dissections to relate the local details of the measured isochrones and the conduction velocities to a single parallel fiber orientation. For the purpose of the computations, however, it was necessary to approximate the complex anisotropy by adjusting the conductivities to approximate an idealized state of the changing values of the effective \( R_a \) along the major and minor axes of the preparation. Therefore, we used the following procedure to derive the value of \( R_a \) in the \( x \) (fast) and \( y \) (slow) directions at each site:

1. We used the measurements of Clerc (1976) in calf ventricular trabeculae at 25°C for assigning \( R_a \), a value of 402 \( \Omega \cdot \text{mm}^2/\text{msec} \) when the conduction velocity was 0.48 m/sec. From these data the constant, \( K_a \), of Equation 8 has a value of 926 \( \Omega \cdot \text{mm}^2/\text{msec}^2 \).

2. The local conduction velocity at each location 300 \( \mu \text{m} \) apart was measured as the distance traveled, normal to the orientation of the isochrone, per unit of time. These values were transcribed to a map of the preparation, which was divided into "slow" and
"fast" velocity areas. For the experimental results to be presented, the average velocities in the respective slow and fast areas were 0.2 and 0.4 m/sec. We used Equation 8 \((K = 926 \, \Omega \cdot \text{mm}^2/\text{msec}^2)\) to derive the \(R_i\) values associated with these two velocities. The \(R_i\) value of 2315 \(\Omega \cdot \text{cm}\) was assigned to the \(y\) (slow) axis at all sites, and the value of 578 \(\Omega \cdot \text{cm}\) was assigned to the \(x\) (fast) axis at each site. Thereby, each site was assigned two \(R_i\) values, one corresponding to intracellular currents along the \(y\) axis and the other corresponding to currents along the \(x\) axis (Fig. 1).

3. Finally, to account for deviations beyond the range of the average velocities, the computer program interpolated the original velocity values at locations 300 \(\mu\text{m}\) apart to each site 25 \(\mu\text{m}\) apart. The conduction velocity at each site was evaluated to identify those with values below or above the average range of 0.2 to 0.4 m/sec. For each of these sites, Equation 8 was used to calculate the associated \(R_i\) value. It was found that those velocities that exceeded 0.4 m/sec were directed more toward the fast axis as compared to the slow axis and, conversely, sites with velocities less than 0.2 m/sec were directed more toward the slow axis. Therefore, the new value of \(R_i\) was assigned to the appropriate site on the \(y\) axis when the velocity was less than 0.2 m/sec and on the \(x\) axis when the velocity was greater than 0.4 m/sec.

The intracellular potential and the effective resistivities along the \(x\) and \(y\) axes at each site were used to compute the longitudinal intracellular current density in the \(x\) and \(y\) directions using Equation 2. The transmembrane current of each site was computed as the first derivative of the intracellular potential waveforms at numerous locations. The experimental problem was to find a preparation with properties that would allow us to use realistic assumptions for the calculations and to make accurate measurements for comparison with the theoretical results. First, the preparation had to be free of electrical irregularities produced by insulating boundaries which prevent continuous cell-to-cell current flow in any direction. For the purposes of this study we use the term "uniform anisotropy" to mean tissue of this type. Our extracellular measurements indicated that many preparations, especially the atrium, had electrical separation of adjacent parallel fibers for distances of several hundred microns ("nonuniform anisotropy"). When potential gradients exist across these separations, they present small insulating boundaries for intracellular current flow (Katz and Miledi, 1965; Goldstein and Rail, 1974). These multiple "end-effects" (Plonsey, 1977) throughout the tissue presented intractable computational problems for us. Second, to compute the extracellular potential, one has to account in detail for all of the currents entering and leaving extracellular space. This requires a reasonable estimate of the thickness of the active tissue plus electrical measurements that account for all of the wavefront of depolarization at each instant. Third, a desirable feature was that the preparation have the same intracellular action potential throughout, since this assumption simplifies the calculation of the intracellular current flow to obtain the transmembrane current. For this study, we thought that the foregoing requirements could be adequately fulfilled and satisfactorily evaluated only in two-dimensional, rather than three-dimensional, cardiac muscle.

