Propagating Depolarization in Anisotropic Human and Canine Cardiac Muscle: Apparent Directional Differences in Membrane Capacitance

A Simplified Model for Selective Directional Effects of Modifying the Sodium Conductance on \( V_{\text{max}} \), \( \tau_{\text{foot}} \), and the Propagation Safety Factor

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As yet there is no model or simulation that accounts for the anisotropic difference in the shape of the upstroke and safety factor of propagating cardiac action potentials: fast upstrokes occur with slow transverse propagation and slow upstrokes occur with fast longitudinal propagation. The purpose of this paper is to demonstrate, however, that a simplified cable model based on directional differences in the effective membrane capacitance predicts in detail the experimentally measured directionally dependent behavior of the upstroke in response to modification of the sodium conductance. Quinidine and lidocaine produced greater relative decreases in \( V_{\text{max}} \) and conduction velocity with longitudinal propagation than with transverse propagation, as predicted on the basis that the shape differences should produce an anisotropic distribution in the membrane uptake of sodium channel binding drugs. The simulation predictions of the effects of positive shifts of the take-off potential due to premature action potentials were also confirmed experimentally: there was a greater relative decrease in conduction velocity, \( V_{\text{max}} \), and \( V_{\text{amp}} \) with a greater increase in \( \tau_{\text{foot}} \) during longitudinal propagation than with transverse propagation. The major anisotropic differences in shape occurred when the take-off potential approached the least negative value that produced a propagated response. The extensive experimental verification of the results of a simplified model based on directional differences of effective membrane capacitance, combined with directional differences in effective axial resistivity, provides an initial quantitative basis for the anisotropic behavior of propagating depolarization in response to modification of the sodium conductance in cardiac muscle. Circulation Research 1987;60:206-219

When the conduction velocity of activation is modified by changing the internal resistance of a cell, classical continuous medium theory predicts that the shape of the action potential should not change. 1,2 For this reason, the mechanism underlying differences in the shape of the upstroke, excitability, and the safety factor of the cardiac action potential has long been attributed to a “depressed segment” or to a “spatial difference in the refractory period” (see Mines,4 applied in the “leading circle concept” of Allessie et al5). Recently, however, a major new mechanism of cardiac conduction disturbances was introduced with new evidence that the anisotropic passive electrical properties of cardiac muscle have an independent role in determining the shape of the upstroke, dependent on the direction of propagation to the orientation of the fibers.6,7 Fast upstrokes were found to be associated with low propagation velocities (in a direction transverse to the long cell axis) and slow upstrokes with high propagation velocities (in the direction of the long cell axis). Consequently, the usual association of high velocity and high safety factor for propagation is reversed by this anisotropic phenomenon: Propagation at a low velocity is actually more resistant to disturbances in the membrane ionic properties than is propagation at a higher velocity.

To account for this previously unrecognized direction-dependent behavior of depolarization, we proposed the idea of anisotropic discontinuous propagation. In addition to the classical influence of the membrane ionic properties on conduction, this hypothesis proposes that anisotropic structural complexities have an independent role in determining the functional properties of cardiac muscle and thereby provide new mechanisms for the conduction disturbances that cause reentry, e.g., very slow conduction in the transverse direction. We suggested that recurrent discontinuities of effective axial resistivity can produce results that change the shape of the action potential in the right direction.6 The presence of notches at the peak of the depolarization phase of action potentials when propagation occurs in the transverse direction (but not in the longitudinal direction) suggests that propagation is dis-
continuous in that direction. Notches might be expected when the intracellular current has to pass through localized regions (cell junctions) of high resistance relative to the cytoplasm. We argued that such junctions would separate the active membrane into patches loosely coupled to each other, each patch generating an action potential that would be expected to be closer to a membrane action potential than those propagating in a continuous structure.

The Problem

Thus far, however, computer stimulations by ourselves and others have failed to explain the anisotropic behavior of the upstroke of the cardiac action potential. One example is the use of a discretized cable to represent the discrete pattern of electrical coupling between cells. This model uses a large spatial increment (i.e., large size for each isopotential patch of membrane) with a high value of internal resistance to increase $V_{\text{max}}$ above that of a continuous cable.\[6\] Similar theoretical results are produced by a cable model of cylindrical cardiac cells interconnected by an intercalated disk structure.\[1-10\] As noted previously, both discretization and a recurrent disk structure are quite incomplete models of anisotropic discontinuous propagation.\[12,13\]

Although $V_{\text{max}}$ increases, the foot of the action potential is prolonged, just the opposite of the change observed experimentally.

Experimental evidence that fits such a one-dimensional discretized model has been obtained recently by exposure of sheep Purkinje strands to heptanol, which increases $r_i$ (resistance per unit length) by producing electrical uncoupling between cells. Sicouri et al\[14\] observed that when heptanol produced a marked reduction in the conduction velocity, it also induced "foot" potentials; i.e., the foot of the action potential prolonged markedly. This experimental change of the foot is also the opposite of that observed when the velocity decreases due to a change in the direction of propagation. Thereby, the results of Sicouri et al\[14\] provide evidence that the mechanism that changes the shape of depolarization when the velocity decreases due to increased resistance of the intercellular connections is different from the mechanism that changes the shape when the velocity decreases due to the increase in $r_i$ associated with a change in the direction of propagation.\[6\]

Another possible mechanism for the directional differences in the shape of the upstroke is that of direction differences in axial impedance, which could result from the presence of a capacitance parallel to the internal resistance at the intercalated disk.\[15\] We performed simulations to determine the effect of differences in internal impedance vs. a pure internal resistance by adding a capacitance parallel to the internal resistance: the added capacitance increased the conduction velocity, but it had no effect on the shape of the action potential (J.D. Sloan and M.S. Spach, unpublished observations).

