Brief Communication

Effects of Increasing Intercellular Resistance on Transverse and Longitudinal Propagation in Sheep Epicardial Muscle

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Propagation in cardiac muscle is faster in the longitudinal than in the transverse axis of the cells. Yet, as a result of the larger upstroke velocity of action potentials propagating transversely, it has been suggested that longitudinal propagation is more vulnerable to block. To study the relation between conduction velocity and maximal upstroke velocity ($V_{\text{max}}$), as well as the time course of conduction delay and block in the transverse vs. longitudinal direction, thin square pieces of sheep epicardial muscle were superfused with the cellular uncoupler heptanol (1.5 mM). Action potentials were recorded with microelectrodes at opposite corners of the preparation while stimulating alternately in the longitudinal or transverse direction with bipolar electrodes located at contralateral corners. In all cases, block occurred more promptly for transverse than for longitudinal propagation. The decrease in conduction velocity was greater than expected for $V_{\text{max}}$ decay and, in some cases, $V_{\text{max}}$ increased while conduction velocity decreased. In the presence of high grade conduction impairment, foot potentials appeared and the upstrokes became "notched." We conclude that when intercellular coupling is impaired, transverse propagation is more vulnerable to block, and need not be dependent on changes in $V_{\text{max}}$. (Circulation Research 1987; 60: 780-785)

A ction potential propagation in cardiac muscle is faster along the longitudinal axis of the cells than in the transverse direction. This anisotropy can be explained on the basis of a higher intercellular resistivity for the spread of current in the direction perpendicular to the long axis of the muscle cell bundles. 1-3

In 1981, Spach et al 1 demonstrated that this anisotropic property of cardiac muscle is also associated with differences in action potential configuration, depending on whether the excitation wavefront propagates transversely or longitudinally. More importantly, greater action potential upstroke velocity ($V_{\text{max}}$) and amplitude, together with a faster foot potential, were seen during transverse propagation. Based on experiments in which the active properties of the cell membrane were altered by the application of premature stimulation, these authors 1,4 have also suggested that, even though under normal conditions propagation velocity is faster in the longitudinal direction, the higher $V_{\text{max}}$ provides a greater safety factor for transverse propagation; that is, longitudinal conduction is more vulnerable to block.

In the experiments presented here the role of cell-to-cell coupling on anisotropic conduction was tested by exposing thin sheep epicardial muscle preparations to the uncoupler heptanol, 1,6 while alternately stimulating in the transverse and longitudinal directions. Our results demonstrate that the effects of changing the specific nexal resistance are different from those observed when conductance is decreased (see ref. 7); i.e., transverse propagation is more sensitive to electrical uncoupling, regardless of the directional differences in $V_{\text{max}}$ observed.

Materials and Methods

Nine epicardial muscle preparations were used for the study. Young sheep (7-20 kg) were anesthetized with sodium pentobarbital (36.5 mg/kg i.v.). The hearts were rapidly excised and placed in warm, oxygenated Tyrode solution. After clearly identifying the fiber orientation by gross anatomical inspection, small, rectangular pieces of epicardium (about 5 x 5 x .6 mm) were cut with a razor blade, carefully following the longitudinal and transverse axes of the fibers. Suitable preparations were immediately transferred to a Plexiglas chamber and continuously superfused with oxygenated Tyrode solution. The composition of the solution was (in mM): NaCl 130.0, KCl 4.0, NaHCO3 24.0, NaH2PO4 1.2, MgCl2 1.0, MgCl2 1.0, CaCl2 1.8, and glucose 5.6. Solutions were saturated

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with a gas mixture of 95% O₂ and 5% CO₂, temperature was maintained at 37 ± 0.5°C, and the pH was 7.4.

Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl and connected to a WPI 750 dual microprobe system. V\textsubscript{max} was obtained by feeding the amplified membrane potential signal to an electronic differentiator (Tektronix, Beaverton, Ore., model AM501). Two pairs of thin (100 μm) bipolar electrodes (S\textsubscript{1} and S\textsubscript{2}) were connected to separate stimulators (Frederick Haer, Brunswick, Me., model p6i) and placed on the surface of the tissue, at opposite corners from each other (see Figure 1, inset). An intracellular microelectrode (M\textsubscript{1}) was used to record in a third corner, thus forming an imaginary triangle (S\textsubscript{1} - M\textsubscript{1} - S\textsubscript{2}). In this manner, the relation of S\textsubscript{1} to the site of recording (M\textsubscript{1}) was parallel to the orientation of the fibers (longitudinal propagation), whereas S\textsubscript{2} - M\textsubscript{1} propagation was transverse. The two stimulators were synchronized so that each unit had a basic cycle length (BCL) of 1,500 milliseconds but their firings were out of phase from each other by 750 milliseconds, therefore the effective BCL was 750 milliseconds but the site of stimulation alternated in a beat-to-beat manner. All signals were displayed on a Tektronix 565 oscilloscope and photographed with a Grass C4R (Quincy, Mass.) kymographic camera.

