Active Modulation of Electrical Coupling between Cardiac Cells of the Dog

A Mechanism For Transient and Steady State Variations in Conduction Velocity

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SUMMARY. Propagation velocities of action potentials were measured simultaneously along the longitudinal and transverse axes of cardiac fibers in ventricular muscle. The anisotropic distribution of propagation velocities was found to be altered transiently and in the steady state by the rate and pattern of stimulation and by ouabain. The relative amount of velocity change varied with the direction of propagation and was greatest in the direction perpendicular to the long fiber axis. None of the variables usually associated with the membrane ionic mechanism of depolarization—resting potential, Vmax, and Tfoot—showed enough variation to account for the observed changes in velocity. A simplified anisotropic propagation model representing the internal current pathway as an alternating sequence of cytoplasmic and junctional resistance is presented, taking into account the larger contribution to the internal resistance made by the cell couplings in the transverse direction than in the longitudinal direction. On the basis of this model, it was concluded that the observed changes in velocity were due to changes in cell coupling. Both transient and steady state velocity changes were found to correspond to changes in the action potential duration, suggesting that there is a common factor, such as the internal calcium and/or sodium concentrations, linking the control of the action potential duration and the coupling resistance between cardiac cells. (Circ Res 51: 347-362, 1982)

IT IS well known that the conduction time through the atrioventricular (AV) node increases with increasing stimulus frequency. It is also generally believed that the propagation velocity of action potentials in atrial or ventricular muscle is independent of the stimulus frequency, provided the frequency is not so high that depolarization encroaches on the repolarization phase of the preceding action potential (Brooks et al., 1955). There is however, some evidence that the propagation velocity in the rabbit atrium does decrease at high rates with little or no change in the rest or "takeoff" potential (Vaughn Williams, 1959; Viersma et al., 1968a; Pasmooij et al., 1976). These changes in velocity with stimulus frequency, including those in AV node, have been associated with changes in the maximum rate-of-rise of the action potential (Vmax) and have therefore been attributed to alterations in the membrane ionic mechanism of depolarization, i.e., altering the activation kinetics of the sodium conductance, gNa.

The factors that change the propagation velocity of the action potential can be divided into two classes: changes in membrane ionic mechanisms and changes in electrotonic coupling. Most investigators have assumed that interventions modifying the conduction velocity operate by changing the rate of depolarization intrinsic to the membrane while the resistance between cells (intercellular coupling) and other core conductor properties remain unchanged (Hoffman and Cranefield, 1960). Examples of this interpretation include the effects of drugs such as ouabain (Moe and Mendez, 1951; Viersma et al., 1968b) and quinidine (Vaughn Williams, 1958), elevation of the extracellular potassium concentration (Dominguez and Fozzard, 1970), premature stimulation (Gettes and Reuter, 1974), and variation of the takeoff potential in Purkinje fibers that exhibit diastolic depolarization (Peon et al., 1978). Hunter et al. (1975) and Khodorov and Timin (1975) have shown that the relationship between Vmax and gNa is complex for propagated action potentials even in a relatively simple cable.

Mechanisms in the second class—variations in the velocity of propagation due to changes in electrotonic coupling—have received little experimental study in cardiac muscle. Weingart (1977) showed that ouabain increases the internal longitudinal resistance of ventricular muscle and that the increase in resistance contributes to the associated decrease in conduction velocity (in addition to the role played by other factors such as the decrease in the rate of rise and amplitude of the action potential). He assumed that the myoplasmic resistivity remained constant and concluded that the increased internal resistance reflected an increase in intercellular resistance. He also noted that the accompanying increase in muscle contractility that he observed provided evidence for the involvement of [Ca++] in the control of cell-to-cell resistance. He attributed the mechanism of the ouabain-dependent...
decoupling to an increase of [Ca\(^{++}\)], secondary to an increase in [Na\(^+\)], (Reuter and Seitz, 1968; Baker et al., 1969; Glitsch et al., 1970; Carafoli et al., 1974). In this case brought about by blocking of the Na-K pump (Langer and Serena, 1970; Ellis, 1977). Ando et al. (1981) also suggested that this mechanism might account for the ouabain-dependent increase in axial resistance they demonstrated in rabbit atrial muscle.

There is additional evidence linking changes in intracellular ionic concentrations with altered intercellular coupling. De Mello (1975) demonstrated a decrease in junctional conductance between heart cells following intracellular injection of calcium, a result consistent with the work of Rose and Loewenstein (1975) in salivary glands. De Mello (1976) also found decoupling of cardiac cells when there is an increase of [Na\(^+\)], and he suggested that at least a part of the effect of cardiac glycosides on impulse conduction in the AV node might be due to a decrease in electrical coupling between the cells. Further, Kositsky et al. (1972) have suggested that during cardiac fibrillation there may be a change in intercellular coupling, and evidence that this occurs has recently been presented by Bredikis et al. (1981). By driving rabbit atria at very high frequencies (600 to 900/min) for prolonged periods, simulating fibrillation, they found that the resistivity of the intercellular pathway increased. Assuming that the cytoplasmic resistivity did not change, they attributed the increase in internal resistance to decreased cellular coupling. They interpreted the increase in coupling resistance to be due to an increase in [Ca\(^{++}\)], associated with prolonged activity (Langer, 1965).

Other information relating propagation velocity and cell-to-cell coupling comes from experiments on anisotropic cardiac muscle where directional differences in velocity are best accounted for by differences in effective axial resistivity with direction due largely to the spatial distribution of cell-to-cell connections (Clerc, 1976). We recently presented evidence that the axes perpendicular and parallel to the fiber orientation differ not only in the magnitude of internal resistance, but also in the distribution of this resistance (Spach et al., 1981). The interrelationship of propagation velocity, \(V_{\text{max}}\), and the time constant of the foot of the action potential in the longitudinal direction was consistent with a model having a uniform distribution of properties (Hunter et al., 1975; Khodorov and Timin, 1975), but the relationship of these quantities in the transverse direction could only be accounted for by assuming recurrent discontinuities of internal resistance. Fast propagation was associated with slow upstrokes and a low safety factor along the longitudinal axis of the fibers, and slow propagation was associated with fast upstrokes and a high safety factor along the transverse axis. These results are consistent with the anatomical finding that cells are grouped into unit bundles with a high density of cell-to-cell connections (nexuses) within the bundles and a smaller number connecting the bundles (Sommer and Dolber, 1982).