Finally, a two-dimensional model based on the complex distribution of cellular connections has been suggested by Geselowitz and Miller.\[16\] They noted that during transverse propagation "each cell will be excited by colliding waves moving in opposite directions along the cell axis" (thus increasing $V_{\text{max}}$). The results of our recent simulations and experiments failed to justify a model of collisions at a microscopic level. During a real collision, the foot of the upstroke does not change, and the shape of the phase-plane plot of $V_n$ vs. $V_m$ of a collision does not fit that of an action potential measured during transverse propagation.\[13\] That is, during a collision the rate of rise of the upstroke does not increase until approximately the time of turn-on of the sodium current, while during transverse propagation the rate of rise increases from the onset of the foot.

Thus, it now seems clear that models based solely on recurrent discontinuities of intracellular resistance due to the nonhomogeneous distribution of the cellular connections do not give a full accounting of the anisotropic behavior of the depolarization phase of the cardiac action potential. We have found, however, by means of repeated computer simulations and experimental measurements of propagating depolarization on a microscopic scale that the direction-dependent shape changes behave as though there is a directional difference in the "effective" membrane capacitance.

In this paper, we test the hypothesis that there are anisotropic differences in the effective membrane capacitance that is filled by the upstroke of action potentials propagating in different directions in cardiac muscle. For this, we performed a combined experimental-theoretical analysis of the anisotropic propagation behavior of action potentials under normal conditions and under conditions that modify the sodium conductance. We measured directional differences in the shape of the upstroke and in conduction velocity in cardiac muscle bundles when the preparations were exposed to use-dependent sodium channel blocking drugs and when premature action potentials were initiated at progressively less negative take-off potentials.

We also performed computer simulations in an attempt to fit the measured directional differences in shape with the results of a simplified theoretical model using continuous cables that differed in the values of membrane capacitance and in the values of internal resistance. We reduced $G_{\text{Na}}$ to mimic drug blockade of the sodium channels and changed the take-off (holding) potential to simulate early premature action potentials. The results show that an anisotropic propagation system with directional differences of the effective membrane capacitance, combined with directional differences in effective axial resistivity, provides an excellent theoretical fit of the nonhomogeneous propagation response found when the sodium conductance is modified by use-dependent drug blockade of the sodium channels and by changes in the take-off potential.

Materials and Methods

Experiment

We studied specimens from 29 human subjects whose ages varied between 1 and 72 years. These age
ranges provided preparations with uniform and non-uniform anisotropic electrical properties. After approval of investigational protocols by an institutional committee for guidelines for human subject research, right atrial specimens were obtained at cardiac surgery prior to artificial pumping of blood for circulatory assist. Surgery was performed for the following conditions: coronary artery disease (12, ages 43–68 years), Wolff-Parkinson-White syndrome (8, ages 8–43 years), and congenital heart disease (9, ages 1–62 years). Right atrial hypertrophy was evidenced in the P waves of the electrocardiogram in 4 patients (ages 15–62 years) with an atrial septal defect. None of the remaining subjects had evidence of hemodynamic or electrophysiologic dysfunction of the right atrium; i.e., the electrocardiographic P waves were normal and the right atrial pressure was normal at cardiac catheterization.

Successful experiments were conducted in 21 of the human specimens, 9 from younger subjects (1–28 years) and 12 from older subjects (43–68 years). All of these viable specimens were larger than 1 cm² in area; none of the other preparations responded to stimulation and all were less than 1 cm² in size. In 4 preparations, data beyond that presented here were obtained for a recent analysis of the relation between extracellular and transmembrane potential derivatives during anisotropic propagation at a microscopic level. To make certain that the results had general applicability (i.e., they were not species specific), we also studied atrial and ventricular specimens from 24 adult dogs.

Each human specimen was transported to the laboratory in cooled perfusate solution at 2–5°C, pinned to the floor of a rectangular tissue bath (4 cm × 5 cm), and maintained at 35°C. The composition of the perfusate, in mM, 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 mixture of 95% O₂–5% CO₂ and perfused through a cannula to produce a high flow rate. To measure the anisotropic conduction velocities, we used extracellular electrodes made of flexible tungsten wires, 50 μm in diameter, and insulated except at the tip. The flexible nature of the electrodes was important so that the tip did not shift position when vigorous contractions occurred during drug exposure (11 human and 12 dog preparations) and after premature stimuli (14 human and 12 dog preparations). A dissecting microscope equipped with a Nikon F250 35-mm camera was used to document each recording position. Comparison of normal and premature action potentials depended on propagation being initiated at the stimulus site; therefore, we used additional electrodes to verify that propagation began within 200 μm of the tip of the stimulus electrode.

Each extracellular electrode was connected to an AC-coupled differential amplifier, having a frequency response flat between 0.1 and 30,000 Hz. Intracellular potentials were recorded with conventional glass microelectrodes filled with 3 M KCl and having resistances between 15 and 25 MΩ. The separate reference electrodes for each extracellular and intracellular electrode were located 7 cm from the recording area. The mechanical motion of the preparations remained vigorous for 4 to 10 hours. To measure the effects of sodium channel-blocking drugs on conduction velocity, quinidine gluconate 5–10 μg/ml or lidocaine HCl 5–10 μg/ml was added to the solution in the reservoir and the conduction times between 2 electrodes aligned along the long axis of the fibers and 2 along an axis transverse to the long fiber axis were monitored simultaneously as the drug concentration at the preparation increased over 20 minutes (the equilibration time of the tissue bath). To measure directional differences in the shape of the upstroke of the action potential at different stimulus rates during drug exposure and with premature beats, 1 stimulus electrode was positioned to produce propagation in the longitudinal direction and another electrode was positioned to produce transverse propagation at the intracellular impalement site. Thereby, although the velocities in the longitudinal and transverse directions were measured simultaneously during an intervention, the directional differences in the shape of the upstroke were measured asynchronously by switching from one stimulus electrode to the other and by repeated measurements to make certain that reproducible shape differences occurred. (We were not able to maintain 2 stable intracellular impalments for simultaneous measurements of $V_n$ with longitudinal and transverse propagation; one or the other intracellular impalement dislodged before a complete drug or premature beat sequence could be finished.)