We used the extracellular waveforms to identify tissue of uniform anisotropy. Two pacemaker and two recording electrodes were arranged at fixed positions as shown in Figure 2. The stationary recording electrodes were positioned so that each would be located alternately in the fast area of the excitation wave (along the axis of fiber orientation) and then in the slow area (perpendicular to fiber orientation) as the excitation sequences were altered by changing the pacemaker site (Fig. 2, top). Uniform anisotropy was designated when the waveform remained free of small rapid deflections as the waveform shape was changed from a large biphasic...
deflection (fast axis) to a smaller triphasic deflection (slow axis). All tissues demonstrated a large biphasic waveform of smooth contour along the fast axis. However, electrical irregularities were apparent in tissue of nonuniform anisotropy from the multiple small, rapid deflections that were superimposed on the triphasic waveforms when the excitation sequence was changed. These multiple small deflections would be expected from asynchronous excitation of electrically separate fiber bundles (Spach et al., 1973). The irregular waveforms occurred frequently on the papillary muscle of the right ventricle, especially in areas where small fat droplets occurred in between the endocardial fibers. Ventricular epicardial waveforms indicated uniform anisotropy; however, the superfused in situ preparation of epicardial tissue became nonresponsive to the pacemaker stimulus after 40–60 minutes.

The only preparations of uniform anisotropy that remained electrically stable for a long time were those of the right ventricular endocardial surface of the ventricular septum, an area studied by Myerburg et al. (1978). Initially, we attempted to remove the endocardium by gentle dissection (Myerburg et al., 1978). However, once the endocardium was removed, persistent electrical irregularities appeared in the triphasic waveforms in the slow conduction areas. By extensive electrical exploration, we found nine preparations that were free of Purkinje fibers, and we studied these with the endocardium intact.

Intracellular action potentials were measured in six preparations with the same findings in all. Although there was marked similarity in the symmetrical shape and duration (2.0–2.5 msec) of the upstroke at different locations, there were minor asymmetrical shape differences similar to those in the published waveforms of Clerc (1976). However, when the intracellular electrode remained stationary and we changed the excitation sequence, the upstrokes of the intracellular action potentials remained virtually superimposable, while there were marked changes in the amplitudes and shapes of the associated extracellular waveforms (Fig. 2, top).

All excitation sequences were measured after 3 hours of superfusion by which time mechanical motion occurred only on the endocardial surface. To determine the depth of active tissue at that time, we used a multipolar plunge electrode in five preparations (Fig. 2, bottom). Active depolarization did not extend further than 1 mm beneath the surface. We could not resolve the thickness of the active tissue to less than 1 mm (interelectrode spacing). Therefore, for the purpose of the calculations, we used 0.3 mm as an approximation. The S-T segment was negative on the entire surface of the preparation. Positive S-T segments occurred at a depth of 1 mm or more. That the positive injury currents were generated in the region 1–2 mm beneath the endocardial surface was evidenced by the large amplitude S-T segment there, with diminishing amplitude at greater depths in the preparation.

Relation of the Shape of the Extracellular Waveform to the Excitation Sequence

The sequence to be analyzed shows the changes that occur when the wavefront of depolarization propagates from a single stimulus site. The waveforms had different shapes that were divided into four general categories, and each category was distinctive for a specific area (Fig. 3). The borders of each area, within which all of the waveforms had the same features, expanded symmetrically in relation to the enlarging isochrones. In the immediate vicinity of the onset site, the waveforms were negative (Fig. 3, area A, nos. 1 and 4). This small area expanded in the slow area but extended only 100–200 μm along the fast axis.

In the large area along the fast axis (Fig. 3, area B), the waveforms were biphasic in shape with an initial positive deflection followed by a prominent negative peak (nos. 2 and 3). The magnitude of the positive peak increased, and the negative peak decreased over a distance of 2 mm from the onset site; at greater distances, the magnitude of the peaks was variable. The third area (C) occupied a broad region along the slow axis. In this region all of the waveforms were triphasic in shape; i.e., negative potentials preceded the biphasic positive-negative deflection (nos. 5 and 6). As noted along the fast axis, there was a change in the relative magnitudes of the positive and negative peaks along the slow axis as a function of distance from the onset site. Although there was a small initial negative deflection in all of the waveforms, the positive component developed from an initial notch in the negative deflection at position 4 to a more prominent positive peak at more distant positions (nos. 5 and 6). However, the positive peaks remained less prominent than the associated negative peaks, and the peak-to-peak amplitude of the biphasic component of the waveforms in the slow areas (C) was less than that of the waveforms in the fast area (B).