In 3 experiments, a second microelectrode (M\textsubscript{2}) was placed in the corner opposite to M\textsubscript{1} (Figure 1 inset). Under these conditions, action potentials induced by S\textsubscript{1} propagated longitudinally toward M\textsubscript{1} but transversely to M\textsubscript{2}, whereas the reverse was true for S\textsubscript{2} (i.e., longitudinal to M\textsubscript{1}, but transverse to M\textsubscript{2}). Propagation was constantly monitored in the control and during superfusion with 1.5 mM heptanol. In 6 experiments, V\textsubscript{max} was also recorded.

Conduction time was measured as the interval between the stimulus artifact and the peak of the V\textsubscript{max} signal of M\textsubscript{1} or M\textsubscript{2}. In those experiments where V\textsubscript{max} was not recorded, the time at which the action potential upstroke reached half its maximal amplitude was used as the point of reference. Distances between the stimulating electrodes and the sites of impalement were measured with a calibrated stereomicroscope eyepiece.

**Results**

*Anisotropic Conduction Block*

In all heptanol experiments, conduction block occurred in the transverse direction, while longitudinal propagation was maintained for several minutes. Indeed, although the time course of heptanol (1.5 mM) effects varied from one experiment to another, the time to transverse block was 27.8 ± 2.6 (mean ± SEM) minutes, whereas longitudinal block was observed at 43.8 ± 3.2 minutes (p < 0.01; n = 9). Figure 1 illustrates an example. Panels A and B show superimposed recordings of V\textsubscript{max} (upper trace) and transmembrane potential (lower trace) recorded at M\textsubscript{1} in response to S\textsubscript{1} (action potential labelled L) and S\textsubscript{2} (action potential labelled T); Panel A is the control. Conduction velocity was 0.603 m/sec in the longitudinal direction (i.e., from S\textsubscript{1} to M\textsubscript{1}) and 0.135 m/sec in the transverse direction (i.e., from S\textsubscript{2} to M\textsubscript{1}). As previously reported by Spach et al., control V\textsubscript{max} was greater during transverse propagation. This was true for all our experiments in which V\textsubscript{max} was recorded. Panel B shows 4 superimposed oscilloscope traces obtained at 59 minutes of heptanol superfusion, several beats before T block occurred. Conduction velocity (θ), measured at the longest stimulus–response intervals, decreased to 0.246 m/sec (59.2%) and 0.068 m/sec (49.6%) for L and T, respectively. Changes in V\textsubscript{max} varied widely from one experiment to another, and also from time to time within the same experiment (see Figure 3). In the case shown in Figure 1, V\textsubscript{max} decreased more for T than for L. Just prior to T block (longest S\textsubscript{2}-M\textsubscript{2} interval), V\textsubscript{max} increased in L but slightly decreased in T. A similar behavior was observed in 3 of our experiments. In 5 of the experiments, V\textsubscript{max} was higher for T than for L just before transverse block occurred.

To demonstrate the homogeneity of the heptanol-
induced effects on transverse vs. longitudinal propagation, a second microelectrode (M2; Figure 1, inset) was inserted in the corner opposite M1. The records presented in Panel C of Figure 1 were taken 15 minutes after those in Panel B. The upper trace is from M2 and the lower trace from M1, and two superimposed sweeps are shown. When S1 fired, propagation occurred only in the L direction, as demonstrated by the simultaneous recording of an action potential by M1 and resting potential by M2. When S2 fired, the reverse situation was observed; a propagated action potential was recorded by M2 (longitudinal to S2), but no activity was recorded at M1, i.e., transverse block occurred.