A pacemaker stimulus of 1.0 msec in duration and 1.5 times threshold was applied to the endocardial surface of each muscle bundle at a basic cycle length varying between 250 and 1,000 msec. A PDP-11/44 computer system controlled the rate and synchronized the regular pacing and premature stimuli with the data recording. The outputs of the recording amplifiers were sampled at rates between 7,000 and 50,000 per second (12-bit samples). The computer stored the data and displayed the waveforms concurrently on a Tektronix 4014 unit (Beaverton, Ore.) with a persistent screen. The outputs of each amplifier were also displayed on a Tektronix 565 analog oscilloscope.

After each experiment, the measured waveforms stored digitally were redisplayed and photographed for initial analysis. After appropriate waveforms were selected, they were transferred to a HP-9000 computer for detailed analysis and automatic plotting of the waveforms and their derivatives, as well as for constructing digital phase-plane plots. The transmembrane potential $V_n$ was calculated by subtracting the extracellular potential from the intracellular potential as described previously. The first time derivative of $V_n$ was obtained numerically and the values were plotted in time steps of 20, 25, or 50 μsec. To minimize the sensitivity of the derivatives to the slight noise in the original waveforms, we used established techniques for finding the derivatives of slightly smoothed data. The maximum value of the first time derivative
Theory

The goal of the theoretical analysis was to determine if a simplified cable model based on differences in the membrane capacitance could predict the observed directionally dependent behavior of the upstroke (\(V_{\text{up}}\), \(V_{\text{max}}\), and the effective conduction velocity) in response to modification of the sodium conductance. For the purposes of this study, therefore, our model does not include the details of discontinuities of resistance that produce notches at the peak of the upstroke during transverse propagation; rather, it represents an averaged description of the tissue.

We simplified the problem as much as possible by using a single transient sodium current mechanism with the equivalent electrical circuit of a continuous cable. This conduction model is representative of a plane wave propagating in a two-dimensional group of tightly coupled cells. We used a macroscopic rather than a single-channel description of the sodium current because propagated action potentials are determined by the macroscopic currents as a function of time and space. As noted in a recent analysis, we considered that models in the form of the Hodgkin-Huxley equations for the macroscopic sodium current were most appropriate because of the small time scale they describe and fit. We chose the Ebihara-Johnson description of the fast sodium current because it is based on voltage clamp data in cardiac muscle under normal conditions.

For propagation in one dimension along a uniform structure, the time course of \(V_m\) is a function of time or space and given by the cable equation:

\[
I_n = (a/2R_c)(\partial^2 V_m/\partial x^2) = C_m \partial V_m/\partial t + I_{on},
\]

(1)

where \(a\) is the radius of the cylinder (in cm), \(R_c\) is the internal resistivity (ohm-cm), and \(C_m\) is the specific membrane capacitance (\(\mu F/cm^2\)). We approximated the ionic current \(I_{on}\) (\(\mu A/cm^2\)) during the depolarization phase of the action potential by the fast transient sodium current parallel with a leakage (repolarizing) current:

\[
I_{on} = g_{Na}(V_m - V_{Na}) + g_L(V_m - V_L),
\]

(2)

where \(g_{Na}\) is the sodium conductance \(mS/cm^2\), \(g_L\) is the leakage conductance \(mS/cm^2\), and \(V_{Na}\) and \(V_L\) are the sodium and leakage equilibrium potentials, respectively.

The sodium conductance \(g_{Na}\) per unit area was given by

\[
g_{Na} = \bar{G}_{Na} m h,
\]

(3)

where \(\bar{G}_{Na}\) is the maximum sodium conductance, and the dimensionless activation and inactivation variables \(m\) and \(h\) were assumed to follow the kinetics described by Ebihara and Johnson.

For the results to be presented here, we chose a value of 33.4 mV for \(V_{Na}\). There were no qualitative differences in the results when \(V_{Na}\) was varied from 25 mV to 76 mV, a range consistent with observations following changes in internal sodium concentration in ventricular muscle and in Purkinje fibers. \(g_L\) was held constant at 0.05 mS/cm² and \(V_L\) was adjusted to the individual values of the resting potential; i.e., the leakage current was zero at the take-off (hold) potential. To simulate the effect of use-dependent drug blockade of the sodium channels, the take-off potential was held constant at \(-80\) mV, and the value of \(G_{Na}\) was reduced in steps from 35 mS/cm² to 9 mS/cm². To mimic early premature action potentials, \(G_{Na}\) was maintained constant at a value of 35 mS/cm² and the hold potential was varied between \(-80\) mV and \(-50\) mV.

A membrane capacitance of 1.0 \(\mu F/cm^2\) and an internal resistivity of 190 ohm-cm were used in a continuous cable to simulate propagation in the longitudinal direction. Another cable with values of 0.5 \(\mu F/cm^2\) and 4,990 ohm-cm for \(C_m\) and \(R_c\), respectively, was used to represent transverse propagation. We selected the longitudinal \(R_c\) value from experimental results available from other species and preparations.

Since there is no appropriate model to base an extraction of \(R_c\) values in the transverse direction, we selected transverse \(R_c\) values to produce the appropriate low conduction velocities in that direction.

Equation 1 was solved over a distance considerably greater than 15 to 20 resting space constants of a cylinder with a length of 3.6 mm and a radius of 5 \(\mu m\) (approximate to a cell diameter). The time course of \(V_m\), \(g_{Na}\), and \(I_{on}\), along with the conduction velocity, were computed at the midpoint of the cable where there was uniform conduction (i.e., no end effects).