The final area (D) was a small one with a variable location from sequence to sequence. However, it always occurred distal to the onset site and formed an intervening region between a rapid area on one side and a slow area on the other. These waveforms (area D, nos. 7 and 8) were characterized by fluctuating low-level potentials (7) or slowly increasing positive potentials (8) prior to the prominent intrinsic deflection. These waveforms occurred in regions near which there were significant deviations in the contours of the isochrones from the purely elliptical shape that should occur throughout a preparation with parallel fiber orientation. These deviations can be seen in the contours of the isochrones bordering each area D in Figure 3.

The accompanying computed waveforms are shown at the bottom of Figure 3. The position of each computed waveform was within 300 μm of the associated measured waveform. Although there are detectable differences between the two, they show
Figure 3. Relation of the extracellular waveforms to the excitation sequence. The excitation sequence shown was constructed from the waveforms recorded at 110 positions within an area of 12 mm x 10 mm. Excitation was initiated by the pacemaker stimulus at electrode 1 (onset site) in area A. Each isochrone (broken line) represents a 2-msec interval so that the latest time represented is at the lower border at 20 msec. The areas within which all of the waveforms had the same general shape are indicated by the letters A-D within the circles. The bold dark lines demarcate the “fast” area (B) from the “slow” area (C). A small transitional area is indicated by D. The numbers (within boxes) indicate the locations of the measured and computed waveforms (bottom). The potentials of the computed waveforms were calculated at 1-msec intervals.

Good agreement in regard to the general shape and amplitude changes that occurred from location to location. Note that the theoretical waveforms reproduced the increase in magnitude of the positive peak in the waveforms along the fast axis (nos. 1–3) and the same progression in the positive component of the triphasic waveforms along the slow axis (nos. 4–6). Also, the computed waveforms reproduced the low-level fluctuations in the waveforms in area D (nos. 7 and 8). The resemblance is striking between the progressive change in shape of the measured and computed waveforms along the slow axis and the “embryonic r waves” originally described by Durrer et al. (1954) for the in situ ventricular wall.

The measured and computed waveforms of Figure 3 indicated that those with the greatest amplitudes occurred in the fast area (B) and those with the smallest amplitude occurred in the slow area (C). Figure 4 shows the statistical relationship between the peak-to-peak amplitude of waveforms measured throughout the preparation and the local conduction velocity of the isochrone. Although there is considerable scatter in the measurements, the analysis indicates that a definite relationship exists between the two for both the measured and computed waveforms. A note of caution is indicated in evaluating the peak-to-peak voltage of waveforms. Whereas most waveforms have a prominent positive peak, some do not (Fig. 3, D, no. 8) and in others the notch in the negative deflection (no. 4) represents the appropriate measurement point as an index of the local maximum extracellular potential gradient associated with the upstroke of the underlying intracellular action potential.
Potential Distributions on the Surface of the Preparation

Associated with the movement of the isochrones shown in Figure 3, the pattern of the potential distributions changed continuously during the first 9 msec and thereafter the major features remained constant. Figures 5 and 6 show the potential distributions with the accompanying isochrones. For each instant the associated theoretical result can be compared with the measured isopotential map.

Initially the isochrone was elliptical in shape and there were two maxima, one at each end of the ellipse (Fig. 5, 2 msec). A single minimum was at the onset site, and the magnitude of the minimum considerably exceeded that of either maximum. Low-level potentials occupied most of the preparation with positive potentials in the fast area and negative potentials in the slow area, a pattern resembling that produced by an ectopic stimulus site in the in situ ventricle. (Corbin and Scher, 1977; Spach and Barr, 1975b). As the area of the elliptical-shaped isochrone increased, the magnitudes of the extrema increased, and two minima developed from the initial single one and they shifted toward the associated maxima (3 msec). At each end of the ellipse, the portion of the isochrone located between the maxima and minima formed the demarcation line between positive and negative potentials; however, the portion of the isochrone in the slow area occurred in the region of negative potentials with a steep negative gradient along the trailing edge. The computed isopotential maps were similar to the measured ones in regard to the location and the changes in magnitude of the extrema, as well as the associated regions of low-level positive and negative potentials (Fig. 5, 2 msec and 3 msec).

As the area of the isochrone enlarged further, the pattern became more complex (Fig. 5, 5 msec). In the slow areas, which were occupied by negative potentials, a “saddle point” developed along the leading edge of the isochrone. At the same time, the two maxima in the fast areas maintained their location along the leading edge of the wavefront, and the two minima continued to accompany the maxima as they shifted apart. Steep negative gradients occurred in the trailing edge along the entire
circumference of the ellipse formed by the isochrone. Thus, the pattern at 5 msec consisted of multiple maxima and minima; i.e., there were potential extrema in each fast and slow area. In each fast area there was a prominent maximum and minimum. In each slow area there was a low-level isolated minimum, distal to the relative maximum and minimum that occurred along the leading and trailing edges of the isochrone. A final development was the relative maximum in the negative potential area surrounding the onset site. Note that the associated theoretical result (fig. 5, 5 msec) reproduced all of the above features of the measured potential distribution.