In this paper, we use the anisotropy of ventricular muscle as a tool to distinguish between membrane and cell-coupling mechanisms for altering the propagation velocity. The relative contributions of cytoplasmic and junctional components of axial resistance will change with the direction of propagation, depending on the cell geometry and packing and the locations of cell-to-cell connections. (The same set of connections, of course, joins the cells regardless of the direction of propagation; what changes is the relative area of membrane and volume of cytoplasm between connections.) Insufficient morphological data are available to support the construction of a highly detailed model of cell geometry and interconnections in cardiac tissue, but approximations can be made from studies of strands of conducting fibers (Johnson and Sommer, 1967) and ventricular muscle (Sommer and Dolber, 1982). Since it is known that [Na\(^+\)] varies with the frequency of stimulation (Conn and Wood, 1959; Langer, 1967; Fozzard and Sheu, 1981), that [Ca\(^{++}\)] changes with [Na\(^+\)], and that cell-to-cell coupling is influenced by [Ca\(^{++}\)], it might be expected that propagation would be influenced by the stimulation frequency—particularly where cell-to-cell couplings make a large contribution to the internal resistance. We not only have investigated this relationship, but also have tested this explanation by other interventions known to change internal ionic connections, such as the application of ouabain (Lee and Klaus, 1971).

### Methods

#### The Preparation and Experimental Setup

In vitro preparations from the hearts of 17 dogs (weight 15–25 kg) were studied. Each dog was anesthetized with pentobarbital sodium (30 mg/kg, iv). The hearts were excised rapidly and the preparations were pinned to the floor of a circular tissue bath, 15 cm in diameter. Preparations included papillary muscles from the right ventricle. The millimolar composition of the perfusate was as follows: NaCl, 128; KCl, 4.69; MgSO\(_4\), 1.18; NaH\(_2\)PO\(_4\), 0.41; NaHCO\(_3\), 20.1; CaCl\(_2\), 2.23; and dextrose, 11.1. The solutions were aerated in a reservoir with a gas mixture of 95% O\(_2\)-5% CO\(_2\) and superfused through three separate cannulas to produce a brisk flow rate (100 ml/min) across the preparation. A YSI thermistor (diameter 1 mm, time constant 0.2 sec) was used to maintain the temperature of the preparation at 35°C (it did not vary more than 0.1°C).

The extracellular and intracellular electrodes and recording techniques have been described in detail previously for in vitro measurements of anisotropic propagation in atrial and ventricular muscle (Spach et al., 1979, 1981). Intracellular potentials were recorded with conventional glass microelectrodes filled with 3 M KCl and having resistances between 5 and 20 megohms. The extracellular electrodes were made of flexible tungsten wire, 50 μm in diameter, and insulated except at the tip. Each extracellular electrode was connected to one input of an AC-coupled differential amplifier, having a frequency response flat between 0.1 and 30,000 Hz. The (separate) reference electrodes for each extracellular or intracellular electrode were positioned 7 cm away from the recording site.

A pacemaker stimulus 0.5–1.0 msec in duration and of amplitude 1.5–2.0 times threshold was applied to the surface...
of the preparation by a unipolar electrode. A PDP-11/20 computer system (Barr et al., 1976) controlled the stimulus frequency, the time of premature stimuli, and the duration of periods when there was cessation of stimulation (pauses). The computer system also synchronized the pacing stimuli with the data recording. The outputs of the recording amplifiers were sampled at a rate between 6,600 and 20,000 per second (12-bit samples). The data were recorded in blocks of time varying in duration from 240 to 500 msec. Simultaneously, the depolarization waveforms of the initial 20 to 50 m sec of each block of data were displayed on a Tektronix 4002 unit with a persistent screen so that each new waveform could be viewed superimposed on the waveforms of previous beats. This provided a way to immediately detect small changes in conduction time between electrodes positioned along the longitudinal and transverse axes of the preparation. It also provided a way to check the reproducibility of slight changes in conduction time and to make selective interventions at the most appropriate times during the experiment. The output of each recording amplifier also was displayed on one channel of a Tektronix 565 analog oscilloscope to monitor both the depolarization and repolarization waveforms. Photographs of both displays were taken to ensure that the digitizing rates were sufficient for accurate digital reproduction of the waveforms. A dissecting microscope equipped with a Nikon F250 35-mm camera was used to document the positions of the stimulus and recording electrodes. All measurements were made after 2 hours of superfusion.

Four to six extracellular electrodes were positioned in two perpendicular lines to simultaneously record the waveforms along the longitudinal and transverse axes of the fibers. The stimulus electrode was positioned at the intersection of the two axes. The basic stimulus frequency was 60/min. Each step change to and from this frequency was recorded as follows: immediately after recording the last beat during the steady state at any given frequency, the interstimulus interval was abruptly changed to a new value. Waveforms at the new frequency were recorded repetitively at intervals varying from 3 to 7 seconds. The intervals of 3 to 7 seconds, depending on the duration of the sampling block, were required for the computer to digitally record the entire action potential (up to 500 msec), store the data, display the depolarization waveforms, and then concurrently transfer the data to digital tape. The stimulus frequency remained at a constant value (usually 60/min) for other interventions, which included the addition of ouabain to the perfusate.

Meticulous care was taken to evaluate the following in each experiment: (1) Frequency-dependent changes in conduction velocity that are related to the takeoff potential can occur in the presence of diastolic depolarization of the fibers (Péon et al., 1978). That this did not affect our results was apparent from the fact that without stimulation the preparation did not beat; also, all transmembrane potentials remained at a constant value during diastole. (2) Extracellular waveforms, rather than intracellular, waveforms were used as a sensitive index of changes in the excitation sequence and to ensure the absence of Purkinje fibers in the area of study (Spach et al., 1973, 1979). If there was any change in the configuration of the extracellular waveforms, such as the appearance of irregularities, the experiment was stopped. (3) To make sure that the wavefronts approximated a plane wave propagating along the longitudinal and transverse axes of the fibers at the surface of the preparation and that there were no macroscopic irregularities in the wavefronts, periodically throughout each experiment an extracellular electrode was used to map local excitation in the region surrounding the most distal recording electrode along each axis. (4) Measurements in these and previous ventricular preparations demonstrated that the conduction velocity was greatest close to the stimulus electrode and became relatively constant 1.0 mm distant in the longitudinal direction and 0.5 mm distant in the transverse direction. The location of the nearest measuring electrode along each axis exceeded these distances from the stimulus electrode by at least a factor of two to three.

All measurements were made after 2 hours of superfusion. After this time we did not record any evidence of active depolarization extending more than 1–2 mm beneath the surface (Spach et al., 1979), so that the active layer was only 1–3 depolarization wavelengths thick. We did not find evidence for a different excitation sequence in the viable tissue below the surface. Further, we obtained the same directional changes in different areas of the same preparation as well as in different preparations. We thus concluded that excitation at the surface was well approximated by a plane wave and that changes in the deep subsurface tissue did not contribute to our conclusions about directional differences in propagation velocity. The location of the site for cell impalement was chosen to be midway between the two major axes of propagation as defined by the locations of the stimulus and extracellular electrodes. Care was taken to impale cells only at the surface of the preparation. Constant recording conditions, along with documented fixed electrode positions, were achieved for repetitive intervals lasting from 5 to 45 minutes in 14 ventricular preparations.