The ordinary differential equations for \(m\) and \(h\) were solved by the predictor-corrector or modified Euler method, with a time increment of 2 \(\mu sec\). The cable equation (Equation 1) was solved by the Crank-Nicolson implicit method, using a length increment (\(\Delta x\)) of 0.014 resting space constants.

The values were computed and stored on an HP-9000 computer for detailed analysis and plotting of the waveforms. The first-time derivatives of \(V_m\) were obtained numerically from the computed values of the transmembrane potential. The time course of \(V_m\), \(V_{Na}\), \(g_{Na}\), and \(I_{on}\) were plotted for each time instant at 20 \(\mu sec\) intervals. To determine the total sodium conductance and the total sodium current, the areas of the curves were obtained by numerical integration. For additional analysis of the waveforms, phase-plane graphs were made of \(V_m\) vs. \(V_{Na}\), and the trajectories of...
Anisotropic Differences in Shape of Upstroke

Results

We present experimental results primarily for human preparations; we found no species differences, and the changes in the shape of the upstroke were similar in both uniform and nonuniform anisotropic preparations in response to the same interventions. The terms "uniform" and "nonuniform" anisotropic properties are used as described previously for the electrical properties of human and dog preparations.\(^1\)\(^7\)\(^\textit{a}\)\(^\textit{b}\)\(^\textit{c}\) Uniform anisotropic properties characterized \(9\) human atrial preparations, all from subjects less than \(29\) years old, and \(12\) dog right ventricular papillary muscles.

The \(12\) human preparations from subjects between \(43\) and \(68\) years of age all demonstrated nonuniform anisotropic properties, and the \(68\) crista terminalis atrial preparations from adult dogs. All of the preparations had resting potentials more negative than \(-77\) mV before interventions. The least negative take-off potential that produced a propagated response was \(-63\) mV in both human and dog preparations, thus allowing us to interpret the mechanism of depolarization of all action potentials to be the fast sodium current.

"LP" is used to mean propagation in the direction along the longitudinal axis of the fibers and "TP" to mean propagation in the direction along an axis transverse to the long axis of the fibers. To facilitate comparison of the experimental and theoretical results, typical anisotropic changes in the experimentally measured upstrokes are presented with the corresponding simulation results for the two theoretical cables, one with a \(C_m\) value of \(1\) \(\mu\)F/cm\(^2\) to mimic longitudinal propagation \((C_m.1)\) and the other with a \(C_m\) value of \(0.5\) \(\mu\)F/cm\(^2\) to mimic transverse propagation \((C_m.5)\).

\[\text{Anisotropic Differences in Shape of Upstroke of } V_u \text{ and Associated Effects on Nonmeasurable Variables } g_{\text{Na}} \text{ and } I_{\text{Na}}\]

Figure 1A shows a typical experimental result from a single-cell impalement during longitudinal and transverse propagation in an atrial bundle from a 49-year-old subject. The rate of rise and amplitude of the upstroke were greater during transverse propagation than during longitudinal propagation, as expected.\(^6\) Also, initial repolarization was more rapid with transverse propagation (Panel A, column 1). The phase-plane plots of the measured upstrokes (Panel A, column 3) show that the slope of the initial trajectory of \(V_u\) was greater with transverse than with longitudinal propagation; i.e., the rate of rise of the foot of the action potential was greater with transverse than with longitudinal propagation. An initial linear trajectory of the phase-plane plot (dashed lines in Panel A, column 3) indicates a process that is exponential in time.\(^19\)\(^20\) A close approximation of this behavior occurred during transverse propagation in all preparations. However, in the preparations with nonuniform anisotropic properties from subjects over \(42\) years old, there were small but definite deviations (concavity up) from a straight line during longitudinal propagation, as can be seen for LP in Figure 1A3.

The simulation results for the upstrokes of the theoretical action potentials and the associated time course of the sodium conductance and sodium current are shown in Panels B and C of Figure 1. The experimental directional differences of \(V_{\text{max}}\), \(V_{\text{peak}}\) and \(I_{\text{rep}}\), as well as the initial phase of repolarization, were all reproduced by the computed action potentials of the two theoretical cables with different values of the membrane capacitance (Figure 1B). The more rapid initial repolarization associated with cable \(C_m.5\) was accounted for by the greater repolarization current \(I_{\text{rep}}\) that occurred with the more positive peak of the upstroke; i.e., \((V_u - V_r)\) was greater during initial repolarization when the peak value of the upstroke was more positive. (When \(g_{\text{Na}}\) was assigned a value of zero, there was no repolarization of \(V_u\) from the peak value of the upstroke.)

Figure 1C shows the influence of the different shapes of the upstroke on the nonmeasurable internal membrane variables \(g_{\text{Na}}\) and \(I_{\text{Na}}\). Although the peak values of \(g_{\text{Na}}\) showed little difference, the area of the \(g_{\text{Na}}\) curve was \(16\)\% greater for the cable that had the slower upstroke \((C_m.1)\) in Panel C, column 1). Also, in shifting from cable \(C_m.1\) to cable \(C_m.5\), the increase in \(V_{\text{peak}}\) was associated with a decrease in peak \(I_{\text{rep}}\) (\(-27\)% and in the area of the \(I_{\text{rep}}\) curve (\(-44\)% (Panel C, column 2). This relationship between the sodium current and \(V_{\text{max}}\) is opposite to that of the classical one; i.e., decreases in the sodium current are generally considered to be associated with decreases in \(V_{\text{max}}\) such as occur with ischemia, drugs, and premature beats.