The general features of the potential gradients that developed at 5 msec persisted throughout the time interval analyzed. However, major changes occurred in the pattern of the potentials in the slow areas (Fig. 6). As the circumference of the total wavefront increased, a thin projection of positive potentials extended along the leading edge of the isochrone into the region of negative potentials in each slow area (7 and 8 msec) until positive potentials occupied the leading edge of the total excitation wave (9 msec). At this time (9 msec), the spatial potential gradients of the excitation wave (i.e., potential gradients directed normal to the orientation of the isochrone) varied in a systematic manner. The absolute maxima and minima were located at the most distal part of the excitation wave in each fast area. The magnitude of the positive and negative peaks, as well as the distance separating the two, decreased gradually along the length of the isochrone as one progressed from the maximum in each fast area to a location along the isochrone near the middle of the slow area. The distance between the maximum and minimum in each fast area was approximately 0.9 mm, and the distance between the positive and negative peaks in the middle of the slow area was approximately 0.2 mm (Fig. 6, 9 msec). Deviations from this progression were related to asymmetry and shifts in the major axis of the elliptical-shaped isochrone, as well as to irregularities in the smooth contour of the isochrone. Although the total isochrone was surrounded by positive potentials at 9 msec, negative potentials persisted distal to the wavefront in each slow area with a local minimum positioned approximately 1 mm in front of the approaching isochrone. Finally, as these changes occurred between 7 and 9 msec, the magnitude of the negative potentials surrounding the onset site continued to decrease.

All of the major features of the measured maps were reproduced in the computed maps between 7 and 9 msec (Fig. 6). Along the length of the isochrone, the progressive change in the magnitude of the peak-to-peak potential and the distance between the peaks of the excitation wave in the fast and slow areas were apparent in the theoretical results. There was general agreement in the location of the maxima and minima, including the isolated minimum in the region toward which the excitation wave was propagating in each slow area and the relative maximum that persisted in the region of the onset site. Note that from 7 to 9 msec (Fig. 6), the major sequential changes in each slow area represent the spatial counterpart of the "embryonic r waves" of waveforms 4 to 6 in Figure 3.

Relation between Extracellular Potentials and Transmembrane Currents

To enhance the interpretation of the systematic change in the spatial potential gradients along the isochrone, we analyzed the computed isopotential map at 8 msec (Fig. 6) in terms of the transmembrane current. Our idea was to relate the details of the potential distribution at that instant to the transmembrane currents. The isochrone representation of the excitation wave at 8 msec is reproduced in Figure 7A with the accompanying measured velocities of each part. The associated transmembrane
Current distribution is shown in panel B in the form of an isocurrent map. It was drawn from a large montage that contained the computed net transmembrane current values of each 25-μm segment through the preparation. It is evident from comparing panels A and B of Figure 7 that the positive and negative peak values of the transmembrane current and the distance between the peaks, as well as the spatial extent of the positive and negative currents, are related to the different conduction velocities of each part of the isochrone.

The reflection of the transmembrane current distribution in the measured and computed waveforms along the entire wavefront can be seen in Figure 7C and compared with the details of the potential distribution at 8 msec in Figure 6. The waveforms with the greatest peak-to-peak amplitude occurred in the areas of highest velocity, and those with the smallest amplitude occurred in the slowest areas. Computed and recorded waveforms clearly agree in both amplitude and waveshape. Also, note the effect of the transmembrane current distribution on the spatial extent of the extracellular potentials in the computed map (Fig. 6, 8 msec) where the distance separating the maximum and its associated minimum was much greater in each fast area than in each slow area. Although the shape of the isochrone and the locations of the highest and lowest velocities were, in general, consistent with that expected from a parallel fiber orientation, there were noticeable digressions from this relationship that were reflected in the extracellular potentials. Inter-

![Figure 7](http://circres.ahajournals.org/)