After the conclusion of an experiment, the waveforms stored digitally were redisplayed and photographed for detailed inspection. Conduction velocity was calculated as the distance traveled per unit time, taking the difference in the time of the peak negative derivative (intrinsic deflection) of the extracellular waveforms at two electrode sites along a given axis. The time constant of the foot of the action potential (\(t_{\text{foot}}\)) was calculated graphically by plotting the first 8 mV of depolarization on semilogarithmic paper and obtaining an approximate straight line fit by eye. \(V_{\text{mem}}\), the transmembrane potential was obtained by numerical differentiation of the digitally recorded action potentials (Fig. 5). The preparations were examined histologically by light microscopy to verify the fiber orientation in the region of measurement, as was done previously (Spach et al., 1981).

Linear fits of the data were made using linear regression. Student’s \(t\)-test was used with the level of significance taken as 0.01%. Exponential fits were made using a nonlinear regression procedure that produced least-squares estimates of the parameters of nonlinear models using the Gauss-Newton method (Gallant, 1975).

Changes in the duration of the action potential were measured either from intracellular recordings or from extracellular electromgrams. In the intracellular recordings, the duration of the action potential was measured from the onset of depolarization to a point of intersection of the baseline with the tangent to the steepest part of phase 3 repolarization. In the extracellular electromgrams, most of the
T waves had terminal positive deflections that coincided with the end of the action potential. The duration of the action potential was measured from the onset of the depolarization waveform to the baseline crossing of the terminal positive deflection. Linear regression analysis showed an excellent correlation between the area of the action potential (Gibbs et al., 1963) and the duration of the action potential (r = 0.97, P < 0.0001, t = 23.2), as well as between the action potential durations measured intra- and extracellu-
larly (r = 0.99, P < 0.0001, t = 24.8).

The Anisotropic Propagation Model

In all of the measurements described above, we studied action potential propagation under circumstances where the excitation pattern could be well represented by a plane wave traveling either parallel to the long cell axis or per-
pendicular to it. Under these circumstances, a one-dimen-
sional (cable) model is appropriate for each axis because the spatial derivative of the transmembrane potential is non-
zero only along one or the other of these axes. Furthermore, correlating the two axes with cell orientation makes it possible to relate electrical properties to the cell geometry and the distribution of interconnections. We did not attempt to measure the geometrical quantities necessary to calculate actual resistances or resistivities; this would have required rapid measurements of both cell geometry and axial resistance in two directions during and after a variety of inter-
ventions. Since it was not possible for us to make these observations in a single preparation in a short time interval, we attempted, instead, to estimate the relative contributions of cytoplasmic and junctional resistances along the two axes from velocity measurements. The model described below, therefore, address only the relative velocities along the two perpendicular axes.

Figure 1 shows a simplified equivalent circuit for the intracellular current in elongated cardiac cells for wave-
fronts propagating along either the longitudinal or the trans-
verse axis. The representation of cellular geometry and interconnections is greatly oversimplified; however, certain essential features are included. The axial current must traverse a finite length of cytoplasm between connections along the pathway, causing the axial resistance in each direction to consist of recurrent discontinuities of resistance produced by alternating cytoplasmic and junctional contribu-
tions. The relative contribution of junctional resistance to the total axial resistance is different in the transverse and longitudinal directions because of the anisotropic cell ge-
ometry and distribution of junctions. Our equivalent circuit is based on the following assumptions and approximations: (1) We assume that we are observing plane wave propaga-
tion of action potentials on a two-dimensional sheet of cells (see Methods for justification). As noted above, such propa-
gation is essentially one-dimensional in nature, provided that the single axis of variation is chosen in the direction of propagation. We therefore represented our preparation by a one-dimensional (cable) equation; since there are no voltage gradients perpendicular to the axis of propagation, there are no currents in that direction and cell couplings need not be considered. (2) Since we are considering propaga-
tion only parallel to and perpendicular to the fiber axis, we can represent the internal current pathway as an alter-
nating sequence of cytoplasmic resistance and junctional resistance. The relative contributions of these resistances will be estimated from an idealized, regular, and constant cell geometry. We will assume an elongated cell shape with junctions only near the ends of cells. As a result, the relative contribution of junctions to the axial resistance is greater and the distance between junctions is smaller in the trans-
verse direction than in the longitudinal direction. (3) We assume that the resistance to current flow in extracellular space is negligible compared to internal resistance. (4) We assume that the axial impedance is essentially resistive, i.e., that the time constant of the nexus membranes is so short that capacitive current through these membranes (Freygang and Trautwein, 1970; Chapman and Fry, 1978) does not affect the calculation of changes in velocity.

We have previously presented evidence that action po-
tential propagation in cardiac muscle is often discontinuous at a cellular level because of the elongated cell shape and the complex distribution of intercellular connections (Spach et al., 1981). The evidence came from measurements of propagation velocity, the maximum rate of rise, and the time constant of the foot of the action potential; the ob-
erved relationship was opposite to that expected from continuous cable theory. In this paper, we describe changes in velocity and look for their origin; we looked for related changes in the maximum rate of rise and time constant of the foot, but could not resolve any changes in the param-
eters. Our model, therefore, does not include the effects of discontinuities at a cellular level, but attempts to represent an averaged description of the tissue. Clerc (1976) used a similar approximation and found agreement between measured velocities and internal resistances and the predictions of continuous cable theory. He further stated that the action potential shape stayed constant, but his analysis did not depend on constant shape and, in addition, we see in his published records the same evidence for discontinuities as we found, i.e., the same changes in the shape of depolar-
ization with transverse and longitudinal propagation (see Fig. 5 of Clerc's paper). Our model, therefore, makes use of the inverse square relationship between propagation veloc-
ity and axial resistance predicted by continuous cable theory since we could not resolve small changes in the shape of depolarization for propagation in a fixed direction.

![Figure 1: Equivalent circuit of the simplified propagation model used to interpret the velocity measurements along the longitudinal and transverse axes of the fibers showing the axial contributions of cytoplasmic (R_c) and junctional (R_j) resistance.](http://circres.ahajournals.org/DownloadedFrom)
Longitudinal Propagation

For a plane wave propagating along the longitudinal axis of the cells, the total resistance contributed by each cell is given by:

\[ R_{\text{cell}} = \frac{R_l + \frac{R_j}{L}}{\pi a^2} \]

where \( L \) is the equivalent length of the cell with radius \( a \), \( R_c \) is the cytoplasmic volume resistivity, and \( R_j \) is the specific junctional resistivity. This resistance can be expressed in terms of a longitudinal resistivity

\[ R_{\text{L}} = \frac{R_c + \frac{R_j}{L}}{\pi a^2} \]

The number of cell-to-cell junctions per unit length \( n \) is equal to \( 1/L \), so the effective axial resistivity during longitudinal propagation becomes

\[ R_{\text{an}} = R_c + nR_j \]

Transverse Propagation

It is difficult to define the cross-sectional area of the cell for the current flow in the transverse direction, since it depends on the specific geometric arrangement of the cell-to-cell connections. However, for the idealized geometry model shown in Figure 1, the equivalent cross-sectional area of the cell for current flow in the transverse direction is \( 2aL \). The total resistance contributed by each cell is then

\[ R_{\text{cell}} = \frac{R_c + \frac{R_j}{L}}{\pi a^2} \]

Once again this can be expressed in terms of a transverse resistivity

\[ R_{\text{an}} = \frac{R_c + \frac{R_j}{L}}{\pi a^2} \]

Equations 3 and 6 are the same as those given by Clerc (1976), although the specific arrangement of the cell-to-cell connections of Figure 1 is different from Clerc's representation.