The phase-plane plots of \(V_u\) vs. \(V_u\) (Figure 1, A3 and B3) and the trajectory of \(g_{\text{Na}}\) as a function of \(V_u\) (Panel C, column 3) provide insight to the mechanism of the differences in the shape of the upstroke and in the time course of the internal membrane variables. The major difference was that the total electrical load in cable \(C_m.1\) was greater than in cable \(C_m.5\). That is, the sodium current in cable \(C_m.1\) had to discharge more local as well as more downstream capacitance through local circuit currents (electrotonus). Thereby, the area of the \(I_{\text{rep}}\) curve serves as an index of the total capacitance (local and downstream) discharged by the sodi-
FIGURE 1. Typical experimentally measured upstrokes of $V_m$ during longitudinal and transverse propagation (A), computed upstrokes for two theoretical cables identical except for different membrane capacitances of 1 and 0.5 $\mu F/cm^2$ (B), and influence of different shapes of upstroke on sodium conductance and sodium current (C). In A and B, time course of $V_m$ is shown in column 1 and of $V_m$ in column 2, and phase-plane plots of $V_m$ vs. $V_m$ are shown in column 3. Action potentials in A were measured in atrial bundle from 49-year-old subject. LP = longitudinal propagation; TP = transverse propagation. In C, columns 1 and 2 show time course of sodium conductance and sodium current, and trajectory of $g_{Na}$ as a function of $V_m$ is shown in column 3.

Evidence for Anisotropic Distribution in Membrane Uptake of Use-Dependent Sodium Channel Binding Drugs

Theoretical Considerations. The difference in the areas under the $g_{Na}$ curves associated with upstrokes of different shapes (Figure 1) suggests that there should be an anisotropic distribution in the membrane uptake of use-dependent sodium channel binding drugs which, in turn, should produce selective directional effects on conduction. This prediction arises from the fact that the area under the $g_{Na}$ curve reflects the total open time of the sodium channels. Thus, the greater the total open time of the sodium channels, the greater the access to the binding sites within sodium channels by use-dependent channel blocking drugs. It follows that the uptake of quinidine should be greater with longitudinal propagation than with transverse propagation because the total open time of the sodium channels would be greater during the slower upstroke than during the faster upstroke (cf. $C_m1$ with $C_m.5$ in Panel C, column 1 of Figure 1).

Drug blockade might amplify the directional differences in the shapes of the upstroke, and the greater drug binding with longitudinal propagation due to the longer duration of depolarization might in itself cause further prolongation of depolarization. A simulation of propagation incorporating the drug binding process would presumably show this increased prolongation. To simplify our calculations, we assumed that the processes were independent and approximated the combined effect by a two-step process. We computed the ratio of areas under the $g_{Na}$ curves as a function of $G_{Na}$ and, assuming drug binding proportional to the area, multiplied the velocity curve (dotted curve in Figure 2A2) by this additional correction factor to obtain the upper solid curve in the figure. The simulation results (Figure 2A1) show that the relative difference between the areas under the $g_{Na}$ curves increased as $G_{Na}$ decreased in each cable; e.g., the $C_m1/C_m.5$ ratio of the $g_{Na}$ areas increased from 1.16 to 1.36 as the normalized value of $G_{Na}$ decreased from 1.0 to 0.29 (35 mS/cm^2 to 10 mS/cm^2). Propagation did not occur in cable $C_m1$ when $G_{Na}$ was reduced below 10 mS/cm^2, but stable propagation continued in cable $C_m.5$ when $G_{Na}$ was reduced to 9 mS/cm^2. (This is a simple theoretical illustration of the greater safety factor due to a lower effective membrane capacitance.)

The simulation results of Figure 2A2 show that for
Experimental analysis. We used quinidine gluconate to test the above theoretical predictions that this use-dependent sodium blocking drug should alter the ratio $\theta_L/\theta_T$, reflecting a selective distribution of drug blockade of the sodium channels (due to the different shapes of the upstrokes). Changes in this ratio also occur with changes in stimulus rate, presumably due to intracellular ionic changes that alter nexal resistance. Therefore, before exposing each preparation to drugs, we measured the effective conduction velocity in the longitudinal and transverse directions ($\theta_L$ and $\theta_T$) when the stimulus rate was increased from 60 to 150/min. $V_m$ remained constant (<4% change) while there was a greater relative decrease in $\theta_L$ (7–12%) than in $\theta_T$ (0–4%). The time constants of conduction velocity change during the recovery process varied between 25 and 90 seconds, values similar to our previous results in canine ventricular muscle.

Figure 2B shows a representative result during exposure to quinidine gluconate (10 $\mu$g/ml) of an atrial preparation from a 66-year-old subject. After a steady state was reached at a constant stimulus rate of 60/min, the rate was abruptly increased to 120/min (time zero). Both $\theta_L$ and $\theta_T$ initially decreased in an exponential manner (solid lines of Panel B, column 1), and the relative decrease in conduction velocity was greater in the longitudinal direction than in the transverse direction. With increasing time at the higher rate, $\theta_L$ did not decrease beyond 88% of its original value while $\theta_T$ continued to decrease to 74% of its original value at 85 seconds (Figure 2B2). This difference in the relative decreases in longitudinal and transverse conduction velocities was more than could be accounted for by an equal decrease in the number of sodium channels in each direction; i.e., by the same decrease in the value of $G_{Na}$ for the 2 theoretical cables (lower 2 curves of Figure 2A2). However, when the $g_{Na}$-area-effect was included for selective directional differences in drug uptake due to the directional differences in the shape of the upstroke, the considerably greater relative decrease in longitudinal than transverse velocity was accounted for ("area effect" in Figure 2A2).

Another consistent feature following a step change in the stimulus rate was that the time constant of conduction velocity change was greater for longitudinal than transverse propagation. For example, in Figure 2B1 the time constants were 5.9 and 7.4 seconds, respectively, for the $\theta_L$ and $\theta_T$ decreases following a step increase in stimulus rate from 60 to 120/min. During the recovery process at 60/min, the time constants were 4.5 seconds for $\theta_L$ and 14.3 seconds for $\theta_T$ (not shown). These directional differences in the time constants of velocity change provide evidence for selective regional differences in the kinetics of use-dependent sodium channel binding due to the anisotropic differences in the shape of the upstroke.