**Figure 7** Transmembrane current distribution of the activation wavefront. The local conduction velocities at different parts of the isochrone (A) and the computed membrane current distribution (B) are shown for the time instant at 8 msec for comparison with the potential distribution at that instant in Figure 6. The values of conduction velocity shown for each part of the isochrone in A were measured in the direction of the accompanying arrows. In the isocurrent map in B, positive current (current flowing from intracellular to extracellular space) is represented by the darkly stippled area and negative current (current flowing from extracellular to intracellular space) by the lightly stippled area. The currents represented in the stippled areas are the net transmembrane currents that exceeded ±0.1 mA/mm². The values of the isocurrent lines in the negative (broken lines) and positive (solid lines) areas are shown beneath the isocurrent map. The measured and computed waveforms at varying positions along the isochrone are shown in C. The locations of the waveforms are designated by the numbers in A. Note the correspondence between the spatial extent of the positive and negative currents and the distance between their peak values in comparison to the potential distribution at 8 msec in Figure 6.
estingly, even though the general position and shape of the waveforms at locations 3 and 8 (Fig. 7, A and C) were along the same slow axis, the considerable differences in local velocity were reflected in the amplitude of the peak-to-peak deflections of the measured and computed waveforms.

Although the leading edge of the entire excitation wave consisted of positive transmembrane current, negative extracellular potentials were associated with the positive currents in the region of lowest velocity in the upper slow area in the computed map at 8 msec (Fig. 6). This illustrates the complex effects of the currents in extracellular space and is a specific example of the way the potential at any point is determined by the currents of the total excitation wave; i.e., in Equation 7 there is a 1/r relationship in the way the transmembrane currents affect the extracellular potential. The 1/r relationship between distance and the negative transmembrane current can be used to explain the gradual emergence of positive potentials along the leading edge of the excitation wave in each slow area. Only after the total wavefront had enlarged considerably were the prominent negative currents in each fast area sufficiently distant to have a diminished effect on the less prominent positive currents in the leading edge of the excitation wave in each slow area. Thus, the gradual increasing circumference of the total excitation wave accounted for the origin of the "embryonic r waves," which occurred only in slow areas (Fig. 7C).

Relationship between Conduction Velocity and Intracellular Current

The above analysis assumes that the thickness of the active tissue is constant and that the local membrane properties, including the excitability of the membrane (Peon et al., 1978), are not affected by changes in the conduction velocity. Thereby, the intracellular current spread that determines the conduction velocities is regulated by the local spatial intracellular potential gradient and the effective intracellular resistivity. Measurements in each preparation showed that the velocity was entirely dependent upon the direction of propagation of the excitation wave, such as shown in Figure 2. Table 1 shows the quantitative intracellular electrical changes that were computed for the range of conduction velocities of the wavefront of Figure 7A. The values of the intracellular current were determined for a single bundle with a cross-sectional area of 0.01 mm².

As the conduction velocity decreased from 0.5 to 0.1 m/sec, the spatial extent of the upstroke of the intracellular action potential decreased from 1.2 to 0.24 mm (Table 1), values that are reproduced in the variations of the spatial extent of the transmembrane current distribution shown in Figure 7B. While velocity and the length of the upstroke of the action potential decreased, both the intracellular potential gradient and the effective intracellular resistivity, increased, the relative increase in being greater than that of the intracellular potential gradient (Table 1). Note the magnitude of change in R, and in the intracellular potential gradient was large when the conduction velocity decreased from 0.2 to 0.1 mm/msec as compared to a much smaller change when the conduction velocity decreased the same amount from 0.5 to 0.4 mm/msec. The computed results in Table 1 are consistent with the analysis of conduction velocity and R, in a synthetically grown strand of cardiac muscle by Lieberman et al. (1973). Also, confidence in the quantitative values of R, in Table 1 is enhanced by comparison of a measured R, value of 3620 Ω·cm at a velocity of 0.16 mm/msec by Clerc (1976) with the computed R, value of 3616 Ω·cm derived from Equation 8 for that velocity.

Although the spatial intracellular potential gradient and the effective intracellular resistivity varied in a nonlinear manner with respect to velocity, the maximum intracellular current was directly proportional to the conduction velocity (Table 1). The transmembrane currents in the leading edge of the propagating action potential are determined by the intracellular currents, as shown in the Appendix. As a result, the total amount of current entering extracellular space in the leading edge of each part of the excitation wave, Iₘ⁺, is directly proportional to the local conduction velocity.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relation between Conduction Velocity and Intracellular Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity</td>
<td>Spatial length of upstroke of intracellular action potential, φ</td>
</tr>
<tr>
<td>(mm/msec)</td>
<td>(mm)</td>
</tr>
<tr>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>0.4</td>
<td>0.96</td>
</tr>
<tr>
<td>0.3</td>
<td>0.72</td>
</tr>
<tr>
<td>0.2</td>
<td>0.48</td>
</tr>
<tr>
<td>0.1</td>
<td>0.24</td>
</tr>
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</table>