Results from 1 of 2 preparations in which two microelectrode impalements (M1 and M2) were maintained throughout the experiment are illustrated graphically in Figure 2. Conduction velocity (m/sec) is plotted on the ordinates as a function of time of heptanol superfusion (minutes), which is plotted on the abscissae. Panel A shows M1 results (i.e., S1 generates L conduction and S2, T conduction), and Panel B presents data collected by M2. Clearly, the shapes of the curves in both panels are nearly the same. For both recording sites, transverse block occurred at similar times (64 minutes in M1 and 60 minutes in M2), while L propagation persisted for several more minutes. Additionally, at both sites conduction velocity decayed in a stepwise manner with changes being separated by intervals in which relatively brief steady levels were maintained. Similar results were obtained in all single and double impalement experiments.

Conduction Velocity and $V_{\text{max}}$

Cable theory predicts a linear relation between the squared conduction velocity and $V_{\text{max}}^2$, and some authors have suggested that such a relation holds in mammalian ventricular muscle under several circumstances. If a linear relation indeed exists under the experimental conditions, then the ratio $V_{\text{max}}^2/\theta^2$ (considering the relative values of those variables to their respective controls) should remain constant throughout the stepwise development of heptanol-induced block. A ratio greater than 1 would mean that the decrease in $\theta^2$ (or increase in $V_{\text{max}}$) is proportionately greater than the changes in the other variable, or that both variables changed in opposite directions (as in Figure 1B). In Figure 3, Panel A shows the time course of heptanol-induced changes of $V_{\text{max}}$ during longitudinal (open squares) and transverse (closed squares) propagation; Panel B illustrates the respective $V_{\text{max}}^2/\theta^2$ ratios also as functions of time. The changes in $V_{\text{max}}$ were somewhat unpredictable (panel A), and the decrease in conduction velocity was independent of $V_{\text{max}}$ (Panel B). Indeed, as in the experiment of Figure 1B, $V_{\text{max}}$ changes for L and T were opposite to each other. In Figure 3A, $V_{\text{max}}$ of the longitudinally propagated action potential showed a gradual increase, whereas it decreased for T. As shown in Figure 3B, the relation between $\theta^2$ and $V_{\text{max}}$ did not follow the linear relation described by other authors. In fact, in all experiments, the final $V_{\text{max}}^2/\theta^2$ ratio was greater than one for both L and T propagation.

Heptanol-Induced “Notches” in Action Potential Upstroke

The presence of “notches” in the action potential upstroke has been associated with conditions of high grade block and high axial resistivity in cardiac muscle. Such notches also developed in our experiments during heptanol superfusion, as illustrated in Figure 4. Panel A shows control action potential upstrokes for both L and T propagation. In Panel B, at 10 minutes of heptanol, conduction time had increased to 7.7 and 41.5 milliseconds for L and T, respectively, and a notch (arrow) was clearly apparent on the transverse action potential. Immediately thereafter (Panel C), transverse conduction was blocked. Finally, in Panel D, obtained at 35 minutes of heptanol superfusion and 20 minutes after T block had occurred, a two-step upstroke was clearly seen at a time when longitudinal propagation began to fail.
Discussion

There are two technical limitations to be considered in the interpretation of the results. First, in these experiments, conduction times were estimated from the interval between the stimulus artifact and the peak of the $V_{\text{max}}$ signal at the $M_1$ or $M_2$ recording site. This method probably introduced quantitative inaccuracies when attempting to calculate propagation velocity since the excitation wave actually starts at an unknown distance from the stimulating electrode, and there was some uncertainty about possible delays in activation at the stimulus site caused by heptanol-induced reduction in electrical coupling and current spread. Second, from the measurements it was impossible to verify the precise site of block or whether the direction of propagation remained constant with respect to fiber orientation during the development of uncoupling. The stepwise conduction velocity changes (see Figure 2) observed in the course of heptanol superfusion would lead one to speculate that the wavefront may have followed an increasingly tortuous pathway. However, additional measurements with multiple intracellular and extracellular recording sites will be required to confirm these findings and to determine accurately the site of block.

In spite of these limitations, the results show that an increase in intercellular resistance induced by heptanol can delay, and finally block, action potential conduction in sheep epicardial muscle. This effect is not causally related to a decrease in maximal upstroke velocity or to a heptanol-induced change in excitability, as has been demonstrated also in Purkinje fibers during superfusion with either heptanol or hypertonic solutions, or even with ouabain at a marginally toxic concentration ($1 \times 10^{-7} \text{M}$). In this paper, we further demonstrate that conduction block can occur in a clearly anisotropic manner and can possibly set the stage for longitudinal dissociation. Indeed, when cell-to-cell uncoupling occurs, propagation in the transverse direction can be blocked at a time when longitudinal propagation is maintained; that is, the margin of safety for propagation is greater along the longitudinal axis of the fibers.