Kadish et al recently showed that procainamide produces a greater relative decrease in longitudinal than transverse conduction velocity in dog ventricular muscle, which they suggest may result from greater drug-induced changes in $r$, for longitudinal than trans-
verse propagation secondary to "an effect of procainamide on junctional resistivity." We have previously predicted such directional differences on the basis of anisotropic differences in the upstroke rather than differences in $r_c$. The present findings support this hypothesis:

1. The "$g_{Na}$-area-effect" at increasing levels of drug blockade is large enough to account for the anisotropic differences of conduction velocity change; i.e., additional mechanisms were not required.

The cytoplasmic resistivity rather than the nexal resistance is the major determinant of $r_c$ along the long axis of the fibers. To explain a greater effect of the drug on conduction in the longitudinal than in the transverse direction, a drug-induced change in cytoplasmic resistivity would be required rather than one of nexal resistance. The converse would seem more likely.

2. Lidocaine HCl (10 $\mu$g/ml) produced use-dependent anisotropic differences in propagation velocity similar to those of quinidine. With lidocaine, the time constants of $\theta_1$ and $\theta_2$ change following step increases or decreases in stimulus rate were less than 2-3 seconds. During quinidine exposure of 21 preparations, the time constants of use-dependent recovery changes of $\theta_1$ and $\theta_2$ varied between 4 and 15 seconds. These time constants of conduction velocity change were in the range of those previously reported for the use-dependent effects of quinidine and lidocaine on the sodium carrying system ($V_{Na}$). Thus, all the time constants of recovery following drug exposure was much shorter than those following changes in rate (25 to 90 seconds), the latter being in keeping with changes in $r_c$.

Moreover, predictions based on directional differences of $C_m$ fit the measured anisotropic differences of $V_{max}$ during use-dependent drug uptake. Figure 3A shows a representative experimental result of the directional differences in $V_{max}$ measured during a single-cell impalement in a dog papillary muscle exposed to quinidine gluconate. When the stimulus rate was increased to 120/min at a concentration of 7 $\mu$g/ml, both longitudinal and transverse $V_{max}$ values decreased to new steady-state values (Figure 3A1). However, $V_{max}$ consistently decreased relatively more with longitudinal than transverse propagation; i.e., the average $V_{max}$ TP/LP ratio increased from 1.77 to 2.03 (Figure 3A2). When the concentration of quinidine gluconate was increased to 10 $\mu$g/ml at a stimulus rate of 60/min, $V_{max}$ continued to decrease more in the longitudinal direction than it did transversely, and the average $V_{max}$ TP/LP ratio increased to 2.37 (Figure 3A2). After the stimulus rate was increased to 133/min at this higher drug concentration, there was a continued greater relative decrease in $V_{max}$ in the longitudinal direction as the average $V_{max}$ TP/LP ratio increased to 2.70.

Figure 3B shows that the measured directional differences in $V_{max}$ were reproduced by the simulated action potentials of the two theoretical cables with different values of $C_m$. Without the $g_{Na}$-area-effect, the lowest value of $G_{Na}$ that sustained propagation in both cables resulted in a maximum increase from 1.52 to 1.78 in the $V_{max} C_m$ 5/C_m 1 ratio, an increase too small to account for the measured differences in $V_{max}$. However, when the $g_{Na}$-area-effect was included to account for selective regional drug blockade, the computed $V_{max} C_m$ 5/C_m 1 ratio increased by 0.97, an amount comparable to the 0.93 increase in the experimental $V_{max}$ TP/LP ratio. Hence, we conclude that the directionally different response of the conduction velocity and $V_{max}$ was due to greater drug blockade of the sodium channels during longitudinal propagation than during transverse propagation which, in turn, could be attributed to the directional difference in the shape of the upstrokes.

**Anisotropic Response to Modification of Sodium Conductance by Changing Take-off Potential**

Figure 4 shows typical directional differences in longitudinal and transverse conduction velocities measured in uniform (A) and nonuniform (B) anisotropic...
preparations. As the basic cycle length remained constant at a value between 1,000 and 800 msec, reductions in the premature interval produced little to no change in the directionally different conduction velocities until the premature interval was in the range of 400 to 440 msec. Any further shortening of the premature interval produced a decrease in both $\theta_0$ and $\theta_T$ (column 1). However, there was consistently a greater decrease in the normalized values of longitudinal than transverse conduction velocity (column 2). In both types of preparations, the difference between the two became progressively greater as the premature interval approached the absolute refractory period, longitudinal propagation failure occurred while stable propagation continued in the transverse direction (Figure 4B), a phenomenon previously described for nonuniform anisotropic dog cardiac muscle.\(^5\) In these preparations, the longitudinal conduction velocities of the earliest propagated responses were 47 to 55% of the control values, while simultaneously the transverse velocities were 60 to 70% of their original values. As the premature interval continued to decrease, slow but stable transverse propagation persisted in the absence of longitudinal propagation until $\theta_T$ decreased to approximately 50% of the control value; any further shortening of the premature interval resulted in transverse propagation failure (Figure 4B).