The assumption used to compute the maximum intracellular current, I, of a single bundle with a cross-sectional area of 0.01 mm² was that the membrane properties were constant everywhere; i.e., the same time course of the intracellular action potential occurred at each site. Vₘ⁺, of the digitized intracellular action potential was 150 mV/msec.
Potentials in the Volume Conductor at Varying Distances from the Surface

The foregoing analysis still did not explain the initial negative deflection in the triphasic waveforms (Fig. 7C). The problem was that the negative deflection was associated with an isolated minimum that occurred in advance of the excitation wave in each slow area (Fig. 6), and the minimum was located where the calculated net transmembrane current was zero. The significance of the isolated minimum was that it indicated that the extracellular currents in the plane of the preparation would flow from all of the surrounding areas to the location of the minimum. Since the net transmembrane current at this point was zero, the currents in the volume conductor at that point could only flow away from the surface of the preparation. Therefore, we measured and computed the potentials that exist in the volume conductor at varying distances above the surface. The computed results along the fast and slow axes are shown for three time instants in Figure 8.

The magnitude of the potential decreased with increasing distance from the surface at all locations along the fast axis. Thereby, the pattern of current flow in the volume conductor along the fast axis was the same as the classic one for a one-dimensional structure (Lorente de Nó, 1947). Along the slow axis, however, in the leading edge of the excitation wave, the direction of the potential gradient in the volume conductor reversed near the surface (Fig. 8). At any point in the leading edge of the wavefront, or ahead of that, the potential became more negative for a short distance above the surface (50 µm), and then it became more positive with increasing distance. Note in Figure 8 that at all time instants along the slow axis the shape of the spatial potential curve at 50 µm above the surface had a unidirectional gradient of increasing magnitude of the negative potentials that extended from the region considerably ahead of the excitation wave to the minimum in the trailing edge of the excitation wave, a spatial gradient different from that on the nearby surface. Thus, a unidirectional negative potential gradient existed from the apparent minimum on the surface up into the volume conductor at 50 µm and therefrom to the potential minimum in the trailing edge of the excitation wave.

The above theoretical results were verified by...
FIGURE 9  Measured and computed waveforms at varying distances from the surface in the slow area. The measured triphasic waveform at the surface (0 μm) changed to a totally negative waveform within 100 μm of the surface. Similar shape changes in the computed waveforms can be seen, although the rapidity with which the potential magnitudes changed as a function of distance from the surface was different.

Discussion

Perhaps the most remarkable aspect of our results was that the general relationship between the extracellular potential and the intracellular potential gradients of propagating wavefronts in anisotropic muscle was found to be the opposite of the classical relationship in isotropic tissue. In anisotropic tissue, larger spatial intracellular potential gradients of activation wavefronts produced smaller extracellular potentials due to the overriding influence of the decrease in the effective intracellular conductivity. In contrast, in isotropic tissues, the larger intracellular potential gradients produce larger extracellular potentials. The reason for the difference is that the extracellular potential is determined by the transmembrane current which, in turn, is determined by the intracellular current, not potential. In the isotropic case, the effective intracellular conductivity is constant throughout. Thereby, changes in the spread of intracellular current are determined by the spatial intracellular potential gradients; i.e., when the intracellular gradient increases, the intracellular current increases and produces an increase in the extracellular potential nearby. The results showed that, although the increase in the spatial intracellular potential gradient that occurred with a decrease in conduction velocity would be expected to augment intracellular current flow, in fact, the intracellular currents decreased because of the large decrease in intracellular conductivity. This resulted in an associated decrease in the extracellular potential nearby.

In our extracellular measurements the spatial extent of the extracellular potential gradients and the distances between the positive and negative peaks along the length of the excitation wave varied considerably. These variations were accounted for by differences in the distribution and spatial extent of the transmembrane currents which occurred over distances that included scores of cells (Fig. 7). Woodbury and Crill (1961) showed by their intracellular measurements that local circuit current flow (i.e., intracellular current flow through cell-to-cell connections) extends over numerous cells. This concept provides the basis for the theoretical analysis in our study. The gratifying similarity between the measured and theoretical results in extracellular space support their measurements. Furthermore, the results show that in anisotropic muscle the local circuit current flow can be described quantitatively in terms of the distribution of current that flows between intracellular and extracellular space. The basis for this quantitative description is the spread of the intracellular currents, which are determined by the interaction of the spatial intracellular potential gradient and the effective intracellular conductivity.