For tissue of uniform anisotropy, the ratio of the junctional resistance in the transverse direction to that of the longitudinal direction can be expressed as an anisotropic coupling ratio \( A \):

\[ A = \frac{\text{Equivalent junction resistance per unit length}_{\text{transverse}}}{\text{Equivalent junction resistance per unit length}_{\text{longitudinal}}} \]

Comparing Equations 3 and 6, the anisotropic coupling ratio \( A \) is equal to \( L^2/\pi a^2 \) for the idealized geometric arrangement of Figure 1. Thus, Equation 6 becomes

\[ R_{\text{an}} = R_c + A(nR_j) \]

Numerical values for the model parameters are shown in Table 1. We chose a value of \( R_c \) similar to the range of values (282-324 \( \Omega \)·cm) reported by Chapman and Fry (1978). Values reported for \( R_c \) in cardiac muscle range between 1 \( \Omega \)·cm\(^2\) (Woodbury and Crill, 1961) to 4 \( \Omega \)·cm\(^2\) (Chapman and Fry, 1978). For \( R_j = 1 \) ft·cm\(^2\) and \( n = 70 \), \( R_{\text{an}} \) is 400 ft·cm, a value almost the same as the measured value of the specific internal resistivity in the longitudinal direction of ventricular muscle reported by Clerc (1976). The values shown for \( L \) and \( A \) were calculated from assumed values of \( n \) and \( a \). We chose a relatively large cell radius in proportion to cell length as a “worst case” example of the anisotropy effect on propagation; a more realistic (thinner) cell shape would produce larger differences between velocities along the two axes.

| TABLE 1 |
|---------------------|---------------|-------------------|
| **Numerical Values for the Model Parameters** |
| \( R_c \) | Specific cytoplasmic resistivity | 330 \( \Omega \)·cm (100%) |
| \( R_j \) | Specific junctional resistivity | 1.0 \( \Omega \)·cm\(^2\) (100%) |
| \( n \) | Number of equivalent cell-to-cell connections per cm in longitudinal direction | 70 |
| \( L \) | Length of cell | 142.8 \( \mu \)m |
| \( a \) | Radius of cell | 15.9 \( \mu \)m |
| \( A \) | Anisotropic coupling ratio | 25.6 |

Predictions of the Anisotropic Propagation Model

Figure 2 shows the changes in propagation velocity and effective axial resistivity calculated from the anisotropic propagation model for separate increases in \( R_c \) or \( R_j \) and for equal relative increases in both, assuming that the propagation velocity varies in proportion to the reciprocal of the square root of the axial resistivity. The figure shows the calculated changes along each axis (longitudinal and transverse), as well as the resulting change in the ratio of the velocities. Note that when both \( R_c \) and \( R_j \) are increased in the same proportion, the effective resistivities and velocities in both directions change together so that the velocity ratio does not change. However, when either \( R_c \) or \( R_j \) is increased independently, the resistivities and velocities along the two axes change in opposite and characteristic directions; the velocity ratio decreases with increasing \( R_c \) and increases with increasing \( R_j \). Thus, on the basis of the simplified model, a clear interpretation of the origin of a velocity change can be made by comparing the relative changes along the longitudinal and transverse axes (\( \theta_l \) and \( \theta_T \)) after an intervention.

Results

Experimental Justification of the Anisotropic Propagation Model

It is a major assumption of our propagation model that the anisotropic velocity ratio \( \theta_l/\theta_T \) is not affected by uniform changes in membrane properties. We have tested this assumption by measuring \( \theta_l/\theta_T \) for premature stimuli when the fast sodium mechanism presumably is and is not fully recovered. Weidmann (1970) demonstrated that the internal resistance of ventricular muscle does not change throughout the time course of repolarization of a single action potential. His result suggests that neither the junctional nor cytoplasmic resistivity changes perceptibly during the
first several hundred milliseconds following depolarization. Therefore, $\theta_L/\theta_T$ should remain constant when the velocity is altered by stimulating at a more depolarized membrane potential during the repolarization phase of the previous action potential. The relative values of $R_a$ and $R_{a,r}$ can be derived from the measured values of $\theta_L$ and $\theta_T$ at the basic frequency of the steady state (Spach et al., 1979). For any subsequent premature beat, it should be possible to predict the propagation velocity along the transverse axis from the measured velocity along the longitudinal axis (or vice versa).

Figure 3 shows a typical result for premature action potentials. To ensure that the membrane properties of the preparation were uniform, the functional refractory period (Rosenblueth, 1958) was measured at multiple sites and was found to vary by not more than 3 msec throughout the region of measurement. The premature stimulus was injected at variable delays after every 15th regular stimulus occurring at a constant frequency of 1/sec. Measurements in other anisotropic preparations demonstrated that interventions that decrease the rapid inward sodium current produced a monotonic decrease in $V_{max}$ (not shown) with decreasing velocity as long as there were no local changes in the direction of propagation with respect to the cell orientation, as previously described in Purkinje strands (Spach et al., 1981). The sequence of excitation remained constant for beats of varying prematurity. The absolute values of conduction velocity changed more in the longitudinal than in the transverse direction (panel A). However, the relative change was the same, as shown by the lack of change in the $\theta_L/\theta_T$ ratio. A comparison of measured values of $\theta_T$ and the theoretically derived values of $\theta_T$ (derived from the known values of $\theta_L$) are shown in panel B. We interpreted the excellent correlation between the measured and computed values of $\theta_T$ (reflecting the constancy of $\theta_L/\theta_T$) to indicate that changes in membrane ionic properties either do not affect the cytoplasmic or junctional resistivities, or that they are changed in the same proportion. Combining our results with those of Weidmann (1970), we conclude that neither resistivity is altered, and that

FIGURE 2. Predictions of the anisotropic propagation model. The relative changes in the specific axial resistivities (A), the longitudinal and transverse conduction velocities (B), and the $\theta_L/\theta_T$ ratio (C) are shown for separate relative increases of the cytoplasmic resistivity (column 1), the junctional resistivity (column 2), and for equal relative increases in both the cytoplasmic and junctional resistivities (column 2).
the assumptions of the anisotropic propagation model are justified. We also concluded that, following an abrupt increase in the stimulus frequency, there is no change in the \( \theta_L/\theta_T \) ratio of the first beat at the new frequency even if that beat occurs before there is full recovery from inactivation of the sodium mechanism (Gettes and Reuter, 1974).