To simulate the effects of premature beats on the conduction velocities, it was assumed that shortening the premature interval produced a decrease in the conduction velocities by shifting the take-off potential to a less negative value.\(^5\) The following theoretical fit of the experimental results can be seen in Figure 4C: 1) The large difference in the computed values of the conduction velocities for the same take-off potential in the 2 theoretical cables (Figure 4CI) was due to the difference in the respective $R_e$ values of 190 ohm-cm (cable $C_m$) and 4,990 ohm-cm (cable $C_m$.5). Changing the value of $R_e$ between 100 and 6,000 ohm-cm in either theoretical cable altered the conduction velocity in relation to the reciprocal of the square root of $R_e$, but it had no effect on the shape of the upstroke nor did it alter the relative changes in conduction velocity in response to changes in the resting potential and to
changes in $G_m$. 2) The decrease in the normalized conduction velocity values was greater in the cable with the inherently slower upstroke ($C_m.5$ in Figure 4C2). 3) The greatest difference in the normalized conduction velocities of the 2 cables occurred at the least negative take-off potential that produced a propagated response. 4) Propagation did not occur in cable $C_m.1$ at take-off potentials less negative than $-55$ mV, but stable propagation continued in cable $C_m.5$ at less negative take-off potentials up to $-52$ mV. 5) The lowest conduction velocity that occurred in either theoretical cable was approximately 50% of the original highest conduction velocity. Also, when conduction failed at 50% of its original conduction velocity in cable $C_m.1$, the velocity in cable $C_m.5$ was 62% of its original value, values similar to the experimental longitudinal and transverse conduction velocity values when unidirectional longitudinal conduction failure occurred in the nonuniform anisotropic preparations (cf. Figure 4B).

Typical anisotropic changes in $V_{max}$ secondary to changes in the take-off potential are shown in Figure 5A for premature action potentials induced in an atrial preparation from a 43-year-old subject. At all take-off potentials, $V_{max}$ was greater with transverse propagation than with longitudinal propagation. As the take-off potential became less negative, the normalized values of $V_{max}$ decreased slightly more with longitudinal than with transverse propagation (Figure 5A2). Large changes in the take-off potential produced small changes in the $V_{max}$ TP/LP ratio at take-off potentials more negative than $-74$ mV. However, small positive shifts of the take-off potential at less negative values produced large increases in the $V_{max}$ TP/LP ratio (i.e., the slope was greater), and the increases became more pronounced as the take-off potential approached the value at which propagation failed (Figure 5A3). The least negative take-off potential that produced both longitudinal and transverse propagation was $-63$ mV; at take-off potentials between $-64$ and $-63$ mV, transverse propagation continued in the absence of propagation in the longitudinal direction (Figure 5A1), as occurred in the other preparations with nonuniform anisotropic properties (Figure 4B).

The simulation results (Figure 5B) reproduced all of the above features of the experimentally measured directional differences in $V_{max}$: 1) $V_{max}$ was greater in cable $C_m.5$ than in cable $C_m.1$ at all take-off potentials. 2) The decrease in the normalized values of $V_{max}$ was slightly greater in the cable with the inherently slower upstroke ($C_m.1$ in Figure 5B2). 3) The slope of the curve of the $V_{max}$ $C_m.5/C_m.1$ ratio vs. take-off potential was small at $-65$ mV, becoming quite steep at $-55$ mV. 4) Propagation did not occur in cable $C_m.1$ at take-off potentials less negative than $-55$ mV, but stable propagation continued in cable $C_m.5$ at more positive take-off potentials. In the theoretical curves, the take-off potential was $10-12$ mV more positive than in the experimental data. This probably reflects a shift in the voltage dependence of the kinetic parameters of the membrane model from the preparation in which they were originally measured (cultured chick embryonic heart cells).23

A similar experimental-theoretical test of the time constant of the foot of the upstroke is shown in Figure 6 for the same action potentials analyzed in Figure 5. The time constant of the foot was greater with longitudinal than transverse propagation at all take-off potentials (Figure 6A1). Large changes in the take-off potential at values more negative than $-74$ mV produced little change in the normalized values of $\tau_{foot}$ and in the $\tau_{foot}$ LP/TP ratio. However, at take-off potentials less negative than $-74$ mV, small positive shifts in the take-off potential produced greater increases in the normalized values of $\tau_{foot}$ with longitudinal propagation than with transverse propagation (Panel A, column 2). Further, the $\tau_{foot}$ LP/TP ratio increased progressively as the take-off potential approached the value at which propagation failed (Panel A, column 3).

The simulation results (Figure 6B) demonstrated the following fit of the $\tau_{foot}$ experimental results: 1) At all take-off potentials, $\tau_{foot}$ was greater in the cable with the greater membrane capacitance. 2) There was little change in the relative values of $\tau_{foot}$ of the action potent-

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Anisotropic changes in $V_{max}$ secondary to changes in take-off potential: Experimental results (Panel A) with corresponding theoretical predictions based on directional differences in $C_m$ (Panel B). Panel A shows effects of changes in take-off potential on the absolute (1) and normalized (2) values of $V_{max}$ during longitudinal and transverse propagation, as well as effect on ratio of $V_{max}$ values (3). Experimental results were obtained in atrial preparation from 43-year-old subject. Panel B shows corresponding theoretically predicted changes in $V_{max}$ as a function of take-off potential in two cables with different values of $C_m$ (changing $R$, had no effect on $V_{max}$).
A final test of the simplified model of directional differences of $C_m$ is shown in Figure 7 for the amplitude of the upstroke of premature action potentials measured during a single-cell impalement in a preparation from a two-year-old child. The amplitude of the upstroke was greater with transverse propagation than with longitudinal propagation at all take-off potentials (Panel A, column 1). As the take-off potential became less negative, the normalized values of $V_{m}^{\text{L}}$ decreased more with longitudinal than with transverse propagation. As occurred with $V_{m}^\text{max}$ and $\tau_{\text{foot}}$, large shifts in the take-off potential at more negative values produced little change in the relative difference in the $V_{m}^{\text{L}}$ values. At less negative values, however, the $V_{m}^{\text{L}}$ increased progressively with positive shifts of the take-off potential as the take-off potential approached the least negative value that resulted in a propagated response (Figure 7A3). In this preparation with uniform anisotropic properties, longitudinal and transverse propagation failed at the same take-off potential ($-67$ mV); i.e., there was no unidirectional longitudinal propagation failure as occurred in the older nonuniform anisotropic preparations.