Studies of Propagation in Anisotropic Cardiac Muscle

The results emphasize the importance of extracellular, rather than intracellular, recordings for the purpose of studying propagation in anisotropic muscle. Although the complexity of the extracellular waveforms may initially suggest additional difficulty in measuring excitation sequences, in fact, the
reverse is so. A single record could be interpreted to indicate not only the presence of beat-to-beat changes in conduction velocity, but also the waveform shapes could be interpreted with respect to the location of the recording site in relation to conduction in other areas, e.g., the triphasic excitation waves in each slow area. That is to say, the shape of the extracellular waveform was determined by the currents of the total excitation wave. Such power is relinquished with the intracellular technique.

In Purkinje strands, which approximate one-dimensional isotropic cylinders, Peon et al. (1978) recently showed that when the resting intracellular potential was more negative than $-70 \text{ mV}$, there was no correlation between $V_{\text{max}}$ and conduction velocity due to changes in the membrane properties; i.e., membrane excitability. Our results in multidimensional anisotropic muscle with constant membrane properties were particularly intriguing in this regard since they showed that the time course of the intracellular action potential (e.g., $V_{\text{max}}$) had no relation at all to changes in conduction velocity (Fig. 2). The extracellular potential, however, was quite sensitive to local changes in conduction velocity, as well as to different conduction velocities that occurred from area to area.

**Evaluation of Electrocardiographic Bioelectric Sources**

Sano et al. (1959) pointed out that in the theory of electrocardiography the direction of myocardial fibers had been almost ignored. Subsequently, potential distributions measured in intact animals showed that the potential maxima and minima were not distributed evenly along the isochrone, and the isochrone was challenged as a valid representation of the bioelectric sources because the assumption required for its use, i.e., a constant current density along the isochrone, was questionable (Spach and Barr, 1975a). Recently, Corbin and Scher (1977) have strongly challenged the uniform double-layer hypothesis as a useful electrocardiographic equivalent generator based on studies with their axial theory model that consists of parallel dipoles to represent longitudinal current flow in parallel fibers.

Even with the above questions about the cardiac generator, the essential information needed has been lacking, i.e., a quantitative description of the factors that determine the characteristics of the cardiac sources. The intracellular-extracellular approach described in this study defines the bioelectric sources directly in terms of intracellular current flow which determines the transmembrane currents that produce the potentials in the volume conductor. Defining the bioelectric sources directly in terms of the transmembrane current distribution represents a major shift from the way extracellular dipoles (e.g., Holt et al., 1971; Corbin and Scher, 1977) have been used; i.e., such dipole sources have required assumptions about the current density distribution that have been difficult to verify. Our results are encouraging in this regard since the determinant of the extracellular sources, the transmembrane currents, could be calculated in detail in anisotropic tissue starting from a knowledge of intracellular action potentials. This same general approach can be used to study many different phenomena, such as the relation between the origin of cardiac currents and the potentials measured on the body surface (Miller and Geselowitz, 1978).

The formulations presented for the derivation of intracellular and transmembrane currents are applicable to the general case of multidimensional anisotropic tissue. However, Equation 6 describes the extracellular potential resulting from current sources in an unbounded homogeneous medium. In the general case, the extracellular conductivity might be anisotropic and inhomogeneous (i.e., in vivo condition), and Equation 6 would not apply. For our conditions, the tissue bath fluid level was sufficiently distant to the preparation to justify the assumption of an unbounded medium. Moreover, the possible effects of the boundary between the active tissue and the underlying inactive muscle of the preparation were not taken into consideration.

A salient observation was that not only was there no uniform current density along the isochrone, it was not possible in anisotropic tissue to have a "uniform layer" of current when there were differences in conduction velocity (i.e., intracellular current) along the length of the wavefront. The experimental measurements and theoretical analysis provide definitive proof that a "uniform double layer" does not exist when the wavefront has components that propagate in different directions in anisotropic tissue. As shown in the formulation of Equation 7, only when the effective intracellular conductivity remains constant in the direction of the propagating action potential can the transmembrane current density remain the same along the length of the wavefront. This condition might be approximated in the normal left ventricular wall when there is a normal sequence of excitation from endocardium to epicardium; however, even if this were so, the transmembrane current density of the wavefront would be different with a different excitation sequence.