**Steady State Variation of Conduction Velocity with Stimulus Frequency**

Figures 4 and 5 show the measurement of the steady state variation of conduction velocity with stimulation frequency in ventricular muscle along axes parallel to ("longitudinal") and perpendicular to ("transverse") the long cell axis. In this and in the following experiments, the stimulus interval was varied between 1000 and 300 msec (60 to 200/min) in 50- to 200-msec steps. At each value, the stimulus interval was held constant for at least 3 minutes. It was assumed that the process(es) altering the velocity had reached steady state by this time, since there were no visible changes in the recorded waveforms during the following 30 seconds. The velocities along both axes decreased monotonically as the stimulus interval was shortened (Fig. 4A), with a much larger percentage decrease in the transverse direction as compared with the longitudinal direction. The relative variation of \( \theta_T \) with stimulus interval was well fitted by a single exponential; the variation of \( \theta_L \) was so small we were unable to define its functional dependence.

Changes in conduction velocity produced by various interventions (including increased stimulus rates higher than those used in this study) usually have been attributed to mechanisms involving the membrane ionic mechanisms of depolarization. We therefore checked to see whether the observed velocity

![Figure 3](image-url)

**FIGURE 3.** Anisotropic conduction velocities of premature action potentials in ventricular papillary muscle. In panel A, the functional refractory period (FRP) is identified by the vertical bar. In panel B, the experimental values of \( \theta_T \) are plotted against the theoretically derived values of \( \theta_T \) that were computed from the measured values of \( \theta_L \).

![Figure 4](image-url)

**FIGURE 4.** Steady state variation of the anisotropic conduction velocities as a function of the stimulus interval. Each experimental point represents an average value from three measurements. The longitudinal and transverse conduction velocities, \( \theta_L \) and \( \theta_T \), are shown in A. Quantities associated with depolarization are shown below: B = resting potential, C = \( V_{max} \) during longitudinal (L) and transverse (T) propagation, and D = \( V_{max} \) during longitudinal and transverse propagation.
change could be accounted for by changes in quantities associated with depolarization: the rest potential, the maximum rate of rise of the action potential $V_{\text{max}}$, and the time constant of the foot of the action potential $T_{\text{foot}}$ (Vaughn Williams, 1959; Viersma et al., 1968a; Dominguez and Fozzard, 1970; Chen and Gettes, 1976). A single microelectrode impalement provided the intracellular potentials necessary to measure these quantities; two extracellular stimulating electrodes were arranged to initiate propagation either along the longitudinal or transverse axes at the single impalement site. Figure 5A shows typical intracellular records from which the rest potential, $T_{\text{foot}}$, $V_{\text{max}}$, and action potential duration were extracted. Panel B shows the numerically derived (unsmoothed) first derivative of the upstrokes for propagation in the transverse ($\theta_T$) and longitudinal ($\theta_L$) directions. No change was visible in the derivative as the cycle length was varied. As can be seen in panels B–D of Figure 4, we observed no significant change in the rest potential, $T_{\text{foot}}$, or $V_{\text{max}}$ that could account for the change in the transverse velocity. The lack of change in the rest potential and in the upstroke of the action potential is consistent with the results of Hoffman and Suckling (1954) in dog ventricular muscle for stimulus intervals longer than 272 msec (stimulus frequencies less than 220/min).

**Figure 5.** Steady state action potentials and their derivatives at different stimulus intervals. Two stimulating electrodes were positioned to produce propagation along the longitudinal and transverse axes at a single intracellular recording site. The action potentials in panel A propagated in the transverse direction. In panel B, the derivatives of the upstrokes at each steady state are shown for propagation in the transverse ($\theta_T$) and longitudinal ($\theta_L$) directions; note the constant amplitude. The waveforms are photographs of an analog display of waveforms recorded digitally (10,000 samples/sec); derivatives were obtained by numerical differentiation without smoothing. All the records in the figure are from a single impalement.

Whereas the frequency-dependent changes in conduction velocity occurred without any significant change of depolarization, there were changes in the repolarization phase of the action potential. We therefore investigated the relationship between the anisotropic propagation velocities and repolarization as a function of stimulus frequency. Colatsky and Hogan (1980) demonstrated in dog Purkinje fibers an exponential relationship between the action potential duration and the diastolic interval at steady state with different frequencies. They analyzed their data as a function of diastolic interval because voltage clamp experiments by Hauswirth et al. (1972) have suggested that the duration of the action potential depends most directly on the preceding diastolic period. To compare our results with those of Colatsky and Hogan, we measured both the steady state action potential duration and conduction velocities as a function of the diastolic interval, defined as the time from the end of the action potential to the onset of the next depolarization.

Figure 6 shows a typical result, representing data from the same experiment illustrated in the preceding two figures. The absolute values of action potential duration, $\theta_T$, and $\theta_L/\theta_T$ are plotted as a function of diastolic interval (column 1) and of stimulus frequency (column 2). We found an exponential relationship between the action potential duration and diastolic interval and a linear relationship between the action potential duration and stimulus frequency, relationships (in ventricular muscle) of the same form as those found in Purkinje fibers by Colatsky and Hogan (1980). The conduction velocity in the transverse direction (panel B) changed in the same way as the action potential duration; $\theta_T$ also varied exponentially as a function of the diastolic interval and linearly as a function of the stimulus frequency. Also, the velocity ratio (panel C) decreased exponentially as a function of increasing diastolic interval and linearly as a function of decreasing stimulus frequency (reflecting the minimal change in velocity along the longitudinal axis of the fibers). There was a high correlation between the steady state velocity in the transverse direction and the action potential duration ($r = 0.98$, $P < 0.0001$, $t = 28.5$), as would be expected from their similar dependence on stimulus interval (Fig. 6, A and B).

**Effects of Sudden Changes in Stimulus Frequency**

As we performed the preceding experiments, it became apparent that—when the stimulus frequency changed abruptly—the velocity along the transverse axis of the fibers (as well as the action potential duration) underwent considerable change before stabilizing at the new frequency. Typical results of the time course of these transient changes are demonstrated in Figures 7 and 8. At each new frequency, the first digital sampling of the waveforms occurred 3–7 seconds after the abrupt change in frequency. As noted above (Fig. 3), other measurements...
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Figure 6. Steady state action potential duration (A) in ventricular muscle, propagation velocity along the transverse axis of the fibers (B), and the anisotropic velocity ratio $\theta_L/\theta_T$ (C) as a function of diastolic interval (column 1) and as a function of stimulus frequency (column 2). The data are shown as a function of both diastolic interval and stimulus frequency for purposes of reference with the data of Colatsky and Hogan (1980).