In a normally excitable tissue immersed in a homogeneous extracellular fluid with low resistivity, successful propagation should occur if the amount of current offered to a given patch of membrane is large enough to bring that patch to threshold. This condition might not be fulfilled if the depolarizing current offered by the source to the recipient patch is too weak and/or the longitudinal resistance is too high. Hence, on the basis of our experiments, we suggest that increases in coupling resistance can have opposite directional effects to those attributed to decreases in the conductance of the ionic depolarizing currents. Accordingly, if starting conditions are such that coupling resistance is low and $V_{\text{max}}$ is higher for T than for L, it is
conceivable that block can occur more easily for an action potential propagating longitudinally than for one propagating transversely. Such a possibility has been partially supported by experimental results showing L block and T propagation in cardiac muscle exposed to high concentrations of potassium. On the other hand, if coupling resistance is increased homogeneously throughout the tissue, conduction must depend primarily on the ability of the current to flow from one cell to the next, regardless of the change in $V_{\text{max}}$. In this regard, computer simulations using one-dimensional arrays of Hodgkin and Huxley-type cells have shown that electrical uncoupling can lead to a conduction velocity decrease even in the presence of an increased $V_{\text{max}}$. Further simulations using two-dimensional arrays of simple double-conductance model cells have predicted that, when coupling resistance is increased homogeneously throughout the array, propagation should be more vulnerable to block transversely than longitudinally. Both theoretical predictions are borne out by our experimental results, which demonstrated additionally that transverse block could be achieved even in the presence of relatively small longitudinal propagation changes (see Figure 3). This anisotropic blockade can be explained readily by the higher initial level of axial resistance in the transverse direction.

Spach et al. have postulated that the higher $V_{\text{max}}$ value observed during transverse propagation is the result of the higher side-to-side resistivity of the cardiac muscle. Under these conditions, the electronic propagation of inward current associated with the action potential at each patch of membrane would be reduced, thus reducing the “leak” and leading to increases in the amplitude and velocity of discharges of the membrane capacitors of cells acting as current source. Computer simulations support this hypothesis. Theoretically, $V_{\text{max}}$ should increase as a result of electrical uncoupling. Yet the variations in $V_{\text{max}}$ that we observed during the superfusion with the uncoupler heptanol did not follow a constant pattern. In fact, in some experiments, $V_{\text{max}}$ tended to decrease during transverse propagation while increasing longitudinally in the course of heptanol superfusion, although occasionally the reverse was also observed (not shown). We attribute these variations in $V_{\text{max}}$ to several mutually antagonistic changes occurring simultaneously. First, from voltage clamp experiments in nerve, it is known that heptanol produces a 30% decrease in the sodium current by displacing the inactivation curve toward less negative levels. Second, the increase in axial resistivity itself should produce a decrease in the amplitude and rate of rise of the depolarizing electrotonic current offered to cells distal to the source, thus giving rise to “foot potentials” (observed in some of our experiments) and partially inactivating sodium channels in those cells. Both factors acting together would counteract the increase in $V_{\text{max}}$ associated with higher axial resistivity. The resulting $V_{\text{max}}$ change should therefore depend on the balance of all parameters involved. Accordingly, in the case of transverse propagation, since the initial side-to-side resistivity is already high, the predominant effect on $V_{\text{max}}$ could be a progressive decrease (see Figure 3) resulting mainly from heptanol-induced foot potentials and sodium channel blockade, whereas during longitudinal propagation a gradual $V_{\text{max}}$ increase could be observed. In either case, when coupling resistance increases to a critical range, propagating inputs arriving from different sources into a given cell would have different delays and produce “notches” in the action potential upstroke of that cell (see Figure 4).

In conclusion, changes in electrical coupling of ventricular cells have a markedly different effect on anisotropic propagation from those observed when active generator properties are altered. This may have important implications in the mechanisms of cardiac rhythm and conduction disturbances.

References

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Delmar et al. Coupling Resistance and Anisotropic Conduction


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