The simulation results (Figure 7B) again reproduced the major features of the experimental data: 1) At all take-off potentials, the amplitude of the upstroke was less in the action potential with the inherently slower upstroke ($C_{m}^{\dagger}$ in Figure 7B1). 2) A given shift of the take-off potential to a less negative value produced a greater decrease in the normalized values of $V_{m}^{\text{L}}$ of the action potential with the slower upstroke ($C_{m}^{\text{dagger}}$ in Figure 7B2). 3) The slope of the relationship between the $V_{m}^{\text{L}}$ and the take-off potential increased with less negative take-off potentials, again becoming quite steep as the take-off potential approached the value at which propagation failed.

Discussion

The major point established by these combined experimental-theoretical results is that propagating depolarization in anisotropic cardiac muscle behaves as though there are directional differences in the effective membrane capacitance to be filled by the upstroke of the action potential. The results describe a relationship between the magnitude of the sodium current and $V_{m}^{\text{max}}$ for anisotropic (multidimensional) propagation that is diametrically opposite to the classical relationship for one-dimensional propagation where decreases in the magnitude of the sodium current reduce $V_{m}^{\text{max}}$ in proportion to the square of the conduction velocity.40 The results provide an anisotropic mechanism for the relationship between the sodium current, $V_{m}^{\text{max}}$, and the safety factor of propagation that is different from the traditional mechanism of spatial differences in the membrane ionic properties, e.g., differences in the refractory period4 and depressed segments of membrane.3 In addition, recent in vitro experiments suggest that our proposed anisotropic mechanism is important in establishing the directional differences necessary for unidirectional propagation block that leads to reentry at a small-size scale,13 and that spatial variations in the refractory period become more important in reentrant circuits on a large-size scale.5

Because of known cardiac structural differences between species,6,41,42 the question arises as to whether anisotropic differences in the shape of the upstroke only occur in a few species. Since originally reporting this phenomenon, termed "anisotropic discontinuous propagation,"6 we have performed additional experiments in cardiac preparations not only from dogs and humans but from cats, rabbits, and hamsters. Measurements in all of these preparations have confirmed that $\tau_{\text{foot}}$ is smaller and $V_{m}^{\text{max}}$ and the amplitude of the upstroke are greater during transverse propagation than during longitudinal propagation.

The major anisotropic differences in the shape of the upstroke occurred when the take-off potential approached the least negative value that produced a propagated response. Theoretically, this is explained by directional differences in the electrical load; there was...
a difference in the amount of local and downstream capacitance to be discharged for sufficient turn-on of the sodium current for stable propagation. We conclude that in the uniform anisotropic preparations, the tight electrical coupling between all groups of cells caused propagation to fail simultaneously in all directions when the take-off potential shifted beyond the one that produced a propagated response. In the non-uniform anisotropic preparations, however, electrical insulating boundaries exist between groups of cells, which may provide a way for propagation to continue in the transverse direction after failure in the longitudinal direction; i.e., to continue in the direction with the greatest safety factor (associated with the greater rate of rise and amplitude of the upstroke).

We have been unsuccessful in our attempts to make direct estimates of $C_m$ in the longitudinal and transverse directions in cardiac preparations, as done previously by Clerc, if for no other reason than the details of the cardiac structure in uniform and nonuniform anisotropic preparations have not been sufficiently resolved. However, we have performed computer simulations (unpublished) in an attempt to provide a full accounting for the directional differences in the shape and behavior of depolarization in response to modification of the available sodium conductance. In these simulations, we altered each parameter of the cable equation (Eq. 1) and in the equation describing the membrane ionic currents (Eq. 2 includes the Hodgkin-Huxley equations for the fast sodium current and the term for the leakage or repolarization currents). Changes in each variable produced changes in the conduction velocity and in the shape of the upstroke, but only when differences in the membrane capacitance were introduced did the theoretical predictions fit the experimental data.

We also tested the behavior of models of the discrete pattern of cell-to-cell coupling, such as the discretization model of Joyner and the model of Diaz et al., in which cylindrical cells are interconnected by an intersected disk structure. Both of these models increase $V_{m}$ above that of a continuous cable and thereby might have the same relationship to the continuous case that transverse propagation has with longitudinal propagation in experimental preparations. However, positive shifts of the take-off potential produced a greater relative decrease in the larger $V_{m}$ values of the discretized models than in the smaller $V_{m}$ values of the continuous case. Also, at a take-off potential of $-55$ mV, decremental conduction to extinction occurred in the transverse direction in the continuous case. Thus, models that include only discretization and recurrent disk structures produce results that go in the wrong direction compared to the experimental results when the sodium conductance is modified.

Having evaluated every possibility within the limits of our simulation capabilities, including models that involve multiple cables with discontinuities introduced by connecting the individual cables together at numerous sites, we have not found any way to account for the anisotropic differences in the shape and behavior of propagating depolarization in cardiac muscle other than by directional differences in the effective membrane capacitance. We believe that the extensive experimental verification of the theoretical predictions of our simplified model of directional differences of effective membrane capacitance, combined with the directional differences in effective axial resistivity, provides an important initial quantitative basis to account for the anisotropic behavior of propagating depolarization in response to modification of the sodium conductance.

In this paper, we have started with the simplest possible model, clearly an oversimplification of the actual tissue structure. We believe more realistic models will have to be developed in the future. The major question now is: how can the effective membrane capacitance be different in the same tissue when the direction of propagation is altered? No model or simu-
loration based on corresponding cardiac structural anisotropic differences is available in the literature. Although work in progress on such a model, the results of this paper indicate that directional differences in the effective membrane capacitance (or its equivalent) will have to be included, along with directional differences and discontinuities of effective axial resistivity, for a full accounting of the anisotropic behavior of propagation depolarization in uniform and nonuniform anisotropic cardiac muscle.

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