Demonstrated no change in the $\theta_L/\theta_T$ ratio of the first beat at a higher frequency of stimulation.

Typical plots of the action potential duration, $\theta_T$, and $\theta_L/\theta_T$ vs. time following an abrupt increase in the stimulation frequency of the papillary muscle are shown in Figure 7. Each increase in frequency followed an interval of 3–6 minutes during which the preparation was allowed to reach steady state at the basic frequency of 60/min. During the first 20 seconds after the shift to a higher frequency, the action potential duration shortened rapidly, more shortening accompanying greater increments in the stimulation frequency (panel A). Thereafter, the action potential duration decreased slowly or did not change measurably; the prolonged slow decrease was more prominent for the larger increments in stimulation frequency. The experimental points were fitted with exponential curves of the form $y = A + Be^{Ct}$ in a manner similar to that of Gibbs et al. (1963) for their measurements of the time course of the area of the action potential following an abrupt change of stimulation frequency in rabbit ventricular muscle.

Changes in velocity along the longitudinal axis were minimal, and frequently no change in $\theta_L$ was detected. However, $\theta_T$ decreased rapidly following a step increase in frequency (panel B). The rapidity with which $\theta_T$ decreased and the extent of the decrease were related to the magnitude of the increment in the stimulation frequency. During the initial 20–40 seconds at the new frequency, there was a time lag in the relative decrease of $\theta_T$ in comparison to the rapid and immediate decrease in the action potential duration. However, both decreased slowly during the remainder of the 3-minute measurement period. At high frequencies (e.g., 218/min), two components were apparent and the data in the 3-minute interval were fitted with two exponentials. Since there was little change in $\theta_L$ when the stimulation frequency was increased suddenly, the $\theta_L/\theta_T$ ratio increased by an amount and time course similar to that of $\theta_T$ (Fig. 7C).

Figure 8 shows the recovery of the action potential duration, $\theta_T$, and the $\theta_L/\theta_T$ ratio after the stimulus frequency was reduced abruptly to 60/min. The preparation had achieved a steady state at each of the prior elevated frequencies before the step reduction. During the first 20 seconds, the action potential duration lengthened rapidly (panel A). The magnitude of the rapid increase in action potential duration was related to the prior elevated frequency. Thereafter, the action potential duration continued to increase slowly until it reached a new steady state at the original value corresponding to the basic frequency of 60/min. Two exponentials were required to fit the recovery of the action potential duration. This result differs from that of Gibbs et al. (1963), who found only a single exponential in the recovery of the action potential area after an abrupt reduction of stimulation frequency in rabbit ventricular muscle.
Increments in velocity along the longitudinal axis were small or not measurable (0 to 3%) in contrast to clear increases in velocity along the transverse axis of the fibers. \( \theta_T \) increased rapidly during the first 20–60 seconds (Fig. 8B) and, thereafter, more slowly, until it reached a steady state velocity at the basic 60/min frequency. The change in \( \theta_T \) with time after the reduction in frequency was fitted by a single exponential. Although the duration of the action potential increased quickly to near baseline values, it took many more cycles to restore \( \theta_T \). The \( \theta_L/\theta_T \) ratio (panel C) decreased in association with the increase in \( \theta_T \), and followed a similar time course since there was such a small relative change in \( \theta_L \).

Comparing the behavior of the velocity ratio \( \theta_L/\theta_T \) (Fig. 6C) with the predictions of our simplified propagation model (Fig. 2), we concluded that both the steady state and transient changes in anisotropic conduction velocities must have been due to a decrease in the coupling resistance between cells with decreasing stimulus frequency. We also concluded that the anisotropic velocity changes were not caused by a modification of the membrane ionic process causing depolarization, but were, instead, more closely related to repolarization. We therefore proceeded to test other interventions known to affect repolarization to see whether the correlation between the velocity ratio and action potential duration held for them as well.

Effects of Inactivity

Cohen et al. (1976) showed that when repetitive stimulation is initiated after prolonged pauses in rabbit ventricular muscle, regional differences in the shortening of the action potential develop rapidly. They attributed the differences in shortening to regional differences in the response of the Na-K pump to repetitive activity, since the differences became smaller after blocking the pump activity by cooling and ouabain. Hence, it was of interest to determine whether periods of inactivity also affect the anisotropic conduction velocities, and, if so, to see if there is again a close relationship between the changes in velocity and in action potential duration after the initiation of repetitive activity.

Figure 9 shows characteristic plots of action potential duration, \( \theta_T \), and the \( \theta_L/\theta_T \) ratio vs. time following the onset of stimulation after pauses of 30–90 seconds. Except for the periods of inactivity, the papillary muscle was stimulated at a frequency of 60/min. The greatest duration occurred with the first beat (panel A), and that duration increased in relation to the length of the preceding pause. The duration decreased

\[ \frac{\theta_L}{\theta_T} \] (C) are plotted as a function of time after an increase in the rate of stimulation to various rates shown in the figure after a steady state had been reached at 60/min. In each plot, the experimental points represent values normalized to the original steady state value at 60/min (time zero). The exponential equations that fit the experimental points (solid lines) accompany each plot. The action potential durations were measured from the extracellular electrograms (see Methods).
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\[
y(t) = 99.06 - 5.92e^{-3.74e-8.75e^{-0.020t}} \\
y(\tau) = 101.53 - 15.51e^{-0.095\tau} - 9.61e^{-0.010\tau} \\
y(\xi) = 330.05 - 26.44e^{-1.09e-240.39e^{-0.009\xi}}
\]