We do not believe that the implication of these results can be limited to the in vitro study of propagation in small preparations. The waveforms and potential distributions showed striking similarities to the "embryonic r waves" of Durrer et al. (1954) in the in situ ventricular wall and to the epicardial potential distributions produced by ectopic beats in intact chimpanzees (Spach et al., 1978).

Our results agree with the emphasis given by Corbin and Scher (1977) to the influence of parallel fiber orientation on electrocardiographic potentials; however, they do not agree with their measurements or the predictions of their axial theory model.
in an important respect. In the axial theory model, the extracellular dipoles are aligned for an idealized parallel fiber orientation so that the theory does not allow for currents to spread along the slow axis; i.e., it predicts that no extracellular gradients can occur ahead of the approaching excitation wave along the slow axis. Thereby, the measured and computed triphasic waveforms that occurred only in the slow areas, waveforms similar to the "embryonic r waves," should not occur during excitation according to the predictions of the axial theory model. In addition, the axial extracellular dipole model predicts that no extracellular potentials would result from wavefronts oriented perpendicular to the fiber axis.

The results emphasize the importance of specifying the assumptions involved when making predictions about cardiac muscle from electrocardiograms. The solid angle theorem, as it is often used to relate activation wavefronts to the electrocardiogram, assumes that the current per unit area is constant everywhere on the boundary used to compute the solid angle. The results show that for anisotropic muscle this assumption frequently does not hold.

The use of multiple dipole models to predict an increase in ventricular muscle mass (Holt et al., 1971) is based on the assumption that a specified volume of muscle produces the same quantity of current; i.e., an increase in the time integral of the computed heart dipole strength for a specified muscle area is equated with a concomitant increase in muscle volume. Two assumptions are required for this relationship to be valid: (1) The direction of spread of the wavefronts through the hypertrophied muscle should be the same as that of the control state. Otherwise, if hypertrophy is accompanied by changes in the local excitation sequence, our results indicate that it is possible for the potential to increase disproportionately or even decrease. (2) The anisotropic properties of the muscle should not be changed by the increase in the total mass of muscle. This assumption warrants close scrutiny in view of the marked variation in the degree of cardiac muscle fiber disarray that is known to occur in common heart diseases (van der Ble-Kahn, 1977), as well as cardiac tissue swelling (Lepeschkin, 1976).

Appendix

The following is a formulation that demonstrates the proportionality between conduction velocity and the total transmembrane current entering extracellular space.

For bundles of uniform geometry and membrane properties (i.e., the same cross-sectional area and the same action potential shape at all locations) the conduction velocity, \( \theta \), in each will vary as a function of the effective intracellular conductivity. If we assume that each bundle has uniform effective intracellular conductivity, \( \bar{\sigma}_i \), throughout, but each with a different value, the total transmembrane current entering extracellular space, \( I_m^+ \), is

\[
I_m^+ = \int_{-\infty}^{+\infty} A \bar{\sigma}_i \frac{\partial^2 \phi}{\partial x^2} dx,
\]

where \( "0_{m}" \) is the spatial location of the point of transition between the net positive and negative transmembrane current, \( A \) is the cross-sectional area of the bundle, and \( x \) is the longitudinal distance along each bundle. In this equation, it is assumed that \( \theta \) is constant at all times; i.e., it does not change when depolarization occurs.

If we assume constant conduction velocity over time period \( t \), then

\[
dx = \theta dt,
\]

and the second spatial derivative of the intracellular potential becomes

\[
\frac{\partial^2 \phi}{\partial x^2} = \frac{\partial^2 \phi}{\theta^2 \bar{\sigma}_i^2}.
\]

By combining Equations 9, 10, and 11 the positive transmembrane current, \( I_m^+ \), can be expressed as

\[
I_m^+ = \int_{-\infty}^{+\infty} A \bar{\sigma}_i \frac{\partial^2 \phi}{\theta^2 \bar{\sigma}_i^2} \theta dt,
\]

From Equations 3 and 8 the intracellular conductivity, \( \bar{\sigma}_i \), becomes

\[
\bar{\sigma}_i = K \theta^2,
\]

where \( K \) represents the passive membrane properties and the geometry of the tissue. By combining Equations 12 and 13, we have

\[
I_m^+ = KA \theta \int_{-\infty}^{+\infty} \frac{\partial^2 \phi}{\theta^2 \bar{\sigma}_i^2} dt,
\]

thus showing that the total positive transmembrane current, \( I_m^+ \), is directly proportional to conduction velocity in the presence of constant membrane properties.

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