Effects of Ouabain

Three papillary muscle preparations were exposed to a gradual increase in ouabain concentration by switching the flow of perfusate entering the tissue bath from the control solution without ouabain to one containing \(2 \times 10^{-6}\) M ouabain, a concentration of ouabain known to produce an irreversible increase in axial resistance (Weingart, 1977; Ando et al., 1981). The preparations were stimulated at a constant rate of 60/min, and waveforms were recorded periodically as the ouabain concentration increased over a 20-minute period (the equilibration time of the bath). Photographs showed that the distance between the extracellular electrodes did not change as the contractions became more vigorous with the infusion of ouabain. A typical response to the ouabain is shown in Figure 10. During the first 20 minutes, only minimal changes could be detected in \(\theta_L\), \(\theta_T\), or in the action potential duration and velocity. However, at 20-25 minutes in all preparations, both the action potential duration and the velocity along the transverse axis of the fibers began to decrease, while the velocity along the longitudinal axis of the fibers showed little change. For the next several minutes, the action potential duration and \(\theta_L\) continued to decrease and the \(\theta_L/\theta_T\) ratio increased rapidly during the first 20-30 seconds of repetitive stimulation, and thereafter it continued to decrease slowly toward the original steady state value. There were no observable changes in the foot of the action potential or in the rate of rise of the upstroke as a result of the periods of inactivity. For the first beat, \(\theta_T\) increased slightly above the steady state value (panel B), but the magnitude of the increase was not systematically related to the length of the preceding pause. After the first beat, however, \(\theta_T\) either increased rapidly or remained stable, the magnitude of the increase being related to the length of the preceding pause. \(\theta_T\) reached its peak value during the first 10-20 seconds of repetitive activity, while the action potential duration decreased rapidly. Depending upon the magnitude of the \(\theta_T\) increase, \(\theta_T\) then decreased rapidly during the next 20-40 seconds and thereafter declined slowly toward the original steady state value. There were minimal (1-2%) or no changes in the velocity along the longitudinal axis of the fibers following periods of inactivity (not shown). Accordingly, the time course and magnitude of the changes in \(\theta_L/\theta_T\) (panel C) were governed by the time course and magnitude of \(\theta_T\).

The velocity ratio \(\theta_L/\theta_T\) (C) are shown as a function of time during the 3-minute interval following the reduction of the stimulus frequency from various initial rates to 60/min. The values at time zero are slightly higher than the values at the end of the 3 minutes at a high rate in Figure 7C. The slight increase reflects a continued change in \(\theta_L/\theta_T\) that occurred during an additional 3-minute interval before the stimulus frequency was decreased. The data points for each quantity are normalized to the steady state value at the stimulus frequency of 60/min. The action potential durations were measured from the extracellular electrograms (see Methods).
Figure 9. Action potential and velocity changes produced by initiating repetitive activity after pauses in the stimulation of ventricular papillary muscle. Before and after the pauses, the rate of stimulation was maintained constant at 60/min. In each plot, the experimental points are normalized to the steady state value at 60/min. The first experimental point in each plot (time zero) is the first beat after the pause. The action potential durations were measured from intracellular action potentials.

markedly. Irregularities then appeared in the extracellular waveforms, indicating a local change in the sequence of impulse spread. In one preparation, an increase in the stimulation frequency from 60 to 120 beats/min after 23 minutes of perfusion resulted in an abrupt change from a normal pattern of excitation with smooth extracellular waveforms to a dissociated pattern of local excitation with irregularities in the waveforms; the normal pattern returned when the frequency was returned to 60/min.

Figure 10. Changes in the anisotropic conduction velocities (A), the \( \theta_1/\theta_2 \) ratio (B), and the action potential duration (C) in ventricular papillary muscle produced by ouabain. The preparation was exposed to a gradual increase in ouabain concentration over a 20-minute period (the equilibration time of the bath) by switching the perfusate entering the bath at time zero from the control solution to one containing 2 \( \times 10^{-6} \) M ouabain. The rate of stimulation was maintained constant at 60/min throughout the experiment. Action potential durations were measured from intracellular recordings.

Experimental points are normalized to the steady state value at 60/min. The first experimental point in each plot (time zero) is the first beat after the pause. The action potential durations were measured from intracellular action potentials.
Discussion

The measurements described above show that the distribution of propagation velocities of action potentials in cardiac muscle—a distribution known to be anisotropic (Clerc, 1976; Spach et al., 1981)—is altered transiently and in the steady state by the rate and pattern of stimulation and by other interventions such as the application of ouabain. The relative amount of velocity change varies with direction of propagation in the tissue and is greatest in the transverse direction, perpendicular to the long fiber axis. Assuming a simplified anisotropic propagation model representing the internal current pathway as an alternating sequence of cytoplasmic and junctional resistance, the relative contributions of each depend on the direction of propagation; e.g., the cell couplings make a larger contribution to the internal resistance in the transverse direction than in the longitudinal direction. The observed increase in the ratio \( \theta_L/\theta_T \) with increased stimulation rate could then be due either to a decrease in cytoplasmic resistance (by approximately one half) or to an increase in cell coupling resistance (by approximately two times). A change of this magnitude in cytoplasmic resistance is unlikely (Weingart, 1977), whereas the cell coupling resistance is known to be variable (Loewenstein, 1966; De Mello, 1975). We therefore concluded that the velocity changes we observed were due to changes in cell coupling resistance.

We have assumed in the interpretation of our results that there were no significant changes in cell volume as the rate of stimulation was changed. This conclusion is consistent with the theoretical analysis of Jakobsson [(1980) see his Fig. 2A], who demonstrated that the resting potential and the cell volume remain constant for increases of [Na\(^+\)] up to 20 mM/liter, a value above the maximum concentration of Na\(^+\) measured by Fozzard and Sheu (1981) in ventricular muscle with increases of the rate of stimulation similar to those reported in this paper.

Brediktis et al. (1981) observed an increase in input resistance and a decrease in the space constant following 15 minutes of stimulation at very high rates (600-900/min) in atrial muscle. They attributed the changes they observed to alterations in cell coupling, but at these prolonged very high rates one might expect changes in rest potential and cell volume. By the use of autonomic blocking agents, they concluded that electrorelease of autonomic transmitters was not responsible for the effects observed. We did not apply autonomic blocking agents but did use point stimulation (50 μm diameter unipolar stimulating electrode) and measured waveforms several hundred micrometers away from the stimulation site, i.e., beyond the expected region of electrorelease of neural transmitters.

None of the variables usually associated with the membrane ionic mechanism of depolarization—resting potential, \( V_{\text{max}} \), or \( T_{\text{foot}} \)—showed enough variation to account for the observed changes in velocity. These associations are not dependent on the specific ion responsible for depolarization and, thus, the conclusion applies equally to sodium and calcium currents. Both transient and steady state velocity changes did, however, correspond to changes in action potential duration in response to several kinds of interventions. For example, the anisotropic velocity ratio \( \theta_L/\theta_T \) varied with the rate of stimulation in steady state, following an abrupt change in rate, or following a pause in a manner similar to the variation of action potential duration in similar experiments (Gibbs et al., 1963).

Is it possible that there is a common factor linking the control of action potential duration and the coupling resistance between cardiac cells? First, it is well known that cell-to-cell coupling is influenced by \( [\text{Ca}^{++}] \), (Loewenstein, 1966; De Mello, 1975) or a soluble intermediate for \( [\text{Ca}^{++}] \), (Johnston and Ramon, 1981; Perachia et al., 1981). Steady state changes in the action potential duration are also associated with changes in \([\text{Na}^+]\) and \([\text{Ca}^{++}]\) (Bassingthwaighte et al., 1976; Carmeliet, 1977; Colatsky and Hogan, 1980). Changes in \([\text{Na}^+]\) and \([\text{Ca}^{++}]\), are known to be linked, probably through a Na-Ca exchange mechanism (Reuter and Seitz, 1968; Carafoli et al., 1974), and an increasing frequency of stimulation of cardiac muscle results in an increase of \([\text{Na}^+]\) (Conn and Wood, 1959; Langer, 1967; Cohen and Fozzard, 1979) and of \([\text{Ca}^{++}]\) (Fozzard and Sheu, 1981). Fozzard and Sheu (1981) also showed that the internal concentrations of sodium and calcium required 2-3 minutes to come to a new equilibrium after a change in stimulation frequency, approximately the same time taken for the velocity changes in our experiments.

The internal calcium and/or sodium concentrations may therefore be common factors between the changes in velocity and action potential duration. Theoretical calculations have suggested that the changes in internal sodium concentration with stimulation rate are due to a shift in the balance between passive sodium leakage into the cells and active transport out of the cells by the Na-K pump. Using a thermodynamically-constrained model of Na-K active transport (Chapman et al., 1979), Johnson et al. (1980) showed that the action potential duration and internal sodium concentration would require 2-5 minutes to come to a new steady state after an abrupt change in stimulation rate, about the same equilibration time we observed for the propagation velocities. Eisner et al. (1981) observed a time constant of 51 seconds for the decay of internal sodium concentration following a voltage clamp step. Our observation of velocity changes with the application of ouabain is further evidence for the involvement of the Na-K pump, since this intervention is known to inhibit Na-K ATPase activity (Lee and Klaus, 1971). Weingart (1977) observed a biphasic change in axial resistance following the application of ouabain at doses similar to those we applied. It is difficult, however, to correlate his results with ours, since he did not indicate the direction of propagation with respect to the cell orientation. He also did not map excitation locally at the sites of measurement, which we found necessary to identify...
the appearance of changes in the activation sequence.

Implications for Intracellular Events

Based on the anisotropic propagation model, the rapid changes in the $h_t/h_T$ ratio indicate that the coupling resistance between cardiac cells can be altered within the course of a few action potentials, within a second or two. There was, however, a time lag in the change of the $h_t/h_T$ ratio compared to that of action potential after each shift to a new frequency. This time lag was most prominent following the onset of repetitive activity after long pauses (Fig. 9). The existence of the time lag suggests that, in addition to the activity of the Na-K pump, there may be other steps in the intracellular communication between the surface membrane and the intercellular junctional membrane. In a variety of physiological processes, Ca++ seems to be an intermediate between events at the cell surface and those in the cell interior (Loewenstein and Rose, 1978). Rose and Loewenstein (1975) demonstrated by a local rise in Ca++ concentration in Chironomus salivary glands that diffusion of Ca++ through the cytosol is slow. The increase of the $h_t/h_T$ lag after a quiescent period suggests that the normal repetitive mechanical motion of the heart may speed up the intracellular mixing of Ca++.

Calmodulin may also be involved as an intracellular mediator between the surface membrane and the junctional membrane, similar to its known role in the regulation of other intracellular events (Cheung, 1980). Johnston and Ramón (1981) demonstrated, in internally perfused crayfish segmented axons, that electrotonic uncoupling depends on the existence of an intracellular intermediate for Ca++ or H++. Peracchia et al. (1981) suggested that the cytoplasmic intermediate is calmodulin, since a calmodulin inhibitor prevents gap junction crystallization and electrical uncoupling. Calmodulin is a known enzyme activator, so changes in the configuration of the tubules of the junctional membrane also may involve an enzyme located along the nexus. Indeed, an intense reaction product to ATPase activity has been demonstrated along the nexus in dog ventricular muscle (Sommer and Spach, 1964) in contrast to some of the other parts of the intercalated disc.

Implications for Rate-Dependent Conduction Disturbances

We wish to suggest that the cumulative effects of increased stimulation frequency on the conduction velocity, termed “fatigue” by Lewis and Master (1925), is due to the activity of the Na-K pump as a common metabolic factor for the control of the conduction velocity by the regulation of the internal cellular ionic concentrations which, in turn, affect both the membrane currents (passive and active) and the coupling resistance between cardiac cells.

The effects of the past history of action potentials on conduction velocity are most pronounced in the AV node. Billette et al. (1976) noted that it has been difficult to establish an explanation for the cycle

length-dependent delay of the AV node, and Heethaar et al. (1981) pointed out that the anatomic basis for the time-dependent delay is not clear. The delay at high rates in the AV node has been attributed entirely to cell membrane effects, most probably a marked prolongation in the recovery of the depolarizing currents from inactivation (Haas et al., 1971). The "passive" electrical properties, including the coupling resistance between cells, have been thought to remain constant (Billette et al., 1976). However, De Mello (1977) found that acetylcholine reduces the space constant of the AV node secondary to a reduction in the resistance of the surface cell membrane. Ideka et al. (1980) recently demonstrated that ouabain and high calcium perfusate reduce electrical coupling between AV node cells. Our results suggest that transient and steady state changes in the coupling resistance between cells is a generalized property of cardiac muscle. Since the changes in velocity were greatest where there was a large contribution to the internal resistance by the coupling resistance between cells, it is likely that similar coupling changes contribute to the cumulative effects on the conduction time across the AV node (fatigue) when there is an increase in the stimulus frequency.

Finally, the results indicate there may be circumstances in which a slow heart rate may be necessary for maintaining cell-to-cell coupling in the heart. In disease states in which there is diminished capacity of the Na-K pump, the intracellular concentration of Na+ and Ca++ may rise higher than normal, producing an abnormally high cell-to-cell coupling resistance, with the excitation pattern being especially vulnerable to increases in heart rate. Clinically, in the presence of acute ventricular ischemia, maintenance of a slow heart rate is a major factor in the prevention of cardiac conduction disturbances, and, in general, cardiac arrhythmias associated with ventricular ischemia are highly rate dependent (Scherlag et al., 1974; EI-Sherif et al., 1977). The dynamic changes in cell-to-cell coupling resistance indicated by the present results may be an important factor contributing to the cumulative rate-dependent depressive effect on conduction that is considered important in the development of conduction block secondary to myocardial ischemia (Lazzara et al., 1975).

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References


Kositsky GI, Khaspekova NB, Kobrin VI (1972) Synchronous recording of action potentials of separate myocardial cells in frog cardiac muscle. Cardiology 9: 18-24

Langer GA (1975) Calcium exchange in dog ventricular muscle: Relation to frequency of contraction and maintenance of contractility. Circ Res 17: 78-89


Moe GK, Mendez R (1951) The action of several cardiac glycosides on conduction velocity and ventricular excitability in the dog heart. Circ Res 4: 729-734
Sommer JR, Spach MS (1964) Electron microscopic demonstration of adenosinetriphosphatase in myofibrils and sarcoplasmic membranes of cardiac muscle of normal and abnormal dogs. Am J Pathol 44: 491-505

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